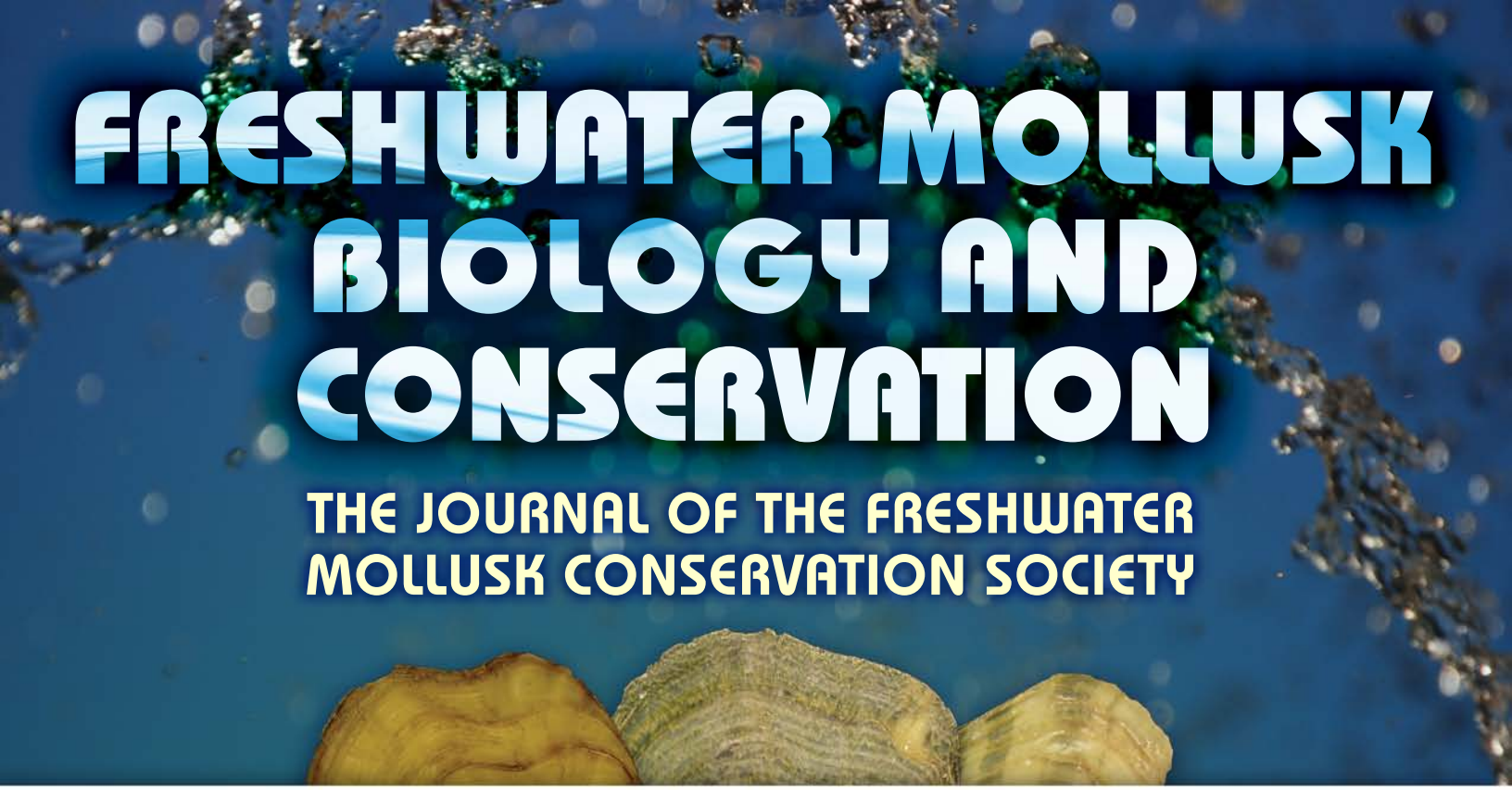

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REGULAR ARTICLE

BASELINE QUALITATIVE AND QUANTITATIVE MUSSEL SURVEYS OF THE MILL RIVER SYSTEM, MASSACHUSETTS, PRIOR TO FINAL DAM REMOVAL

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ABSTRACT

Dam removal is a common conservation tool that has many potential benefits for freshwater mussels. We conducted qualitative and quantitative mussel surveys in the Mill River system, Massachusetts, where four dams have been removed or modified to benefit aquatic organisms. These data represent a baseline for future monitoring of the effects of dam removal or modification. Mussel assemblages were composed of six species and were dominated by *Elliptio complanata*; *Lampsilis radiata* was the second most abundant species. Two species of Special Concern in Massachusetts, *Ligumia nasuta* and *Leptodea ochracea*, were rare, as were *Pyganodon cataracta* and *Utterbackiana implicata*. We conducted catch-per-unit-effort (CPUE) surveys at 77 sites; mussels occurred throughout much of the watershed except for the lower portion of the Mill River. The highest CPUE values were found immediately downstream of the two lakes in the system. We conducted quadrat-based surveys at nine sites, including one site in each of the lakes. Precision of estimates of total mussel density was $\geq$80% at most sites, which will allow detection of moderate to large changes over time. Monitoring of changes for rarer species may require a watershed-based approach based on CPUE because quantitative estimates had wide confidence intervals.

KEY WORDS: freshwater mussels, dam removal, population and assemblage size estimates, sampling adequacy and precision, stream habitat

INTRODUCTION

Dams are one of the major contributors to imperilment of freshwater mussels and their host fishes (Watters 1996; Vaughn and Taylor 1999; Gangloff et al. 2011). There are more than 75,000 dams in the United States and about 4,000 in New England (Graf 1999). Most Massachusetts dams were built in the 1700s and 1800s as small mill dams, and many are now obsolete and pose human and environmental risks (Division of Ecological Restoration 2018). The Massachusetts Department of Fish and Game Division of Ecological Restoration has removed at least 40 obsolete dams since 2005 (Division of Ecological Restoration 2018).

The Taunton River, a 1,295 km^2 watershed in southeastern Massachusetts, hosts one of the largest river herring runs (*Alosa* spp.) in New England and was designated a National Wild and Scenic River in 2009 (https://www.rivers.gov/rivers/taunton.php). The main stem of the Taunton River is free-flowing, but many tributaries are blocked by obsolete mill dams that impact river processes and habitat. Four such dams blocked the Mill River, a tributary of the Taunton River. The Mill River Restoration partnership is a collaboration of government agencies, nonprofit organizations, and others working to remove these dams and other fish passage barriers.

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Table 1. Site data for qualitative mussel survey sites in the Canoe (CR), Snake (SR), and Mill (MR) rivers. GPS coordinates indicate the upstream and downstream boundaries of each site. Sites with a single set of GPS coordinates were sampled with a transect-based approach, and coordinates indicate location of transect (see text). Macrohabitat codes: Gl = glide; Lsp = lateral scour pool; Mcp = midchannel pool; Po = pool; Ri = riffle; Ru = run. Substrate codes: Bo = boulder; Co = cobble; Fi = fines; Gr = gravel; Lwd = large woody debris; Sa = sand; Si = silt; Swd = small woody debris; Tra = trash. Vegetation codes: Av = aquatic vegetation; Ba = benthic algae.

Stream	Site Number	Start GPS	End GPS	Mean Depth (m)	Mean Width (m)	Habitats	Substrates	Vegetation
CR	1	42.00266, −71.15771	42.00310, −71.15868	0.2	6	Ri, Ru, Po	Bo Co, Gr, Sa	
CR	2	42.00120, −71.15687	42.00266, −71.15771	0.2	8	Ri, Ru, Po, Gl	Gr, Sa, Co	
CR	3	42.00074, −71.15672	42.00129, −71.15687	0.3	5	Ri, Rn	Co, Gr, Sa, Si	
CR	4	41.99940, −71.15711	42.00074, −71.15672	0.3	2	Mcp, Ri	Sa, Si	Av
CR	5	41.99899, −71.15614	41.99940, −71.15711	0.5	3	Mcp	Sa, Si	Av
CR	6	41.99730, −71.15694	41.99899, −71.15614	1.0	5	Mcp	Sa, Si,	Av
CR	7	41.99578, −71.15881	41.99730, −71.15694	1.0	8	Mcp	Si, Fi, Sa	Av
CR	8	41.99534, −71.15958	41.99578, −71.15881	0.3	10	Mcp, Lsp	Sa, Gr, Co, Si	Av
CR	9	41.99507, −71.15953		0.2	5	Mcp	Co, Gr, Sa	Av
CR	10	41.99371, −71.16022		0.3	3	Gl	Sa, Co	Av on margin
CR	11	41.99262, −71.16052		0.4	3	Mcp	Sa, Si, Co	Av on margin
CR	12	41.99150, −71.16039		0.3	6	Mcp	Sa, Co	Ba, Av on margin
CR	13	41.99075, −71.16129		0.3	8	Lsp	Sa, Co, Si	
CR	14	41.98992, −71.16235		0.4	5	Mcp	Si, Swd	Av
CR	15	41.98959, −71.16331		0.3	4	Mcp	Sa, Si	Av
CR	16	41.98861, −71.16422		0.3	5	Lsp	Sa, Gr, Si	
CR	17	41.98774, −71.16481		0.6	5	Mcp	Gr, Si, Sa	
CR	18	41.98643, −71.16595		0.7	7	Mcp	Gr, Si, Sa	
CR	19	41.98648, −71.16786		0.4	5	Lsp	Sa, Si, Co	
CR	20	41.98541, −71.16941		0.3	4	Lsp	Sa, Si	Av on margin
CR	21	41.98436, −71.16940		0.2	6	Gl	Sa, Gr	Av on margin
CR	26	41.98249, −71.16341		0.2	7	Ru	Co, Sa, Si	
CR	27	41.98172, −71.16199		0.1	9	Ru	Co, Sa, Bo	
CR	28	41.98172, −71.16050		0.2	5	Mcp	Co, Sa	
CR	29	41.98214, −71.15871		0.3	5	Mcp	Co, Sa	
CR	30	41.98183, −71.15645		0.2	6	Mcp	Sa, Gr	
CR	31	41.98077, −71.15672		0.3	4	Mcp	Sa, Si	
CR	32	41.97949, −71.15527		0.2	6	Mcp	Sa, Si	
CR	33	41.98022, −71.15369		0.6	4	Mcp	Sa, Gr	
CR	34	41.98054, −71.15208		0.3	4	Ru	Sa	Av
CR	35	41.98024, −71.15084		0.3	2	Mcp	Sa, Si	Av
CR	36	41.97963, −71.14767		0.3	4	Mcp	Si	Av
CR	37	41.97794, −71.94449		0.4	4	Mcp	Sa, De	Av
SR	38	41.96706, −71.12579	41.96692, −71.12473	2.0	40	Mcp	Sa, Gr, Co	
SR	39	41.96760, −71.12251	41.96743, −71.12208	0.8	10	Mcp	Si, Swd	
SR	40	41.96743, −71.12208	41.96300, −71.11773	0.4	12	Ru	Sa, Si, Swd	
SR	41	41.96653, −71.12209		0.2	2	Mcp	Sa, Si, Fi	
SR	42	41.96624, −71.12072		0.4	3	Mcp, Lsp	Sa, Cl, Si	Av on margin
SR	43	41.96550, −71.11987		0.4	3	Mcp	Sa, Si, Swd	
SR	44	41.96475, −71.11873		0.5	8	Mcp, Lsp	Sa, Si, Fi	
SR	45	41.96429, −71.11772		0.4	6	Lsp	Sa, Si, Swd	
SR	46	41.96300, −71.11773	41.96264, −71.11718	0.3	7	Mcp	Sa, Si	Av
SR	47	41.96264, −71.11718	41.96286, −71.11581	0.4	6	Mcp	Sa, Si	Av
SR	48	41.96286, −71.11581	41.96497, −71.11355	0.4	7	Mcp	Sa, Cl,	Av
SR	49	41.96309, −71.11355	41.96497, −71.11153	0.8	7	Mcp	Sa, Si, Swd	
SR	50	41.96451, −71.11328	41.96497, −71.11530	0.4	5	Ru	Sa	Av
SR	51	41.96497, −71.11153	41.96533, −71.11010	0.9	5	Mcp	Sa, Si	Av
SR	52	41.96533, −71.11010	41.96595, −71.10915	0.3	5	Mcp	Sa,	Av
SR	53	41.96595, −71.10915	41.96667, −71.10719	0.7	6	Mcp	Sa, Si	Av

Table 1, continued.

Stream	Site Number	Start GPS	End GPS	Mean Depth (m)	Mean Width (m)	Habitats	Substrates	Vegetation
SR	54	41.96667, −71.10719	41.96612, −71.10533	0.6	4	Mcp	Sa, Si	Av
SR	55	41.96612, −71.10533	41.96473, −71.10483	1.5	5	Mcp	Sa, Si	Av
SR	56	41.96473, −71.10483	41.96392, −71.10565	0.4	6	Mcp	Sa, Si	Av
SR	57	41.96392, −71.10565	41.96252, −71.10279	1.3	7	Mcp	Sa, Si	Av
SR	58	41.96252, −71.10279	41.95989, −71.10010	1.5	10	Mcp	Sa, Si	Av
SR	59	41.95989, −71.10010	41.95840, −71.10017	1.5	10	Mcp	Sa, Si	Av
SR	60	41.95840, −71.10017		0.8	8	Mcp	Sa	Av
MR	61	41.92811, −71.10641	41.93374, −71.10789	0.5	8	Mcp, Lsp, Ri, Ru	Sa, Cl	Av
MR	62	41.92310, −71.10610	41.92811, −71.10641	0.5	10	Mcp, Ri, Ru, Gl	Bo, Co, Gr	
MR	63	41.92177, −71.10369	41.92310, −71.10610	0.5	10	Mcp, Ri, Ru	Co, Sa	
MR	64	41.92118, −71.10296	41.92177, −71.10369	0.5	10	Mcp	Co, Sa, Gr	
MR	65	41.91901, −71.10152	41.92118, −71.10296	1.5	15	Mcp	Sa, Co, Gr	
MR	66	41.91648, −71.10033	41.91902, −71.10142	0.4	10	Mcp, Ri, Ru	Co, Sa, Si, Bo	
MR	67	41.91460, −71.09669	41.91643, −71.10033	0.3	12	Mcp, Lsp, Gl, Ri, Ru	Co, Sa, Bo, Si	Av
MR	68	41.90996, −71.09785	41.91460, −71.09669	0.3	12	Mcp, Ri, Ru, Gl, Lsp	Sa, Gr, Co, Bo	
MR	69	41.90690, −71.09999	41.90996, −71.09785	1.0	12	Mcp, Lsp	Sa, Cl	
MR	70	41.90459, −71.09836	41.90690, −71.09999	0.25	15	Ri, Ru, Mcp, Lsp, Gl	Co, Sa, Gr, Si, Bo	
MR	71	41.90354, −71.09769	41.90459, −71.09836	0.25	15	Mcp, Ri, Ru	Co, Gr, Sa, Si	
MR	72	41.90009, −71.09267	41.90364, −71.09760	0.25	10	Ri, Ru, Lsp, Mcp	Co, Gr, Sa,	
MR	73	41.90014, −71.09113	41.90009, −71.09267	0.25	12	Ri, Ru	Co, Gr Sa,	
MR	74	41.89822, −71.08938	41.90014, −71.09113	0.5	8	Mcp, Lsp, Ri, Ru	Co, Gr, Sa,	
MR	75	41.89730, −71.08926	41.89822, −71.08938	0.25	8	Mcp	Sa, Co	
MR	76	41.89693, −71.08656	41.89730, −71.08926	0.5	10	Mcp, Lsp, Ri, Ru	Sa, Co, Gr, Lwd	
MR	77	41.89646, −71.08528	41.89693, −71.08656	0.5	9	Mcp, Lsp	Sa, Bo, Gr, Tra	

The partnership is dedicated to monitoring the impacts of dam removals on stream habitats and on fish and invertebrate populations, including mussels. From 2012 to 2013, two dams were removed on the Mill River (Hopewell Dam, 2012; Whittenton Dam, 2013), and a fish ladder and eelway were installed at a third dam (Morey's Bridge Dam, 2013), and the last and most downstream dam in the system (West Britannia Street Dam) was removed in January 2018.

Coincident with the above partnership activities, the Massachusetts Chapter of the Nature Conservancy, the Massachusetts Division of Ecological Restoration, and the Massachusetts Natural Heritage and Endangered Species Program evaluated approaches to monitoring the effects of dam removal on mussel assemblages in the Mill River (Hazelton 2014). They considered two major questions. (1) How does dam removal alter habitat for the Eastern Pondmussel (*Ligumia nasuta*)? The Eastern Pondmussel is listed as a species of Special Concern in Massachusetts and occurs in low gradient and lotic habitats such as those present in impounded areas (Natural Heritage and Endangered Species Program 2015a). (2) Will dam removal allow recolonization by the Alewife Floater (*Utterbackiana implicata*; no state status) as increased passage and rearing habitat become available for migratory hosts such as river herring and shad (Natural Heritage and Endangered Species Program 2015b)? Hazelton (2014) concluded that both questions are best answered by a long-term monitoring scheme, to be conducted every four years, that includes an initial qualitative survey of the Mill River system and the establishment of permanent quantitative monitoring sites. Hazelton (2014) also recommended establishing a quantitative monitoring site in Winnecunnet Pond and Lake Sabbatia, two natural lakes within the watershed.

Our goal was to conduct baseline qualitative and quantitative surveys of mussel assemblages in the Mill River system as recommended by Hazelton (2014). The resulting baseline data will allow monitoring of areas affected by dam removal or modification in 2012 and 2013 (Hopewell, Whittenton, and Morey's Bridge dams), and they provide a pre-dam-removal baseline for West Britannia Street Dam, which was removed after this study was completed. In addition to evaluating the effects of dam removal or modification on *U. implicata* and *L. nasuta*, these data also provide information on *Leptodea ochracea*, the Tidewater Mucket, a species of Special Concern in Massachusetts that occurs in the region (Natural Heritage and Endangered Species Program 2015c). We identified two specific objectives associated with the study goal. Our first objective was to conduct qualitative mussel surveys in 2015 throughout the Mill River system from the upstream sections of the Canoe River to the confluence of the Mill River with the Taunton River (17 river km) to document species composition, mussel abundance (catch per unit effort),

Table 2. Results of 2016 qualitative mussel surveys in the Canoe, (CR), Snake (SR), and Mill (MR) rivers. EC = *Elliptio complanata*; LN = *Ligumia nasuta*; LO = *Leptodea ochracea*; LR = *Lampsilis radiata*; PC = *Pyganodon cataracta*; UI = *Utterbackiana implicata*. CPUE = catch-per-unit-effort.

	Species						Time Searched	Species	Total Number	CPUE
Site	EC	LR	LO	LN	PC	UI	(min.)	Richness	of Mussels	(number/min.)
CR1	0	0	0	0	0	0	51	0	0	0.0
CR2	41	1	0	0	0	0	84	2	42	0.5
CR3	29	0	0	0	0	0	48	1	29	0.6
CR4	9	1	0	0	0	0	24	2	10	0.4
CR5	6	0	0	0	0	1	24	1	7	0.3
CR6	17	3	0	1	0	0	41	3	21	0.5
CR7	16	11	0	1	0	1	54	3	29	0.5
CR8	29	0	0	0	0	0	75	1	29	0.4
CR9	0	0	0	0	0	0	5	0	0	0.0
CR10	0	0	0	0	0	0	2	0	0	0.0
CR11	0	0	0	0	0	0	2	0	0	0.0
CR12	25	1	0	0	0	0	4	2	26	6.5
CR13	2	0	0	0	0	0	3	1	2	0.7
CR14	0	0	0	0	0	0	3	0	0	0.0
CR15	5	0	0	0	0	0	3	1	5	1.7
CR16	2	0	0	0	0	0	4	1	2	0.5
CR17	3	0	0	0	0	0	3	1	3	1.0
CR18	0	0	0	0	0	0	3	0	0	0.0
CR19	11	0	0	0	0	0	2	1	11	5.5
CR20	9	0	0	0	0	0	3	1	9	3.0
CR21	6	1	0	0	0	0	4	2	7	1.8
CR22	0	0	0	0	0	0	2	0	0	0.0
CR23	4	2	0	0	0	1	2	2	7	3.5
CR24	5	1	0	0	0	0	4	2	6	1.5
CR25	9	0	0	0	0	0	19	1	9	0.5
CR26	0	0	0	0	0	0	2	0	0	0.0
CR27	0	0	0	0	0	0	4	0	0	0.0
CR28	0	0	0	0	0	0	4	0	0	0.0
CR29	0	0	0	0	0	0	4	0	0	0.0
CR30	19	0	0	0	0	0	5	1	19	3.8
CR31	1	0	0	0	0	0	3	1	1	0.3
CR32	0	0	0	0	0	0	1	0	0	0.0
CR33	21	2	0	0	0	0	4	2	23	5.8
CR34	0	1	0	1	0	0	2	2	2	1.0
CR35	0	0	0	0	0	0	3	0	0	0.0
CR36	1	0	0	0	0	0	3	1	1	0.3
CR37	0	0	0	0	0	0	3	0	0	0.0
SR38	100	10	0	5	0	5	18	3	120	6.7
SR39	3	1	0	0	0	1	4	2	5	1.3
SR40	12	4	0	0	0	0	4	2	16	4.0
SR41	0	0	0	0	0	0	2	0	0	0.0
SR42	0	0	0	0	0	0	5	0	0	0.0
SR43	0	0	0	0	0	0	2	0	0	0.0
SR44	1	0	0	0	0	0	3	1	1	0.3
SR45	11	2	0	0	0	0	3	2	13	4.3
SR46	6	0	0	0	0	0	3	1	6	2.0
SR47	3	0	0	0	0	0	4	1	3	0.8
SR48	3	1	0	0	0	0	4	2	4	1.0
SR49	6	1	0	0	0	2	4	2	9	2.3

Table 2, continued.

Site	Species						Time Searched (min.)	Species Richness	Total Number of Mussels	CPUE (number/min.)
	EC	LR	LO	LN	PC	UI				
SR50	8	0	0	0	0	0	2	1	8	4.0
SR51	9	0	0	0	0	0	2	1	9	4.5
SR52	3	0	0	0	0	0	2	1	3	1.5
SR53	1	2	0	0	0	0	3	2	3	1.0
SR54	1	0	0	0	0	0	3	1	1	0.3
SR55	0	0	0	0	0	0	3	0	0	0.0
SR56	8	1	0	0	0	0	4	2	9	2.3
SR57	7	0	0	0	0	1	4	1	8	2.0
SR58	2	0	0	0	0	2	4	1	4	1.0
SR59	1	0	0	0	0	0	4	1	1	0.3
SR60	3	0	0	0	0	0	2	1	3	1.5
MR61	885	228	0	2	2	0	80	4	1,117	14.0
MR62	782	10	0	0	0	0	144	2	792	5.5
MR63	264	1	0	1	0	0	16	3	266	16.6
MR64	132	2	0	0	0	0	62	2	134	2.2
MR65	52	4	0	0	0	0	112	2	56	0.5
MR66	0	0	0	0	0	0	50	0	0	0.0
MR67	0	2	0	0	0	0	56	1	2	0.0
MR68	31	2	0	0	0	0	112	2	33	0.3
MR69	12	1	0	0	0	2	84	2	15	0.2
MR70	0	0	0	0	0	0	58	0	0	0.0
MR71	0	0	0	0	0	0	24	0	0	0.0
MR72	0	0	0	0	0	0	114	0	0	0.0
MR73	0	0	0	0	0	0	22	0	0	0.0
MR74	0	0	0	0	0	0	88	0	0	0.0
MR75	0	0	0	0	0	0	24	0	0	0.0
MR76	0	0	0	0	0	0	70	0	0	0.0
MR77	0	1	0	0	0	0	44	1	1	0.0
Totals	2,616	297	0	11	2	16	1,756	5	2,942	1.7

and distributions of freshwater mussel assemblages relative to existing (i.e., West Britannia) and historical dams. Our second objective was to establish nine long-term quantitative mussel-monitoring sites in the Mill River system, including one site each in Winnecunnet Pond and Lake Sabbatia. We quantitatively sampled these nine sites in 2016.

METHODS

Study Area

The Mill River watershed is located within the Taunton River watershed in the Northeastern Coastal Zone Ecoregion of southeastern Massachusetts (Fig. 1). The Mill River watershed drains 113 km^2 and is covered by 49% forest, 17% wetlands, 3% lakes and ponds, and 33% developed land, of which 12% is considered impervious (United States Geological Survey 2018, based on NLCD 2011 data). The Mill River system is made up of three segments, the Mill, Snake, and Canoe rivers, which are delineated by Lake Sabbatia and Winnecunnet Pond. Both are natural lakes, but water level in Lake Sabbatia is raised substantially and regulated by Morey's Bridge Dam. Most of the Canoe and Snake rivers are associated with extensive wetlands. These sections have abundant aquatic vegetation, and there is no defined stream channel in some places. In contrast, the Mill River is more consistently riverine and characterized by typical riffle/run/pool development. Morey's Bridge Dam is upstream of site 61 at the outflow of Lake Sabbatia, Whittenton Dam was located near site 61, West Britannia Street Dam was located near site 65, and Hopewell Dam was located near site 67 (see subsequent discussion for information about site selection).

Objective 1: Qualitative Mussel Survey

We conducted qualitative surveys between July 1, 2015, and August 15, 2015, on approximately 17 km of the Mill

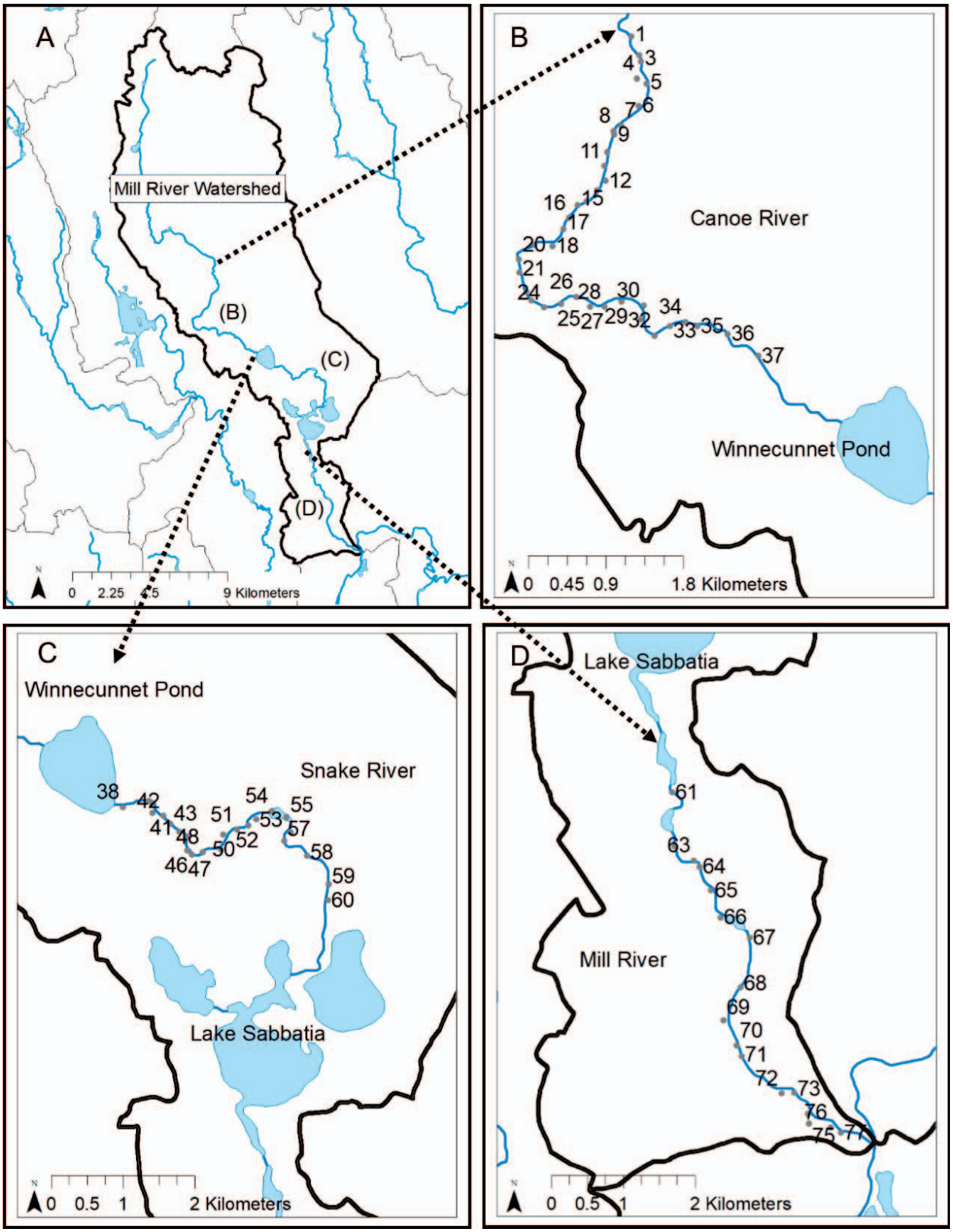

Figure 1. (A) Map of the Mill River watershed showing location of the Canoe (B), Snake (C), and Mill River (D) segments. Numbers on panels B–D indicate 2015 qualitative sampling sites. Some site numbers are not shown due to overlapping labeling format rules in ArcMap. Dams and dam removal areas are in the Mill River (D) segment: Morey's Bridge Dam is located at the outflow of Lake Sabbatia upstream of site 61; Whittenton Dam was located near site 61; West Britannia Street Dam was located near site 65; and Hopewell Dam was located near site 67.

Table 3. Site data and sampling precision for quantitative mussel sampling sites in the Mill River system. Site codes for streams represent the dam-removal effect category (e.g., USRS; see text) followed by the site number (see Table 1). Site codes for lakes are WP = Winnecunnet Pond; LS = Lake Sabbatia. GPS coordinates represent the upstream (US) and downstream (DS) boundaries of the 100-m reach at each stream site or the location of transects at lake sites. The columns "*n* required" indicate the number of samples necessary to achieve 80% and 90% precision (Downing and Downing 1992). NA = not applicable, cannot be calculated.

Site	Location	Coordinates	Area (m^2)	Samples (*n*)	% Area Sampled	*n* Required for 80% Precision	*n* Required for 90% Precision
USRS 07	US	−71.15877, 41.99578	700	25	3.6	11.0	43.9
	DS	−71.15950, 41.99530					
WP	Transect 1	−71.12676, 41.97088	3,100	21	0.7	8.2	32.6
	Transect 2	−71.12680, 41.97056					
	Transect 3	−71.12682, 41.97020					
USRS 38	US	−71.12597, 41.96706	2,000	13	0.7	4.2	16.7
	DS	−71.12479, 41.96695					
LS	Transect 1	−71.11089, 41.93848	3,100	21	0.7	23.8	95.3
	Transect 2	−71.11095, 41.93739					
	Transect 3	−71.10915, 41.93452					
DRRS 61	US	−71.10748, 41.93129	1,200	25	2.1	9.3	37.1
	DS	−71.10708, 41.93078					
DRRS 65	US	−71.10288, 41.92183	1,000	25	2.5	25.0	100.0
	DS	−71.10304, 41.92110					
DRRS 67	US	−71.09820, 41.91566	1,200	25	2.1	NA	NA
	DS	−71.09738, 41.91540					
DSRS 70	US	−71.09836, 41.90459	1,000	25	2.5	79.1	316.2
	DS	−71.09766, 41.90355					
DSRS 76	US	−71.08647, 41.89680	1,100	25	2.3	NA	NA
	DS	−71.08530, 41.89664					

River system from the mouth of the Mill River upstream into the Snake and Canoe rivers (Fig. 1). We examined the entire study section for suitable mussel habitat and the presence of live mussels or relic shells. We delineated qualitative sample sites based on changes in habitat or the spatial extent of mussel aggregations (Table 1). At each qualitative site, we conducted timed searches for mussels with view scopes and snorkeling and by touch. Timed searches were from 1 to 144 minutes (Table 2); in general, we spent more time at sites with higher mussel abundance and at larger sites. At riverine sites, we attempted to search the entire sample area. In sections of the Canoe and Snake rivers associated with extensive wetlands (sites 9–37 and 39–60), it was impractical to delineate and sample sites as for lotic sections because much of the stream was a complex mosaic of terrestrial and aquatic habitats. In these sections, we established sites in areas of localized lotic habitat and conducted timed searches at each site within a single haphazardly placed transect that traversed the stream width. We calculated catch-per-unit-effort (CPUE) for each site based on total search time. We recorded GPS coordinates and macrohabitats (riffle, run, pool, glide, mid-channel pool, lateral scour pool), substrate (boulder, cobble, gravel, sand, silt, fines), and vegetation (rooted aquatic vegetation, benthic algae) at each site. We identified and counted all live mussels and then returned them to the substrate.

Objective 2: Quantitative Sampling Sites

Site selection.—We selected nine long-term quantitative mussel sampling sites to encompass the range of potential effects likely associated with dam removal. These effects were categorized as follows: (1) upstream reference sites (USRS), representing conditions upstream of direct dam effects; (2) dam removal and restoration sites (DRRS), representing conditions directly influenced by dam removal; and (3) downstream of dam removal and restoration (DSRS), representing conditions downstream of dam removal. We grouped all qualitative sites into one of these three categories. We selected sites in each category based in part on the occurrence of diverse and abundant mussel assemblages identified in the qualitative samples (Table 2), but because all sites in the DRRS and DSRS categories had low mussel CPUE, we were forced to select sites with low mussel abundance so that these categories were represented. As a result, we had two USRS sites, three DRRS sites, and two DSRS sites (Table 3). In addition, we selected one site each in Winnecunnet Pond (WP) and Lake Sabbatia (LS).

Quantitative mussel survey methods.—At each quantitative stream site, we established a 100-m reach representative of the site. In May and June 2016, we sampled 13–25 1-m^2 quadrats at randomly selected X,Y coordinates within each reach (Table 3). At quantitative lake sites, we established a 100-m reach of

Table 4. Results of 2016 quantitative mussel sampling in the Mill River system. See Table 3 for site code definitions. Number = number of individuals; % = percentage of total mussels at the site; Density = number of individuals/m^2; SD = standard deviation of density estimates; Population = estimated number of individuals at site; ±95% CI = ±95% confidence interval around the population estimate. EC = *Elliptio complanata*; LN = *Ligumia nasuta*; LO = *Leptodea ochracea*; LR = *Lampsilis radiata*; PC = *Pyganodon cataracta*; UI = *Utterbackiana implicata*.

		Species						
Site	Parameter	EC	LR	LO	LN	PC	UI	Total
USRS 07	Number	127	3	0	0	0	0	130
	%	97.7	2.3	0.0	0.0	0.0	0.0	100.0
	Density	5.1	0.1	0.0	0.0	0.0	0.0	5.2
	SD	4.8	0.3	0.0	0.0	0.0	0.0	5.0
	Population	3,556	84	0	0	0	0	3,640
	±95% CI	1,169	76	0	0	0	0	1,204
WP	Number	187	9	1	0	3	0	200
	%	93.5	4.5	0.5	0.0	1.5	0.0	100.0
	Density	8.8	0.4	0.0	0.0	0.1	0.0	9.4
	SD	6.9	0.9	0.2	0.0	0.4	0.0	7.2
	Population	27,605	1,329	148	0.	443	0	29,524
	±95% CI	8,177	1,066	119	0	377	0	8,616
USRS 38	Number	406	54	2	5	0	0	467
	%	86.9	11.6	0.4	1.1	0.0	0.0	100.0
	Density	31.2	4.2	0.2	0.4	0.0	0.0	35.9
	SD	34.5	5.0	0.4	1.1	0.0	0.0	40.2
	Population	62,462	8,308	308	769	0	0	71,846
	±95% CI	34,404	4,948	315	1,137	0	0	40,129
LS	Number	15	4	0	2	2	0	23
	%	65.2	17.4	0.0	8.7	8.7	0.0	100.0
	Density	0.7	0.2	0.0	0.1	0.1	0.0	1.1
	SD	1.3	0.4	0.0	0.3	0.3	0.0	1.7
	Population	2,214	590	0	295	295	0	3,395
	±95% CI	1,553	533	0	377	377	0	1,194
DRRS 61	Number	166	6	0	3	0	1	176
	%	94.3	3.4	0.0	1.7	0.0	0.6	100.0
	Density	6.9	0.3	0.0	0.1	0.0	0.0	7.3
	SD	9.8	0.5	0.0	0.4	0.0	0.2	10.3
	Population	7,968	288	0	144	0	48	8,448
	±95% CI	4,073	229	0	187	0	42	4,284
DRRS 65	Number	22	2	1	0	0	0	25
	%	88.0	8.0	4.0	0.0	0.0	0.0	100.0
	Density	0.9	0.1	0.0	0.0	0.0	0.0	1.0
	SD	4.3	0.5	0.3	0.0	0.0	0.0	4.9
	Population	880	80	40	0	0	0	1,000
	±95% CI	439	155	35	0	0	0	466
DRRS 67	Number	0	0	0	0	0	0	0
	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Density	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Population	0	0	0	0	0	0	0
	±95% CI	0	0	0	0	0	0	0
DSRS 70	Number	2	0	0	0	0	0	2
	%	100.0	0.0	0.0	0.0	0.0	0.0	100.0
	Density	0.1	0.0	0.0	0.0	0.0	0.0	0.1
	SD	0.3	0.0	0.0	0.0	0.0	0.0	0.3
	Population	80	0	0	0	0	0	80
	±95% CI	110	0	0	0	0	0	110

Table 4, continued.

Site	Parameter	Species						Total
		EC	LR	LO	LN	PC	UI	
DSRS 76	Number	0	0	0	0	0	0	0
	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Density	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Population	0	0	0	0	0	0	0
	±95% CI	0	0	0	0	0	0	0

shoreline and used a weighted line to demarcate three transects running perpendicular to the shoreline at 25, 50, and 75 m. We sampled a 1-m^2 quadrat every 5 m beginning 1 m from shore along each transect.

We collected mussels from quadrats by excavating the substrate to about 10 cm depth and placing all individuals into a nylon mesh dive bag. We identified all individuals and returned them to the substrate. We calculated mean mussel density and standard deviation for each species based on simple random sampling and extrapolated total population size (and 95% confidence intervals) based on site area (Huebner et al. 1990; Harris et al. 1993; Christian and Harris 2005). We calculated the precision of our estimates (total mussel abundance, all species) and the number of samples needed for 80% and 90% precision at each of our sites (Downing and Downing 1992).

RESULTS

Objective 1: Qualitative Mussel Survey

We found five mussel species and a total of 2,942 individuals across all 77 qualitative sites (Table 2). Mean CPUE across all sites was 1.7 individuals/min. The highest CPUE values were found in the Mill River (14.0 and 16.6), but the Canoe and Snake rivers each had sites with CPUE >5.0 individuals/min. We found no mussels at 26 sites, which occurred in all three stream segments. Across all sites, the relative abundance of the five species was *Elliptio complanata* (89%), *Lampsilis radiata* (10%), *U. implicata* (0.5%), *L. nasuta* (0.4%), and *Pyganodon cataracta* (<0.1%). *Ligumia nasuta* was observed at six sites and represented by 11 individuals. *Utterbackiana implicata* was observed at nine sites and represented by 16 individuals. We did not detect *Le. ochracea* in qualitative samples.

We found four species and 300 individuals in the Canoe River (Table 2). Mean CPUE across all sites was 1.1 individuals/min. Mussel CPUE showed no clear upstream to downstream pattern, and sites with higher CPUE were scattered throughout the stream. Species relative abundance was *E. complanata* (90%), *La. radiata* (8%), *U. implicata* (1%), and *L. nasuta* (1%). We found a total of three *L. nasuta*, one each at sites 6, 7, and 34. We found a total of three *U. implicata*, one each at sites 5, 7, and 23.

We found four species and 226 individuals in the Snake River (Table 2). Mean CPUE across all sites was 1.8 individuals/min. Mussel CPUE showed no clear upstream to downstream pattern, and sites with higher CPUE were scattered throughout the Snake River segment. Species relative abundance was *E. complanata* (83%), *La. radiata* (10%), *U. implicata* (5%), and *L. nasuta* (2%). We found five *L. nasuta* at a single site (38). We found a total of 11 *U. implicata* distributed across sites 38, 39, 49, 57, and 58.

We found five species and 2,416 individuals in the Mill River (Table 2). Mean CPUE across all sites was 2.3 individuals/min. The highest CPUE was found at sites immediately downstream of Lake Sabbatia (sites 61 and 63), but mussels were conspicuously absent or rare downstream of site 69. Species relative abundance was *E. complanata* (89%), *La. radiata* (10%), *L. nasuta* (<1%), *U. implicata* (<1%), and *P. cataracta* (<1%). We found a total of three *L. nasuta* at sites 61 and 63 and one *U. implicata* at site 69.

Objective 2: Quantitative Sampling Sites—Mussels

Estimates of mean mussel density across quantitative sites ranged from 0.0 to 35.9 individuals/m^2 (Table 4). Population estimates at sites where mussels were detected ranged from 1,000 mussels at DRRS65 to 71,846 mussels at USRS38. Species richness ranged from zero at DRRS67 and DSRS76 to four at WP, USRS38 and LS, and we observed a total of six species across all quantitative sites. As with qualitative samples, *E. complanata* dominated mussel assemblages at all quantitative sites, but we found *Le. ochracea* only in quantitative sampling; we found a total of four individuals of *Le. ochracea* at three sites. Precision of mussel density estimates at sites where mussels were detected was ≥80% except at USRS38 and DSRS70, where precision was 69% and 40%, respectively (Table 3). At site DSRS38, only six additional samples were required to achieve 80% precision (31 samples); in contrast, a large number of samples (225) were required at DSRS70 because of the low mussel density at this site. The number of samples required to achieve 90% precision was 316 at DSRS70 and between 17 and 100 at the other sites where mussels were detected.

DISCUSSION

Mussel assemblages in the Mill River system were dominated by *E. complanata*, which is typical of New England streams (e.g., Raithel and Hartenstine 2006). *Ligumia nasuta*, *Le. ochracea*, and *U. implicata* were rare throughout the system. *Utterbackiana implicata* appears to be a specialist on anadromous fishes such as herrings and Striped Bass (Kneeland and Rhymer 2008). The rarity of this species is probably related to the fact that dams formerly blocked the movement of these fishes into the system. Improved fish passage for anadromous fishes after dam removal and installation of fish ladders at Morey's Bridge Dam may result in increased abundance of *U. implicata* (see Smith 1985). It is more difficult to predict the response of *L. nasuta* and *Le. ochracea* to dam removal. These species typically occur in low-gradient streams and lakes, and *Le. ochracea* appears able to parasitize a number of nonmigratory fishes; hosts of *L. nasuta* are unknown (Kneeland and Rhymer 2008; Nedeau 2008). The rarity of *P. cataracta* in the Mill River was surprising because this species appears able to adapt to a wide range of habitats, including impounded streams, and it is a host generalist (Nedeau 2008).

Mussel CPUE showed no clear upstream to downstream pattern in the Canoe or Snake rivers, and substantial mussel aggregations occurred irregularly throughout these streams. Typical riffle/run/pool stream habitats occurred in these streams only in the upper reaches of the Canoe River (sites 1–8) and in the Snake River immediately downstream of Winnecunnet Pond (site 38). Riverine sites in the Canoe River were not associated with conspicuously higher mussel CPUE than wetland-influenced sites, but the highest CPUE in the Snake River was observed at site 38. Similarly, the highest CPUE in the Mill River was observed immediately downstream of Lake Sabbatia. Higher abundance at these sites may be due to increased food availability associated with high primary productivity in the lakes and geomorphological stability of the sites (Ward and Stanford 1983; Gangloff et al. 2011). The rarity or absence of mussels in the Mill River downstream of site 69 may be due to the effects of urban development associated with the city of Taunton (Walsh et al. 2005). The former presence of four dams near this section and backwater effects from the confluence with the Taunton River also may be factors in reducing mussel abundance (Ward and Stanford 1983; Ashmore 1993; Christian et al. 2005).

We were unable to directly examine the effects of former dam presence or recent dam removal on mussel assemblages because of the heterogeneous nature of the system, the concentration of dams in a relatively short stretch of the Mill River, and the recent removal of dams. Quantitative sites associated with West Britannia Dam site (DRRS65), Hopewell Dam site (DRRS67), and the downstream-most sites (DSRS70 and DSRS76) all had low mussel density and species richness. Similar to qualitative sites, we cannot specify the factors that limit mussel occurrence at these sites, but future monitoring will be valuable for examining mussel responses in these areas.

Most of our quantitative estimates of total mussel density had precision sufficient to allow detection of moderate changes in density over time. Because of low mussel density at site DSRS70, a prohibitively large number of samples were required to achieve 80% precision. However, changes may be statistically detectable if mussel abundance increases dramatically at this site. Except for DSRS70, achieving 90% precision required up to a 10-fold increase in sample effort above our effort, but 90% precision could be achieved at some sites with a more modest increase in effort. Future monitoring efforts will need to weigh study goals against resources available for sampling at those times. Although our samples were adequate to detect moderate changes in total mussel density, the power to detect changes in density of target species such as *L. nasuta*, *Le. ochracea*, and *U. implicata* will be very low because of their rarity and the wide confidence intervals associated with their density estimates. Such changes might be detectable at quantitative sites if restoring access for migratory host fishes of *U. implicata* results in dramatic increases in the abundance of this mussel. Detecting more modest changes in abundance or distribution of rarer species may require a watershed-scale approach based on CPUE (e.g., Strayer and Smith 2003).

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REGULAR ARTICLE

HOST FISH ASSOCIATIONS FOR TWO HIGHLY IMPERILED MUSSEL SPECIES FROM THE SOUTHWESTERN UNITED STATES: *CYCLONAIAS NECKI* (GUADALUPE ORB) AND *FUSCONAIA MITCHELLI* (FALSE SPIKE)

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ABSTRACT

Most freshwater mussels (Unionidae) require a specific host fish to advance their life cycle. Currently, hosts are known for only one-third of the mussel species endemic to the United States and Canada. Texas boasts the greatest diversity of freshwater mussels in the southwestern United States. However, information on mussel-host relationships for ~52 species known to occur within the state is either lacking or incomplete, including two species, *Cyclonaias necki* (Guadalupe Orb) and *Fusconaia mitchelli* (False Spike), currently under review for listing under the U.S. Endangered Species Act. To address this deficiency, we conducted laboratory trials that tested 12 fish species (four families and 11 genera) for *C. necki* and eight species (four families and seven genera) for *F. mitchelli*. For *C. necki*, we identified four host species, *Ictalurus punctatus* (Channel Catfish), *Pylodictis olivarus* (Flathead Catfish), *Noturus gyrinus* (Tadpole Madtom), and *Ameiurus natalis* (Yellow Bullhead). The transformation period was 11 to 22 d for *I. punctatus* (peak metamorphosis at 15 d), 16 d for *P. olivaris* and *A. natalis*, and 10 d for *N. gyrinus*. For *F. mitchelli*, we identified two host species, *Cyprinella lutrensis* (Red Shiner) and *Cyprinella venusta* (Blacktail Shiner); for both, the transformation period was 18 d. Current information on the status of these six host species within the Guadalupe River suggests that imperilment of *C. necki* and *F. mitchelli* may be partly related to the status of their host fishes. Our results also provide critical information for informing recovery activities, such as translocation and captive propagation, if deemed necessary for one or both mussel species.

KEY WORDS: Unionidae, host fish, glochidia, juveniles, freshwater mussels, Guadalupe River

INTRODUCTION

North America boasts the greatest diversity of freshwater mussels (hereafter, mussels) with approximately 300 species (Haag 2012; Williams et al. 2017), but over the course of the last century, anthropogenic impacts have resulted in widespread declines, making mussels among the most imperiled group of organisms in North America (Master et al. 2000). Freshwater mussels provide a range of ecosystem services, including cycling nutrients (Vaughn et al. 2008), filtering suspended sediments (Spooner and Vaughn 2008), stabilizing substrates (Vaughn and Hakenkamp 2001), and providing microhabitats for aquatic macroinvertebrates (Vaughn and Spooner 2006). Accordingly, their decline will likely have long-term negative consequences for the ecological function of riverine systems.

Freshwater mussels have a unique life history in that, to successfully reproduce, most require a fish to briefly host their parasitic larvae (glochidia) (Watters and O'Dee 1998). Male mussels release sperm into the water column, and it is filtered from the water by females; fertilization is internal (Haag 2012). The fertilized eggs are brooded to mature larvae (glochidia) within the modified gills (marsupia) of the female

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mussels. After the glochidia mature, female mussels may attract their host(s) by using modified mantle tissue lures, disguising their larvae in packages (i.e., conglutinates) that resemble food items, or passively release their glochidia into the water column (Barnhart et al. 2008; Sietman et al. 2012). This entire process can last several months, and its success depends on adequate flows, water quality (e.g., temperature), food availability, and fish host availability (Roe et al. 1997; Galbraith and Vaughn 2009).

The nature of mussel-host fish relationships varies by species and can be general (multiple fish host species for a single mussel species) or specific (a single host fish species for a single mussel species). To date, host use is known reasonably well for ~130 North American mussel species, but it remains poorly described for the remaining two-thirds of native species (Haag 2012). Texas boasts the greatest diversity of freshwater mussels in the southwestern United States; unfortunately, for 13 of the 52 species that occur in the state, researchers do not know or have not confirmed host fishes (Haag 2012; Ford and Oliver 2015). *Cyclonaias necki* (Guadalupe Orb) (Burlakova et al. 2018) and *Fusconaia mitchelli* (False Spike) (Dall 1896) are two of these unstudied species, and many questions regarding their reproductive biology and host fish associations remain unanswered (Howells et al. 1996; Howells 1997; Ford and Oliver 2015). This lack of information is problematic because both species are currently being reviewed for listing under the U.S. Endangered Species Act (USFWS 2011).

Knowledge of host fish associations is important for conservation efforts because this information can be used to determine whether a species' imperilment is related to the loss of its host fish (Kelner and Sietman 2000), which in turn can help focus recovery activities. For species that do become listed and/or are the focus of restoration programs, knowledge of host associations can guide captive propagation techniques for population augmentation and reintroduction (Jones et al. 2004). Finally, a knowledge of mussel-host fish relationships can help us develop a more complete understanding of how host fish abundance and dispersal impact freshwater mussel population and community ecology, information unknown for the vast majority of mussel species (FMCS 2016).

Given the role that host fish information plays in conservation and management of rare mussel species and the potential listing of *C. necki* and *F. mitchelli*, our objectives were to (1) identify primary and marginal hosts of *C. necki* and *F. mitchelli* and (2) use the resulting information to discuss management and conservation implications and to identify potential future research opportunities.

METHODS

Mussel Species

The focal species of this study were *C. necki* and *F. mitchelli*, which are endemic to central Texas and considered imperiled (USFWS 2011). The historical range of *C. necki* is believed to include only the Guadalupe River basin (Randklev et al. 2017; Johnson et al. 2018), although recent studies have mistakenly described it as occurring in the San Antonio River basin (see Burlakova et al. 2018). Current live collections of this species are known from the Cypress, Blanco, San Marcos, and Guadalupe rivers (Randklev et al. 2017; Johnson et al. 2018). *Fusconaia mitchelli* historically ranged throughout the Brazos, Colorado, and Guadalupe river basins in Texas (Strecker 1931; Stansbery 1971; Pfeiffer et al. 2016). To date, live collections of *F. mitchelli* have been made in the lower Guadalupe, lower San Saba, Llano, San Gabriel, and Little rivers as well as in Brushy Creek (Howells 2010; Randklev et al. 2013, 2017).

Study Site

Our study was conducted in the Guadalupe River basin of central Texas. Located in the floodplains and low terraces of the Western Gulf Coastal Plain ecoregion (Griffith et al. 2007), the Guadalupe basin is characterized by underlying karst geology, with limestone bedrock in the upper reaches and alluvial sediments in the lower reaches (Blum et al. 1994). Flow within the basin is derived from groundwater and spring inputs and impoundment release, primarily from Canyon Lake reservoir (Young et al. 1972; Perkin and Bonner 2011). The Guadalupe River has seven mainstem impoundments, which were constructed between 1928 and 1962; the largest impoundment is a bottom-release dam forming Canyon Lake reservoir, and the rest are run-of-the river reservoirs (Young et al. 1972). The region is susceptible to hydrologic extremes, ranging from intense precipitation and flooding events to severe droughts. Gravid female *C. necki* and *F. mitchelli* were collected from the Guadalupe River between Gonzales and Cuero, Texas, and potential host fish were predominantly collected from sites on the Guadalupe, San Marcos, and Blanco rivers, all of which are part of the Guadalupe basin (Fig. 1), with the exception of *Noturus gyrinus* (Tadpole Madtom), which was collected from a single site on the Brazos River.

Collection

We collected gravid individuals of the two focal species during the spring (mid-March through late April 2017). Because neither species is sexually dimorphic, females were identified based on visual inspection for the presence of inflated and discolored gills, which is characteristic of gravid females. Because the handling of gravid mussels for some species, particularly those belonging to the tribes Pleurobemini and Quadrulini, can induce brood abortion, we placed collected individuals into individual plastic bags filled with river water to retain aborted gill contents (Yeager and Neves 1986; Bruenderman and Neves 1993). Following collection, we transported mussels in insulated coolers to the Texas A&M AgriLife Research & Extension Center in Dallas, Texas. In the laboratory, we visually inspected the contents of each plastic

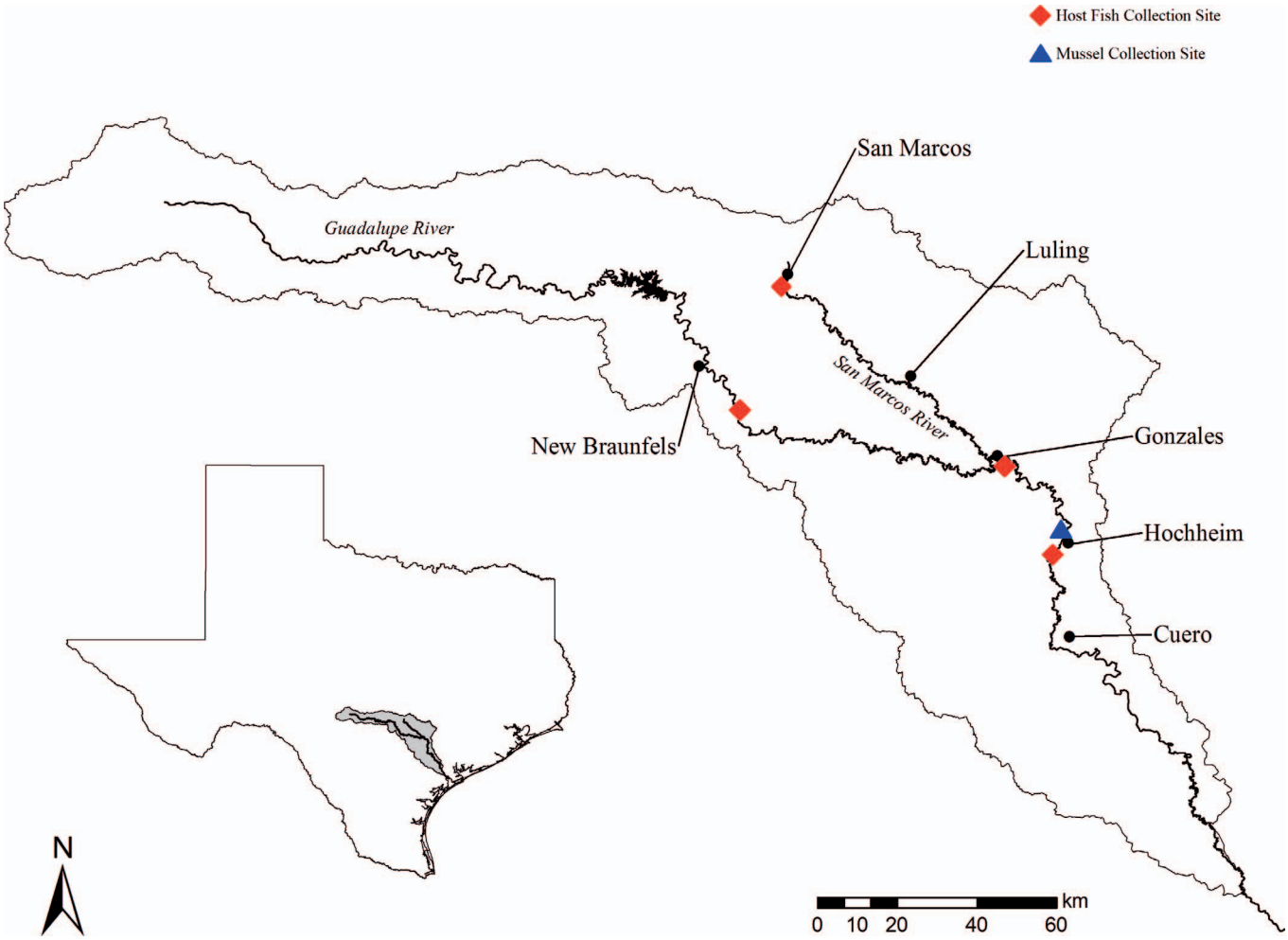

Figure 1. Map of the Guadalupe River basin of Texas showing the collection site for gravid *Cyclonaias necki* (Guadalupe Orb) and *Fusconaia mitchelli* (False Spike) and host fish collection sites. *Noturus gyrinus* (Tadpole Madtoms) were collected from a single site on the Brazos River, Texas, which is not shown on this map.

bag for aborted gill contents (i.e., glochidia, conglutinates, or undeveloped embryos). We placed gravid females into 55 µm mesh-lined containers in recirculating flow-through systems, with temperature (21–25°C) and water chemistry matching those of the Guadalupe River.

Potential host fishes were collected from sites where mussels were not present and at least 30 d prior to the observed brooding period to minimize the chance of using fish with prior glochidia infestation or acquired immunity to glochidia (Zale and Neves 1982; Rogers and Dimock 2003). We attempted to collect as many species of fish as possible while also making sure to collect suspected hosts. Fishes were collected via seine and electrofishing with a goal of collecting at least five individuals of each species (see below for experimental design). We visually inspected all fish to ensure no current infestation from glochidia. Following collection, we transported fish to the laboratory in covered stock tanks under aeration with water from the collection site, which was treated with NaCl to maintain a 3–5 ppt salinity to reduce handling stress and disease outbreak. Upon arrival, we separated fish by species into recirculating holding systems with water temperature and chemistry matching the collection site at time of sampling. We held potential host fishes for a 30-d quarantine period to allow any encysted glochidia to drop off.

Experimental Design: Host Testing

We conducted laboratory host suitability trials using standard methods (Zale and Neves 1982), inducing glochidial infections in potential host fishes and monitoring for rejection of glochidia or metamorphosis of juvenile mussels. Specifically, we flushed released glochidia from containers holding gravid females and suspended them in 100 mL of water. While vigorously stirring with a large rubber-bulb syringe to ensure glochidia were evenly distributed in the container, we used a pipette to remove ten 200-µL subsamples. We evaluated these subsamples under a dissecting microscope to ensure that glochidia were mature (i.e., developed valves and presence of an adductor muscle) and viable (ascertained by introducing a

Table 1. Results of the host trials for *Cyclonaias necki* (Guadalupe Orb) including the list of fish species tested (Trial), number of replicates (No. Rep: number of tested individuals for a given species of fish; number in parentheses denotes number of individuals that produced juveniles), total number of juvenile mussels collected (No. Juv), total number of glochidia attached (No. Glch), days to juvenile mussel transformation (Trans), and mean metamorphosis rate (% M) with standard errors (±1 SE) in parentheses.

Species Trial	No. Rep	No. Juv	No. Glch	Trans (d)	% M (SE)
Ameiurus natalis (Yellow Bullhead)	3 (3)	8	378	15	2.51 (0.52)
Ictalurus punctatus (Channel Catfish)	2 (2)	183	459	11–22	38.24 (8.99)
Pylodictis olivaris (Flathead Catfish)	2 (2)	130	388	16	34.08 (2.09)
Noturus gyrinus (Tadpole Madtom)	3 (3)	194	697	10	27.56 (2.88)
Cyprinella lutrensis (Red Shiner)	5 (0)	0	7	0	0
Cyprinella venusta (Blacktail Shiner)	5 (0)	0	14	0	0
Lepomis macrochirus (Bluegill)	5 (0)	0	29	0	0
Micropterus treculii (Guadalupe Bass)	5 (0)	0	225	0	0
Macrhybopsis marconis (Burrhead Chub)	5 (0)	0	29	0	0
Campostoma anomalum (Central Stoneroller)	5 (0)	0	35	0	0
Etheostoma spectabile (Orangethroat Darter)	5 (0)	0	36	0	0
Pimephales vigilax (Bullhead Minnow)	5 (0)	0	0	0	0

saturated NaCl solution to observe the closure of valves) (Zale and Neves 1982; ASTM 2006). Viability was enumerated as

$$\frac{(\text{No. open initially}) - (\text{No. open after exposure})}{\text{Total no. of glochidia}}.$$

Broods with viability of $\geq$ 70% were used to infect fish. Depending on the number of available glochidia, we used glochidia from one or multiple females to infect fish.

To infect fishes with glochidia, we placed them into a bath containing ~4,000 glochidia L^{-1}. The bath was aerated and vigorously stirred with a turkey baster to keep glochidia suspended. Fishes were exposed in the bath for 15 min and then transferred to individual 2.75 L tanks using dip nets. We monitored transformation success of glochidia on individual fish in a recirculating (AHAB) system. Each trial consisted of five replicate tanks, each containing a single infected fish. For some of the species tested, we did not have enough individuals for five replicates, so we used two to four replicates. Each of the replicate tanks was self-cleaning; water exited from the bottom rather than the top, ensuring that the glochidia and/or juveniles were removed from the tank. The water from each tank passed through a 55 μm mesh filter cup, which we examined every other day for sloughed glochidia or juvenile mussels. We also flushed each tank with an increased flow rate for 15 min prior to monitoring the filter cups to remove any glochidia or juveniles that may not have made it into the filter cup at standard flows. Water temperature was maintained at 23°C, matching average water temperatures of the Guadalupe River during the period of glochidia release. Fishes were fed bloodworms and brine shrimp daily. We continued trials until no further glochidia were found in filter cups for four consecutive monitoring events.

Analyses

We empirically determined host suitability through visual observation and by calculating metamorphosis rate by species. Specifically, successful glochidial metamorphosis was defined by the presence of juveniles, which showed valve growth beyond the original glochidial valve, the presence of a fully formed and active foot, and paired adductor muscles. We calculated the metamorphosis rate (% M) as follows for each individual fish:

$$\frac{\text{No. juveniles}}{(\text{No. juveniles}) + (\text{No. sloughed glochidia})} \times 100.$$

RESULTS

Cyclonaias necki

We collected 29 gravid females (marsupium appeared inflated and had a grainy appearance relative to noninflated individuals) of *C. necki* for use in host fish trials. Water temperature at the time of collection ranged from 21.1 to 31.6°C (mean = 25.8°C). Of those individuals, only 11 released mature glochidia that could be used for host fish trials (i.e., viability ≥70%). Most gravid females (~60%) aborted immature embryos, and for those individuals we were unable to quantify viability. We used a total of 12 fish species in host trials, but juvenile metamorphosis was observed in only four species, all of which were ictalurids: *Ictalurus punctatus* (Channel Catfish), *Pylodictis olivaris* (Flathead Catfish), *Ameiurus natalis* (Yellow Bullhead), and *Noturus gyrinus* (Tadpole Madtom) (Table 1). *Ictalurus punctatus* (n = 2) produced 183 juveniles with a metamorphosis rate of 38.24% (± 8.99 SE), followed by *P. olivaris* (n = 2), which produced 130 juveniles, an average metamorphosis rate of 34.08% (± 2.09 SE). *Noturus gyrinus* (n = 3) produced 194 juveniles with an average metamorphosis rate of 27.56% (±2.88 SE). We recovered only eight juveniles from *A. natalis* (n = 3), a metamorphosis rate of 2.51% (±0.52 SE). The period for juvenile metamorphosis was 11 to 22 d for *I. punctatus* (peak

Table 2. Results of the host trials for *Fusconaia mitchelli* (False Spike) including the list of fish species tested (Trial), number of replicates (No. Rep: number of tested individuals for a given species of fish; number in parentheses denotes number of individuals that produced juveniles), total number of juvenile mussels collected (No. Juv), total number of glochidia attached (No. Glch), days to juvenile mussel transformation (Trans), and mean metamorphosis rate (% M) with standard errors (±1 SE) in parentheses.

Species Trial	No. Rep	No. Juv	No. Glch	Trans (d)	% M (SE)
Ameiurus natalis (Yellow Bullhead)	5 (0)	0	45	0	0
Cyprinella lutrensis (Red Shiner)	3 (3)	36	156	18	32.51 (9.11)
Cyprinella venusta (Blacktail Shiner)	3 (3)	12	54	18	34.49 (3.51)
Lepomis macrochirus (Bluegill)	5 (0)	0	12	0	0
Gambusia affinis (Western Mosquitofish)	5 (0)	0	20	0	0
Pimephales vigilax (Bullhead Minnow)	5 (0)	0	22	0	0
Campostoma anomalum (Central Stoneroller)	5 (0)	0	5	0	0

metamorphosis at 15 d), 10 d for *N. gyrinus*, 15 d for *A. natalis*, and 16 d for *P. olivaris*.

Fusconaia mitchelli

We collected 34 gravid females for use in host fish trials. Water temperature at the time of collection ranged from 21.1 to 31.6°C (mean = 25.8°C). Of the individuals collected, only 10 released mature glochidia that could be used for host fish trials (i.e., viability ≥70%). Most gravid females (~60%) aborted immature embryos, and for those individuals we were unable to quantify viability. Of the eight species evaluated, two cyprinid species, *Cyprinella lutrensis* (Red Shiner) and *Cyprinella venusta* (Blacktail Shiner), successfully transformed glochidia (Table 2), yielding a total of 48 juveniles. *Cyprinella lutrensis* ($n = 3$) produced 75% ($n = 36$) of metamorphosed juveniles, while *C. venusta* ($n = 3$) produced the remaining 25% ($n = 12$). The average metamorphosis rate for *C. lutrensis* was 32.51% (±9.11 SE), while the average metamorphosis rate for *C. venusta* was 34.49% (±3.51 SE). For both *C. lutrensis* and *C. venusta*, transformation was observed in three of the five trials, and the transformation period for *F. mitchelli* was 18 d.

DISCUSSION

Our results show that *C. necki* and *F. mitchelli* are likely specialists, with host use restricted to a single family or genus of fishes, which matches similar findings of laboratory host trials of closely related congeners (see Supplemental Table 1). Specifically, for *C. necki*, we found that it uses *I. punctatus*, *P. olivaris*, *N. gyrinus*, and *A. natalis* as hosts. However, high transformation rates on *I. punctatus*, *P. olivaris*, and *N. gyrinus* suggest that these fish species are likely the primary hosts, while low transformation rates on *A. natalis* suggest that this species is likely a marginal host. Other *Cyclonaias* and *Quadrula* species also have been shown to use ictalurids as hosts (Haggerty et al. 1995; Hove et al. 2011, 2012; Harriger et al. 2015), and our findings for *C. necki* provide additional support for this inference. For *F. mitchelli*, we found that it uses *C. lutrensis* and *C. venusta* as hosts, corroborating previous studies identifying Cyprinid fishes as primary hosts for the genus *Fusconaia* (Neves 1991; Bruenderman and Neves 1993; White et al. 2008). Taken together, our findings provide further evidence that phylogeny may be used to predict host use for other threatened species for which the host is unknown (Haag and Warren 2003).

Freshwater mussels are sessile (Allen and Vaughn 2009; Gough et al. 2012), and as a result, host fish are the primary means of dispersal, which can affect mussel population and community structure (Mansur and da Silva 1999; Barnhart et al. 2008; Horký et al. 2014). Generally, smaller freshwater fishes (e.g., darters and sculpin) have reduced home ranges compared to larger fishes (e.g., ictalurids) (Funk 1957; Freeman 1995; Minns 1995; Rodriguez 2002; Petty and Grossman 2004), and such information may provide insight into the conservation status of a given mussel species. Similarly, fish size influences upstream and downstream movement, with smaller fish moving less than larger fish, a characteristic likely tied to their reproduction and larval dispersal (Gerking 1950; Hall 1972; Minns 1995). The ictalurids we found to serve as hosts for *C. necki* exhibit potamodromous migratory behavior (Pellet et al. 1998), suggesting greater dispersal capacity and perhaps resiliency to human impacts. That behavior might explain why *Cyclonaias* and *Quadrula* mussel species in Texas appear to be more broadly distributed with multiple stronghold populations spread throughout their range (Randklev et al. 2017). However, we also identified *N. gyrinus* as a host for *C. necki*. This species of fish is diminutive, maintains a small home range (often a single riffle), and is rare within the Guadalupe basin (Perkin and Bonner 2011; GBRA and TPWD 2014). If *N. gyrinus* proves to be the primary ecological host (see below) for *C. necki*, then our findings would suggest that this species' decline could be associated with the conservation status of its host fish. If this turns out not to be the case, ongoing declines in ictalurid fishes within Texas rivers (Anderson et al. 1995) may still be evidence that *C. necki's* decline is related, in part, to its host fish. For *F. mitchelli*, we found that it uses cyprinids as hosts, which typically have a small home range and dispersal capacity and are generally sensitive to anthropogenic impacts (Irmscher and Vaughn

2015). Thus, its host fish relationship may explain *F. mitchelli's* patchy distribution within its presumptive range and the fact that stronghold populations are aggregated in reaches away from human impacts (Brittain and Eikeland 1988; Watters 1992; McLain and Ross 2005). However, *C. lutrensis* is known to be tolerant of poor water quality and habitat, which could mean that the imperilment of *F. mitchelli* is unrelated to its host fish. In this study, we were able to test only four of the 10 minnow species known to occur in the lower Guadalupe due to the fact that the remaining six species have become increasingly rare (e.g., *Notropis buchanani*, Ghost Shiner; Perkin and Bonner 2011). Because we did not test these other species, conservationists and managers should not assume that *F. mitchelli's* imperilment is unrelated to the status of its host fish.

Host specificity for species like *C. necki* and *F. mitchelli* is important because it may minimize competition for host fish (Bauer 2001; Rashleigh and DeAngelis 2007) and potentially increase reproduction success via host attraction and successful metamorphosis (Barnhart et al. 2008). However, high host specificity comes with a cost in human-dominated landscapes, as it ties the fate of the mussel species with that of the fish, such that extirpation of the host fish results in recruitment failure for the mussel (McNichols et al. 2011). Habitat fragmentation and impoundments inhibit host fish dispersal, alter fish assemblages and community structure, and displace or extirpate the host fish necessary for mussel populations to persist (Watters 1996; Vaughn and Taylor 1999). The consequence of these impacts to mussels are diminished gene flow and reduced colonization, which over time can lead to extirpation or extinction (Watters 1996; Bogan 2008; Newton et al. 2008). For example, declines in *Reginaia ebenus* (Ebonyshell) in the Upper Mississippi River have been attributed to the extirpation of its host fish, *Alosa chrysochloris* (Skipjack Herring), caused by river impoundment (Kelner and Sietman 2000; Hart et al. 2018). Similarly, declines in *Elliptoideus sloatianus* (Purple Bankclimber) are thought to coincide with decline of the *Acipenser oxyrinchus desotoi* (Gulf Sturgeon) in the Apalachicola-Chattahoochee-Flint basin in southeastern North America (Georgia, Alabama, Florida) (Fritts et al. 2012). For *C. necki* and *F. mitchelli*, it is unknown whether their declines are associated with impoundments, either directly through habitat loss or indirectly by loss of host fish. Impoundments cannot be ruled out because the Guadalupe River is highly managed with seven mainstem impoundments, including Canyon Lake reservoir, which is a deep storage reservoir that significantly alters mainstem discharge and water temperatures via hypolimnetic releases (Young et al. 1972; Edwards 1978). Recent studies of fish assemblage structure within the Guadalupe River have demonstrated significant shifts in fish assemblages following mainstem impoundment (Perkin and Bonner 2011).

We were successful in identifying a suite of hosts for two mussel species of high conservation concern (*C. necki* and *F. mitchelli*) from the southwestern United States, which to our knowledge is novel. Based on these results we provide the following recommendations for future host studies for these and other mussel species from this region. First, low fecundity and difficulty collecting viable glochidia limited our capacity to test a broader range of fish species, a common issue for most host fish studies, especially those focused on rare species. That said, additional host testing may yield further insights into host suitability and better determination of primary and marginal hosts. For example, the association between *C. necki* and *N. gyrinus* should be further evaluated given that *N. gyrinus* was collected from the Brazos basin. Laboratory host studies have shown that mussels tested with fish species from the same river system have higher metamorphosis success than laboratory trials that use fish from a different basin than where mussels are collected (Haag 2012). Thus, it is possible that *N. gyrinus* collected from the Guadalupe would have had higher metamorphosis success and juvenile production rates than what we observed in this study. Second, if large-scale production of juveniles is desired, pipetting glochidia directly onto the host gills instead of using glochidia baths might use glochidia more effectively. In our study, cyprinids were more diminutive compared to other fishes we tested, meaning their gills had a smaller surface area for glochidia attachment compared to other tested species. Thus, if we had pipetted glochidia onto the gills, we possibly would have seen greater attachment success. However, it is important to note that this method is unlikely to change which hosts are primary versus marginal. Third, our study entailed identifying hosts through laboratory infections (termed physiological hosts), which may not be the same in natural settings (termed ecological hosts) (Levine et al. 2012). Thus, future host studies for our focal species should reconcile this knowledge gap by identifying ecological hosts and then comparing those to the results presented in this study. A "DNA barcoding" approach could be a way to do this, particularly in river systems with more than one species; it entails collecting naturally infested fish from the wild, chosen, in part, based on information from laboratory trials like our study. Collected individuals are transported back to the laboratory and held in an AHAB system or aquaria until glochidia or juveniles are released from the fish. Glochidia and juveniles are then identified using a molecular approach (e.g., Boyer et al. 2011).

The fish hosts we identified in this study will enable captive propagation programs to begin recovery reintroduction efforts, although comprehensive genetic management plans should be developed before captive-raised animals are released into the wild (McMurray and Roe 2017). When assessing population viability and developing recovery goals, future management and conservation efforts regarding *C. necki* and *F. mitchelli* should take into consideration host fish abundance and habitat and population connectivity (now that this information is known) in addition to other metrics, such as mussel demography and abundance.

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REGULAR ARTICLE

A COMPARISON OF GLOCHIDIAL SHELLS OF THE FRESHWATER MUSSELS *ANODONTA CALIFORNIENSIS*, *ANODONTA KENNERLYI*, *ANODONTA NUTTALLIANA*, AND *ANODONTA OREGONENSIS*

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ABSTRACT

Only recently have substantial efforts been made to understand phylogenetic relationships among freshwater mussels of the western United States and Canada. Genetic studies show the existence of two divergent clades in western *Anodonta*, one containing *Anodonta californiensis* and *Anodonta nuttalliana*, and another containing *Anodonta oregonensis* and *Anodonta kennerlyi*, but relationships within these two clades remain unclear. For example, some authors have placed *A. californiensis* in the synonymy of *A. nuttalliana*, but additional taxonomic information is needed to resolve these issues. We examined glochidial shell size and fine structure of these four species to assess the taxonomic utility of these characters. Glochidia of *A. oregonensis* and *A. kennerlyi* were similar in size and fine structure, which supports their proposed close relationship. Glochidia of *A. californiensis* and *A. nuttalliana* were smaller in all dimensions than *A. oregonensis* and *A. kennerlyi*, which supports the existence of two divergent clades. However, shell size and fine structure also differed between *A. californiensis* and *A. nuttalliana*, which supports the distinctiveness of these two taxa. Glochidial characters may help to clarify evolutionary relationships among western U.S. *Anodonta* and other problematic groups.

KEY WORDS: glochidia, *Anodonta*, Anodontinae, scanning electron microscopy

INTRODUCTION

Until recently, six species of freshwater mussels in the genus *Anodonta* were recognized from western North America (Turgeon et al. 1998): Yukon Floater, *Anodonta beringiana* A. Middendorf, 1851; California Floater, *Anodonta californiensis* I. Lea, 1852; Woebegone Floater, *Anodonta dejecta* Lewis, 1875; Western Floater, *Anodonta kennerlyi* I. Lea, 1860; Winged Floater, *Anodonta nuttalliana* I. Lea, 1838; and Oregon Floater, *Anodonta oregonensis* I. Lea, 1838. A recent taxonomic revision reassigned *A. beringiana* to the genus *Sinanodonta* based on its closer genetic relationship to Asian *Sinanodonta woodiana* Lea, 1834, than to North American *Anodonta* (Chong et al. 2008; Lopes-Lima et al. 2017). Williams et al. (2017) also synonymized *A. dejecta* under *A. californiensis* based primarily on adult shell morphology (see Bequaert and Miller 1973; AZGFD 2017).

Genetic studies show that the remaining four species represent two highly divergent clades: *A. oregonensis*/*kennerlyi* and *A. californiensis*/*nuttalliana* (Chong et al. 2008; Mock et al. 2010). Both species within each clade are genetically similar, and their distinctiveness is unclear. Blevins et al. (2017) suggested synonymizing *A. californiensis* under *A. nuttalliana* based on overlapping adult shell morphology. However, adult shell morphology in western *Anodonta* is highly variable, and additional characters are needed to evaluate the status of these taxa (Mock et al. 2010).

Gross glochidial shell morphology has been used to inform mussel taxonomy since the early 1900s (LeFevre and Curtis 1910; Surber 1912). More recently, glochidial fine structure as revealed by scanning electron microscopy (SEM) has been

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used to inform phylogenetic hypotheses (Sayenko et al. 2005; Pimpão et al. 2012; Sayenko 2016a, Sayenko 2016b). We examined glochidial shell morphology of *A. californiensis*, *A. kennerlyi*, *A. nuttalliana*, and *A. oregonensis* to assess whether these characters may be useful for better understanding relationships among these taxa.

METHODS

We collected two to four gravid females of each species from the following locations (Fig. 1): *A. californiensis*, Wildhorse Creek, tributary of the Umatilla River, Oregon (*A. californiensis* is extirpated at the type locality); *A. kennerlyi*, Lake Chilliwack, British Columbia (type locality); *A. nuttalliana*, Columbia Slough, near Portland, Oregon (type locality); and *A. oregonensis*, Walla Walla River, Washington (*A. oregonensis* was unavailable at the type locality). The specimens of *A. kennerlyi* and *A. nuttalliana* we used for this project were the same specimens whose identification was described by Chong et al. (2008) based on mitochondrial cytochrome c oxidase subunit I (COI) sequencing. We verified our specimens of *A. californiensis* and *A. oregonensis* based on adult shell shape and examination of mitochondrial COI sequences that allow assignment to the clades described by Chong et al. (2008).

Upon collection, we examined the gills of each mussel to assess gravidity; gravid gills were identified as having a puffy or swollen appearance. We transported gravid mussels to the laboratory where we collected glochidia by rupturing the gill and flushing out glochidia with a wash bottle filled with water. We used only fully mature glochidia for analysis. Maturity was determined by introducing several grains of salt into a subsample of glochidia; fully mature glochidia snapped shut after exposure to salt (Zale and Neves 1982).

We preserved and examined glochidia in two ways. We preserved one subsample of glochidia from each female in 70% ethanol and measured the size of 20 glochidia from each subsample. For each glochidium, we measured the following shell dimensions under a light dissecting microscope using ImageJ image analysis software (NIH 2004): height (the widest point from the dorsal to ventral shell edge), length (the widest point from the anterior to posterior shell edge), and hinge length (Fig. 2).

We preserved a second subsample of glochidia from each female for examination of shell fine structure with SEM. We removed glochidial tissue by soaking glochidia in a 5% sodium hypochlorite solution for 2 min, followed by five rinses in tap water and preservation in 70% ethanol (Kennedy et al. 1991; O'Brien et al. 2003). Glochidial shell samples were shipped to the Interdisciplinary Center for Biotechnology Research at the University of Florida, Gainesville, for SEM, where several hundred shells of each species were mounted on double-sided carbon tape, air dried for 15 min, and coated with gold. Photos were taken of the exterior and interior valve; the flange region, a flattened area along the ventral margin of the glochidial valve; and shell sculpture.

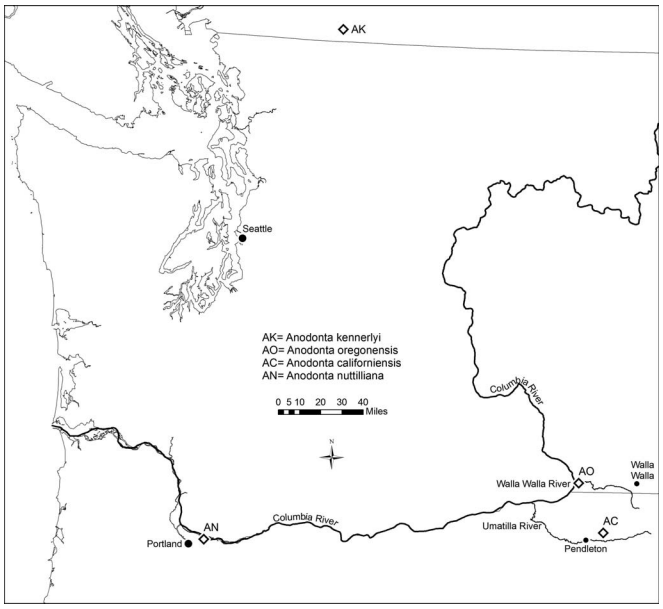

Figure 1. Map of Oregon and Washington, USA, and southern British Columbia, Canada, showing sites where mussels were collected for this study.

We examined the following fine structures: the styliform hook, the projection from the ventral edge of the valve; microstylets, larger (>1.0 μm) toothlike projections located on the styliform hook; micropoints, smaller points (<1.0 μm) located along the ventral valve edge; and exterior shell sculpture, the fine surface texture on the valve (Fig. 3; see Clarke 1981; Hoggarth 1999).

We used multivariate analysis of variance (MANOVA) to examine how glochidia size varied among the four species. All linear combinations of the dependent variables were approximately normally distributed based on examination of scatter

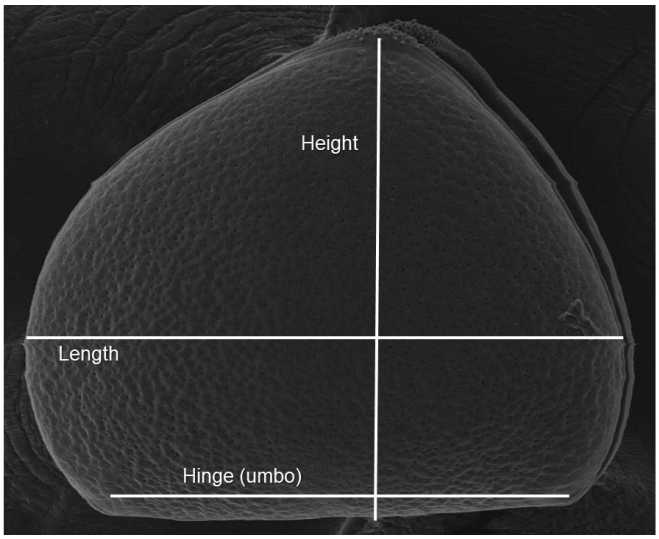

Figure 2. Scanning electron micrograph of an anodontine glochidium showing size dimensions used in this study. Photo by K. Backer-Kelley.

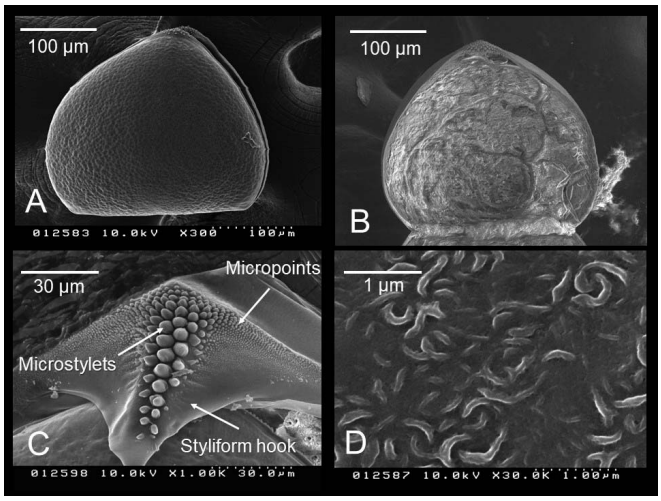

Figure 3. Scanning electron micrographs of *Anodonta californiensis* glochidia. A, exterior of valve (300×); B, interior of valve (300×); C, flange region with styliform hook and associated structures (1,000×); D, exterior valve sculpture (30,000×). Photos by K. Backer-Kelley.

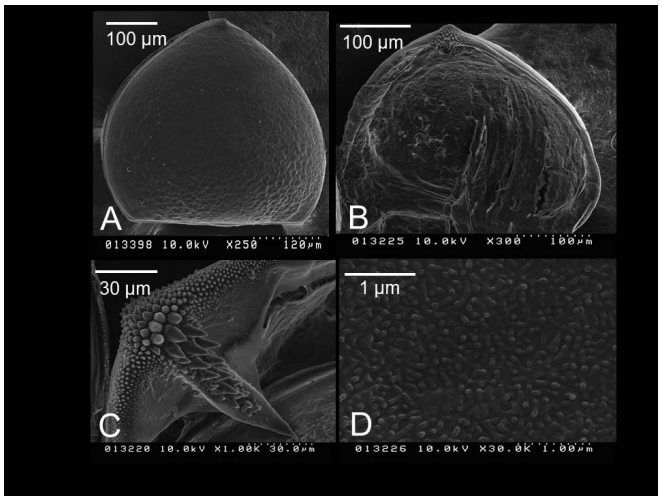

Figure 4. Scanning electron micrographs of *Anodonta kennerlyi* glochidia. See Fig. 3 for details. Photos by K. Backer-Kelley.

plots, and there were no departures from normality or homogeneity of variance (Shapiro-Wilks test; Bartlett's test for homogeneity of variance). Because the MANOVA overall F test was significant, we examined the three size variables separately using ANOVA and Tukey's HSD post-hoc tests. All analyses were conducted with JMP 11 (SAS Institute, Cary, NC, USA).

We made qualitative comparisons of fine structure morphology among species.

RESULTS

There were significant overall differences in shell size among the four species (Wilks $\lambda = 0.05$, $F_{177,81} = 45.3$, $P < 0.001$). Shell length, height, and hinge length each differed significantly among the four species ($F_{3,78} = 94.76$, 143.37, 167.03, respectively; $P < 0.0001$ for all tests; Table 1). Shell length was greatest in *A. kennerlyi* and smallest in *A. californiensis*, and length differed among all four species. Shell height and hinge length were not significantly different between *A. kennerlyi* and *A. oregonensis* but were significantly larger than in *A. nuttalliana* or *A. californiensis*. Shell height and hinge length differed significantly between *A. nuttalliana* and *A. californiensis*, with *A. californiensis* having the smallest values.

Microstylet morphology was similar within each clade, but it differed between the two clades. However, microstylet arrangement was similar between the *A. oregonensis/kennerlyi* clade and *A. nuttalliana*, but it differed in *A. californiensis*. Shell sculpture differed between the *A. californiensis/nuttalliana* and *A. oregonensis/kennerlyi* clades, but this character was similar within clades (Table 2; Figs. 3–6). Shell sculpture of *A. oregonensis* and *A. kennerlyi* was intermediate between two previously described sculpture patterns, beaded and loose-looped (Hoggarth 1999). Shell shape and micropoint morphology did not provide consistent discrimination of clades or species.

Table 1. Glochidial shell measurements of four western North American *Anodonta*. Values are means ± standard deviation (µm) and are based on univariate ANOVA for each size variable. Values within a column with different superscripted letters are significantly different ($P < 0.05$, Tukey's HSD post-hoc tests).

Species	Length	Height	Hinge Length
A. californiensis	252.6 ± 10.2[a]	230.5 ± 16.4[a]	162.3 ± 13.6[a]
A. nuttalliana	265.0 ± 10.5[b]	260.4 ± 10.1[b]	204.1 ± 10.2[b]
A. oregonensis	299.6 ± 13.6[c]	301.1 ± 14.4[c]	234.9 ± 12.1[c]
A. kennerlyi	317.7 ± 19.2[d]	312.5 ± 14.8[c]	242.9 ± 14.5[c]

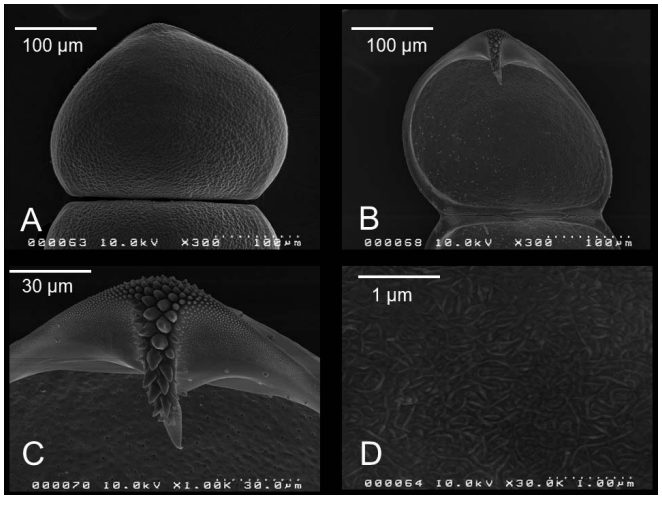

Figure 5. Scanning electron micrographs of *Anodonta nuttalliana* glochidia. See Fig. 3 for details. Photos by K. Backer-Kelley.

Table 2. Glochidial fine structure characters of four western North American *Anodonta*.

Species	Shape	Microstylets	Micropoints	Shell sculpture
A. californiensis	Subtriangular	Blunt; in rows	Numerous; along ventral rim and microstyle	vermiculate
A. nuttalliana	Subtriangular	Blunt; in broken rows	Few; along ventral rim and microstyle	vermiculate
A. oregonensis	Subtriangular	Sharply pointed; in broken rows	Few; along ventral rim and microstyle	intermediate beaded/ loose-looped
A. kennerlyi	Subtriangular	Sharply pointed; in broken rows	Numerous; along ventral rim	intermediate beaded/ loose-looped

DISCUSSION

The similar glochidial size of *A. oregonensis* and *A. kennerlyi* supports the close genetic relationship between these species (Chong et al. 2008). The smaller size of *A. californiensis* and *A. nuttalliana* potentially supports the close relationship between these two species and their distinctiveness from the *A. oregonensis/A. kennerlyi* clade. However, the consistent and marked differences in size between *A. californiensis* and *A. nuttalliana* do not support placement of *A. californiensis* in the synonymy of *A. nuttalliana* (Blevins et al. 2017).

Patterns of glochidial shell fine structure among the four species were similar in most respects to patterns of size. *Anodonta oregonensis* and *A. kennerlyi* had similar patterns of shell sculpture, which supports their close genetic relationship (Chong et al. 2008). This pattern, which was intermediate between beaded and loose-looped sculpture, has been described in one other North American anodontine, *Utterbackiana implicata* (Hoggarth 1999), and in an Asian species, *Kunashiria haconensis* (Sayenko 2016a). Shell sculpture also was similar between *A. californiensis* and *A. nuttalliana*, which supports their close genetic relationship, and the difference in sculpture between these species and *A. oregonensis/kennerlyi* supports the existence of two divergent clades in western *Anodonta* as proposed by Chong et al. (2008). Sculpture similar to that of *A. californiensis* and *A. nuttalliana* also is present in North American *Utterbackiana suborbiculata* (Hoggarth 1999) and Asian *Anodonta cygnea* and *Cristaria tuberculata* (Sayenko 2016a, 2016b). Microstylet morphology also supported differences between the two clades, but microstylet arrangement (continuous versus broken rows) of *A. californiensis* differed markedly from *A. nuttalliana*, which does not support the placement of *A. californiensis* in the synonymy of *A. nuttalliana* as proposed by Blevins et al. (2017). However, microstylet arrangement of *A. nuttalliana* was more similar to the more distantly related *A. oregonensis/kennerlyi* than to its apparent close relative, *A. californiensis*. Micropoint morphology did not appear to be useful for diagnosing clades or species.

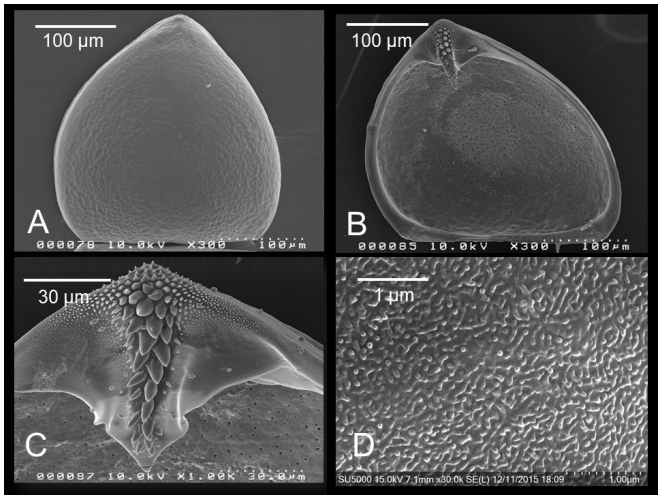

Figure 6. Scanning electron micrographs of *Anodonta oregonensis* glochidia. See Fig. 3 for details. Photos by K. Backer-Kelley.

Patterns of glochidia shell size and fine structure among these four species of western *Anodonta* largely support proposed phylogenetic relationships based on genetic data (Chong et al. 2008; Mock et al. 2010), but they provide additional information about the potential distinctiveness of *A. californiensis* and *A. nuttalliana*. Glochidial shell features provide less ambiguous and less variable characters than notoriously vague and highly variable adult shell characters, which can be influenced to a large extent by environmental factors. Our conclusions are based on glochidia from a single population for each species. Within-population variation in glochidial size generally is low, but little is known about among-population variation (Kennedy and Haag 2005), and our data do not reflect this latter potential source of variation. Nevertheless, use of glochidial characters in conjunction with genetic data, adult anatomical characters, reproductive traits, and other data may help to clarify evolutionary relationships among western North American *Anodonta* and other problematic groups.

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