

Early life history of the sheepnose (*Plethobasus cyphus*) (Mollusca: Bivalvia: Unionoida)

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ABSTRACT

Managing a rare species can be improved with knowledge of its natural history. The sheepnose (*Plethobasus cyphus*) is a freshwater mussel recently listed by the US as federally endangered. We used standard methods to study *P. cyphus* brooding behaviour, host fishes in the laboratory and under natural conditions, and glochidial morphology. We monitored a population of *P. cyphus* in the Chippewa River, WI during spring and summer 2007–2009 and 2011 and found brooding animals between mid-May and early August. Gravid individuals ranged between 5 and 27 yr (mean age \pm 1 s.d. = 13 ± 4 yr). *Plethobasus cyphus* brooded glochidia in outer gills, which varied in colour from red, orange, pink, cream, or white. We observed mature glochidia more commonly in individuals with cream or white gills and these glochidia were released in a clear, adhesive, mucus matrix. In laboratory trials we found several minnow and topminnow species (29 spp.) served as productive suitable native hosts. The mean number of juvenile mussels released per cyprinid per day was significantly higher for trials conducted at 22–25°C compared with those at 18–20°C, and 83% of trials conducted at 18–20°C using suitable host species produced no juveniles. Glochidia had a unique outline and shell morphometrics that distinguished *P. cyphus* from seven other Chippewa River mussel species that produce similar sized glochidia. Using morphometrics we determined that mimic shiners (*Notropis volucellus*) were natural hosts for *P. cyphus*, round pigtoe (*Pleurobema sintoxia*), and Wabash pigtoe (*Fusconaia flava*). Releasing mucus-bound glochidia has evolved in a variety of mussel species and may be more common than is currently realized. Our data show that *P. cyphus* is a cyprinid host specialist, and propagation efforts for this species can be strengthened through improved access to mature glochidia by using females with cream-coloured gills and increased juvenile production through warmer fish holding temperatures.

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Introduction

The biodiversity of freshwater bivalves (Unionoida) is broad (est. 840 spp.) with approximately one-third of known species living in North America (Graf and Cummings 2007). Habitat loss, degraded water and habitat quality, overharvesting, land-use changes, and invasive species have led to the extinction of several species and listing of many others as threatened or endangered (Strayer et al. 2004; Williams et al. 2008). All three species in the genus *Plethobasus* (*P. cicatricosus*, *P. cooperianus*, and *P. cyphus*) (Turgeon et al. 1998) are federally endangered. *Plethobasus cyphus* (Rafinesque, 1820), the sheepnose, is the most widespread and abundant member of the genus, and although more is known about this species than the others, little is known about its natural history.

Plethobasus cyphus was recently listed as endangered by the US federal government (USFWS 2012) reflecting the need to conserve the species through, among other things, improved understanding of its biology, which is a top priority of a national strategy for conserving rare freshwater unionoids (National Native Mussel Conservation Committee 1998). The life cycle of most North American unionoids involves larvae (glochidia) briefly attaching to a host fish during which time glochidia metamorphose into juveniles and then leave the fish. *Plethobasus cyphus* is reported to brood glochidia in their outer gills between May and early August and release pink or red lanceolate conglutinates (packages of glochidia produced by the female) (Ortmann 1911, 1912; Lefevre and Curtis 1912; Watters et al. 2005), which are simple, non-elastic and do not contain unfertilized eggs (Watters 2008). Ortmann (1911) observed minnows eating *P. cyphus* conglutinates in a stream. Sauger (*Sander canadensis*) were shown to be naturally infested with *P. cyphus* glochidia and these glochidia had developed two adductor muscles (Surber 1913; Wilson 1916), a characteristic of juvenile mussels. Laboratory trials have shown that *P. cyphus* glochidia will metamorphose on a minnow species, the central stoneroller (*Campostoma anomalum*) (Watters et al. 2005). This scant information is inadequate for assessing glochidia host requirements, which makes *P. cyphus* population viability determination nearly impossible. This mussel species may live nearly 30 years and often occurs in large rivers (Ortmann 1919; Baker 1928; Watters et al. 2009). Little additional information about *P. cyphus* can be gleaned from its congeners. Like *P. cyphus*, *P. cooperianus* and *P. cicatricosus* are generally large river species (Parmalee and Bogan 1998), and *P. cooperianus* brood glochidia in outer gills, which were yellow in colour, during early June (Wilson and Clark 1914; Ortmann 1919; Parmalee and Bogan 1998).

Glochidia morphometrics have applications in systematic studies and can be used to identify unknown juvenile mussels recovered from naturally infested fishes. Glochidia morphology has been used to classify unionoid genera and species (Ortmann 1912; Williams et al. 2008). Species-specific glochidia characters are used to identify juvenile mussels recovered from naturally infested fishes (Kennedy and Haag 2005; Allen et al. 2007). A few early studies described *P. cyphus* glochidia valves as having a semicircular outline, an obliquely rounded ventral margin, and a long and straight or slightly curved hinge line (Surber 1912; Ortmann 1919; Coker et al. 1921). Shell length ranged between 0.21 and 0.22 mm with a height of 0.20 mm, but more accurate dimension measurements are needed to identify unknown juvenile mussels.

We undertook the following research objectives to improve our understanding of *P. cyphus* life history: (1) describe *P. cyphus* brooding behaviour, (2) identify suitable

P. cyphus glochidia hosts using laboratory studies, and (3) determine those mussel species naturally infesting fishes collected near a *P. cyphus* population.

Materials and methods

Brooding behaviour

We used snorkelling gear and SCUBA to study *Plethobasus cyphus* brooding behaviour in the Chippewa River, WI during the spring and summer of 2007–9 and 2011. The lower Chippewa River has a diverse mussel assemblage that includes several protected species (Balding 1992; Hove et al. 2013). We visited a *P. cyphus* population in Rusk County monthly between May and July 2007, and another population in Dunn County monthly between May and September 2008, and bimonthly between April and August 2009 and 2011. A visual cue we used to find *P. cyphus* was the branched papillae around the incurrent aperture (Wolf et al. 2012), a characteristic shared only with a few of the other mussel species. We measured water temperatures each visit during 2008–2009, and during 2011 with an iBCod[®] recording thermometer placed at the sediment surface. We collected 10–28 *P. cyphus* during each visit, briefly peered between the valves to determine gill colour and visually estimate gill thickness, and returned mussels to the collection site. If mussels had gills inflated at least three times normal thickness we considered them gravid. During 2011 we also collected a small number of glochidia from each gravid mussel and viewed them with a dissecting microscope to determine glochidia maturity. Glochidia with fully formed valves and where tissue occupied less than one-third the volume between the valves were considered mature. We estimated adult mussel age by counting annual rings on the periostracum (Haag 2009). Between 19 May and 23 July 2009 we measured the orientation of brooding mussels with respect to the river current and valve height above substrate. Orientation was described with reference to aperture position, where at 0° the incurrent aperture was directly upstream of the excurrent aperture, and at 180° the excurrent aperture was upstream of the incurrent. During 2013 we brought six gravid *P. cyphus* into the laboratory to observe glochidia release behaviours and to conduct host trials. Four females were half-buried in sand and gravel in aerated buckets and two females were similarly placed in a recirculating raceway with a clear wall and monitored daily. We photographed glochidia released in buckets and shot time-lapse video set at 2-second intervals that was viewed frame by frame to record the time and behaviour of glochidia release events. We conducted statistical analyses (two-sample *t*-test, Chi-square analysis, and Tukey test (Zar 1996)) using JMP v. 9.2 statistical software (SAS Institute, Cary, NC).

Hosts

We followed standard methods to conduct glochidia host suitability trials during 2008, 2009, 2011, and 2013 (Zale and Neves 1982; Hove et al. 2000). Gravid *P. cyphus* were collected from the Chippewa River in Dunn County, WI, and fishes from streams and rivers in Minnesota and southeastern Missouri. We conducted host suitability trials at the University of Minnesota Wet Laboratory (UMN) or Minnesota Pollution Control Agency Biomonitoring Test Laboratory (MPCA) where fishes were held at least 2 weeks prior to

glochidia inoculation. Water temperatures were held close to Chippewa River weekly average (18–24°C) at the UMN and between 23°C and 26°C at the MPCA. We used glochidia released in beakers or buckets for most trials but flushed glochidia from the gills of a few *P. cyphus*. We followed standard methods to determine glochidia health, infest and hold fishes, and recover juvenile mussels (Hove et al. 2011; Bloodsworth et al. 2013). To test glochidia health via salt exposure we agitated mucus-bound glochidia to facilitate salt penetration. Fish and mussel nomenclature follows Turgeon et al. (1998) and Page et al. (2013), respectively. We deposited voucher specimens from the Dunn County site at the James Ford Bell Museum of Natural History (UMN) and Illinois Natural History Survey mollusk collections.

We studied glochidial morphology of *P. cyphus* and co-occurring mussel species (Balding 1992; Hove et al. 2013) with similar sized glochidia to assist us in identifying juvenile mussels recovered from naturally infested fishes collected near the Dunn County site. During 2009, 2010 and 2011 we collected and examined fishes for attached glochidia and infested individuals were transported to Grantsburg High School biology laboratory and held in species-specific aquaria. We searched aquaria for juvenile mussels using standard methods (Hove et al. 2011). With scanning electron microscopy we used standard methods to photograph, measure, and describe characteristics of glochidia shells from known mussel species and juvenile mussels recovered from naturally infested fishes (Hove et al. 2011). Glochidia morphometrics used to identify unknown juveniles were generated from glochidia from known mussel species obtained from the Chippewa River (Dunn County), St. Croix River (Chisago County, MN), and Kohlman Creek (Ramsey County, MN). We collected glochidia from gravid mussels in the Chippewa River when possible because the glochidia from some species (*Amblema plicata* and *Obliquaria reflexa*) collected from a nearby river (St. Croix River) were significantly different in size ($t = 9.04$ and 9.46 , respectively, $P < 0.0001$). We used Hoggarth (1999) and Watters et al. (2009) to help us measure, describe, and identify glochidia.

Results

Brooding behaviour

We observed a variety of *Plethobasus cyphus* brooding behaviours. *Plethobasus cyphus* were gravid with developing or mature glochidia from mid-May to early August at 15–29°C water temperature (Figure 1). They brooded glochidia in outer gills, which were significantly more inflated (2–5 mm) than non-inflated gills (1 mm) ($t = 21.7$, $P < 0.0001$). Gill colour of non-gravid mussels was the same as adjacent tissue, whereas gills of brooding animals were red, pink or orange (31%), light pink or light orange (39%), or cream or white (30%). Gill colour of brooding females as a group did not change consistently over the 2009 or 2011 brooding periods. Of 13 recaptured mussels brooding during 2009 or 2011 the gills of six remained the same colour, three grew lighter, three grew darker, and one grew lighter during 2009 and during 2011 grew darker. The gills of females bearing mature glochidia were more frequently cream or white (53%) versus those with immature glochidia, which were more frequently (76%) coloured red, orange, light pink, or light orange ($\chi^2 = 4.54$, $P = 0.03$). Of 16 gravid mussels collected in 2009, 15 were gravid when recaptured in

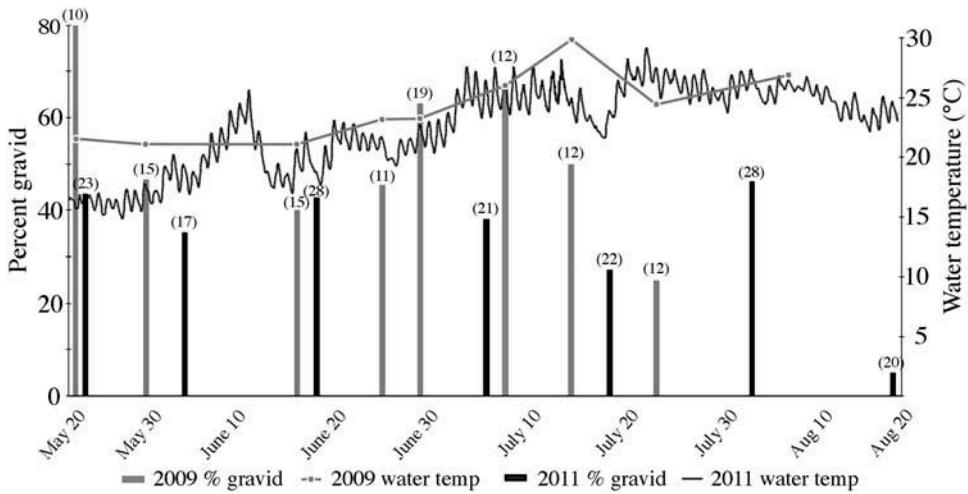


Figure 1. *Plethobasus cyphus* brooding periods in 2009 and 2011. Numbers in parentheses indicate number of mussels examined. Sample dates prior to and after the brooding period are not shown.

2011. There was no significant difference in the amount of shell exposed above the substrate or the orientation of the aperture with respect to river current between brooding and non-brooding *P. cyphus* (parametric two-sample testing of angles) (Zar 1996). We did not observe any mantle structure that resembled a host lure (Barnhart et al. 2008). *Plethobasus cyphus* collected in 2011 ranged in age between 4 and 30 years and gravid individuals ranged between 5 and 26 years (mean age \pm 1 s. d. = 13 ± 4 yr; mean length \pm 1 s.d. (range) = 97 ± 14 (53–126 mm)). There was no significant relationship between gravidity and mussel length or estimated age. Mature glochidia and white ova were released individually or in conglutinate fragments in a clear, cohesive mucus mass (Figure 2). Two individuals held in a recirculating raceway released mucus-bound glochidia via reflexive releases (*sensu* Barnhart et al. 2008), one

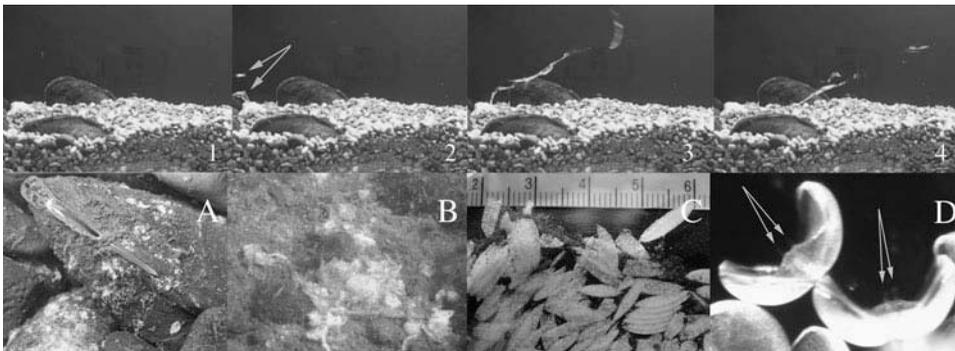


Figure 2. (1–4) *Plethobasus cyphus* releasing glochidia strands in raceway current. (A) mature glochidia released individually or in broken conglutinates in a clear, mucus matrix in the laboratory; (B) mucus matrix released naturally on the Chippewa River floor; (C) immature glochidia in full conglutinates (scale bar: 1 cm numbered increments); (D) glochidia sensory hair cells (at ends of arrows).

individual released 46 times over 11 h 9 min (Figure 2) and the other 23 times over 16 h 9 min. Although we did not measure the number of glochidia released during a single event, the amount of material expelled was variable. *Plethobasus cyphus* sometimes released immature glochidia in whole willow leaf-shaped conglomerates that were solid or paired and ranged in colour similar to gills. When we checked glochidia maturity in 2011 we observed developing glochidia between 20 May and 17 August, and mature glochidia (fully formed valves) between 3 June and 17 August. Gill thickness was not significantly different between females bearing mature versus immature glochidia, nor was there a relationship between glochidia maturity and length of female shell or female age. Several individuals were gravid on multiple dates within the same season, indicating that handling did not cause premature glochidia release for these individuals. Five mussels recaptured once or twice in 2009 or 2011 were brooding for at least 2–10 weeks (ave \pm 1 s.d. = 5 ± 3 wk) and gill colour was similar between capture events.

Hosts

We conducted 379 laboratory host suitability trials in which *P. cyphus* glochidia were exposed to 96 fish species. During inoculations fishes were placed in an aerated bath of glochidia, where fishes would swim among individual glochidia and sticky mucus strands holding glochidia. On two occasions we observed a cyprinid with a strand of glochidia attached to a fin and the trailing end brushed against posterior fins; approximately 100 glochidia were arranged along the strand. We saw glochidia attach to fins (on and between rays), gills, and opercula margins. Although 35 species (five of 20 families) facilitated glochidia metamorphosis, only 29 native species (two of 20 families) produced more than one juvenile (Table 1). Glochidia did not grow while encysted. Cyprinids were the most prevalent laboratory hosts, with 29 of 35 species in 14 genera producing juveniles. Juvenile production varied among species and trials within species, but more productive cyprinids (produced ≥ 20 juveniles/surviving fish for $>$ one trial) included *Campostoma anomalum*, *Cyprinella galactura*, *C. spiloptera*, *C. venusta*, *Luxilus cornutus*, *Macrhybopsis storeriana*, *Notemigonus crysoleucas*, *Notropis nubilus*, *N. topeka*, *Rhinichthys cataractae*, and *Semotilus atromaculatus*. The six non-cyprinid hosts included two species of Fundulidae and Pocelidae, one gasterosteid, and one centrachid, but these species produced few juveniles (Table 1). The mean number of juvenile mussels released per cyprinid per day was significantly higher for trials conducted at 22–25°C compared with those at 18–20°C ($t = 2.12$, $P = 0.037$), and 83% (35 of 42) of trials conducted at 18–20°C using suitable host species produced no juveniles. For one Aug 2011 trial with golden shiners (*Notemigonus crysoleucas*) we counted the number of glochidia on fins and gills throughout the trial and found more glochidia initially attached to fins and more glochidia remained attached to fins. By the time juveniles were being released it appeared as though most glochidia on the gills had been sloughed. Based on our experience conducting host suitability trials with multiple mussel species (Tribes Quadrulini, Lampsilini, Pleurobemini, and Anodontini), mortality of fishes infested with *P. cyphus* was greater than normal, but we were unable to quantify these observations.

Table 1. Fishes that facilitated *Plethobasus cyphus* glochidia metamorphosis. Negative trials are replicates that did not produce juveniles, where 'n' is number of trials and values in parenthesis are means. Positive trials where fish did not survive trial would likely have produced more juveniles.

Fish species	Trial (Ave. water temp. °C)	No. of individuals inoculated/No. survivors	Juvenile/glochidia release period (days)	No. of juveniles recovered
<i>Campostoma anomalum</i>	1 (18–20), 2 (22–24)	4/3, 6/1	20–33, 15–24	90, 57
	3 (*)	*/2	16–20	40
negative trials	n = 6	(2)/(1)	(6)	0
<i>Campostoma oligolepis</i>	1 (23–24)	5/0	11	3
<i>Chrosomus erythrogaster</i>	1 (24)	6/1	14	1
negative trials	n = 3	(5)/(4)	(6)	0
<i>Cyprinella galactura</i>	1 (22–24), 2 (24)	2/1, 1/1	14–17, 7–17	3, 20
negative trials	n = 1	2/2	7	0
<i>C. lutrensis</i>	1 (23–26), 2 (22–24)	10/2, 3/1	10–13, 15–18	9, 7
negative trials	n = 1	2/2	5	0
<i>C. spiloptera</i>	1 (18–20), 2 (23–26)	3/3, 13/9	18–25, 15–24	20, 38
	3 (22–24), 4 (22–23)	5/5, 6/0,	14–17, 12–23	16, 18
	5 (22–23), 6 (24)	42/0, 7/6	11–20, 11–18	46, 121
negative trials	n = 3	(5)/(5)	(7)	0
<i>C. venusta</i>	1 (23–26), 2 (22–24)	6/0, 14/1	9, 9–19	1, 58
	3 (24)	10/4	11–18	26
negative trials	n = 1	2/0	12	0
<i>C. whipplei</i>	1 (22–23), 2 (24)	5/0, 3/3	12, 14	1, 8
negative trials	n = 1	4/3	7	0
<i>Hybognathus hankinsoni</i>	1 (22–24)	2/1	16–25	4
negative trials	n = 1	6/6	5	0
<i>H. nuchalis</i>	1 (23–25)	4/1	11–14	6
<i>Luxilus cornutus</i>	1 (18–20), 2 (23–26)	2/2, 6/6	15–23, 10–25	7, 204
	3 (22–24), 4 (22–24)	7/0, 8/6	12–18, 10–16	7, 74
	5 (23–26), 6 (22–23)	7/0, 2/2	15–17, 14–21	4, 9
	7 (22–23), 8 (22–23)	1/1, 20/6	10–17, 9–16	5, 3
	9 (21)	8/1	14–21	31
negative trials	n = 5	(3)/(2)	(9)	0
<i>L. zonatus</i>	1 (18–20), 2 (22–24)	2/1, 4/0	20–27/12–18	8, 8
	3 (22–24), 4 (24–26)	22/8, 7/3	11–17, 13	72, 11
<i>Machybopsis storeriana</i>	1 (22–24), 2 (24–26)	2/2, 3/2	14–20, 13	79, 5
negative trials	n = 1	2/2	4	0
<i>Margariscus margarita</i>	1 (23–26)	5/4	9–13	10
<i>Nocomis biguttatus</i>	1 (22–23), 2 (22–23)	9/6, 10/9	12–23, 14–18	36, 43
negative trials	n = 5	(4)/(4)	(7)	0
<i>Notemigonus crysoleucas</i>	1 (18–20), 2 (23–26)	6/6, 10/5	16–22, 10–31	53, 360
	3 (23–25), 4 (22–23)	14/9, 10/4	11–22, 13–32	116, 67
	5 (21)	7/2	14–24	340
negative trials	n = 1	5/5	4	0
<i>Notropis atherinoides</i>	1 (23–26)	22/19	11–14	2
negative trials	n = 4	(3)/(2)	(9)	0
<i>N. blennioides</i>	1 (24)	7/5	11–18	24
negative trials	n = 1	7/7	4	0
<i>N. hudsonius</i>	1 (22–23), 2 (22–23)	3/0, 6/0	10, 16–18	1, 8
	3 (22–23)	18/0	11–20	22
negative trials	n = 1	3/0	5	0
<i>N. nubilus</i>	1 (22–24), 2 (22–23)	5/1, 9/8	13–19, 22–24	51, 6
	3 (24)	5/2	10–14	10
negative trials	n = 2	(4)/(4)	(7)	0
<i>N. topeka</i>	1 (23–26), 2 (22–24)	15/10, 10/1	14–18, 14–20	18, 95
negative trials	n = 3	(6)/(6)	(5)	0
<i>N. volucellus</i>	1 (22–24), 2 (23–25)	5/2, 14/2	12, 8	2, 1
negative trials	n = 4	(6)/(4)	(8)	0
<i>Phenacobius mirabilis</i>	1 (23–26), 2 (24)	7/5, 1/1	13, 11–14	6, 4
<i>Pimephales notatus</i>	1 (22–24), 2 (22–23)	4/1, 3/0	12–15, 12	5, 1
	3 (22–23), 4 (24–26)	4/2, 5/4	12, 13	1, 1
negative trials	n = 8	(7)/(6)	(7)	0
<i>P. promelas</i>	1 (22–23), 2 (22–23)	5/2, 17/4	16–18, 16–27	6, 5

(Continued)

Table 1. (Continued).

Fish species	Trial (Ave. water temp. °C)	No. of individuals inoculated/No. survivors	Juvenile/glochidia release period (days)	No. of juveniles recovered
negative trials	3 (24), 4 (*) n = 4	13/1, */17 (5)/(2)	10–18, 16–24 (6)	18, 251 0
<i>Pimephales vigilax</i>	1 (23–26), 2 (24)	6/5, 5/5	13–19, 11–14	14, 11
negative trials	n = 1	1/1	5	0
<i>Rhinichthys atratulus</i>	1 (24)	5/3	14	1
negative trials	n = 4	(3)/(2)	(7)	0
<i>Rhinichthys cataractae</i>	1 (20), 2 (24)	1/1, 4/1	21–25, 10–18	9, 86
	n = 1	1/1	11	0
<i>Semotilus atromaculatus</i>	1 (18–20), 2 (22–23)	3/3, 1/1	22–25, 15–19	4, 24
	3 (22–23), 4 (23–26)	6/3, 11/1	9–14, 6	3, 1
	5 (*), 6 (21)	*/4, 8/8	16–19, 14	30, 1
negative trials	n = 7	(3)/(3)	(10)	0
<i>Fundulus diaphanus</i>	1 (22–23), 2 (22–23)	4/4, 18/2	13–16, 16	5, 1
<i>F. olivaceus</i>	1 (18–20), 2 (23–26)	1/1, 5/4	15–25, 9–12	5, 7
	3 (22–24)	4/2	11	1
negative trials	n = 2	(2)/(1)	(12)	0
<i>Gambusia affinis</i>	1 (24)	10/3	10–14	4
negative trials	n = 1	5/2	11	0
<i>Culaea inconstans</i>	1 (22)	6/6	14	1
negative trials	n = 2	(5)/(4)	(13)	0
<i>Poecilia sphenops</i>	1 (24–25)	15/15	13	1
(non-native species)				
<i>Pomoxis nigromaculatus</i>	1 (22–24)	4/4	15	1
negative trials	n = 3	(3)/(3)	(6)	0

Notes: *Data not collected.

Fish species that did not facilitate glochidia metamorphosis (number of trials, total number of surviving fish, range of days to rejection): *Acipenser fulvescens* (3, 14, 5–6), *Lepisosteus osseus* (3, 4, 4–6), *L. platostomus* (1, 4, 6), *Amia calva* (1, 2, 5), *Carassius auratus* (4, 13, 4–8), *Chrosomus eos* (5, 10, 4–11), *C. neogaeus* (1, 1, 7), *Cyprinus carpio* (4, 6, 4), *Lythrurus umbratilis* (3, 6, 4–9), *Notropis dorsalis* (5, 21, 4–15), *Carpiodes cyprinus* (3, 5, 4–7), *Catostomus commersonii* (5, 10, 3–7), *Erimyzon oblongus* (2, 3, 5–8), *Hypentelium nigricans* (2, 2, 3–4), *Ictiobus bubalus* (1, 1, 4), *Moxostoma anisurum* (3, 5, 4–8), *M. macrolepidotum* (4, 16, 4–7), *Ameiurus melas* (5, 30, 4–7), *A. natalis* (3, 4, 3–5), *A. nebulosus* (1, 1, 4), *Ictalurus furcatus* (2, 9, 4), *I. punctatus* (6, 30, 3–7), *Noturus flavus* (7, 15, 4–7), *N. gyrinus* (4, 8, 5–7), *Pylodictus olivaris* (4, 13, 4–6), *Esox lucius* (1, 9, 12), *Esox niger* (1, 1, 4), *Umbra limi* (2, 6, 5–17), *Aphredoderus sayanus* (3, 24, 4–7), *Elassoma zonatum* (1, 1, 5), *Lota lota* (2, 6, 5–6), *Labidesthes sicculus* (1, 1, 11), *Fundulus catenatus* (2, 2, 10–12), *Cottus bairdii* (1, 1, 4), *Cottus hypselurus* (1, 2, 7), *Morone chrysops* (3, 8, 5–8), *Ambloplites rupestris* (6, 16, 4–5), *Lepomis cyanellus* (5, 28, 4–15), *L. gibbosus* (3, 9, 3–5), *L. gulosus* (1, 1, 5), *L. humilis* (7, 23, 4–6), *L. macrochirus* (7, 37, 3–7), *L. megalotis* (4, 26, 4–7), *Micropterus dolomieu* (3, 7, 4–7), *M. salmoides* (4, 6, 3–7), *Pomoxis annularis* (1, 1, 4), *Etheostoma blennioides* (1, 1, 5), *E. caeruleum* (9, 50, 4–8), *E. exile* (3, 15, 4–6), *E. flabellare* (6, 23, 4–8), *E. nigrum* (10, 48, 4–8), *E. spectabile* (1, 2, 5), *E. zonale* (3, 9, 4–5), *Percia flavescens* (4, 16, 5–11), *Percina caprodes* (7, 25, 3–7), *P. maculata* (9, 23, 4–7), *P. phoxocephala* (7, 34, 4–8), *P. shumardi* (6, 25, 4–7), *Sander canadensis* (4, 19, 4–8), *S. vitreus* (2, 6, 5–8), *Aploidnotus grunniens* (2, 3, 5).

We studied morphological characteristics of glochidia from seven Chippewa River mussel species living in Dunn County, WI, similar in size and shape to sheepnose to identify juvenile mussels recovered from naturally infested fishes. Glochidia height, length and hinge length were significantly different among the seven mussel species studied ($P < 0.0001$). We found that *P. cyphus* valve height (214 ± 8 (mean \pm 1 s.d.)), length (223 ± 10), and hinge length (133 ± 7) were significantly different from most other mussel species (Table 2), and that these glochidia had uniquely round, asymmetric valve outline (Figure 3). *Plethobasus cyphus* glochidia possessed two closely spaced sensory structures (Arey 1924; Jeong et al. 1992) near the centre of the adductor muscle (Figure 2). *Amblema plicata* and *Elliptio dilatata*, and *Pleurobema sintoxia* and *Fusconaia flava* were similar in size and outline, although *F. flava* hinge line length was significantly greater than *P. sintoxia*. Using glochidial shell characters we identified seven *P. cyphus*,

Table 2. Mean glochidia valve heights, lengths and hinge lengths (μm), standard deviations, and ranges observed for Chippewa River mussel species with glochidia similar in size and shape to *Plethobasus cyphus*. Sources for gravid females were from the Chippewa River (^{CR}), St. Croix River (^S), or Kohlman Creek (^K). Valve dimensions with different superscripts were significantly different ($P < 0.05$).

Valve character	<i>Obliquaria reflexa</i>	<i>Elliptio dilatata</i>	<i>Ambliema plicata</i>	<i>Plethobasus cyphus</i>	<i>Toxolasma parvus</i>	<i>Fusconia flava</i>	<i>Pleurobema sintoxia</i>
Height (range)	240 \pm 8 ^a (223–255)	231 \pm 12 ^b (198–250)	219 \pm 4 ^c (210–226)	214 \pm 8 ^c (197–228)	200 \pm 11 ^d (167–212)	166 \pm 4 ^e (158–174)	164 \pm 6 ^e (156–179)
Length	230 \pm 8 ^a (210–248)	220 \pm 8 ^b (206–235)	205 \pm 4 ^c (195–213)	223 \pm 10 ^b (204–237)	181 \pm 10 ^d (158–197)	169 \pm 3 ^e (162–177)	165 \pm 5 ^e (156–177)
Hinge length	133 \pm 8 ^a (117–149)	148 \pm 5 ^b (140–162)	136 \pm 4 ^c (128–147)	133 \pm 7 ^a (120–147)	104 \pm 6 ^d (94–115)	134 \pm 6 ^e (121–147)	118 \pm 6 ^c (106–128)
No. females (no. glochidia)	4 (37) ^{CR}	5 (30) ^S	3 (33) ^{CR}	5 (30) ^{CR}	5 (25) ^K	5 (31) ^{CR}	1 (20) ^{CR} , 5 (26) ^S

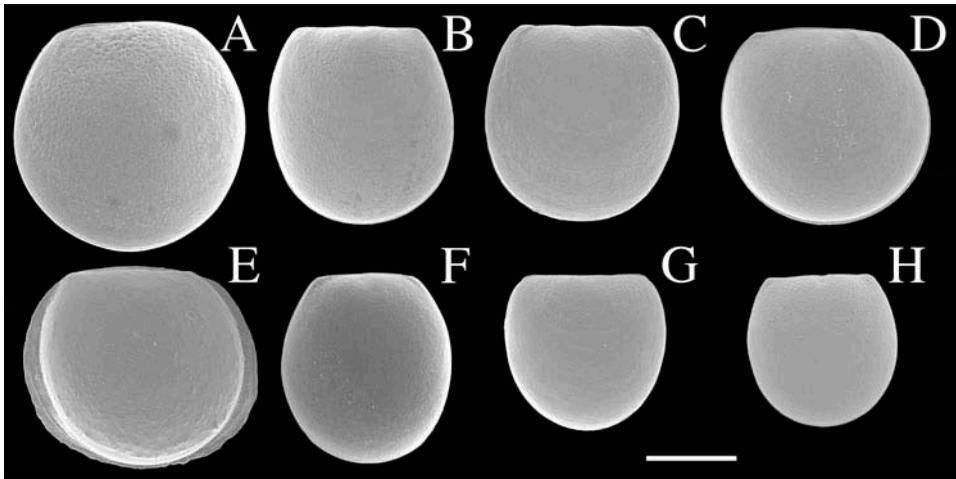


Figure 3. Scanning electron micrographs of glochidia from Chippewa River mussel species similar in size to *Plethobasus cyphus* glochidia. (A) *Obliquaria reflexa*; (B) *Amblema plicata*; (C) *Elliptio dilatata*; (D) *Plethobasus cyphus*; (E) juvenile *Plethobasus cyphus* (valve height = 217 μm) from a naturally infested *Notropis volucellus*; (F) *Toxolasma parvus*; (G) *Fusconaia flava*; (H) *Pleurobema sintoxia*. The scale bar is 100 μm .

Table 3. Glochidial valve dimensions (μm) of juvenile mussels recovered from naturally infested fishes and similar sized glochidia of known mussel species collected near our study site in the Chippewa River, Dunn County, WI.

Infested fish species (known mussel species)	No. juveniles	Glochidial valve		
		Height	Length	Hinge length
<i>Notropis volucellus</i>	7	210 \pm 5	223 \pm 6	135 \pm 7
(<i>Plethobasus cyphus</i>)		(204–217)	(211–229)	(125–142)
	10	214 \pm 8	223 \pm 10	133 \pm 7
		(197–228)	(204–237)	(120–147)
<i>N. volucellus</i> and <i>Cyprinella spiloptera</i>		164 \pm 6	169 \pm 9	141 \pm 8
(<i>Fusconaia flava</i>)		(157–176)	(160–186)	(130–159)
	1	166 \pm 4	169 \pm 3	134 \pm 6
		(158–174)	(162–177)	(123–147)
<i>N. volucellus</i>		157	156	119
<i>Pleurobema sintoxia</i>		162 \pm 3	162 \pm 3	112 \pm 4
		(156–169)	(156–167)	(106–121)

10 *F. flava*, and one *P. sintoxia* from naturally infested mimic and spotfin shiners (Table 3). We had difficulty collecting larger cyprinids, which may have affected our observation of natural hosts.

Discussion

Brooding behaviour

Plethobasus cyphus, like many species in the tribes Amblemini, Pleurobemini, and Quadrulini living in the upper Mississippi River, brood glochidia between mid-May and early August. Upper Mississippi River mussel genera in these tribes that brood glochidia

in the spring or summer include: *Amblema* (May–Aug), *Cyclonaias* (Apr–July), *Elliptio* (Apr–Aug), *Fusconaia* (May–Aug), *Pleurobema* (May–Aug), most *Quadrula* (Apr–Aug), and *Tritogonia* (Apr–June) (Howard 1914; Coker et al. 1921; Heath et al. 2001). The brooding period for *P. cyphus* in our study was similar to reports by others, specifically, May–July in Iowa (Surber 1912), May–June in Ohio (Watters et al. 2005), June–July in Pennsylvania (Ortmann 1919), early summer in Tennessee (Parmalee and Bogan 1998), and at least one individual was gravid in August in Alabama (Williams et al. 2008).

Broadcasting glochidia in mucus occurs infrequently but among a wide variety of North American freshwater mussel species. Reports of glochidia being released in loose strands of mucus or larval threads are most common among the Anodontini (e.g. *Anodonta suborbiculata*, *Anodontoides ferussacianus*, *Pyganodon grandis*, *Strophitus subvexus*, and *Utterbackia imbecillis*) (Utterback 1915–16; Haag and Warren 1997; Watters 2008; Watters et al. 2009). Some of North America’s largest mussel species release glochidia in mucus including *Megaloniaias nervosa* and *Amblema plicata*, as do *A. neislerii*, *Cyclonaias tuberculata*, *Quadrula asperata*, and *Q. pustulosa*, which are Amblemini and Quadrulini species (Stein 1968; O’Brien and Williams 2002; Haag and Warren 2003; Williams et al. 2008; Watters et al. 2009; Sietman et al. 2012). *Plethobasus cyphus* joins *Elliptio arca*, *E. complanata*, *E. dilatata*, and *Popenaias popeii* as Pleurobemini species that release glochidia in mucus (Matteson 1948; Haag and Warren 2003; Levine et al. 2012; Schroeder et al. 2014). Previous reports of pelagic conglutinates released by *P. cyphus* (Ortmann 1911, 1912) could have been puerile and non-infectious (Haag 2012). It is thought that the glochidia-laden mucus released by these species creates a mesh or web that host fishes might swim through or possibly rest upon (Hove et al. 1995; Haag and Warren 2003; Watters et al. 2009). During this study we observed mucus strands attach to a minnow’s fin and the trailing edge of the strand repeatedly brush against posterior fins as the fish swam. Anodontine glochidia are frequently found on fins and gills of fishes (Martel and Lauzon-Guay 2005; McLain and Ross 2005; Rogers-Lowery and Dimock 2006). *Megaloniaias nervosa* glochidia have been observed as frequently on fins of fishes as they have on gills (Weiss and Layzer 1995). Natural infestations of *P. popeii* were observed most frequently on large-bodied catostomids attached to the dorsal and lateral surfaces of the head and less frequently on small-bodied fishes (e.g. red shiner), where glochidia were observed on the gills and occasionally on fins (Levine et al. 2012). Glochidia release behaviours have not been described for many mussel species. The strategy of releasing mucus-encased glochidia has evolved in widely different mussel species. This behaviour may be more common than is currently realized.

Knowledge of female mussel brooding behaviour can help biologists recover mature glochidia for research or propagation purposes. Obtaining mature *P. cyphus* glochidia can be a challenge. We observed roughly one-third of gravid *P. cyphus* bearing immature glochidia during the last 6 weeks of the brooding period. Brooding *P. cyphus* with coloured marsupia (red, pink or orange to light pink or light orange) more often had immature glochidia. When we sought mature glochidia, we collected females with swollen, light-coloured marsupia during the middle or end of the brooding period, and avoided extracting glochidia when possible. When we transferred gravid animals to the laboratory under minimal stress (i.e. held in well-oxygenated river water close to mean daily river temperature) females often released mature glochidia naturally.

Hosts

Plethobasus cyphus glochidia release behaviour appears to be adapted to bring glochidia in contact with a very diverse and successful family of fishes, the cyprinids. This mussel is likely a host specialist, that is, a species that predominantly uses members of one fish family as hosts with occasional use of a few other fish species (Haag 2012). Cyprinidae is the most diverse freshwater fish family in the world and in North America (Berra 2007). Species within this family exhibit a wide variety of habitat preferences, feeding behaviours, and jaw morphologies (Becker 1983; Winfield and Nelson 1991; Ross 2013). The large white masses of glochidia bound in mucus photographed in the laboratory and once in the field (Figure 2) may be unusual, as these masses occurred in static or near-static environments (i.e. laboratory mussels in buckets and backwater field observation). In moderate laboratory current we observed *P. cyphus* release small groups of glochidia in mucus repeatedly over long periods of time that created numerous strands of glochidia distributed at various locations downstream of the gravid female. Cyprinids feeding or resting on the river bed could encounter mucus strands where glochidia might attach to fins or gills and the trailing mucus strand would enable other glochidia to attach to the host. For the trial where we tracked glochidia attachment location throughout the metamorphosis period we found more glochidia on fins than gills, and by the time juveniles were being released most glochidia were on the fins. Sensory structures we observed in *P. cyphus* appear similar to those observed in *Unio* and *Anodonta*, which are thought to facilitate attachment to fins when brushed against (Wood 1974; Wachtler et al. 2001). Although glochidia host analyses show *P. cyphus* is probably a cyprinid specialist, it employs a host generalist glochidia release behaviour to passively entangle (Haag 2012) diverse members of Cyprinidae.

Several observations support the idea that cyprinids are natural hosts for *P. cyphus*. During our field studies we encountered nine fish species, four of which were minnow species (*Cyprinella spiloptera*, *Notropis volucellus*, *Pimephales notatus*, and *P. promelas*), but only *N. volucellus* facilitated metamorphosis of natural *Plethobasus cyphus* infestations. *Notropis volucellus* will facilitate *P. cyphus* glochidia metamorphosis in the laboratory, as will 29 other cyprinid species (Watters et al. 2005; this study). Although *Sander canadensis* has been observed naturally infested with *P. cyphus* (Surber 1913; Wilson 1916) we did not observe glochidia metamorphosis in the laboratory. There may be differences in juvenile production rates among cyprinid species (e.g. *Notropis topeka* and *Cyprinella venusta* seemed more productive in laboratory trials), but due to the low sample sizes and variability we observed this issue remains unclear. The distribution or habitat of the fundulid, gasterosteid, and pocellid species, or 'universal hosts', used in this study and *P. cyphus* probably do not overlap under natural conditions (Pflieger 1975; Becker 1983) and their functionality as hosts is likely ecologically negligible. We recommend additional research to determine which of the numerous suitable hosts we identified serve as functional hosts in nature.

Host use varies among cyprinid specialists. Glochidia host relationship information is limited for most mussel species, and for cyprinid specialists most data come from laboratory host suitability trials. The diversity of minnow species utilized by these specialists seems to vary among mussel genera. *Elliptio* and *Quadrula* species seem to metamorphose on fewer of the cyprinid species tested (mean \pm 1 s.d. (range) number of

studies) ($35 \pm 21\%$ species (20–50%) two studies, and $27 \pm 22\%$ (11–53%) three, respectively) while a broader range of potential hosts were suitable for *Fusconaia* and *Pleurobema* species ($66 \pm 21\%$ (38–88%) four, and $58 \pm 26\%$ (13–100%) nine, respectively) (Hove and Neves 1994; Barnhart and Riusech 1997; Layzer et al. 2003; reviewed by Williams et al. 2008; or Watters et al. 2009; Fritts, Sietman et al. 2012; Johnson et al. 2012). *Plethobasus cyphus* transformed on 83% of cyprinid species tested in our study (78% genera). These studies reveal other trends among cyprinid host specialists. Goldfish and common carp are non-native cyprinids that very rarely facilitate glochidia metamorphosis of cyprinid specialists. *Notropis* species appear to facilitate glochidia metamorphosis less often for *Pleurobema* and *Quadrula* species. Members of Fundulidae, Gasterosteidae, and Poeciliidae have been described as ‘universal hosts’ due to their ability to facilitate glochidia metamorphosis for a mussels in a variety of genera and tribes (Haag and Warren 1997; Haag 2012). However, as observed in this and other studies, comparatively few juveniles are typically recovered from these universal hosts. Their significance as hosts under natural conditions has been shown to be low (Levine et al. 2012) and needs additional study.

Glochidia valve morphometrics, although variable, were useful in identifying juvenile mussels released from naturally infested fishes. We found glochidia valve dimensions as well as qualitative characters (e.g. valve outline) helpful in distinguishing species and identifying juvenile mussels. We observed significant differences in some valve dimensions among females within some mussel species, illustrating the importance of measuring glochidia from several females when generating morphometrics. Glochidia valve dimensions may also vary spatially. For example, although the height and length dimensions of *Fusconaia flava* glochidia from the St. Croix River, WI (110 km to the west) did not differ significantly from those in the Chippewa River ($P = 0.46$ and 0.18 , respectively), height and length dimensions for *Obliquaria reflexa* were different ($P < 0.0001$ and 0.0001 , respectively). Because glochidia dimensions can differ among watersheds we recommend measuring glochidia collected near the study site when identifying juvenile mussels from naturally infested fishes. Future research using glochidia valve dimensions to identify unknown juveniles from naturally infested fishes should include study of whether or not glochidia size or shape changes during metamorphosis.

The kind of data collected in this study could inform unionid systematics, and supports the placement of *Plethobasus cyphus* in the subfamily Pleurobemini. Often 10–15 conchological and 5–6 anatomical characters, or 2–3 gene regions are used to describe unionid genera (Ortmann 1912; Baker 1928; Campbell et al. 2005; Williams et al. 2008; Campbell and Lydeard 2012), but additional information is sometimes needed. In those instances, information about life history and glochidia morphology can provide insight. Characteristics of *P. cyphus* we observed agree with a recent genetic analysis that placed *Plethobasus* in the subfamily Pleurobemini, and supports some genetic evidence that *Plethobasus* is more closely related to *Elliptio* than *Fusconaia* or *Pleurobema* (Campbell and Lydeard 2012). *Plethobasus cyphus* release non-structured conglutinates that presumably passively entangle their hosts similar to four *Elliptio* species (*E. arca*, *E. complanata*, *E. dilatata*, and possibly *E. crassidens*), whereas pelagic conglutinates are released by two *Elliptio* species, *Fusconaia* (11 of 11 species), *Pleurobema* (14 of 14 species), *Pleurobema* (two of two species), and *Hemistena lata* (Figure 4). *Plethobasus cyphus* glochidia are similar in size to *Elliptio* (10 of 12 species)

	Primary host(s)	Infection strategy	Glochidium height (μ)	Glochidium outline	References
<i>Elliptoides sloatianus</i>	sturgeon	broadcast	140	depressed (d.) subelliptical	1,2
<i>Plethobasus cyphus</i>	minnows	broadcast	215	d. subelliptical	3
<i>Hemistena lata</i>	minnows, darters, sculpin	pelagic conglutinates	150	d. subelliptical	4,5,6
<i>Elliptio dilatata</i>	perches, sunfishes	broadcast	210	subelliptical	7,8,9,10,11
<i>E. crassidens</i>	skipjack herring	broadcast?	150	subtriangular	7,12,13,14,15,16
<i>E. mcmichaeli</i>			155	subtriangular	15
<i>E. arca</i>	darters	broadcast?	230	d. subelliptical	4,12
<i>E. complanata</i>	eel, perches, sunfishes, sculpins	broadcast	215	subelliptical	17,18,19,20,21,22
<i>Elliptio</i> spp.		pelagic conglutinates (2 of 2 spp)	>200 (10 of 12 spp)	subelliptical (4 spp), d. subelliptical (7 spp)	4,5,15,23,24,25
<i>Fusconaia</i> spp.	minnows	pelagic conglutinates (11 of 11 spp)	≤ 185 (9 of 9 spp)	subelliptical (5 spp), semicircular (2 spp), d. subelliptical (1 spp)	4,5,14,22,26,27,28,29,30,31,32
<i>Pleurobema</i> spp.	minnows	pelagic conglutinates (14 of 14 spp)	≤ 185 (9 of 11 spp)	subelliptical (5 spp), semicircular (2 spp), d. subelliptical (2 spp), subrotund (1 spp)	2,4,13,14,22,28,29,33,34,35,36,37,38
<i>Pleuroaia</i> spp.	minnows (1 of 2 spp)	pelagic conglutinates (2 of 2 spp)	160-200 (2 of 2 spp)	subelliptical (2 of 2 spp)	4,26,34,39

Figure 4. Systematic relationships among host use, host infection strategies, glochidium size, and glochidium outline in Pleurobemini based on Campbell and Lydeard (2012) and Haag (2012).

Note: ¹Fritts, Fritts et al. (2012), ²O'Brien and Williams (2002), ³This study, ⁴Williams et al. (2008), ⁵Ortmann (1914–1915), ⁶pers. comm. M. Bradley, ⁷Howard (1914), ⁸Wilson (1916), ⁹Luo (1993), ¹⁰Schroeder et al. (2014), ¹¹Hoggarth (1999), ¹²Haag (2012), ¹³Ortmann (1912), ¹⁴Utterback (1915–1916), ¹⁵O'Brien et al. (2003), ¹⁶Ortmann (1911), ¹⁷Matteson (1948), ¹⁸Lellis et al. (2013), ¹⁹Young (1911), ²⁰Watters et al. (2005), ²¹Wiles (1975), ²²Watters et al. (2009), ²³Fuller (1972), ²⁴Porter and Horn (1981), ²⁵Johnson et al. (2012), ²⁶Ortmann (1917), ²⁷Haag and Warren (2003), ²⁸Parmalee and Bogan (1998), ²⁹Bruenderman and Neves (1993), ³⁰Ortmann (1919), ³¹Fuller (1973), ³²Surber (1912), ³³Kennedy and Haag (2005), ³⁴Ortmann (1921), ³⁵Hove and Neves (1994), ³⁶Layzer et al. (2003), ³⁷Culp et al. (2006), ³⁸Surber (1915), ³⁹Kitchel (1985).

(height > 200 μ m), and are larger than glochidia released by *Fusconaia* (nine of nine species) and *Pleurobema* (nine of 11 species) (height ≤ 185 μ m). *Plethobasus*, *Hemistena*, *Fusconaia*, and *Pleurobema* group together in their use of cyprinid hosts, with the occasional fundulid, poecellid, cottid, centrarchid, or percid serving as suitable hosts (Figure 4). *Elliptio* species seem to use a broader range of host fishes, which includes cyprinids, centrarchids, percids, fundulids, ictalurids, catostomids, cottids, and clupeids. Most Pleurobemini glochidia are similar in shape with a near circular outline (subelliptical to subrotund), except for *Elliptio crassidens* and *E. mcmichaeli*, which have subtriangular glochidia. Many phylogenetic relationships among pleurobemine genera are unclear (Campbell and Lydeard 2012), in part due to the diversity of species in *Elliptio*, *Pleurobema*, and *Fusconaia*, and the lack of conchological, anatomical, and genetic information for many species (Williams et al. 2008). Host fish, glochidial release behaviour, and glochidia morphology can be useful characters for unionid systematics, and these data are being reported more frequently (O'Brien and Williams 2002; Haag and Warren 2003; Johnson et al. 2012).

Understanding *P. cyphus* life history helps explain its rarity. Modern conditions make some species attributes favourable and others drawbacks. The influence of habitat and glochidial host requirements on mussel assemblage structure is conceptualized in the 'Host-habitat continuum' (Haag 2012). In this framework mussel species are classified as opportunistic (short life span, stressful habitats, early maturity), periodic (intermediate life span, less stressful habitats, variable maturity but efficient host lures), or equilibrium species (long life, riverine habitat, late maturity). Equilibrium species tend to grow slower, occur in less disturbed habitat (e.g. large streams), mature later, live longer, and have low reproductive investment; attributes characteristic of *P. cyphus*. *Plethobasus cyphus* usually occur in larger rivers (Ortmann 1919; Baker 1928) where they sexually mature around 5 years, bear glochidia only in their outer gills, and may live to approximately 30 years (Ortmann 1912; Watters et al. 2009; this study). These attributes likely served this species in complex, diverse, crowded ecosystems when rivers were less disturbed by anthropogenic activities. *Plethobasus cyphus* comprised a small proportion of Chippewa River mussel communities (Balding and Balding 1996), and this species occurred with other equilibrium species at our study site (Hove et al. 2013). Other attributes influence this species' abundance. *Plethobasus cyphus* recruitment rates may be low due to the species' rarity, passive host encounter strategy, and the unusually strong lethal reaction of cyprinids to heavy *P. cyphus* glochidia infestations. *Plethobasus cyphus* is probably more dependent on frequent host encounters because it releases its glochidia over a short period of time and it lives in a more variable (riverine vs. lacustrine) environment (Haag 2012). Excessive sediment input into streams is a major problem in most US waters (Waters 1995). Fine sediment may lower the adhesiveness of the *P. cyphus*' glochidia mucus strands, or perhaps sediment now smothers strands more rapidly. *Plethobasus cyphus* may have declined more rapidly than other glochidia broadcasters (e.g. *Amblema plicata*, *Megaloniaias nervosa*) because it is a host specialist, probably making it more dependent on benthic, faster current-oriented cyprinid species (e.g. *Erimystax*, *Hybopsis*, *Macrhybopsis* (reviewed in Pflieger 1975; or Becker 1983), which are now rare in several larger streams. Our study site holds one of the largest known *P. cyphus* populations and was located in a coarse substrate reach of a large side channel. If side channel habitats with strong currents are important to this species, then the amount of available habitat has been reduced in large streams like the Mississippi River due to channelization (Anfinson 2003; Knox 2007). A greater understanding of a species' life history requirements allows researchers to ask questions that can test mechanisms causing a species' decline or recovery, leading to improved species management.

Plethobasus cyphus life history requirements revealed by this study can improve conservation efforts for this species and possibly other members of the genus. It is now clear that a wide variety of cyprinids facilitate *P. cyphus* glochidia metamorphosis. Future research can focus on determining if a narrow or broad group of minnows are hosts under natural conditions, and the importance other putative hosts we identified. *Plethobasus cyphus* propagation efforts are improved knowing that females brood glochidia between mid-May and early August, and that gravid animals with light-coloured gills are more likely to hold mature glochidia. Propagation efforts are also strengthened by: (1) having a greater diversity of minnow species to choose from to facilitate *P. cyphus* metamorphosis, (2) increased juvenile production

through holding fishes in warmer waters (between 22°C and 25 C), and (3) knowledge that some minnows are potentially sensitive to over-infestation of glochidia. This study lays the groundwork for future life history studies on *Plethobasus cooperianus* and *P. cicatricosus*, or in situations where they are too rare to be studied effectively (both congeners have smaller ranges and are less abundant than *P. cyphus* (Williams et al. 1993)), these observations can help guide management decisions in the meantime.

Geolocation Information – The states of Minnesota and Wisconsin requested that rare mussel location descriptions be limited to the county in which they were found. This policy is designed to protect rare mollusks from shell collectors, and other threats.

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