



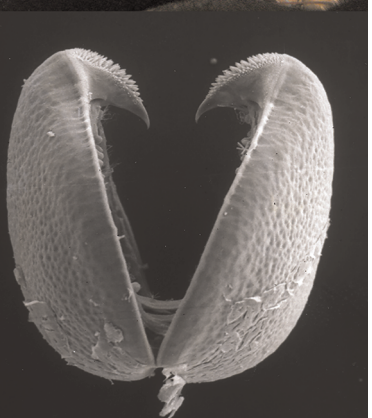

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REGULAR ARTICLE

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

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ABSTRACT

***Paetulunio fabalis* (formerly *Villosa fabalis*) has experienced a significant reduction in its range and is listed as endangered in both the USA and Canada. Little life history or demographic information exists 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 USA 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 (Fonnesbeck and Dodd 2003; Matter et al. 2013; 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.

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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 exists 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.

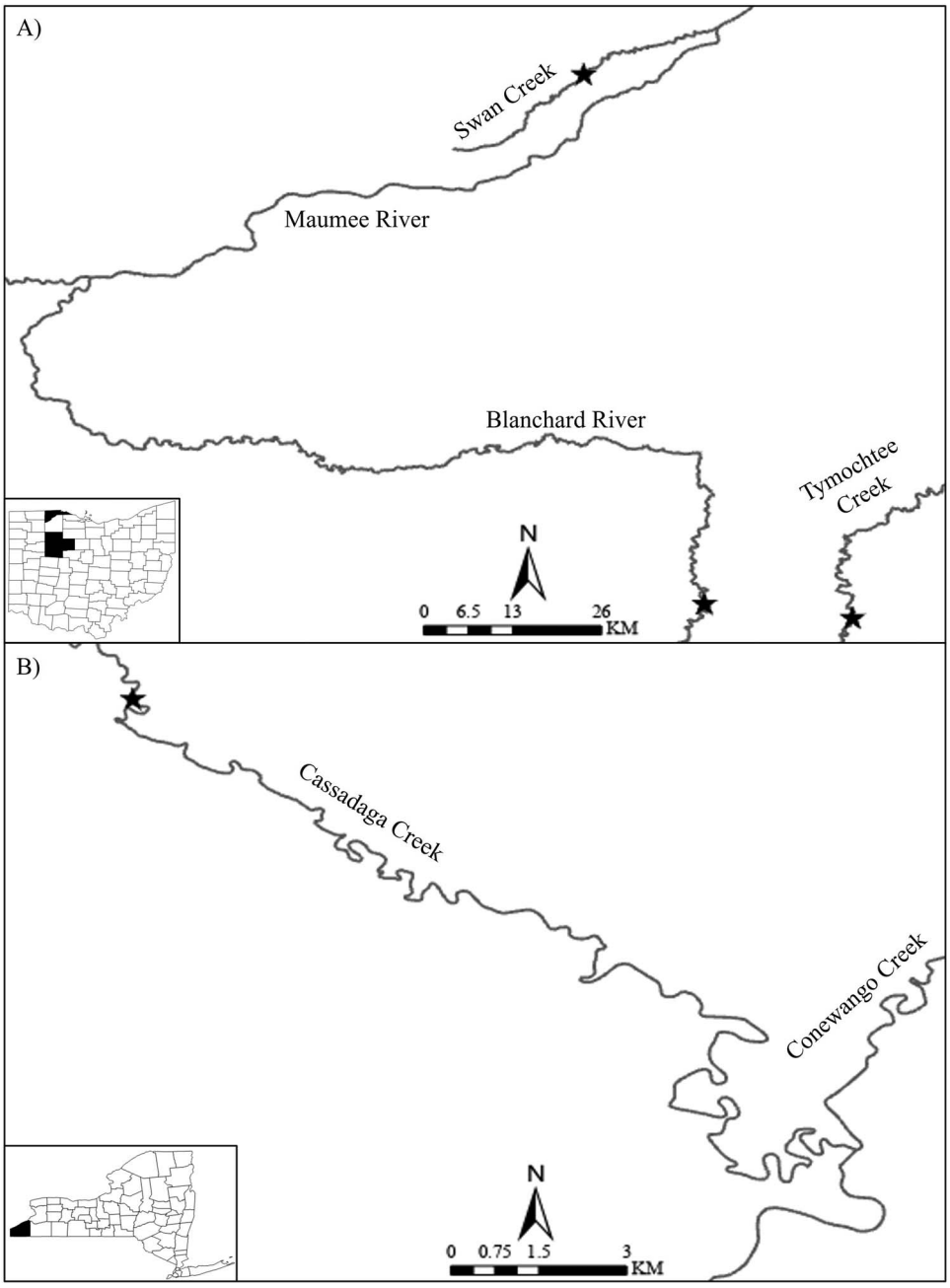

Figure 1. Location of study sites (stars) sampled for *Paetulunio fabalis*. Inset maps show the location of the study areas in (A) Ohio and (B) New York.

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

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 ferussacianus	—	—	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%)	—
Unidentified unionid	—	—	—	—	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

among sites according to habitat conditions and study goals (see below), 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 and 2010.

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 to 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

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

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 made up 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

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

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 Tymochtee 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, whereas 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. Nondetection 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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REGULAR ARTICLE

UNIONID MUSSEL DISTRIBUTIONS IN SOUTH DAKOTA, USA OBSERVED DURING A STATEWIDE SURVEY IN 2014–15

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ABSTRACT

We conducted a statewide survey of freshwater mussels (family Unionidae) in wadeable streams in South Dakota in 2014 and 2015. We conducted timed searches (2 person-hours/site) at 202 sites distributed among all 14 of the state's major river drainages. We collected a total of 605 live mussels and 543 recently dead shells, representing 13 unionid species. We found mussels in each of the 14 river drainages and at 91 of the 202 sites (45%), and we collected live mussels at 22% of the sites. Species richness varied among drainages from one to 10. Mussel species richness and abundance were higher in drainages east of the Missouri River (mean richness/site = 1.2 ± 0.1, mean abundance/site = 5.5 ± 1.5/h) compared with western drainages (mean richness/site = 0.2 ± 0.1, mean abundance/site = 0.4 ± 0.2/h). The Giant Floater was the most widespread and abundant species, occurring in all 14 river drainages and representing 62.1% of all live mussels. Overall, host generalists with an opportunistic life-history strategy dominated mussel assemblages in South Dakota, which may indicate stressful conditions, particularly in western drainages. A compilation of previous records from South Dakota revealed the former presence of 32 species in the state. However, because of differences in sample effort among studies, comparison of our estimates of species richness with estimates from previous surveys at specific sites and in six eastern drainages did not reveal consistent patterns of species loss. Our use of standardized timed-search methods provides a baseline that can be used to better assess future changes in species richness and distribution and mussel abundance.

KEY WORDS: Unionidae, survey, freshwater mussels, South Dakota

INTRODUCTION

Information about freshwater mussel (family Unionidae) distribution in South Dakota is limited. The first mussel surveys in the early 1900s were geographically restricted and provided little data (Coker and Southall 1915; Over 1942). Subsequent surveys focused mostly on larger streams in eastern South Dakota (Perkins 1975, 2009; Hoke 1983, 2003; Frest 1987; Perkins et al. 1995; Skadsen 1998; Perkins and Backlund 2000, 2003; Skadsen and Perkins 2000; Wall and Thomson 2004; Ecological Specialists 2005a, 2005b, 2007, 2012; Shearer et al. 2005). A total of 32 species has been documented east of the Missouri River, including three listed as endangered under the U.S. Endangered Species Act (Higgins Eye, *Lampsilis higginsii*; Scaleshell, *Potamilus leptodon*; Winged Mapleleaf, *Quadrula fragosa*; Table 1). No comprehensive, statewide survey of mussel distributions in South Dakota has been published. Such information is needed to better understand mussel distributions in the state and to serve as a baseline for monitoring future changes in the fauna (Strayer et al. 1994).

We report the results of the first comprehensive, statewide mussel survey of South Dakota. Our study is based on the unpublished survey of Faltys (2016), who sampled 202 sites distributed among all 14 major river drainages in the state. We report the results of this survey and compare our results with past surveys.

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Table 1. Comparison of mussel species occurrence and richness between this study (C = current, 2014–15) and previous surveys (P = 14 previous surveys, 1975–2012) in six river drainages in eastern South Dakota. Fish-host strategies are G, generalist and S, specialist (Haag 2012). Life-history strategies are O, opportunistic, P, periodic, and E, equilibrium (Haag 2012). L indicates species found live, FD indicates species found as recently dead shells, WD indicates species found as weathered dead shells, X indicates species presence but unreported condition, and—indicates that the species was not found. Superscripted numbers represent sources for previous surveys.

			Drainages											
			Big Sioux[1,2,5,7]		James[1,2,9,10]		Minnesota[4]		Missouri[6,8,11–16]		Red[4]		Vermillion[1,2,4]	
Species	Fish Host	Life-History Strategy	P	C	P	C	P	C	P	C	P	C	P	C
Alasmidonta marginata	G	P	X	—	—	—	—	—	—	—	—	—	—	—
Amblema plicata	G	E	L	FD	L	—	—	—	L	—	—	L	L	FD
Anodontoides ferussacianus	G	O	X	—	X	—	L	—	—	—	—	—	L	—
Arcidens confragosus	G	O	WD	—	X	—	—	—	WD	—	—	—	X	—
Cyclonaias pustulosa	S	E	X	—	L	—	—	—	L	—	—	—	—	—
Cyclonaias tuberculata	S	E	X	—	—	—	—	—	—	—	—	—	—	—
Fusconaia flava	S	E	X	—	X	FD	L	L	—	—	—	—	X	FD
Lampsilis cardium	S	P	X	—	X	—	X	—	—	—	—	—	X	—
Lampsilis higginsii	S	P	—	—	—	—	—	—	X	—	—	—	—	—
Lampsilis siliquoidea	S	P	L	L	X	L	L	L	L	—	X	L	X	FD
Lampsilis teres	S	O	FD	—	X	—	—	—	L	—	—	—	X	—
Lasmigona complanata	G	O	L	L	L	L	L	L	L	L	X	L	L	L
Lasmigona compressa	S	O	X	—	—	—	X	—	—	—	—	—	—	—
Ligumia recta	S	P	X	—	X	FD	—	—		—	—	L	X	—
Obliquaria reflexa	S	P	WD	—	FD	FD	—	—	—	—	—	—	—	—
Obovaria olivaria	S	P	FD	—	FD	—	—	—	—	—	—	—	—	—
Pleurobema sintoxia	S	E	X	—	X	—	—	—	—	—	—	—	X	—
Potamilus alatus	S	O	X	—	X	L	L	—	L	L	L	L	L	FD
Potamilus fragilis	S	O	L	FD	L	—	X	—	L	—	—	—	L	FD
Potamilus leptodon	S	O	—	—	—	—	—	—	FD	—	—	—	—	—
Potamilus ohiensis	S	O	L	—	L	—	X	—	L	—	X	—	X	—
Pyganodon grandis	G	O	L	L	L	L	L	L	L	L	L	L	L	L
Quadrula fragosa	S	E	WD	—	WD	—	—	—	—	—	—	—	—	—
Quadrula quadrula	S	E	L	—	L	L	—	—	L	L	—	L	L	—
Sagittunio subrostratus	S	O	FD	—	FD	—	—	—	X	—	—	—	—	—
Strophitus undulatus	G	P	X	—	FD	—	L	L	WD	—	—	—	L	—
Toxolasma parvum	S	O	L	—	X	—	X	—	L	—	—	—	X	—
Tritogonia verrucosa	S	E	L	—	X	—	—	—	—	—	—	—	—	—
Truncilla donaciformis	S	O	WD	—	FD	—	—	—	WD	—	—	—	—	—
Truncilla truncata	S	O	WD	—	L	L	—	—	L	—	—	—	L	—
Utterbackia imbecillis	G	O	—	—	—	—	—	—	L	—	—	—		—
Utterbackiana suborbiculata	G	O	—	—	—	—	—	—	L	—	—	—	—	—
Total Richness			28	5	25	9	12	5	20	4	5	7	18	7

[1]Coker and Southall (1915); [2]Over (1942); [3]Perkins (1975); [4]Perkins et al. (1995); [5]Skadsen (1998); [6]Perkins and Backlund (2000); [7]Skadsen and Perkins (2000); [8]Hoke (2003); [9]Perkins and Backlund (2003); [10]Wall and Thomson (2004); [11]Ecological Specialists, Inc. (2005a); [12]Ecological Specialists, Inc. (2005b); [13]Shearer et al. (2005); [14]Perkins (2007); [15]Ecological Specialists, Inc. (2007); [16]Ecological Specialists, Inc. (2012).

METHODS

Study Area

South Dakota lies entirely within the Great Plains region of North America. It contains 14 major river drainages and is bisected by the Missouri River (Fig. 1; Table 2). All river drainages in the state are within the Missouri River basin except for headwaters of the Minnesota River system (upper Mississippi River basin) and the Red River system (Nelson River basin) in the northeastern part of the state. Substantial

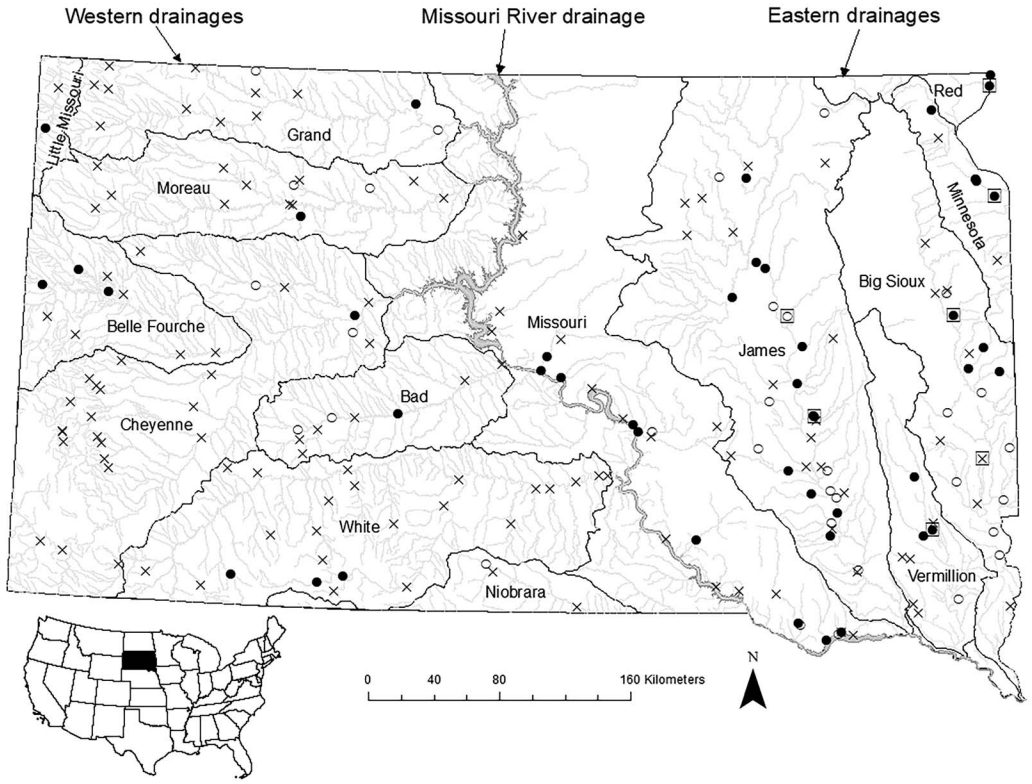

Figure 1. Sites surveyed for freshwater mussels in 14 river drainages in South Dakota in 2014–15. Solid circles indicate sites at which live mussels were found, open circles represent sites at which only recently dead shells were found, and x represents sites at which no evidence of mussel presence was found. Open square indicates historic resurvey site locations ($N = 7$). The inset map shows the location of South Dakota in the continental USA.

environmental and physical differences exist between the eastern and western halves of the state, and strong E-W precipitation and N-S temperature gradients produce distinct regional climates (Johnson et al. 2005). The six river drainages east of the Missouri River (eastern drainages) were glaciated during the Wisconsin glaciation. This area has a continental climate, and most of the original prairie has been converted to row-crop agriculture (Omernik and Griffith 2014; Gewertz and Errington 2015). The eight river drainages west of the Missouri River (western drainages) were not glaciated. This area has a semiarid climate, with rolling plains, buttes, and badlands, dominated by short-grass prairie, which is used primarily for livestock production (Sayler 2014). Streams in western South Dakota are prone to intermittency and flash flooding, whereas eastern South Dakota streams are more hydrologically stable (Chapman et al. 2001).

Mussel Surveys

We surveyed eastern drainages from June 4 to August 14, 2014 and western drainages from May 27 to July 27, 2015. We used ArcGIS (10.1/2012, ESRI, California) to randomly and proportionately select sampling sites on wadeable, perennial main stem (Missouri River) and tributary streams on the basis of watershed area. We sampled 102 sites in the six eastern river drainages, including the Missouri River, and 100 sites in the eight western drainages (Fig. 1). Sites where landowner permission could not be obtained or where there was a lack of flowing water were replaced with another randomly selected site within the same river drainage.

We conducted 2-person-hour timed searches at each site following DeLorme (2011). We began timed searches at the nearest access point and moved upstream. We searched the stream bottom for live mussels and empty shells using tactile searches and visual searches with a mask, snorkel, and viewing buckets. We collected all live mussels and recently dead shells and identified them using Cummings and Mayer (1992) and following taxonomy of FMCS (2021). At each site, we retained as vouchers up to two specimens of each species and deposited them in the South Dakota Aquatic Invertebrate Collection, South Dakota State University, Brookings, South Dakota.

For each site, we calculated species richness as the number of species represented by live individuals or recently dead shells. We expressed abundance as catch per unit effort (CPUE; number live/h). We categorized host use of each species as generalist or specialist, and we categorized life-history strategies as opportunistic, periodic, or equilibrium, both on the basis of Haag (2012).

We compared our results with those of previous surveys in three ways. First, we resurveyed seven previously surveyed sites to evaluate changes in the mussel fauna at those sites. All resurveyed sites were in eastern drainages of the Missouri River. We estimated the rate of change in species richness as (current richness − previous richness)/number of years since the previous

Table 2. Mussel species collected in all 14 river drainages of South Dakota in 2014 and 2015. Numbers in parentheses after drainage name indicate the number of sites sampled. L indicates species found live, X indicates species found only as recently dead shells, and—indicates that the species was not found. CPUE = catch per unit effort (number of live mussels/h). Relative abundance is reported for live mussels. Fish-host use was determined following Haag (2012) where G indicates host generalist and S indicates host specialist. Life-history strategies were determined following Haag (2012) where O indicates opportunistic, P indicates periodic, and E indicates equilibrium.

			Eastern Drainages						Western Drainages											
Species	Fish host	Life history Strategy	Big Sioux (20)	James (39)	Minnesota (6)	Missouri (26)	Red (2)	Vermillion (9)	Bad (9)	Belle Fourche (9)	Cheyenne (27)	Grand (13)	Little Missouri (2)	Moreau (14)	Niobrara (3)	White (23)	Number live & dead	Number live	CPUE	Relative abundance %
Pyganodon grandis	G	O	L	L	L	L	L	L	L	L	X	L	L	L	X	L	784	376	0.931	62.1
Fusconaia flava	S	E	—	X	L	—	—	X	—	—	—	—	—	—	—	—	103	94	0.233	15.5
Lasmigona complanata	G	O	X	L	L	L	L	L	—	L	—	L	—	—	—	—	141	54	0.134	8.9
Potamilus alatus	S	O	—	L	—	L	L	X	—	—	L	—	—	—	—	—	51	35	0.087	5.8
Lampsilis siliquoidea	S	P	L	L	L	—	L	X	—	—	—	—	L	—	—	—	56	20	0.049	3.3
Quadrula quadrula	S	E	—	L	—	L	L	—	—	—	—	—	—	—	—	—	15	13	0.032	2.2
Amblema plicata	G	E	X	—	—	—	L	X	—	—	—	—	—	—	—	—	8	6	0.015	1.0
Ligumia recta	S	P	—	X	—	—	L		—	—	—	—	—	—	—	—	4	2	0.005	0.3
Potamilus fragilis	S	O	X	L	—	—	—	X	—	—	—	—	—	—	—	—	4	2	0.005	0.3
Cyclonaias pustulosa	S	E	—	X	—	—	—	—	—	—	—	—	—	—	—	—	1	0	0.000	0.0
Strophitus undulatus	G	P	—	—	L	—	—	—	—	—	—	—	—	—	—	—	1	1	0.002	0.2
Truncilla truncata	S	O	—	L	—	—	—	—	—	—	—	—	—	—	—	—	1	1	0.002	0.2
Utterbackia imbecillis	G	O	—	—	—	L	—	—	—	—	—	—	—	—	—	—	1	1	0.002	0.2
Drainage richness			5	10	5	5	7	7	1	2	2	2	2	1	1	1	Total	Total		
Drainage CPUE			0.4	3.1	12.9	1.2	14.5	1.4	0.3	1.2	0.02	0.1	0.8	0.1	0	0.3	1148	605		

survey. Second, we compared drainage-wide richness estimates between our survey and 14 previous surveys that provided specific site locations (Table 3). Third, we compared general patterns of species distributions across drainages between our survey and previous surveys (Table 1).

RESULTS

We collected a total of 1,148 mussels (605 live and 543 recently dead shells) across all sites (Table 2; Fig. 1). We detected live or recently dead mussels in all 14 river drainages. Live mussels were observed in all river drainages except the Niobrara and at 45 of 202 sites (22%). We found only recently dead shells at an additional 46 sites (23%) and we found no mussels at 111 sites (55%). We found a total of 13 species, including 12 species represented by living individuals, and one species represented by a single recently dead shell (Pimpleback, *Cyclonaias pustulosa*). Mussel species richness across all sites ranged from zero to seven (mean = 0.7 ± 0.1 SE). We found Zebra Mussels (*Dreissena polymorpha*) at one location in the lower Missouri River (McCook Lake).

Faltys (2016) reported two species not previously documented in South Dakota, the Spike (*Eurynia dilatata*) and the Ellipse (*Venustaconcha ellipsiformis*). After examining photographs and specimens, we determined that both were misidentifications. The specimen identified by Faltys (2016) as a Spike is the Black Sandshell (*Ligumia recta*), and the specimen identified as an Ellipse is the Giant Floater (*Pyganodon grandis*). Additionally, a specimen from the lower Missouri River reported as undetermined by Faltys (2016) is the Paper Pondshell (*Utterbackia imbecillis*).

Mussel species richness and abundance were higher in eastern drainages than in western drainages. All 13 species were found in eastern drainages with total drainage species richness ranging from 5 to 10 (mean richness/site = 1.2 ± 0.1 SE), and abundance of each species ranged from 0 to 81/site (mean CPUE = 2.8/h ± 0.8 SE, all species combined). In contrast, only four species were found in western drainages, with total drainage species richness ranging from one to two (mean richness/site = 0.2 ± 0.1 SE), and abundance of each live species ranged from 0 to 22/site (mean CPUE = 0.2/h ± 0.1 SE, all species combined). The highest species richness was found in the James River drainage in eastern South Dakota (10 species) and the lowest species richness was found in the drainages of the Bad, Moreau, Niobrara, and White rivers in western South Dakota (one species in each drainage). The Red River drainage in northeastern South Dakota

Table 3. Comparisons of mussel species richness between this study (current, 2014–15) and previous surveys in six river drainages in eastern South Dakota. Superscripted numbers represent sources for previous surveys.

Drainage	Period	Number of Sites	Mean Richness/Site (Total Richness)
Big Sioux[3,5]	Previous	75	0.35 (26)
	Current	20	0.25 (5)
James[7,8]	Previous	34	0.68 (23)
	Current	39	0.23 (9)
Minnesota[2]	Previous	56	0.21 (12)
	Current	6	0.83 (5)
Missouri[4,6,9–14]	Previous	233	0.09 (20)
	Current	26	0.19 (5)
Red[2]	Previous	3	1.67 (5)
	Current	2	3.50 (7)
Vermillion[1]	Previous	13	1.00 (13)
	Current	9	0.78 (7)

[1]Perkins (1975); [2]Perkins et al. (1995); [3]Skadsen (1998); [4]Perkins and Backlund (2000); [5]Skadsen and Perkins (2000); [6]Hoke (2003); [7]Perkins and Backlund (2003); [8]Wall and Thomson (2004); [9]Ecological Specialists, Inc. (2005a); [10]Ecological Specialists, Inc. (2005b); [11]Shearer et al. (2005); [12]Perkins (2009); [13]Ecological Specialists, Inc. (2007); [14]Ecological Specialists, Inc. (2012).

had the highest abundance (CPUE = 14.5/h ± 1 SE), and the Niobrara River and Moreau River drainages in western South Dakota had the lowest abundance (CPUE = 0 and 0.1/h ± 0.1 SE, respectively).

The Giant Floater was found in all drainages and was the most abundant species (mean CPUE = 0.931/h ± 0.3 SE), making up 62.1% of all live mussels (Table 2). The Wabash Pigtoe (*Fusconaia flava*), White Heelsplitter (*Lasmigona complanata*), Pink Heelsplitter (*Potamilus alatus*), Fatmucket (*Lampsilis siliquoidea*), and Mapleleaf (*Quadrula quadrula*) were found in three to eight drainages, and each made up 2.2 to 15.5% of live mussels (Table 2). The remaining six species each were found in one to three drainages and represented less than 1% of live mussels.

We observed fewer species than previous studies at four of seven resurveyed sites (Table 4). The largest decrease in the number of species collected occurred at the Whetstone River site with a potential loss of four species; however, the greatest rates of species loss were observed at the Foster Creek and Redstone Creek sites (0.3 species/yr). We observed more species than previous studies at the Bois de Sioux and Vermillion rivers. We observed three new species at the Bios de Sioux River (Threeridge, *Amblema plicata*; Black Sandshell; and Mapleleaf), but we did not find Pink Papershell, *Potamilus ohiensis*, which was reported previously from the site. At the Vermillion River site, we observed four new species (Fragile Papershell, *Potamilus fragilis*, recently dead shells only; Pink Heelsplitter; Threeridge; and Wabash Pigtoe), but we did not find Creeper (*Strophitus undulatus*), which was reported previously from the site. Species richness was unchanged at the Hidewood Creek site.

Table 4. Comparisons of mussel species richness between this study (current, 2014–15) and previous surveys at seven sites in eastern South Dakota. CPUE = catch per unit effort (number of live mussels/h) in this study. Superscripted numbers represent sources for previous surveys.

		Site Richness		
Stream	Drainage	Previous	Current (CPUE)	Change/yr
Vermillion River[1]	Vermillion	3	6 (1)	0.08
Big Sioux River[4]	Big Sioux	1	0 (0)	−0.07
Bois de Sioux River[2]	Red	5	7 (15)	0.11
Foster Creek[5]	James	4	1 (0)	−0.30
Hidewood Creek[3]	Big Sioux	3	3 (0.5)	0.00
Redstone Creek[5]	James	4	1 (0.5)	−0.30
Whetstone River[2]	Minnesota	8	4 (11.5)	−0.21

[1]Perkins (1975); [2]Perkins et al. (1995); [3]Skadsen (1998); [4]Skadsen and Perkins (2000); [5]Wall and Thomson (2004).

Among six eastern drainages, we found lower mean species richness/site than previous studies in three drainages (Big Sioux, James, and Vermillion) and higher richness/site in three drainages (main stems of Minnesota, Red, and Missouri rivers; Table 3). The greatest decline in species richness/site was in the James River drainage (0.68 vs. 0.23 species/site) and the greatest increase in richness was in the Red River drainage (1.67 vs. 3.50 species/site).

General patterns of species distributions across eastern drainages in our study were similar to those of previous studies (Table 1). The four most widely distributed species in our study, Giant Floater (six drainages), White Heelsplitter (six drainages), Fatmucket (five drainages), and Pink Heelsplitter (four drainages), were reported from all six eastern drainages by previous studies. All species that we found in three drainages were reported from four to five drainages by previous studies (Threeridge, Wabash Pigtoe, and Mapleleaf). However, three species that were widespread in previous studies either were not found in our study (Pink Papershell, six drainages previously; Lilliput, *Toxolasma parvum*, five drainages previously) or were found in only one drainage (Creeper, five drainages previously). We did not find four other species that were found in four drainages in previous surveys (Cylindrical Papershell, *Anodontoides ferrusacianus*; Rock-pocketbook, *Arcidens confragosus*; Plain Pocketbook, *Lampsilis cardium*; and Yellow Sandshell, *Lampsilis teres*).

The two most widely distributed species in our study, Giant Floater and White Heelsplitter, are host generalists and opportunistic life-history strategists (Table 2). Together, host generalists and opportunistic strategists made up 72.2% and 77.5% of all live mussels encountered, respectively. In contrast, equilibrium and periodic strategists made up only 18.7% and 3.8% of live individuals, respectively.

DISCUSSION

All unionid species we collected were reported from the state by previous surveys (Table 1). We observed 13 species of unionid mussels, far fewer than the 32 species reported in South Dakota from a compilation of previous surveys. This could be interpreted as a >50% decline in species richness in the state. However, because our survey was designed to cover the entire state, including the largely unsurveyed western drainages, sampling effort in each drainage was substantially lower than that expended by combined previous surveys. Furthermore, our probabilistic sampling design was meant to provide an unbiased depiction of mussel distribution and abundance at a large scale. In contrast, most previous surveys focused on sites or habitats that were considered likely to support mussels. For these reasons, we are unable to conclude whether species richness has declined overall in the state since previous surveys. Our comparisons of species richness at previously surveyed sites and in six eastern drainages indicated possible declines in richness in only about half of the cases, and no change or possible increases in richness in the other cases. These differences in species richness estimates among studies may be due to differences in sampling effort, sampling methods, or other factors (Metcalfe-Smith et al. 1998).

Unionid surveys conducted in states bordering South Dakota have noted declines in species richness (Badra and Goforth 2003; MNDNR 2004; Poole and Downing 2004; Fisher 2006; Obermeyer et al. 2006; Roberts et al. 2008; DeLorme 2011; Grabarkiewicz and Gottgens 2011; Hoke 2011; Stodola et al. 2013). The causes of these declines are unknown, but they have been attributed to degraded water quality and aquatic habitats and hydrologic changes resulting from conversion of grassland to row-crop agriculture (Allan 2004; Downing et al. 2010). Widespread conversion of grassland to row-crop agriculture and accompanying negative effects on streams also has occurred in South Dakota (Johnston 2013; Wright and Wimberly 2013), and it is likely that these factors have negatively affected the state's mussel fauna.

Other factors may pose threats to the mussel fauna of South Dakota. The four dams on the Missouri River and thousands of small impoundments on tributaries alter mussel habitat and host-fish distribution in streams (Watters 2000; Haag 2012). In addition, 22 nonindigenous fish species occur in South Dakota, and they may displace native fish species (Saunders et al. 2002; Hoagstrom et al. 2007). Decreases or changes in host-fish communities could negatively affect mussel recruitment (Douda et al. 2013; Galbraith et al. 2018). However, eight of the mussel species we collected are host specialists, suggesting that changes in the fish fauna would produce species-specific effects on the mussel fauna rather than fauna-wide effects (Haag 2019). Two invasive bivalve species occur in South Dakota, the Asian Clam (*Corbicula fluminea*) and the Zebra Mussel, both of which can pose serious threats to native species (Schneider et al. 1998; Shearer et al. 2005; Huber and Geist 2019; Vanderbush et al. 2021). Finally, changes in temperature, streamflow, runoff, and salinity due to climate change can negatively affect aquatic ecosystems and species, potentially including mussels (Hastie et al. 2003; Ganser et al. 2013; Inoue and Berg 2017).

Overall, the mussel fauna of South Dakota is dominated by species with generalist host use and an opportunistic life-history strategy. Species with those traits generally are considered tolerant of stressful conditions, and their dominance in mussel assemblages can indicate habitat degradation (Morris and Corkum 1996; Metcalfe-Smith et al. 1998; Hornbach et al. 2019). In addition to their lower species richness, drainages west of the Missouri River were composed almost entirely of opportunists or host generalists. This finding probably indicates that mussel populations in that region are limited naturally by arid conditions and hydrologic instability, in addition to human factors. In contrast, host specialists and species with periodic or equilibrium life-history strategies were found predominantly in eastern drainages. This finding could mean that there are fewer environmental stressors and disturbances within these drainages, which allows persistence of life-history strategies that require more stable conditions (Haag 2012).

Timed-search visual and tactile survey methods as used in our study are appropriate for surveys designed to assess patterns of species richness and distribution at large scales. In contrast, quadrat-based methods are more labor intensive and may underestimate species richness, particularly when mussel abundance is low (Hornbach and Deneka 1996), as is often the case in South Dakota. Visual and tactile methods can be biased by habitat or sampling conditions, but standardized application of these methods can provide cost-effective, useful comparisons of mussel abundance and species richness over time (Metcalfe-Smith et al. 1998; Wisniewski 2013). Our ability to assess long-term changes in species richness was limited by the large differences in sampling effort between our study and previous studies. Using standardized timed-search methods can allow more informative assessments of changes in species distribution and richness over time that avoid the difficulties of comparing qualitative, historical records with contemporary surveys (e.g., Angelo et al. 2009). In addition, our estimates of CPUE provide a baseline that can allow assessment of changes in mussel abundance over time.

Because of their relatively sedentary lifestyle, mussel presence and population health are strongly tied to the occurrence of suitable host fish and habitat. Habitat suitability modeling can be used to refine monitoring efforts and conservation planning by identifying priority areas for sampling or conservation efforts (Daniel et al. 2018). Additionally, environmental DNA (eDNA) can be used as a tool to quickly screen wide geographic areas, which is particularly important when the full extent of target species ranges is unknown (Gasparini et al. 2020; Lor et al. 2020; Rodgers et al. 2022). Incorporating habitat suitability modeling and eDNA sampling can augment and guide future monitoring surveys for freshwater mussels in South Dakota.

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REGULAR ARTICLE

SPECIES RICHNESS AND DISTRIBUTION OF SPHAERIIDAE SURVEYED WITH ENVIRONMENTAL DNA METABARCODING

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ABSTRACT

Freshwater bivalves of the family Sphaeriidae (fingernail, pea, and pill clams) are difficult to survey and identify due to their small size and overlapping morphological traits. Environmental DNA (eDNA) metabarcoding offers a cost-effective method for assessing species richness and distributional patterns at large scales. We evaluated sphaeriid species richness and distribution at 15 sites in the Maumee River, Ohio, USA, based on two eDNA metabarcoding assays (broad and targeted), and we compared our results with those from a traditional benthic macroinvertebrate survey. We detected seven molecular operational taxonomic units (MOTUs) in the Maumee River, including *Sphaerium transversum*, five MOTUs representing *Euglesa* spp., and one MOTU representing *Odhneripisidum* sp. *Sphaerium transversum* was widely distributed, occurring at 10 sites, but *Euglesa* and *Odhneripisidum* were restricted to one to four sites in the upper river. Distributional patterns were broadly similar between both metabarcoding assays and benthic surveys. However, eDNA metabarcoding provided species-level identifications, resulting in higher species richness. Environmental DNA sampling augments and enhances traditional benthic surveys, but greater eDNA sample replication is needed to improve detection, and additional sphaeriid reference sequences are needed to improve species-level identification.

KEY WORDS: environmental DNA, metabarcoding, fingernail clam, pea clam, pill clam, biodiversity assessment

INTRODUCTION

The freshwater bivalve family Sphaeriidae Deshayes, 1855 (fingernail, pea, and pill clams) occurs on every continent except Antarctica and currently contains 227 recognized species worldwide (Herrington 1962; Graf 2013; Lee 2019). Sphaeriids are present in virtually all freshwater habitats, including wetlands, lakes, and rivers. Although they often are the smallest freshwater bivalves (<25 mm shell length), they frequently are numerically dominant and ecologically important in nutrient cycling and energy transport (Burch 1975; Kuiper 1983; Lee 2019). Sphaeriidae contains two subfamilies. The Euperinae Heard, 1965, contains 33 species in 2 genera distributed throughout the Americas and the Afrotropics (Graf and Cummings 2023) and includes the invasive *Eupera cubensis* (Prime, 1865), which occurs in the Illinois River, USA, drainage near the Laurentian Great Lakes (Sneen et al. 2009). The Sphaeriinae Deshayes (1820) is widespread and species-rich. An estimated 35 species of Sphaeriinae occur in the Laurentian Great Lakes watersheds, with 24 reported from Lake Erie (NOAA and USEPA 2019; Trebitz et al. 2019).

Accurate morphological identifications of genera and species within Sphaeriidae are difficult due to plasticity of shell characters (Rassam et al. 2021). DNA sequences have been useful for resolving phylogenetic relationships and providing species diagnostics for this group (Lee and Ó'Foighil 2003; Schultheiß et al. 2008; Clewing et al. 2013). Recent phylogenetic studies indicate that Sphaeriinae includes five genera: *Afropisidium* Kuiper, 1962; *Euglesa* Jenyns, 1832; *Odhneripisidium* Kuiper, 1962; *Pisidium* Pfeiffer, 1821; and *Sphaerium* Scopoli, 1777. The genus *Musculium* Link, 1807, was subsumed under *Sphaerium* (Lee and Ó'Foighil 2003), while the genera *Afropisidium*, *Euglesa*, and *Odhneripisidium* formerly were contained in *Pisidium*. Additionally, DNA sequencing studies have identified cryptic

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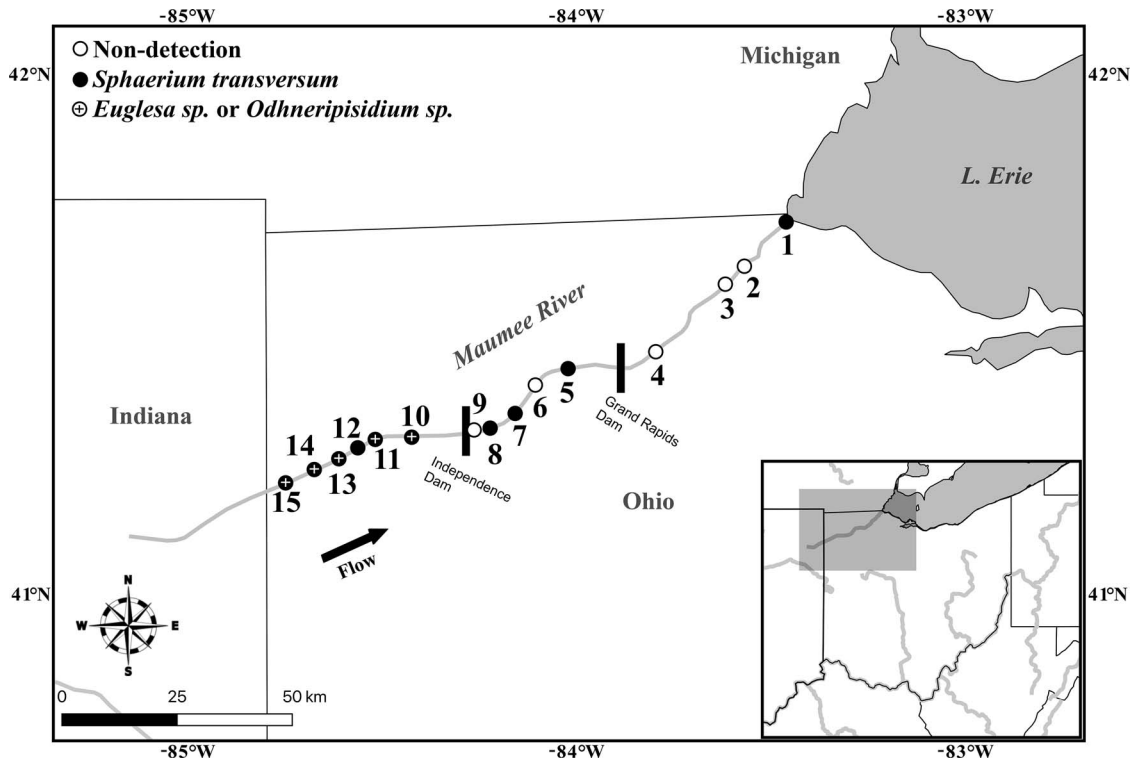

Figure 1. Map of the Maumee River showing sampling sites and eDNA detection of sphaeriid clams. Vertical black lines indication location of low head dams. Inset map shows location of the study area in Ohio.

sphaeriid species (Schultheiß et al. 2008; Clewing et al. 2013; Bößneck et al. 2016; Groh et al. 2020) while providing a better understanding of species distributions (Rassam et al. 2020).

Accurate identification methods and efficient survey approaches are needed to inform assessment of sphaeriid distribution and conservation status. For example, sphaeriid populations across the Great Lakes region have experienced large declines following dreissenid mussel invasions (Lauer and McComish 2001; Burlakova et al. 2018), and continued monitoring is needed for effective conservation. The analysis of environmental DNA (eDNA, genetic material released from urine, waste, mucus, or sloughed cells) provides an efficient method for surveying for a wide range of aquatic taxa (Beng and Corlett 2020; Deiner et al. 2021), including monitoring of invasive bivalves (Gingera et al. 2017; Cowart et al. 2018; Marshall and Stepien 2019; Marshall et al. 2021) and threatened freshwater mussels (Klymus et al. 2021; Marshall et al. 2022). In particular, eDNA may benefit diversity assessments of sphaeriids given the uncertainty surrounding their phylogenetic relationships and their high diversity in North American (Prié et al. 2021).

We compared the detection of sphaeriids using two types of eDNA metabarcoding assays (broad and targeted) versus a traditional benthic macroinvertebrate survey in the Maumee River, Ohio, USA. Benthic macroinvertebrate samples and eDNA samples were collected by the Ohio Environmental Protection Agency (OEPA) in 2012, and we reanalyzed the eDNA samples. We evaluated the ability of eDNA metabarcoding to (1) detect sphaeriids from locations where their presence was previously verified, and (2) characterize species-level diversity in the Maumee River. We discuss the potential of eDNA metabarcoding to facilitate accurate characterization of sphaeriid diversity.

METHODS

The Maumee River begins in Fort Wayne, Indiana, USA, at the confluence of the St. Marys and the St. Joseph rivers and flows 225 kilometers through northeastern Indiana and northwestern Ohio before discharging into Lake Erie (Fig. 1). The river drains 10,620 km^2, making it the largest watershed within the Great Lakes basin. The OEPA conducted a traditional benthic macroinvertebrate survey and collected eDNA samples from August 7 to August 28, 2012, at 15 sites in the Maumee River, Ohio, from river km 0.8 (near the river's mouth; 41.69, −83.47) to river km 158.4 (near the Indiana-Ohio border; 41.18, −84.73; OEPA 2014; Fig. 1). At each site, OEPA staff conducted a macroinvertebrate survey, which consisted of quantitative sampling by placing five modified Hester-Dendy samplers within the river for 6 wk and qualitative sampling with dip nets and hand sampling in different habitats (e.g., riffle, run, or pool) as outlined in OEPA (2008). All sphaeriids were identified to genera recognized at that time (*Sphaerium* or *Pisidium*); therefore, identifications of *Pisidium* may represent taxa from that genus or the now-recognized *Euglesa* or *Odhneripisidium*.

At each site, just prior to performing a traditional benthic macroinvertebrate survey, the OEPA collected a 1-L water sample 10 cm below the surface in a sterilized, bleach-washed Nalgene container, which was placed on ice in a sterile cooler and

transported to the Stepien laboratory at the University of Toledo, where it was frozen at −80°C until DNA was extracted in 2017. At three of the sites, eDNA was isolated and extracted by processing the water through a 0.2-μm PES filter with subsequent DNA extraction using a cetyl trimethyl ammonium bromide (CTAB) protocol (Klymus et al. 2017). At the remaining 12 sites, samples were processed by centrifuging and forming a pellet in 50 mL falcon tubes at 7,500 g for 30 min (Marshall and Stepien 2020). Genomic DNA from the pellets was extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc., Germantown, MD, USA). All samples were processed with a Zymo Research One Step PCR Inhibitor Removal kit (Zymo Research, Irvine, CA, USA). A negative control of 250 ml deionized water was simultaneously extracted to test for possible laboratory contamination.

We examined sphaeriid occurrence in the Maumee River using archived eDNA samples that were previously extracted and processed for other taxonomic analysis. First, we used the results of Marshall and Stepien (2020), who used a broad-range mollusk metabarcoding assay (Mol16S; Klymus et al. 2017) as part of an assessment of overall macroinvertebrate communities. Second, we performed new analyses using a targeted sphaeriid-specific metabarcoding assay (Sph16S; Klymus et al. 2017). The Mol16S assay amplifies a 179–180 bp fragment of the 16S mitochondrial gene for sphaeriids and overlaps completely with the longer 259–260 bp fragment of the 16S gene amplified by Sph16S.

Amplification and library preparation for the Sph16S assay followed that of the Mol16S (Marshall and Stepien 2020) and is described briefly here. We included a short spacer region to increase library nucleotide diversity for enhanced cluster formation. We used a two-step PCR library preparation. The first PCR included 1× PCR buffer, 0.3 mM dNTPs, 0.5 μM of each primer, an additional 1.5 mM $MgCl_2$, 5 U AllTaq (Qiagen), 5 μl template DNA, and ddH_2O to total 50 μl. Conditions were 2 min initial denaturation at 95°C, followed by 40 cycles of 95°C for 5 s, 58°C for 15 s, and 72°C for 10 s, with no final extension. We processed first-step PCR products with a 0.7× HighPrep bead clean-up (MagBio Genomics, Gaithersburg, MD, USA, kit/AC60050), yielding the template for the second step. The second PCR incorporated Nextera paired-end indices (Illumina, San Diego, CA, USA, kit FC-121-1011), p5/p7 adaptor sequences, and eight base sample indices to distinguish among samples. This final reaction contained 1× PCR buffer, 0.2 mM dNTPs, 0.5 μM of each primer, 1.57 U NEB Hotstart Taq polymerase (New England Biolabs Inc., Ipswich, MA, USA), 2.5 μl from the previous PCR cleanup, and ddH_2O to total 25 μl. Conditions were 30 s initial denaturation at 95°C, followed by eight cycles at 95°C for 30 s, 55°C for 30 s, and 68°C for 1 min, with a final 2 min 68°C extension. We sized and quantified PCR products on a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) prior to Illumina MiSeq sequencing conducted at the Ohio State University's Molecular and Cellular Imaging Center in Wooster, Ohio. Each PCR setup included the addition of a negative PCR control, which showed no amplification on gel electrophoresis.

Raw MiSeq data referencing the Mol16S assay used by Marshall and Stepien (2020) are available in the NCBI GenBank repository under BioProject PRJNA600479. We deposited raw MiSeq data for the Sph16S assay in the NCBI GenBank repository under BioProject PRJNA1024515.

We removed forward and reverse primer sequences from the demultiplexed reads using the Cutadapt plugin (Martin et al. 2011) in QIIME 2 (Bolyen et al. 2019). Next, we filtered and trimmed sequence reads using the denoising DADA2 plugin (Callahan et al. 2016) in QIIME 2 to truncate sequence reads based on the quality scores from the forward and reverse read files, estimate error rates, merge and dereplicate sequences into amplicon sequence variants (ASVs), and remove any erroneous or chimeric sequences. We clustered unique ASVs into molecular operational taxonomic units (MOTUs) using the QIIME 2 vsearch *de novo* with a 97% similarity threshold (Rognes et al. 2016).

We used the basic local alignment search tool (Camacho et al. 2009) and the National Center for Biotechnology Information (NCBI) GenBank nonredundant (nr) sequence database to identify MOTUs from sphaeriid taxa. We identified MOTUs to the species level if a sequence had >97% sequence match and to the genus level if it had >90% sequence match. We compared taxonomic classifications obtained from NCBI GenBank with species previously reported from the Great Lakes region (Appendix 1; NOAA and USEPA 2019). We used updated taxonomy for the subfamily Sphaeriinae following the MUSSEL Project database (Graf and Cummings 2023).

We constructed a phylogeny of the identified MOTUs and representative sphaeriid sequences from the NCBI GenBank based on a 259–260 bp region amplified with the Sph16S assay using the Maximum Likelihood method in the program Molecular Evolutionary Genetics Analysis (MEGA11; Tamura et al. 2021). We compared MOTUs produced by the Mol16s and Sph16S assays at each site and against the results of the OEPA benthic macroinvertebrate survey. We obtained sphaeriid occurrence records for the Maumee River and western Lake Erie from three online repositories (IdigBio 2023; GBIF 2023; UM Museum 2023).

RESULTS

All 15 samples were successfully amplified and sequenced using the Mol16S assay, but only 10 samples were successfully amplified and sequenced using Sph16S (Appendix 2). The Sph16S assay resulted in a total of 363,550 raw sequence reads (mean = 36,355.0 ± 1,717.5 standard error [SE]), with 51.70% (187,949 reads) passing through the filtering and merging bioinformatic processing. The Mol16S assay resulted in 1,420,366 raw sequence reads (mean = 94,691.1 ± 16,228.6), with 73.26% (1,040,617 reads) passing through the filtering and merging bioinformatic processing. Sphaeriid MOTU reads accounted for 100% of the final Sph16S dataset, but just 3.6% (± 1.6 SE, range = 0.0–18.2%) of the final Mol16S dataset (Appendix 2). The Sph16S assay resulted in a mean of 18,794.9 (± 1,058.2 SE) sphaeriid reads/sample, but the Mol16S assay resulted in a

Table 1. Taxonomic classification and percent identity for each sphaeriid molecular operational taxonomic unit (MOTU) detected in the Maumee River, Ohio, with the Sph16S and Mol16S metabarcoding assays.

MOTU	Taxonomic Classification	Sph16S	Mol16S
MOTU01	*Sphaerium transversum*	100.00	100.00
MOTU02	*Euglesa compressa*	100.00	100.00
MOTU03	*Euglesa casertana*	99.44	98.07
MOTU04	*Euglesa nitida*	99.44	99.23
MOTU05	*Euglesa fallax*	97.78	97.69
MOTU06	*Euglesa* sp.	96.11	96.54
MOTU07	*Odhneripisidium* sp.	93.33	94.64

mean of only 1,806.4 (± 774.6 SE) sphaeriid reads/sample (Appendix 2).

The Mol16S and the Sph16S datasets detected the same seven MOTUs in the Maumee River (Table 1). These were in three genera of Sphaeriinae: *Euglesa* (5 MOTUs), *Odhneripisidium* (1 MOTU), and *Sphaerium* (1 MOTU; Table 1). The single *Sphaerium* MOTU had 100% genetic match with *S. transversum* (Say, 1829) and was detected at all sites that amplified. Four of the five *Euglesa* MOTUs were identified to the species level as *E. compressa* (Prime, 1852; 100% match), *E. casertana* (Poli, 1791; 98.07–99.44% match), *E. nitida* (Jenyns, 1832; 99.23–99.44% match), and *E. fallax* (Sterki, 1896; 97.69–97.78% match) (Table 1 and Fig. 2; Appendix 3). The MOTU identified as *E. nitida* had high similarity (>97%) to four different species: *E. nitida*, *E. edlaueri* (Kuiper, 1960), *E. maaseni* (Kuiper, 1987), and *E. pseudosphaerium* (Favre, 1927), but only *E. nitida is* reported from North America. We were unable to identify one *Euglesa* MOTU to the species level. This MOTU clustered within a group of *E. fallax* sequences but had only a 96.11% match to any, falling below the 97% species-level threshold (Table 1 and Fig. 2; Appendix 3). We were unable to identify the single *Odhneripisidium* MOTU to the species level. This MOTU matched the Eurasian *O. annandalei* (Prashad, 1925) and the Asian *O. japonica* (Pilsbry and Hirase, 1908), but it had a less than 95% match, and neither of these species is reported from North America (Table 1 and Fig. 2; Appendix 3).

The Sph16S assay yielded positive detections at 10 sampling sites, and the Mol16S assay had positive detections at nine (Table 2 and Fig. 3). The two assays shared 22 of 26 detections (85% overlap), with each assay showing unique detections at two sampling sites. Numbers of read counts for each of the seven MOTUs were similar between the two assays ($R^2 = 0.913$, $P < 0.0001$; Table 2). For both assays, *S. transversum* made up a majority of the sequence reads (Mol16S: 74.7% ± 33.0 SD, Sph16S: 97.0% ± 5.2).

OEPA benthic macroinvertebrate surveys observed *Sphaerium* at ten of our study sites. Our eDNA assays detected *S. transversum* at eight of these ten sites, and at an additional two sites where OEPA did not report *Sphaerium* (Fig. 3). Benthic macroinvertebrate surveys observed species within the "*Pisidium*" group (sensu lato) from two sites, while our eDNA assays detected at least one *Euglesa* or *Odhneripisidium* MOTU at five sites, including one of those in agreement with visual observations (Fig. 3).

DISCUSSION

Our estimates of species distributions in the Maumee River from eDNA metabarcoding were broadly similar to those reported by the OEPA benthic macroinvertebrate surveys. As expected, eDNA metabarcoding improved species-level identifications, going beyond the "*Sphaerium*" or "*Pisidium*" designation. Taxonomic uncertainty associated with vague and overlapping morphological traits typically limits identification to the genus level, resulting in a loss of information about species distribution and status. We described five MOTUs to the species level (*E. compressa*, *E. casertana*, *E. fallax*, *E. nitida*, and *S. transversum*), with only two MOTUs being restricted to genus level identification (*Euglesa* sp. and *Odhneripisidium* sp.) due to lack of reference sequences. These two unidentified taxa illustrate limitations of existing DNA reference databases (Trebitz et al. 2015), as these sequences may belong to species that lack reference sequence data for 16S rDNA or belong to undescribed species. Cryptic species within the subfamily Sphaeriinae have been identified by combining molecular and morphological approaches (Guralnick 2005; Groh et al. 2020). The sequences reported here can be used to determine these identities in the future, as taxonomic advances are made and reference databases improve. The unknown *Euglesa* sp. group occurred within a cluster of *E. fallax* sequences, yet it fell below the 97% species level threshold. This may represent population genetic variation rather than separate species (Marshall and Stepien 2019), and further DNA sequence and morphological data would be needed to confirm.

Based on both eDNA and morphological surveys, *S. transversum* appears widespread throughout the Maumee River. A 2010 benthic survey near the mouth of the Maumee River (Ram et al. 2014) reported four sphaeriids based on morphological identifications including *S. transversum* (as *Musculium*), *E. compressa* (as *Pisidium*), and two taxa not found in our study: *S. securis* (Prime, 1852; as *Musculium*) and *S. simile* (Say, 1817). However, only *S. transversum* and *E. compressa* were confirmed with subsequent DNA analysis of collected specimens (supplementary data in Ram et al. 2014), matching our results. The three online repositories suggest that four species are the dominant sphaeriids within the lower reach of Maumee River and western Lake Erie, including three species we detected with eDNA (*E. casertana*, *E. compressa*, and *S. transversum*) and a fourth nondetected species, *S. striatinum* (Lamarck, 1818). Interestingly, these repositories indicate *S. striatinum* as the first or second most common species. While sphaeriid populations have declined across the Great Lakes region (Lauer and McComish 2001; Burlakova et al. 2018), it is unclear if our failure to detect *S. striatinum* is due to population declines or low eDNA sampling effort.

We did not detect sphaeriids at three sites where they were reported by OEPA benthic macroinvertebrate surveys. Five samples failed to amplify with the Sph16S assay, suggesting low concentration or absence of sphaeriid DNA. These same five samples

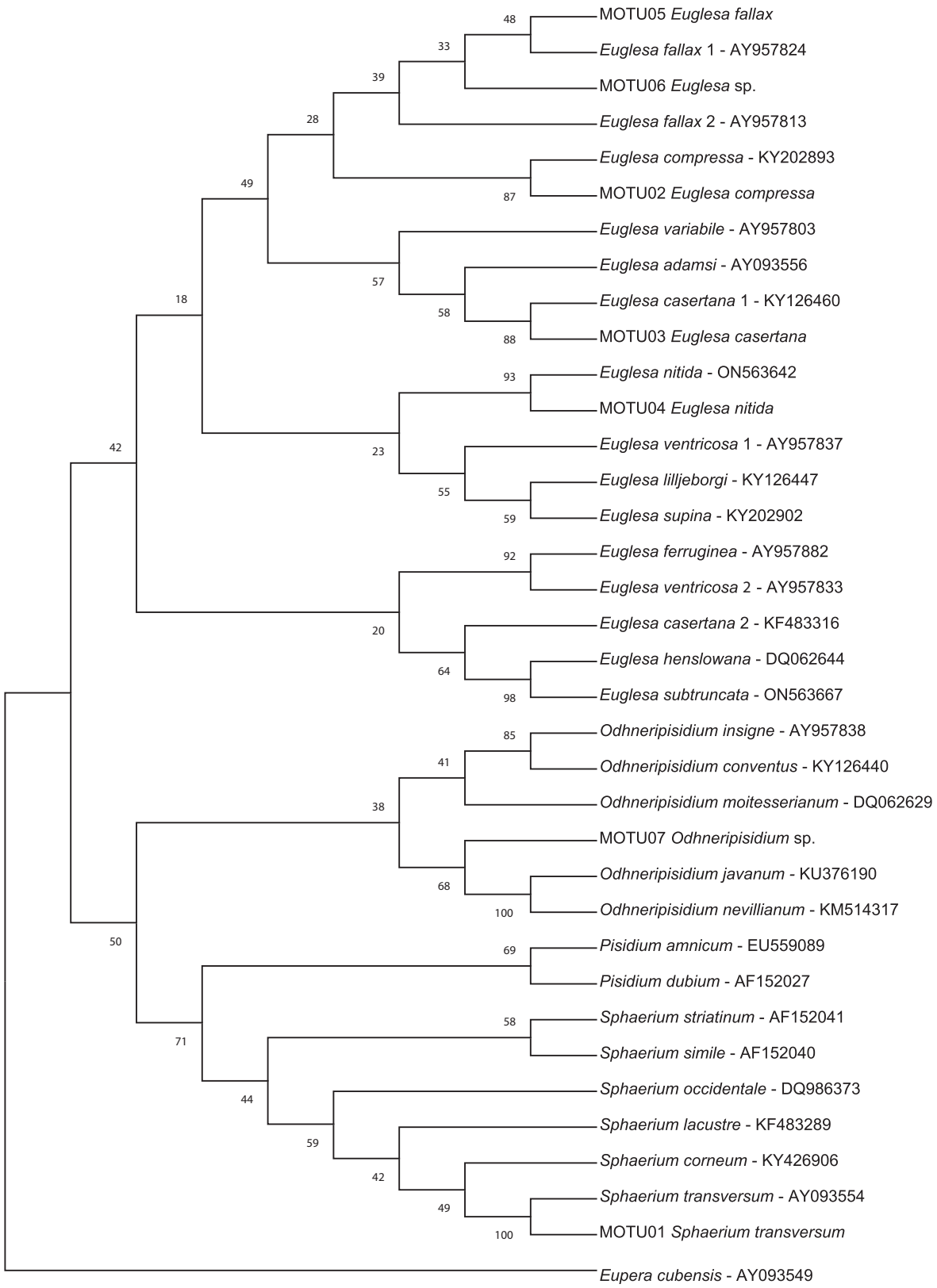

Figure 2. Phylogeny of the identified molecular operational taxonomic units (MOTUs) and representative Lake Erie sphaeriid sequences based on a 259–260 base pair region amplified with the Sph16S assay using the Maximum Likelihood method and Tamura-Nei model within the program Molecular Evolutionary Genetics Analysis (MEGA11). The bootstrap consensus tree is inferred from 500 replicates. Numbers at each node are the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test. Accession numbers represent sequences obtained from NCBI GenBank.

Table 2. Total read counts for each sphaeriid molecular operational taxonomic unit (MOTU) detected in the Maumee River, Ohio, with the Sph16S and Mol16S metabarcoding assays. Bold numbers indicate MOTU detection unique to one assay.

Sph16S	Species	Site 1	Site 5	Site 7	Site 8	Site 10	Site 11	Site 12	Site 13	Site 14	Site 15
MOTU01	*Sphaerium transversum*	21937	**19070**	18077	17854	24168	11853	21025	17146	15061	16336
MOTU02	*Euglesa compressa*	0	0	0	0	0	0	0	195	860	1940
MOTU03	*Euglesa casertana*	0	0	0	0	0	0	0	578	158	825
MOTU04	*Euglesa nitida*	0	0	0	0	0	0	0	28	175	255
MOTU05	*Euglesa fallax*	0	0	0	0	0	0	0	0	118	0
MOTU06	*Euglesa* sp.	0	0	0	0	0	0	0	0	74	0
MOTU07	*Odhneripisidium* sp.	0	0	0	0	136	**41**	0	39	0	0
	Total reads	21937	19070	18077	17854	24304	11894	21025	17986	16446	19356
	MOTU richness	1	1	1	1	2	2	1	5	6	4
Mol16S	Species	Site 1	Site 5	Site 7	Site 8	Site 10	Site 11	Site 12	Site 13	Site 14	Site 15
MOTU01	*Sphaerium transversum*	137	0	220	11	3206	45	1859	5593	6575	2960
MOTU02	*Euglesa compressa*	0	0	0	0	0	**57**	0	520	1147	1216
MOTU03	*Euglesa casertana*	0	0	0	0	0	0	0	1151	289	664
MOTU04	*Euglesa nitida*	0	0	0	0	0	0	0	138	296	214
MOTU05	*Euglesa fallax*	0	0	0	0	0	0	0	0	253	0
MOTU06	*Euglesa* sp.	0	0	0	0	0	0	0	**146**	210	0
MOTU07	*Odhneripisidium* sp.	0	0	0	0	136	0	0	53	0	0
	Total reads	137	0	220	11	3342	102	1859	7601	8770	5054
	MOTU richness	1	0	1	1	2	2	1	6	6	4

were successfully sequenced with the Mol16S assay, yet no sphaeriid sequences were detected. However, the OEPA survey did find sphaeriids at three of these five sites, suggesting that the single 1-L water sample was not always sufficient for collection of sphaeriid eDNA. Our study did not include replicate water sampling, and increasing the number of eDNA samples collected at each site likely would increase detection (Marshall et al. 2022). Along with increasing sample replication, sampling larger volumes of water can increase eDNA detection of bivalves (McKee et al. 2023). It also would be beneficial to sample water nearer the bottom, where sphaeriids occur.

The *Euglesa* and *Odhneripisidium* MOTUs appeared to be restricted to the upper reach of the Maumee River. The drainage area of the Maumee basin increases from 5,985 to 14,356 km^2 after the confluence of the Auglaize River near Independence Dam (our site 9; OEPA 2014). The resulting increase in river discharge may dilute eDNA, reducing the likelihood of sphaeriid detection (Curtis et al. 2021). Additionally, increases in discharge (cubic feet per second) typically result in greater eDNA transport distances (Jo and Yamanaka 2022), which, in turn, adds uncertainty to the determination of source location. Our investigation is limited due to the lack of sample replicates, and studies examining the spatial extent of eDNA recommend collecting several independent replicates throughout each reach (Bedwell and Goldberg 2020).

As expected, sphaeriid MOTUs accounted for a much greater number of read counts using the Sph16S assay compared to the Mol16S assay. Yet the two assays displayed large overlap in site-level sphaeriid MOTU detections. Despite the Mol16S assay amplifying a much broader range of taxa, when a MOTU had a low read count for Sph16S, it usually was likewise detected by the Mol16S assay. This suggests that the use of the family-specific Sph16S assay may not be warranted when interested in monitoring sphaeriids, as the Mol16S assay displayed similar sensitivity and can provide additional information on macroinvertebrate diversity (Marshall and Stepien 2020). On four of 26 occasions, a rarer sphaeriid MOTU was detected at a site with one assay but not the other. Considering the stochastic nature of PCR amplification, processing several PCR technical replicates could improve detection of rare sequences and may increase overlap between the assays (Shirazi et al. 2021).

Obtaining abundance estimates from eDNA metabarcoding datasets is challenging due to species-specific differences in eDNA shedding amounts and rates (e.g., differences in body size, life histories and spawning times, and metabolic activity), behavior, habitat differences, and PCR-based biases such as differential primer annealing and amplification (Ruppert et al. 2019). However, a metanalysis of eDNA metabarcoding studies suggested that sequence read counts often are correlated with species abundance or biomass (Keck et al. 2022). In our study, both assays indicated that *S. transversum* is the dominant species throughout the Maumee River, with *Euglesa* and *Odhneripisidium* being less abundant, based on their lower read counts. However, Klymus et al. (2017) reported lower read counts than expected for *Euglesa* (as *Pisidium*) based on known abundances

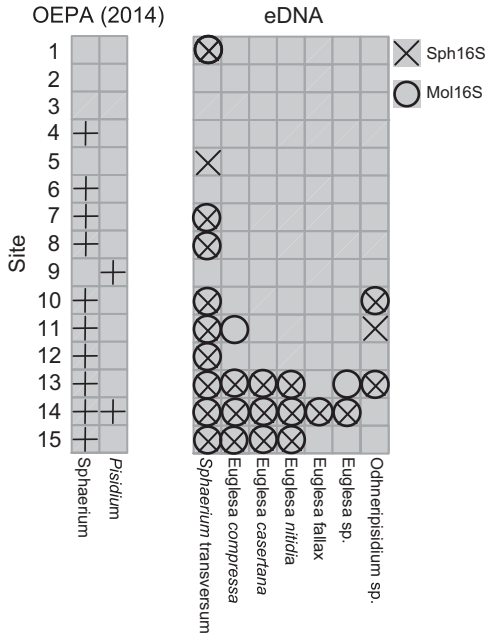

Figure 3. Comparison of sphaeriid clam detection using traditional benthic macroinvertebrate surveys (Ohio Environmental Protection Agency [OEPA]) and two eDNA metabarcoding assays (Sph16S and Mol16S) at 15 sites in the Maumee River, Ohio. Sphaeriids were identified by OEPA only to genus as *Sphaerium* or "*Pisidium*" (sensu lato).

in laboratory mesocosm trials. Because *Euglesa* and *Odhneripisidium* usually are smaller than *Sphaerium*, the former may shed less eDNA, influencing abundance estimates from eDNA sequence read counts.

Environmental DNA sampling is a valuable and cost-effective tool for large-scale, initial assessment of sphaeriid species richness and distributions (Prié et al. 2021). Additional eDNA studies, conducted in concert with traditional benthic surveys, would help to better understand possible sources of bias inherent in this approach. When unidentified MOTU sequences are found, traditional sampling can inform eDNA surveys by providing archived voucher specimens from which reference DNA sequences can be obtained.

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APPENDICES

Appendix 1. List of species in the family Sphaeriidae reported from the Laurentian Great Lakes region. "X" indicates occurrence in the watershed of each major lake. "16s sequences" is the number of reference sequences available for the mitochondrial 16S gene region on the NCBI GenBank database (https://www.ncbi.nlm.nih.gov, accessed September 16, 2023). Species occurrences are based on NOAA and USEPA (2019). Nomenclature follows Graf and Cummings (2023); former genera are given in parentheses.

Species	Superior	Michigan	Huron	Erie	Ontario	16S Sequences
Euglesa (*Pisidium*) *adamsi*	X	X	X	X	X	2
Euglesa (Pisidium) casertana	X	X	X	X	X	75
Euglesa (Pisidium) compressa	X	X	X	X	X	36
Euglesa (Pisidium) equilateralis	—	—	—	—	X	0
Euglesa (Pisidium) fallax	X	X	X	X	X	5
Euglesa (Pisidium) ferruginea	X	X	X	X	X	11
Euglesa (Pisidium) henslowana	X	X		X	X	17
Euglesa (Pisidium) lilljeborgi	X	X	X	X	X	7
Euglesa (Pisidium) milia	X	X	—	—	X	18
Euglesa (Pisidium) nitida	X	X	X	X	X	11
Euglesa (Pisidium) obtusale	—	X	X	—	X	0
Euglesa (Pisidium) rotundata	X	—	—	—	X	0
Euglesa (Pisidium) subtruncata	X	X	X	X	X	32
Euglesa (Pisidium) supina	—	—	—	X	X	18
Euglesa (Pisidium) variabile	X	X	X	X	X	7
Euglesa (Pisidium) ventricosa	X	X	X	X	X	5
Euglesa (Pisidium) walkeri	X	—	X	—	X	1
Ophneripisidium (Pisidium) conventus	X	X	X	X	X	1
Ophneripisidium (Pisidium) insigne	X	—	—	—	—	1
Ophneripisidium (Pisidium) moitessierianum	X	—	—	X	—	4
Pisidium amnicum	X	X	X	X	X	7
Pisidium cruciatum[a]	—	—	—	—	X	0
Pisidium dubium	X	X	X	—	X	1
Pisidium idahoense	X	X	X	X	X	0
Pisidium punctatum (= *P. simplex*)	X	X	X	X	X	0
Sphaerium corneum	X	X	X	X	X	50
Sphaerium (*Musculium*) *lacustre*	—	X	X	X	X	4
Sphaerium nitidum	X	X	X	—	X	0
Sphaerium occidentale	X	—	X	X	X	2
Sphaerium (*Musculium*) *partumeium*	X	X	—	—	X	1
Sphaerium rhomboideum	—	—	X	—	X	2
Sphaerium (*Musculium*) *securis*	X	X	X	X	X	1
Sphaerium simile	X	X	X	—	X	2
Sphaerium striatinum	X	X	X	X	X	5
Sphaerium (*Musculium*) *transversum*	—	X	—	X	X	1

[a] *Pisidium cruciatum* is considered present within Lake St. Clair between the Huron-Erie corridor (NOAA and USEPA 2019).

Appendix 2. Total number of raw sequencing reads per sample and the subsequent number of reads that passed the trimming and merging bioinformatic processing steps for samples collected at 15 sites in the Maumee River, Ohio, using the Sph16S or Mol16S metabarcoding assays.

Sph16S	Raw Reads	Primer Trimmed Reads	Merged Reads	Sphaeriid Reads	Percent Sphaeriid
Site 1	39,510	29,975	21,937	21,937	100
Site 2	—	—	—	—	—
Site 3	—	—	—	—	—
Site 4	—	—	—	—	—
Site 5	38,496	23,864	19,070	19,070	100
Site 6	—	—	—	—	—
Site 7	33,548	21,602	18,077	18,077	100
Site 8	29,896	20,009	17,854	17,854	100
Site 9	—	—	—	—	—
Site 10	34,603	30,131	24,304	24,304	100
Site 11	34,477	22,639	11,894	11,894	100
Site 12	34,643	27,163	21,025	21,025	100
Site 13	34,639	27,116	17,986	17,986	100
Site 14	33,867	23,867	16,446	16,446	100
Site 15	49,871	33,888	19,356	19,356	100
Total	363,550	260,254	187,949	187,949	—
Mean (SE)	36,355.0 (1,717.5)	26,025.4 (1,387.7)	18,794.9 (1,058.2)	18,794.9 (1,058.2)	100

Mol16S	Raw Reads	Primer Trimmed Reads	Merged Reads	Sphaeriid Reads	Percent Sphaeriid
Site 1	293,172	275,819	259,858	137	0.05
Site 2	172,378	163,021	100,799	0	0
Site 3	89,235	83,843	68,068	0	0
Site 4	68,288	63,819	46,101	0	0
Site 5	62,551	57,633	55,064	0	0
Site 6	55,179	50,705	41,726	0	0
Site 7	61,199	55,890	45,574	220	0.48
Site 8	59,527	55,048	36,558	11	0.03
Site 9	75,385	70,325	51,854	0	0
Site 10	95,639	89,361	66,198	3,342	5.05
Site 11	117,126	109,253	70,902	102	0.14
Site 12	83,855	77,426	54,214	1,859	3.43
Site 13	67,870	63,339	47,590	7,601	15.97
Site 14	60,973	56,981	48,156	8,770	18.21
Site 15	57,989	53,908	47,955	5,054	10.54
Total	1,420,366	1,326,371	1,040,617	27,096	—
Mean (SE)	94,691.1 (16,228.6)	88,424.7 (15,359.8)	69,374.5 (14,202.7)	1,806.4 (774.6)	3.6 (1.6)

Appendix 3. sequence data for the mol16s and sph16s assays for each of the seven sphaeriid MOTUS.

>MOTU01 *Sphaerium transversum* Sph16S
ACGTGGGAAAAACTGTCTCTTTTGTATATAAAGAAGTTTATTTTTGAGTGAAAAAGCTTAAATGTTTATAAAAGACGAGAAGACCCTATCGAACTTGAATTATTT
ATTTAAAAATTTAGATAAAGAAAGTTTAGTTGGGGAAACTTAAAGTAAAAAGTAACGCTTTATTTTTGTATCGGGATCCTATATTATAGAAAAATGAAAAAGT
TACCGTAGGGATAACAGCGCTTTCTTCTCTGAGAGGACTAATTAAAGAGTT

>MOTU01 *Sphaerium transversum* Mol16S
ATCGAACTTGAATTATTTATTTAAAAATTTAGATAAAGAAAGTTTAGTTGGGGAAACTTAAAGTAAAAAGTAACGCTTTATTTTTGTATCGGGATCCTATATTAT
AGAAAAATGAAAAAGTTACCGTAGGGATAACAGCGCTTTCTTCTCTGAGAGGACTAATTAAAGAGTTGGTTGCG

>MOTU02 *Euglesa compressa* Sph16S
ACGTGGGAAAAGCTGTCTCTTTTGTATAGAAAGAAGTTTATTTTTGAGTGAAAAAGCTTAAATATTTGTAAAAGACGAGAAGACCCTATCGAACTTGAATTGTG
TGTTTTAGTTTTGGAATACAGAAAGTTTAGTTGGGGAAACTTAAAGTTAAGAAAAACGCTTTTTTGTTATAAAATGATCCTGTATTATAGAAAAATGAAAAAG
TTACCGTAGGGATAACAGCGCTTTCTTCTCTGAGAGGACTAATCAAAGAGTT

>MOTU02 *Euglesa compressa* Mol16S
ATCGAACTTGAATTGTGTGTTTTAGTTTTGGAATACAGAAAGTTTAGTTGGGGAAACTTAAAGTTAAGAAAAACGCTTTTTTGTTATAAAATGATCCTGTATTAT
AGAAAAATGAAAAAGTTACCGTAGGGATAACAGCGCTTTCTTCTCTGAGAGGACTAATCAAAGAGTTGGTTGCG

>MOTU03 *Euglesa casertana* Sph16S
ACGTGGGAAAAACTGTCTCTTTTGTATATAAAGAAGTTTATTTTTGAGTGAAAAAGCTTAAATGTTTATAAAAGACGAGAAGACCCTATCGAACTTGAATTATGT
ATTGTAGATTTATAATGCAGAAAGTTTAGTTGGGGAAACTTAAAGTTAAGAAAAACGCTTTTTTGTTGTAAGATGATCCTGTATTATAGAAAAATGAAAAAGT
TACCGTAGGGATAACAGCGCTTTCTTCTCTGAGAGGACTAATCAAAGAGTT

>MOTU03 *Euglesa casertana* Mol16S
ATCGAACTTGAATTATGTATTGTAGATTTATAATGCAGAAAGTTTAGTTGGGGAAACTTAAAGTTAAGAAAAACGCTTTTTTGTTGTAAGATGATCCTGTATTAT
AGAAAAATGAAAAAGTTACCGTAGGGATAACAGCGCTTTCTTCTCTGAGAGGACTAATCAAAGAGTTGGTTGCG

>MOTU04 *Euglesa nitida* Sph16S
ACGTGGGAAAAGCTGTCTCTTTTATATAAAAAGAAGTTTATTTTTGAGTGAAAAAGCTTAGATGTTTATAAAAGACGAGAAGACCCTATCGAACTTGAATTATG
TGTTTAAGTTTTTAAGTACAAAAAGTTTAGTTGGGGAAACTTAAAGTTAAGAAAAACGTTTTTTTGTTATAAATTGATCCTGTATTATAGAAAAATGAAAAAG
TTACCGTAGGGATAACAGCGCTTTCTTCTCTGAGAGGACTAATTAAAGAGTT

>MOTU04 *Euglesa nitida* Mol16S
ATCGAACTTGAATTATGTGTTTAAGTTTTTAAGTACAAAAAGTTTAGTTGGGGAAACTTAAAGTTAAGAAAAACGTTTTTTTGTTATAAATTGATCCTGTATTAT
AGAAAAATGAAAAAGTTACCGTAGGGATAACAGCGCTTTCTTCTCTGAGAGGACTAATTAAAGAGTTGGTTGCG

>MOTU05 *Euglesa fallax* Sph16S
ACGTGGGAAAAGCTGTCTCTTTTATATAAAAAGAAGTTTATTTTTGAGTGAAAAAGCTTAGATGTTTATAAAAGAGAGAAGACCCTATCGAACTTGAATTATGT
GTTTTAGTTTTGGGGTACAGAAAGTTTAGTTGGGGAAACTTAAAGTTAAGAAAAACGCTTTTTTGTTGTAAAAATGATCCTATATTATGGAAAAATGAAAAA
GTTACCGTAGGGATAACAGCGCTTTCTTCTCTGAGAGGACTAATCAAAGAGTT

>MOTU05 *Euglesa fallax* Mol16S
ATCGAACTTGAATTATGTGTTTTAGTTTTGGGGTACAGAAAGTTTAGTTGGGGAAACTTAAAGTTAAGAAAAACGCTTTTTTGTTGTAAAAATGATCCTATATT
ATGGAAAAATGAAAAAGTTACCGTAGGGATAACAGCGCTTTCTTCTCTGAGAGGACTAATCAAAGAGTTGGTTGCG

>MOTU06 *Euglesa* sp. Sph16S
ACGTGGGAAAAGCTGTCTCTTTTATATAAAAAGAAGTTTATTTTTGAGTGAAAAAGCTTAGATGTTTGTAAAAGACGAGAAGACCCTATCGAACTTGAATTGT
GTGTCTTAGTTTTGGGGTACAGAAAGTTTAGTTGGGGAAACTTAAAGTTAAAAAGAACGCTTTTTTTGTTTAAAAATGATCCTGTATTATAGAAAAATGAAA
AAGTTACCGTAGGGATAACAGCGCTTTCTTCTCTGAGAGGACTAATCAAAGAGTT

>MOTU06 *Euglesa* sp. Mol16S
ATCGAACTTGAATTGTGTGTCTTAGTTTTGGGGTACAGAAAGTTTAGTTGGGGAAACTTAAAGTTAAAAAGAACGCTTTTTTTGTTTAAAAATGATCCTGTATTA
TAGAAAAATGAAAAAGTTACCGTAGGGATAACAGCGCTTTCTTCTCTGAGAGGACTAATCAAAGAGTTGGTTGCG

>MOTU07 *Odhneripisidium* sp. Sph16S
ACGTGGGAAAAACTGTCTCTTTTGCATATGAAGAAGTTTATTTTTGAGTGAAAAAGCTTAGATTATTATAAAAGACGAGAAGACCCTATCGAACTTGAATTAGA
TGTTTGAGTTTTTGAATGTCAAAAGTTTAGTTGGGGAAACTTAAAGTAAAAAAGAACGCTTTATTTTTTTGTAATGATCCTGTAATACGGAAAAACGAAAAAG
TTACCGTAGGGATAACAGCGCTTTCTTCTCTGAGAGGACTAATTAAAGAGTT

>MOTU07 *Odhneripisidium* sp. Mol16S
ATCGAACTTGAATTAGATGTTTGAGTTTTTGAATGTCAAAAGTTTAGTTGGGGAAACTTAAAGTAAAAAAGAACGCTTTATTTTTTTGTAATGATCCTGTAATAC
GGAAAAACGAAAAAGTTACCGTAGGGATAACAGCGCTTTCTTCTCTGAGAGGACTAATTAAAGAGTTGGTTGCG

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REGULAR ARTICLE

EVALUATING THE STATUS AND POPULATION BIOLOGY OF AN IMPERILED FRESHWATER MUSSEL, PURPLE WARTYBACK (*CYCLONAIAS TUBERCULATA*), IN SOUTHERN ONTARIO, CANADA

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ABSTRACT

The Purple Wartyback (PWB; *Cyclonaias tuberculata*) is considered threatened in Canada due to the loss of populations in the Detroit River and Lake Erie and possible declines in remaining populations (Ausable, Sydenham, and Thames rivers). Many aspects of PWB life history and population ecology have not been investigated for Canadian populations. We used data from the Fisheries and Oceans Canada Unionid Monitoring and Biodiversity Observation network to estimate PWB population and life-history parameters in the Sydenham and Thames rivers. This mussel occurred at high density in the Sydenham River, but at low density in the Thames River; however, both populations exhibited positive population growth and strong recruitment. Population growth rate in the Sydenham River was 1.047 (credible interval [CI]: 1.037–1.058) from 1999 to 2015, and population growth rate in the Thames River was 1.157 (CI: 1.100–1.221) from 2004 to 2017. Estimated annual adult survival rate (mean ± SE) from catch-curve analysis of empty shells, with measured ages, across both populations was 0.950 ± 0.007. Estimated survival from catch-curve analysis of live individuals, with estimated ages, was 0.966 ± 0.001 and 0.884 ± 0.009 for the Sydenham and Thames rivers, respectively. Our results show that populations of PWB are robust in the Sydenham River and small but growing rapidly in the Thames River.

KEY WORDS: unionid, freshwater mussels, Purple Wartyback, species at risk, integrated nested laplace approximation, population ecology

INTRODUCTION

Ontario is the center of freshwater mussel biodiversity in Canada, but almost a third of Ontario's species are considered at risk (endangered, threatened, or special concern; Reid and Morris 2017). A better understanding of mussel population ecology is needed to aid recovery planning for at-risk species. Quantifying life-history traits and estimating population size and trajectories are recommended in the recovery strategies for all at-risk mussel species in the Great Lakes basin in Canada (Drake et al. 2021), but addressing gaps in our knowledge of population ecology requires prioritizing nonlethal sampling methods to reduce the impacts of research on at-risk species (Castañeda et al. 2021).

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The Purple Wartyback (PWB; *Cyclonaias tuberculata*) is considered threatened in Canada (COSEWIC 2022). This mussel is widespread in the Mississippi River basin, but in Canada it is restricted to the Lake St. Clair and Lake Huron drainages of the Great Lakes basin in southern Ontario. Populations in the Detroit River and Lake Erie appear to be extirpated after the invasion of dreissenid mussels, and remaining Canadian populations may be declining (Ausable, Sydenham, and Thames rivers; COSEWIC 2022).

The population biology of PWB in Canada is poorly known. Information about population growth rate, survivorship, and recruitment is critical for accurately assessing and monitoring population status (Haag and Williams 2014). Fisheries and Oceans Canada (DFO) conducts regular quadrat-based monitoring (Unionid Monitoring and Biodiversity

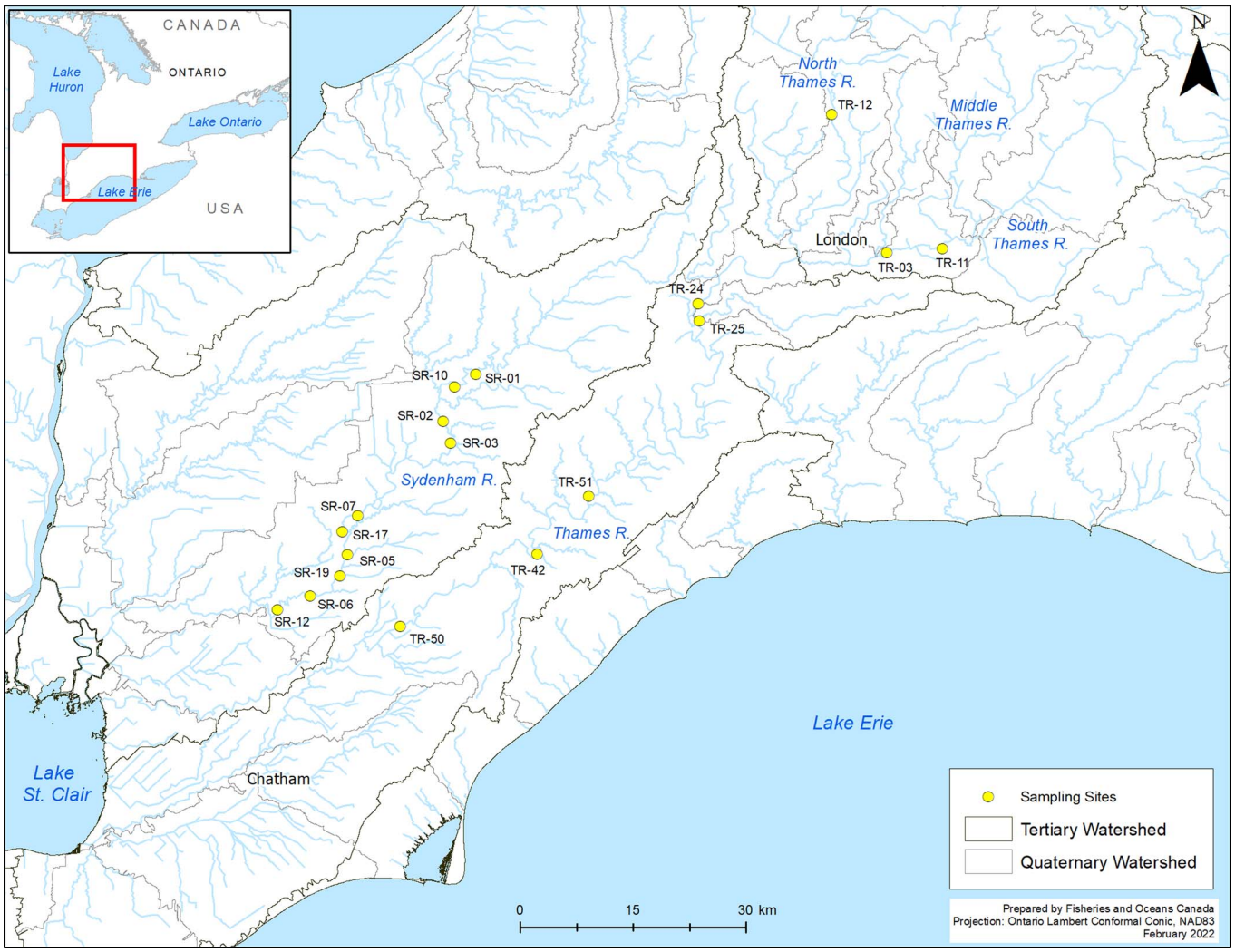

Figure 1. Location of Unionid Monitoring and Biodiversity Observation network sites sampled in the Sydenham (SR) and Thames (TR) rivers. Inset map shows the location of the study area in southern Ontario, Canada.

Observation [UMBO] network) of mussel populations in southern Ontario that includes sampling in the Sydenham and Thames rivers (Metcalfe-Smith et al. 2007; Sheldon et al. 2020; Fig. 1). We used UMBO data to estimate PWB population and life-history parameters in the Sydenham and Thames rivers to improve current understanding of PWB population ecology in southern Ontario.

METHODS

Study Area

The Sydenham and Thames rivers are tributaries of Lake St. Clair that drain approximately 2,700 and 5,800 km^2, respectively (Fig. 1; DFO 2018; SCRCA 2022). Both rivers run through the Carolinian Life Zone, making them among the most biologically diverse areas in Canada (Clarke 1992; Quinlan 2013; Carolinian Canada 2022). Land use in both watersheds is predominantly agricultural, comprising 80–85% of the watershed area (Nürnberg and Lazerte 2015; DFO 2018). Together, the watersheds supported 35 mussel species historically, and >30 species remain in each (Staton et al. 2003; McNichols-O'Rourke et al. 2012; Quinlan 2013). The Sydenham and Thames river watersheds support 14 and 11 mussel species, respectively, that are considered at risk in Canada (Cudmore et al. 2004; Goguen et al. 2022; DFO, unpublished data).

Sampling

We selected, from the UMBO network, 10 sites in the Sydenham River and 8 sites in the Thames River that encompass the distribution of PWB within these watersheds (Fig. 1). We sampled each site twice: we sampled the Sydenham River sites initially between 1999 and 2003 and resampled them between 2012 and 2015; we sampled the Thames River sites initially between 2004 and 2010 and resampled them between 2015 and 2017.

Methods for all sampling events were based on Metcalfe-Smith et al. (2007) and Sheldon et al. (2020). We surveyed

sites by using a systematic sampling design with three random starts (Strayer and Smith 2003). With one exception (see subsequent), we divided each site into approximately 25–3×5 m blocks. Within each block, we randomly selected three 1-m^2 quadrats and hand excavated the substrate to a depth of approximately 10 cm. We identified all mussels detected and measured shell length (maximum anterior-to-posterior distance, nearest 0.1 mm) by using Vernier calipers. We returned substrate and mussels to each quadrat and replaced the mussels in a natural position.

In 2012, we surveyed one site in the Sydenham River (SR-06) following the methods described previously, but we then sampled all remaining quadrats so that the entire 375-m^2 area was excavated (Reid and Morris 2017). We used results of the full excavation at this site as an out-of-sample test of model performance (see Data Analysis).

Empty shells were collected at UMBO sites during targeted surveys in 2018 and 2019 for use in developing a length-at-age relationship. We identified, counted, and collected empty fresh shells (i.e., tissue present, intact ligament, intact periostracum). We measured the shell length of each individual and estimated its age (in years) by interpreting radial thin sections cut from one valve. We prepared thin sections following standard methods for bivalves (Neves and Moyer 1988; Haag and Staton 2003; Haag and Commens-Carson 2008; Haag and Rypel 2011), described herein as follows. We used a Buehler IsoMet 4000 precision saw (Buehler Ltd., Whitby, ON, Canada) with a 1-μm specimen positioning system to cross section each valve from the umbo to the outer margin, intersecting the annual rings at right angles. We set blade speed and feed rate to 1,900 rpm and 16.0 mm/min, respectively, and we programmed cut length to match the shell length. We made a second cut, perpendicular to the first, if the first section was too large to fit on a standard glass slide (7.62 cm $\times$ 2.54 cm). We polished the cut surface with a series of successively finer grit wet sandpapers (e.g., 600, 1200, 2000) and then affixed the cut surface to a glass slide with epoxy, with curing allowed for 48 h. We made a second cut to remove excess shell, leaving a 600-μm thin section epoxied to the slide and polished the thin section as described previously.

We viewed thin sections using either a SMZ800 stereo microscope or an eclipse Ci compound light microscope (Nikon, Mississauga, ON, Canada) at various magnifications. We identified annuli as shell rings that were continuous from the umbo to the shell margin, and they typically included dark and light areas between annuli, which potentially represented variation in growth rate or environmental conditions during the growing season (Haag and Commens-Carson 2008). Three qualified readers independently assessed each thin section. If there were discrepancies between age counts that could not be resolved, we did not include that thin section in the analysis.

Data Analysis

We conducted all analyses using R 4.1.2 (R Core Team 2021). We described growth of PWB by fitting length-at-age data to a von Bertalanffy growth function (VBGF):

$$L_t = L_\infty(1 - e^{-kt}), \tag{1}$$

where L_t is length at age t, L_∞ is asymptotic length, and k is the growth coefficient. We estimated parameters with Bayesian methods by using NIMBLE (de Valpine et al. 2017, 2022). We assumed a log-normal error structure to prevent negative credible intervals (CIs; Ogle 2016), and we used noninformative priors. We collected 61 empty shells across both waterbodies for aging, 31 from the Sydenham River and 29 from the Thames River; for one shell, the river of origin was uncertain. We reached a consensus age for all collected PWB shells.

We generated length–frequency distributions for pooled sites across years for both the Sydenham and Thames rivers by using ridgeline plots (Wilke 2022). We identified putative juveniles based on estimated age at maturity (T_{mat}) and length at maturity (L_{mat}). Neither parameter is known for Ontario populations of PWB. We estimated T_{mat} using the following equation:

$$T_{\text{mat}} = 0.69k^{-1.031} - 1, \tag{2}$$

where k is the VBGF growth coefficient (Haag 2012). We then used equation (1) to estimate L_{mat}. We investigated the change in the proportion of juveniles through time with logistic regressions where individuals were represented as a Boolean, 1: juvenile; 0: adult. Site was included as a random effect if there were improvements in deviance information criterion (DIC).

We estimated annual survival of adult PWB by using the Chapman–Robson method for catch-curve analysis (Chapman and Robson 1960; Smith et al. 2012) based on empty shells collected in 2018 and 2019 and live shells from all sample years where age was estimated based on the VBGF. We estimated survival ($\hat{S}$) by using the following equation:

$$\hat{S} = \frac{\bar{T}}{1 + \bar{T} - \frac{1}{n}}, \tag{3}$$

where n is the total number of fully recruited mussels observed and $\bar{T}$ is the mean age of mussels fully recruited to sampling. We calculated the SE of survival rate using the following equation:

$$\text{SE}_{\hat{S}} = \sqrt{\hat{S}\left(\hat{S} - \frac{\bar{T} - 1}{n + \bar{T} - 2}\right)}. \tag{4}$$

We performed catch-curve analysis on the aged empty shells pooled from both rivers: that analysis assumes that mortality and recruitment are constant, all individuals are equally available to sampling, and there is no error in age estimation (Ogle 2016). If the smaller shells of younger PWB degraded faster or were more likely to be removed from the system (e.g., via predation or water currents) than larger shells of older PWB, the assumption of equal availability to

sampling will be violated and the estimates biased, likely toward estimating greater survival. In addition, if shells persist in the system for a long period of time, the survival estimate may not represent contemporary conditions. As a comparison, we performed an additional catch-curve analysis by assigning ages to the live PWB sampled during the study. We used the Bayesian VBGF fit to generate predicted length distributions for each age-class (Age-0 to the maximum observed age in each river), incorporating parameter uncertainty and residual variance. We used these posterior predictive distributions to generate an age-length key representing the probability that lengths, binned into 10-mm groups, belonged to each age-class. We assigned ages to sampled PWB based on their measured length by using the Iserman and Knight method (Iserman and Knight 2005; Ogle 2016). We assigned live-shell ages based on the river-specific age-length key generated from the VBGF (Figs. A1 and A2). We performed the catch-curve and age-length key analyses using the FSA package (Ogle et al. 2022).

We estimated population density and trajectory by fitting the UMBO data with separate models for the Sydenham and Thames rivers. We used a hierarchical Bayesian approach with integrated nested laplace approximation (INLA; Rue et al. 2009), which uses deterministic methods to make Bayesian inferences allowing for faster computations than Markov Chain Monte Carlo methods. We modelled density (mussels/m^2) as a function of sample year with sample site included as a random effect. We modelled the data using the negative binomial (NB) distribution as preliminary analysis demonstrated using the Poisson distribution resulted in a model that was overdispersed. The model was represented by

$$y_{is} \sim \mathrm{NB}(\mu_{is}, \theta), \tag{5}$$

$$E(y_{is}) = \mu_{is}, \tag{6}$$

$$\mathrm{var}(y_{is}) = \mu_{is} + \frac{{\mu_{is}}^2}{\theta}, \tag{7}$$

$$\log(\mu_{is}) = \alpha + \beta \cdot \mathrm{year}_i + \mathrm{site}_s, \tag{8}$$

where y_{is} represents PWB count from quadrat i and site s, μ_{is} is the expected mean density, and θ is the size parameter of the negative binomial distribution indicating the extent of overdispersion; α is the intercept representing the initial mean density; the covariate year is the year sampled beginning at 0 (survey year subtracted from the first survey year); β is the slope of the year effect representing the instantaneous rate of population growth where population growth rate is $\lambda = e^{\beta}$; *site* represents the site random effect, which was assumed to be independent and identically distributed with a mean of 0 and SD σ_{site}. To determine the importance of including site as a random effect we compared DIC values to the models omitting the random effect. We used the log-gamma prior with shape and rate parameters of 1 and 0.05 for the site hyperparameter (Carroll et al. 2015). We used the default uninformative prior for fixed effects, a normal distribution with a mean of 0 and precision of 0.001.

We used the full excavation of site SR-06 in 2012 as an out-of-sample test of model performance. We compared observed density and the total number of PWB collected to predictions from the fitted model.

RESULTS

In total, 3,275 live PWB were sampled: 3,085 in the Sydenham River and 190 in the Thames River. Density in the Sydenham River was 2.06 mussels/m^2, compared with 0.17 mussels/m^2 in the Thames River. In the Sydenham River, 1,190 PWB were collected in the first sampling period and 1,895 were collected in the second sampling period. An increase in density between sample periods was observed at 8 of the 10 sites in the Sydenham River (Table A1). In the Thames River, 26 and 164 PWB were collected in the first and second sampling periods, respectively, and six of the eight sample sites showed an increase in mean density (Table A1).

Individuals ranged in size from 9.0 to 198.9 mm across both rivers, with a mean length of 80.4 mm in the Sydenham River and 59.8 mm in the Thames River. Mean length in the Sydenham River was significantly greater than that of mussels in the Thames River ($t = 11.9$, $P < 0.001$). The lengths of aged shells ranged from 12.6 to 128.0 mm (mean: 76.3 mm) in the Sydenham River and from 24.4 to 110.6 (mean: 82.3) in the Thames River. The ages ranged from 1 to 92 yr (mean: 26.1 yr) in the Sydenham River and from 2 to 32 yr (mean: 17.6 yr) in the Thames River. There was overlap in the length-at-age data between rivers and no apparent difference in growth; river did not have an important effect on the L_∞ or k parameters when included in the model fit. Consequently, the data were pooled to produce one growth curve common to both rivers, thereby allowing us to retain the "uncertain" shell in the analysis. The VBGF (Fig. 2) coefficient estimates were $L_\infty = 110.9 \pm 3.63$ mm (SE) and $k = 0.091 \pm 0.006$ (SE).

The number of empty shells was highest for age 7 individuals, allowing survival rate to be estimated over ages 7–92. The Chapman–Robson catch-curve survival rate estimate was 0.950 ± 0.007 (SE) or an instantaneous mortality rate of 0.051 ± 0.008 (SE). Length frequency distributions of empty shells and live individuals were similar (Fig. 3). There was a slight bias towards larger size-classes (greater mode) in the empty shell sample, but it appears to be a reasonable approximation of the live individuals sample. The number of live individuals was highest for age 8, allowing survival to be estimated over ages 8–92. The Chapman–Robson catch-curve survival rate estimate was 0.965 ± 0.001 (SE) for rivers combined; the survival rate estimate for the Sydenham River was 0.966 ± 0.001 (SE) for ages 8–92; and the survival rate estimate for the Thames River was 0.883 ± 0.009 (SE) for ages 6–32.

Age at maturity for PWB was estimated to be 7.2 yr, representing a length at maturity of 53.1 mm. Length frequency distributions and mean length of PWB were relatively stable

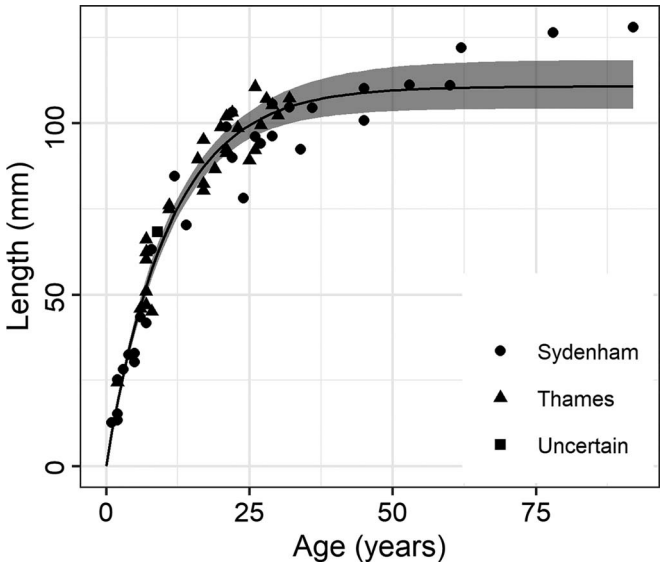

Figure 2. Length-at-age relationship for Purple Wartyback (*Cyclonaias tuberculata*) in the Sydenham and Thames rivers. The solid line represents the fitted von Bertalanffy growth function ($L_t = 110.9(1 - e^{-0.091t})$) and the gray region represents the 95% credible interval.

across years in the Sydenham River (Fig. 4). Juveniles (<53.1 mm) were present in all years and made up 11–18% of the population (mean across years: 13.3%). Logistic regression of juveniles included site as a random effect and indicated a slight decrease in the proportion of juveniles ($P_{juv.}$) over time [$\text{logit}(P_{juv.}) = -0.032\text{year} - 1.57$; $P < 0.001$; $\Delta\text{DIC} = 20.6$]. In the Thames River, length frequency distributions appeared to be bimodal in some years and mean length varied among years. Juveniles were present in all years and made up 14–58% of the population (mean across years: 46.8%). Logistic regression omitted site as a random effect and indicated an increase in the proportion of juveniles [$\text{logit}(P_{juv.}) = 0.13\text{year} - 1.47$; $P = 0.01$; $\Delta\text{DIC} = 0.02$].

The negative binomial model described the quadrat data well with no apparent violations of model assumptions, no overdispersion, and an appropriate number of 0's in model predictions (i.e., no zero-inflation; Table 1). The random site effect was important for both rivers; ΔDIC values of comparisons with models without the site effect were 753 and 139, respectively, for the Sydenham and Thames rivers. The slope of density over time was positive in both rivers (Fig. 5; Table 1). Population growth rate (λ) in the Sydenham River between 1999 and 2015 was 1.047 (95% CI: 1.037–1.058), and λ in the Thames River between 2004 and 2017 was 1.157 (95% CI: 1.100–1.221; Table 2). The expected mean-density estimate for the Sydenham River in 2015 was 1.82 mussels/m^2; for the Thames River in 2017, it was 0.12 mussels/m^2. The estimated population size in the sampled area of the Sydenham River in 2015 was 10,504 (95% CI: 9,563–11,505); for the Thames River in 2017, it was 872 (95% CI: 696–1,091).

During the full excavation of site SR-06, the number of PWB collected ranged from 1 to 36 mussels per quadrat with a mean density of 6.98 mussels/m^2 and the total number of PWB collected was 2,616. The model predicts the expected mean density at site SR-06 in 2012 to be 6.07 mussel/m^2 (95% CI: 5.23–7.04) and the whole-site PWB abundance to be 2,277 mussels (95% CI: 1,960–2,641). The density and total number of PWB collected were within 95% CIs of the model estimates.

DISCUSSION

Populations of PWB in both the Sydenham and Thames rivers experienced positive population growth over the survey time frame. However, these two populations differed in several aspects. The Thames River population had lower abundance and density, but it had a higher population growth rate, than the Sydenham River. The high proportion of juveniles and smaller mean size in the Thames River population support a rapidly growing population. The Sydenham River population had higher abundance and density, lower population growth rate, larger mean size, and more stable length–frequency distributions, all of which suggest a population nearer carrying capacity. In contrast to the Thames and Sydenham populations, the only other remaining population in Canada, in the Ausable River, occurs at low density and shows no evidence of population growth (K. Jean, Ausable Bayfield Conservation Authority, personal communication). It is unknown

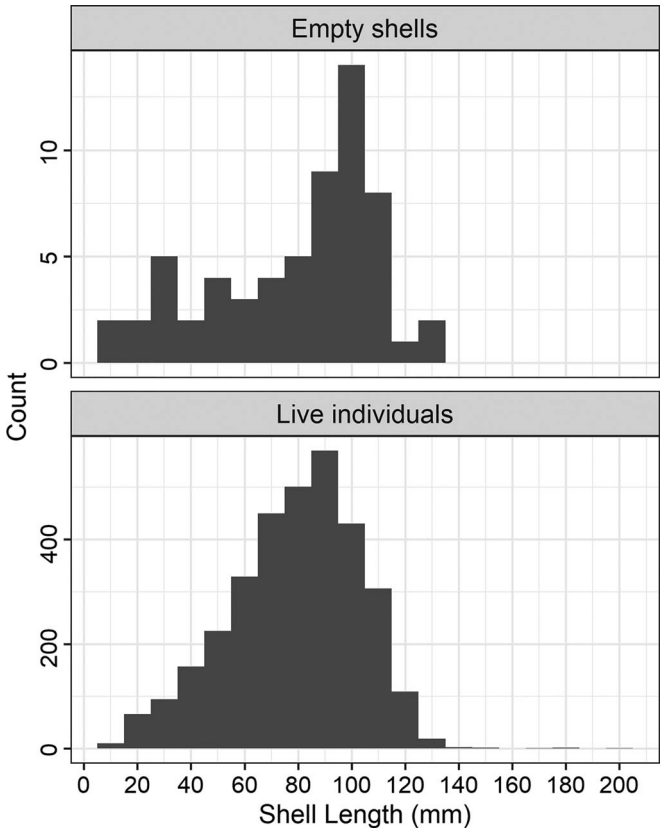

Figure 3. Length frequency distributions of empty shells and live individuals of Purple Wartyback (*Cyclonaias tuberculata*) from the Sydenham and Thames rivers.

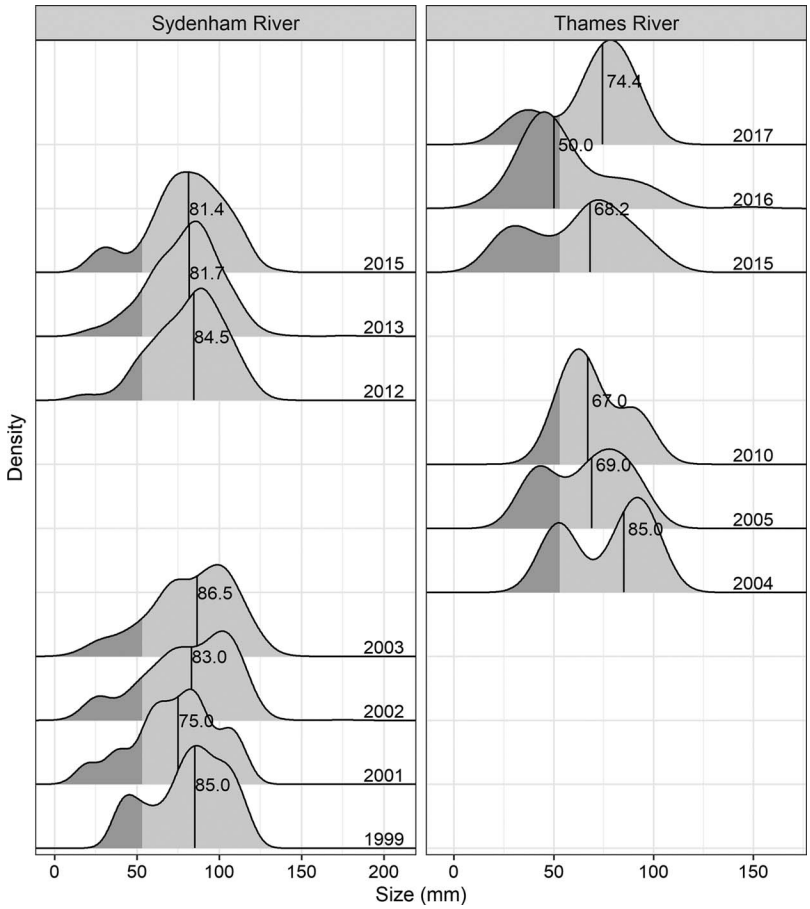

Figure 4. Length distributions of Purple Wartyback (*Cyclonaias tuberculata*) in the Sydenham and Thames rivers over time. Dark gray indicates putative juveniles based on length (<53.1 mm), and light gray indicates adults. Median annual lengths are reported and represented with vertical lines.

why the three Canadian populations of PWB exhibit such different population dynamics.

Density estimates for sparse populations, such as those in the Thames River, can have low precision (Strayer et al. 1997; Lane et al. 2021), which could have influenced our population growth rate estimates. However, our large sample size resulted in a growth rate estimate with a low SE, providing confidence in our estimate. Our conclusion of high population growth rate is supported by the large percentage of juveniles in the population and its low mean mussel size. A population growth rate of >15% is high for most unionids, particularly a long-lived species such as PWB, but growth rates >20% are reported for some species (Patterson 1985; Jones and Neves 2011). Nevertheless, the high population growth rate of PWB in the Thames River is unlikely to be maintained indefinitely as the population approaches carrying capacity.

Purple Wartyback co-occur with many other species at risk (SAR) in the Sydenham and Thames rivers, all of which

Table 1. Parameter estimates for models to estimate density of Purple Wartyback (*Cyclonaias tuberculata*) over time in the Sydenham and Thames rivers. LCI and UCI is the lower and upper 95% credible interval, respectively. θ is the size parameter of the negative binomial distribution, and σ_{site} is the standard deviation of the site random effect.

	Sydenham River				Thames River			
	Median	LCI	UCI	SD	Median	LCI	UCI	SD
Fixed effect								
Intercept	−0.161	−0.863	0.535	0.351	−3.998	−5.575	−2.663	0.730
Year	0.046	0.036	0.057	0.005	0.146	0.096	0.200	0.027
Hyperparameter								
θ	1.864	1.581	2.207	0.160	1.223	0.662	2.535	0.486
σ_{site}	0.942	0.375	2.065	0.440	0.373	0.105	1.127	0.272

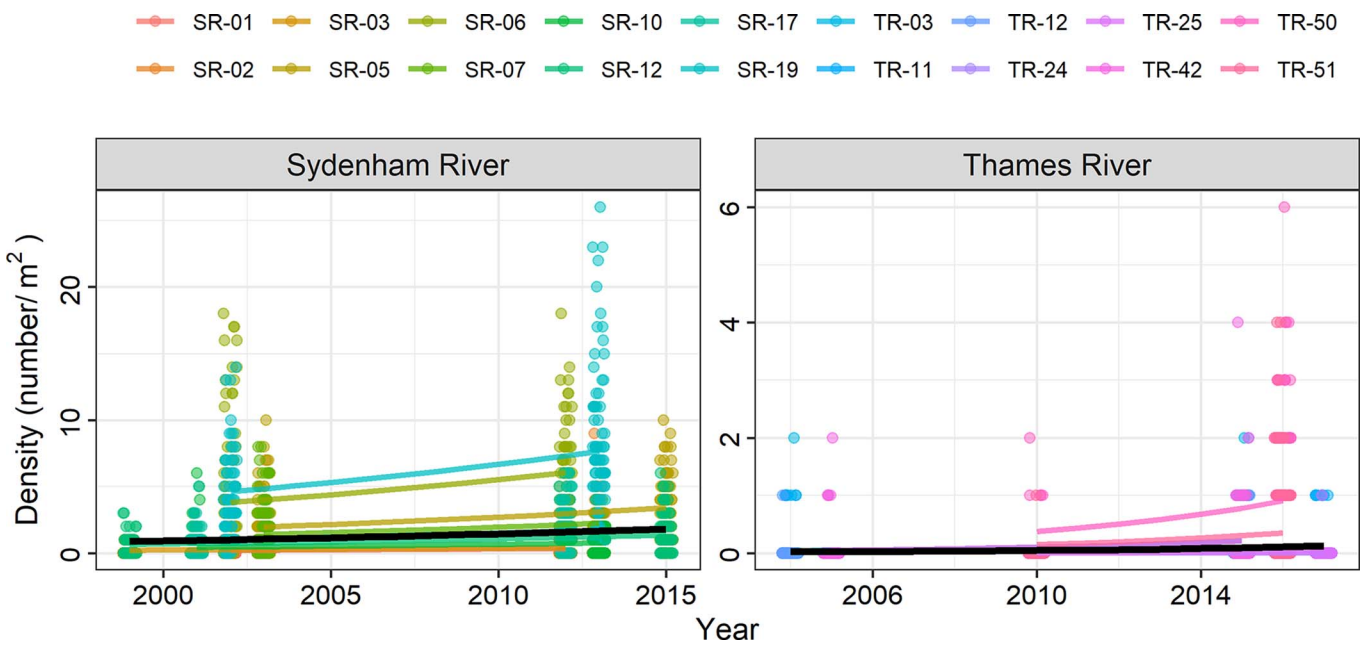

Figure 5. Density of Purple Wartyback (*Cyclonaias tuberculata*) in the Sydenham and Thames rivers (SR and TR, respectively) over time. Colored lines represent fitted relationships for each site, and the black line shows the mean trend for each river across sites.

may have benefitted from conservation actions such as identification and protection of critical habitat and the implementation of mitigation measures to reduce threats within these watersheds (DFO 2018). These actions may have been factors in overall improvement in water quality in both systems, which likely further contributed to increasing populations of PWB. Another at-risk species, the Wavy-Rayed Lampmussel (*Lampsilis fasciola*), showed high population growth and range expansion in the Thames River, leading to a downgrading of its conservation status (COSEWIC 2010).

Our estimates of PWB survival rate from catch-curve analysis of empty shells could be biased to a varying extent based on the shell decay rate. If shells decay slowly and persist in the environment for a long time, our annual survival rate estimates may be underestimated because of the accumulation of individuals that died over several years. Furthermore, if shell decay rates differ across ages, our estimates may be inflated. Unionid shell decay rate relates to both extrinsic factors, such as water chemistry and current, and intrinsic factors, such as shell size and robustness (Strayer and Malcom 2007; Ilarri et al. 2019); however, we have no information about decay rates in our study rivers. Despite the potential for bias, the similarity of our survival estimates from empty shells to survival calculated from live individuals, as well as the similarity of length–frequency distributions for empty shells and live individuals, support the accuracy of our estimates. In the Thames River, our survival estimate from live individuals (0.884) was lower than that from empty shells (0.950). This discrepancy could have been caused by the lower maximum observed age (32 yr), smaller mean size and smaller sample size in the Thames River, resulting in a less precise estimate from live individuals.

No other estimates of PWB survival rate are available, but our estimates are similar to predicted and observed values for other long-lived unionids. Based on a relationship between

Table 2. Summary of population parameters (95% credible intervals) for Purple Wartyback (*Cyclonaias tuberculata*) in the Sydenham and Thames rivers.

Parameter	Sydenham River	Thames River
Abundance at sample sites	10,504 (9,563–11,505)	872 (696–1091)
Density (mussels/m^2)	1.82 (0.94–3.87)	0.12 (0.03–0.42)
Population growth rate (λ; y^{-1})	1.047 (1.037–1.058)	1.157 (1.10–1.221)
% juveniles	14	49
Juvenile trend	↘	↗
Mean length (mm)	80.4	59.8
Length end	→	↘
Survival rate (dead shells)	0.950 (±0.007 SE)	
Survival rate (live individuals)	0.966 (±0.001 SE)	0.884 (±0.009 SE)

instantaneous mortality rate (M) and life span (T_{max}) for 14 species from 15 populations ($M = 4.171 T_{max}^{-1.070}$; Haag 2012), our maximum observed age of 92 for PWB gives a predicted instantaneous mortality rate of 0.033, or a survival rate of 0.968, which is similar to our estimates for empty shells and live individuals from the Sydenham River. Annual survival rates of the long-lived *Amblema plicata* and *Popenaias popeii*, estimated by mark–recapture methods, were 0.97 and 0.98, respectively (Hart et al. 2001; Inoue et al. 2014). Similarly, annual survival was >0.90 for three unionid species in a 4-yr mark–recapture study (Villella et al. 2004). Mark–recapture studies are needed to corroborate the survival estimates we obtained from catch-curve analysis.

The VBGF parameters provide insight into important life-history characteristics such as growth rate, maximum size, life span, age at maturity, and relative shell mass (Haag and Rypel 2011; Haag 2012). The only other published values for PWB growth coefficients are from West Virginia, USA, where L_∞ was 87.0–113.9 mm and k was 0.110–0.164 in the New River and L_∞ was 90.6 mm and k was 0.094 in the Greenbrier River; the maximum age observed in these populations was 91, and length at maturity was 58.6 mm (Jirka 1986). Our estimates of a long life span (92 yr), delayed maturity (7.2 yr), and slow growth ($k = 0.091$) for PWB in Ontario are consistent with the expectation of an equilibrium species adapted to stable habitats (Haag 2012).

The UMBO survey design was not based on randomized site selection; instead, it used prior knowledge to select sites with high mussel density and SAR occurrence. As a result, extrapolation of our density estimates outside of the survey sites is inappropriate (Reid and Morris 2017), and we were unable to make system-wide estimates of density or population size. However, the wide distribution of survey sites within each system means that our estimates of population growth and recruitment may reflect the entire river. In addition, the survey protocol provides accurate and precise estimates of mussel density for common species, such as PWB in the Sydenham River, and it provides reliable detection of large changes in density, even when density is <0.1 mussels/m^2 (Reid and Morris 2017). Although PWB populations in the Detroit River and Lake Erie may be extirpated, populations in the Sydenham and Thames rivers appear to be large and robust or increasing in recent years.

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Appendix

Table A1. Summary of sample data for Purple Wartyback (PWB; *Cyclonaias tuberculata*).

River	Site	Latitude	Longitude	Year	No. of Blocks	No. of Quadrats	No. of PWB	Density (no./m^2)	SE
Sydenham River	SR-01	42.86	−81.79	2002	24	72	14	0.19	0.006
				2012	24	72	23	0.32	0.008
	SR-02	42.806	−81.847	2003	26	78	80	1.03	0.016
				2013	25	75	125	1.67	0.023
	SR-03	42.779	−81.835	1999	23	69	11	0.16	0.005
				2012	23	69	30	0.43	0.012
	SR-05	42.651	−82.01	2003	23	69	139	2.01	0.032
				2015	25	75	251	3.35	0.035
	SR-06	42.604	−82.072	2002	26	78	341	4.37	0.065
				2012	25	75	395	5.27	0.051
	SR-07	42.697	−81.99	2003	27	81	173	2.14	0.025
				2013	25	75	95	1.27	0.021
	SR-10	42.846	−81.825	2001	25	75	47	0.63	0.015
				2013	25	75	41	0.55	0.011
	SR-12	42.589	−82.126	1999	26	78	33	0.42	0.009
				2015	25	75	123	1.64	0.019
	SR-17	42.679	−82.017	2001	27	81	48	0.59	0.011
				2012	25	75	166	2.21	0.023
	SR-19	42.626	−82.023	2002	25	75	304	4.05	0.043
				2013	25	75	646	8.61	0.073
Thames River	TR-03	42.982	−81.114	2004	22	66	9	0.14	0.006
				2015	25	75	10	0.13	0.005
	TR-11	42.983	−81.024	2004	22	66	3	0.05	0.003
				2017	25	75	8	0.11	0.04
	TR-12	43.15	−81.192	2004	21	63	1	0.02	0.002
				2015	25	75	6	0.08	0.004
	TR-24	42.932	−81.424	2010	25	75	0	0	0
				2017	25	75	0	0	0
	TR-25	42.912	−81.424	2010	25	75	0	0	0
				2017	25	75	1	0.01	0.002
	TR-42	42.643	−81.703	2005	23	69	6	0.09	0.005
				2015	25	75	14	0.19	0.008
	TR-50	42.564	−81.93	2010	15	45	6	0.13	0.009
				2016	25	75	85	1.13	0.017
	TR-51	42.709	−81.616	2010	25	75	1	0.01	0.002
				2016	25	75	40	0.53	0.012

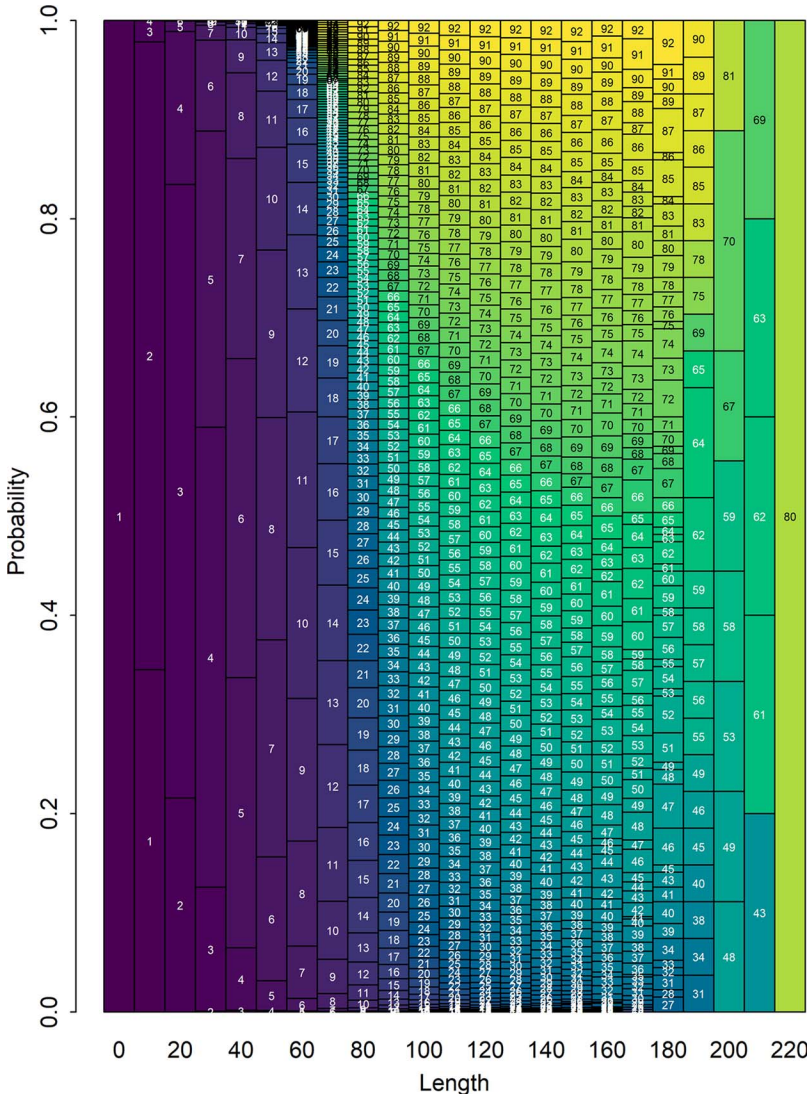

Figure A1. Age-at-length key for Purple Wartyback (*Cyclonaias tuberculata*) from the Sydenham River developed from von Bertalanffy growth function length-at-age predictions. The x axis represents 10-mm length bins, the y axis represents age probabilities, and the tiles represent ages (1–92).

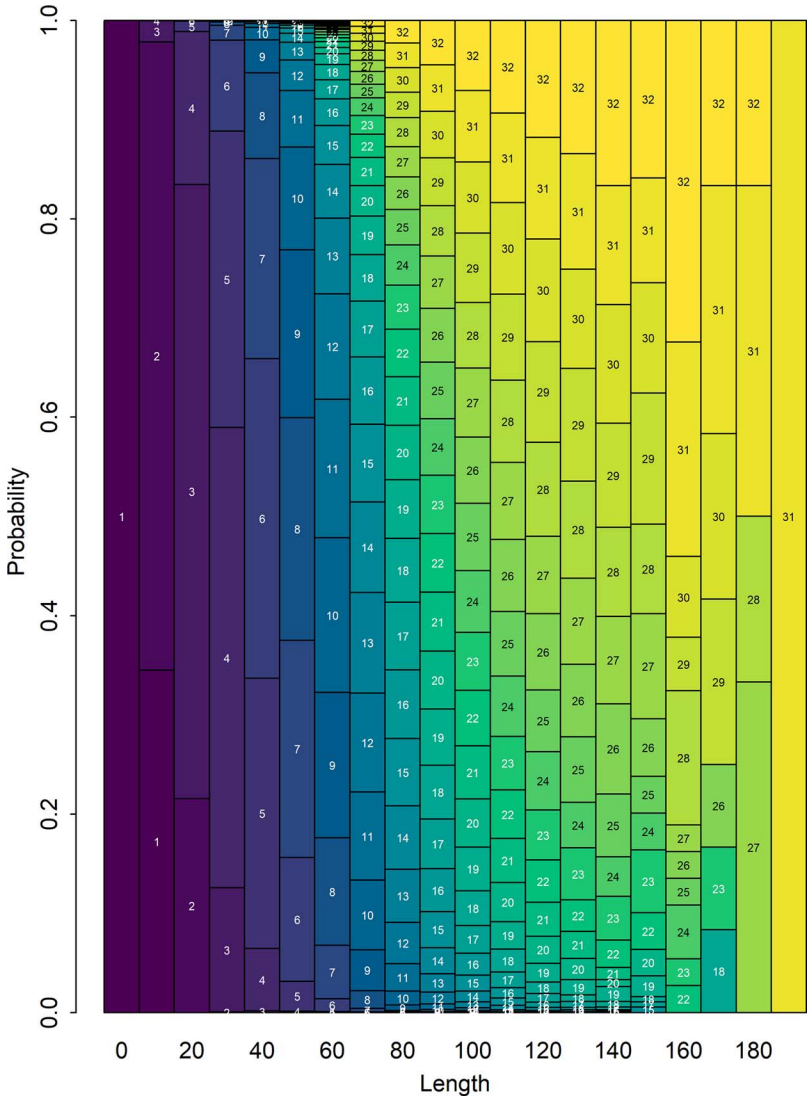

Figure A2. Age-at-length key for Purple Wartyback (*Cyclonaias tuberculata*) from the Thames River developed from von Bertalanffy growth function length-at-age predictions. The x axis represents 10-mm length bins, the y-axis represents age probabilities, and the tiles represent ages (1–32).

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Photo credits (top to bottom): *Hamiota altilis*, GADNR; snorkeler with quadrat, Wendell Haag; *Lithasia obovata*, Monte McGregor; *Margaritifera falcata*, Jeremy Monroe; *Alasmidonta varicosa*, Barry Wicklow