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Early life history and spatiotemporal changes in distribution of the rediscovered Suwannee moccasinshell *Medionidus walkeri* (Bivalvia: Unionidae)

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ABSTRACT: Accurate distribution data are critical to the development of conservation and management strategies for imperiled species, particularly for narrow endemics with life history traits that make them vulnerable to extinction. *Medionidus walkeri* is a rare freshwater mussel endemic to the Suwannee River basin in southeastern North America. This species was rediscovered in 2012 after a 16-yr hiatus between collections and is currently proposed for listing under the US Endangered Species Act. Our study fills knowledge gaps regarding changes in distribution and early life history requirements of *M. walkeri*. Spatiotemporal changes in *M. walkeri* distribution are displayed using a conservation status assessment map incorporating data from 98 historical (1916 to 1999) and 401 recent (2000 to 2015) site surveys from museums and field notes representing records for 312 specimens. Recent surveys detected *M. walkeri* only in the middle Suwannee subbasin (n = 86, 22 locations) and lower Santa Fe subbasin (n = 2, 2 locations), and it appears the species may be extirpated from 67% of historically occupied 10-digit hydrologic unit code (HUC 10) watersheds. In our laboratory experiments, *M. walkeri* successfully metamorphosed on *Percina nigrofasciata* (56.2% ± 8.9) and *Etheostoma edwini* (16.1% ± 7.9) but not on *Trinectes maculatus*, *Lepomis marginatus*, *Notropis texanus*, *Noturus leptacanthus*, *Etheostoma fusiforme*, or *Gambusia holbrooki*. We characterize *M. walkeri* as a lure-displaying host fish specialist and a long-term brooder (bradytictic) that is gravid from fall to early summer of the following year. The early life history and distribution data presented here provide the baseline framework for listing decisions and future efforts to conserve and recover the species.

KEY WORDS: Freshwater mussel · Glochidia · Host fish · Mantle display · Gravidity · Fecundity · Historical distribution · Conservation status

INTRODUCTION

Freshwater mussels (family Unionidae) are prone to extinction due to their complex life cycles, narrow distributions, and intrinsic ecological traits. The southeastern United States harbors 94% of the approximately 300 mussel species known to reside within the country (Williams et al. 1993), including 98% of the country's taxa listed as federally threatened or endangered (Williams et al. 2008). These animals provide valuable ecological services by filtering water and sequestering nutrients (Vaughn 2010) while providing food for migratory birds, small mammals, and turtles (Haag 2012). Recent research and

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survey efforts have resulted in the rediscovery of several taxa (Campbell et al. 2008, Ó Foighil et al. 2011, Randklev et al. 2012, Holcomb et al. 2015, Pfeiffer et al. 2016), including the Suwannee moccasinshell *Medionidus walkeri*. This species is endemic to the Suwannee River basin (SRB) and has been considered threatened (Williams et al. 1993), endangered (Williams & Butler 1994), and extremely rare and critically imperiled (Williams et al. 2014) in previous assessments and is considered Critically Endangered by the International Union for the Conservation of Nature (IUCN; www.iucnredlist.org/details/12930/0). In 2011 the species was petitioned for federal listing (CBD 2011; USFWS 2011) and, after a 16-yr hiatus, 3 live *M. walkeri* were found in 2012. The US Fish and Wildlife Service (USFWS) initiated a 12-mo species status assessment and subsequently proposed *M. walkeri* for listing as a threatened species under the US Endangered Species Act (USFWS 2015).

The SRB is located in north Florida and south Georgia in southeastern North America (see Fig. 1) and represents a unique hydrogeological setting where low nutrient, acidic, tannic water originating from lakes and swamps (e.g. Okefenokee Swamp, Lake Santa Fe) mixes with alkaline, enriched, clear waters discharging from over 250 springs located throughout the watershed below the Cody Scarp (FDEP 2011). The Santa Fe River flows completely underground for a 5 km portion of its course, going underground in O'Leno State Park and emerging at River Rise State Park. This subterranean portion of the river's course acts as a natural barrier limiting dispersal of freshwater mussels at all life stages. The Suwannee River drainage upstream of Swift Creek, Hamilton County, supports few mollusks due to its extremely tannic, highly acidic, low-nutrient water and no unionids have been reported from this portion of the SRB (Williams et al. 2014). Major land use changes in the SRB combined with karst geology have resulted in altered hydrologic flow regimes and increased sediment and nutrient loads (Katz et al. 1999). These environmental perturbations might increase extinction vulnerability of aquatic species, particularly those like *M. walkeri* which are typically found in low abundance and are therefore particularly susceptible to gradual habitat deterioration, catastrophic events, and demographic or environmental stochasticity (Haag & Williams 2014). Increased demands for water resources by domestic, industrial, and agricultural consumers could contribute to dewatered springs and streams, and lower groundwater tables, representing an additional threat to *M. walkeri* (Haag & Williams 2014).

The complex life cycle of freshwater mussels includes an obligate parasitic larval stage (glochidia) typically requiring a vertebrate host to complete metamorphosis into a free-living juvenile mussel (Rogers-Lowery & Dimock 2006). *M. walkeri* host fish, brooding period, fecundity, and host infection strategies are unknown (Williams et al. 2014). Identifying hosts is necessary to determine whether a species is a host specialist (i.e. uses a small number of closely related host species) or host generalist (i.e. suite of hosts from multiple families of fishes). Once a host is determined it is important to consider how physical characteristics such as size, habitat preferences, and dispersal capabilities of the host influence the status and distribution of the mussel species. Understanding the mussels' host infection strategies (i.e. broadcast, conglutinate, or mantle display; see Barnhart et al. 2008) and timing of spawning, brooding, and host attraction are important components of the animal's early life history that can help guide development of management strategies (e.g. instream flows requirements during parasitic larval attachment period).

This study investigates the early life history requirements of *M. walkeri* and provides an in-depth assessment of temporal changes in the distribution of the species throughout the SRB. Our specific objectives were to (1) evaluate spatiotemporal changes in the species' distribution, (2) use frequency and distribution data on *M. walkeri* from mussel surveys to evaluate sampling effort in areas historically known to support the species, and (3) characterize the early life history of *M. walkeri* (i.e. period of gravidity, fecundity, host fish requirements, and host attraction strategy). Our findings provide a foundation of knowledge that could assist resource managers and others interested in the research and conservation of imperiled freshwater species.

MATERIALS AND METHODS

Surveys and distribution

We compiled existing distribution data from museum specimens, field notes, and surveys to map known collection localities for *Medionidus walkeri*. All data (i.e. date of collection, locality, collector, etc.) associated with these specimens are given in Table S1 in the Supplement at www.int-res.com/articles/suppl/n031p163_supp.pdf. Data for museum specimens of *M. walkeri* were compiled and verified from 6 institutions: Academy of Natural Sciences of Drexel University (formerly Academy of Natural Sciences of

Philadelphia [ANSP]), Museum of Comparative Zoology of Harvard University (MCZ), Florida Museum of Natural History (UF), University of Michigan Museum of Zoology (UMMZ), Ohio State University Museum of Biological Diversity (OSUM), and North Carolina Museum of Natural Sciences (NCSM). Field data was evaluated from 3 collections databases: Florida Fish and Wildlife Conservation Commission (FWC) in Gainesville, Florida, USFWS in Panama City, Florida, and US Geological Survey (USGS) in Gainesville, Florida. Issues related to misidentifications (see Shea et al. 2011) were considered negligible because the morphology of *M. walkeri* is distinctive and all specimens were examined by experienced malacologists. Only a single relict specimen was included in our dataset; all other data points were based on live specimens or shell material exhibiting intact periostracum and shiny nacre considered to represent live individuals (Table S1). We have high confidence that our dataset includes data for the vast majority of *M. walkeri* specimens but acknowledge the possibility that some specimens might be lost, held in personal collections, or reside in museums not included in our searches.

We used museum and field collection data to direct surveys and evaluate the current distribution of *M. walkeri*. We aimed to resurvey all historical localities and multiple sites located within USGS 10-digit hydrologic unit code (HUC 10) watersheds (http://water.usgs.gov/GIS/huc.html) known to have previously supported *M. walkeri*. The USGS HUC 10 watershed boundaries were strictly followed except for HUC 311020604, which was expanded downstream to the Santa Fe River Rise, a natural geologic feature that delineates the upper Santa Fe and lower Santa Fe subbasins. This modification influenced 18 data points, 2 of which were historical *M. walkeri* collection sites (see Table S2 in the Supplement at www.int-res.com/articles/suppl/n031p163_supp.pdf). Exact methods for all surveys reported in this study were unattainable. However, surveys conducted from 2010 through 2015 can be generally characterized as involving 2 to 6 searchers conducting timed visual-tactile searches from the stream bank out towards the thalweg to a maximum depth of approximately 2.5 m. The majority of surveys were limited to snorkeling and tactile searches, but some did employ SCUBA. Every effort was made to sample all available habitat types at each survey location. All mussels were identified to species and returned to the river, except for gravid *M. walkeri* used for host trials (see next subsection) and specimens selected for museum vouchering.

We constructed a conservation status assessment map using ArcMap 10.3 (ESRI) following the protocol produced by Georgia Department of Natural Resources (2014) to illustrate the spatiotemporal distribution of *M. walkeri* collections and mussel surveys throughout the SRB at the HUC 10 level. The map was based on 2 related datasets: one that includes all verifiable *M. walkeri* collections from 1916 through 2015 and a second that includes all survey data from 1980 through 2015. Data compiled from field notes of surveys conducted prior to 1980 that did not report captures of *M. walkeri* were not included in our analysis of survey effort due to prevalence of incomplete, vague, or inaccurate locality and collection information. Numbers of *M. walkeri* sampled within each subbasin were used to assess spatial (subbasin level) and temporal (before and after 2000) changes in collections by calculating the average number of *M. walkeri* reported at each location. We confined our level of inference to the subbasin or HUC 10 scale to account for uncertainty in collection locality information and to provide a baseline for future survey and management efforts. The frequency and location of surveys conducted since 2000 were used to evaluate whether data indicate *M. walkeri* has been extirpated from historically occupied HUCs or if perceived extirpations were the result of low survey effort. In this assessment, we operationally define survey effort to be adequate when (1) at least one *M. walkeri* was detected within the HUC, or (2) at least 10 surveys were conducted within the HUC and all historically occupied localities were resurveyed at least once (Table 1).

To facilitate reproducibility of our findings, we have provided an archive of our datasets that includes all available data associated with all *M. walkeri* specimens (Table S1) and all freshwater mussel surveys conducted in the SRB from 1980 to 2015 (Table S2). Also, HUCs throughout the SRB were grouped into 8 subbasins to facilitate presentation of results and discussion (Fig. 1, Table 1).

Early life history investigations

All *M. walkeri* with soft parts found in museum collections and live individuals encountered during surveys were examined for gravidity to characterize timing of glochidial development. To determine size at reproductive maturity, we measured maximum shell length to the nearest millimeter for all gravid mussels using digital calipers. Like other members of the genus, shells of *M. walkeri* are sexually dimorphic,

Table 1. Records of 312 specimens of *Medionidus walkeri* collected in the Suwannee River basin between 1916 and 2015, showing location (subbasin and watershed, identified by 10-digit HUC), date range, and corresponding numbers of specimens collected. Right hand columns show number of surveys conducted in each HUC from 2000–2015 and an assessment of whether or not the survey effort was adequate over this period

Subbasin	HUC	No. of specimens 1916–1999	No. of specimens 2000–2015	No. of surveys 2000–2015	Adequate survey effort? 2000–2015
Upper Suwannee					
	311020108	0	0	5	N
	311020109	0	0	31	Y
	311020101	0	0	7	N
	311020102	0	0	1	N
	311020103	0	0	3	N
	311020105	0	0	2	N
Middle Suwannee					
	311020501	10	1	18	Y
	311020502	0	15	11	Y
	311020503	0	8	6	Y
	311020504	0	7	7	Y
	311020505	40	52	25	Y
	311020506	0	3	16	Y
Lower Suwannee					
	311020507	20	0	23	Y
	311020508	0	0	15	Y
Upper Santa Fe					
	311020601	0	0	12	Y
	311020602	23	0	20	N
	311020603	2	0	18	Y
	311020604	80	0	24	Y
Lower Santa Fe					
	311020605	5	2	32	Y
	311020606	0	0	3	N
	311020607	0	0	23	Y
Upper Withlacoochee					
	311020301	0	0	1	N
	311020302	0	0	3	N
	311020304	0	0	4	N
	311020305	0	0	2	N
	311020306	0	0	2	N
	311020307	0	0	23	Y
	311020401	0	0	3	N
	311020402	0	0	1	N
	311020403	0	0	4	N
	311020404	0	0	2	N
	311020405	0	0	3	N
Lower Withlacoochee					
	311020308	2	0	5	N
	311020309	40	0	32	Y
Alapaha					
	311020201	0	0	6	N
	311020202	0	0	2	N
	311020203	0	0	2	N
	311020204	0	0	2	N
	311020205	0	0	1	N
	311020206	0	0	4	N
	311020207	0	0	2	N
	311020211	0	0	3	N
	311020212	0	0	14	Y

but reliably distinguishing between sexes is difficult based on shell morphology alone. To avoid collecting males or females that were not gravid, each individual was gently pried open and the gills were inspected to determine sex and brooding status. Individuals with inflated gills were recorded as gravid females and in most cases either transported back to the USGS laboratory in Gainesville, Florida (hereafter USGS), or the contents of 1 gill were subsampled with a syringe in the field. We examined gill contents under a dissecting microscope to determine the developmental stage of the eggs or glochidia extracted. Individuals without inflated gills were recorded as not gravid. We defined glochidia developmental stages utilizing a classification system similar to Haag & Staton (2003), by categorizing gill contents as eggs (circular masses lacking glochidia shape, no visible shell or adductor muscle), immature glochidia (shelled glochidia that were free of the egg membrane, but adductor muscle were not fully formed or glochidia were unreactive to saturated sodium chloride [NaCl] solution), or fully developed glochidia (had glochidia shape with a visible adductor muscle or glochidia were reactive to NaCl). The overall morphology of *M. walkeri* glochidia was recorded using a microscope equipped with a digital camera. Measurements of total length, height, width (i.e. degree of inflation), and dorsal margin followed methods of Hoggarth (1999) and were performed using ImageJ software (Rasband 1997).

In January 2013, 4 gravid *M. walkeri* were collected from the Suwannee River near Branford, Florida. Each mussel was placed in a sealed plastic bag without water and transported in a cooler to USGS. At the laboratory, the mussels were placed in separate 1.5 l clear acrylic tanks containing well water held in an environmental chamber (Fitotron Environmental Chambers) at 15°C with automated water

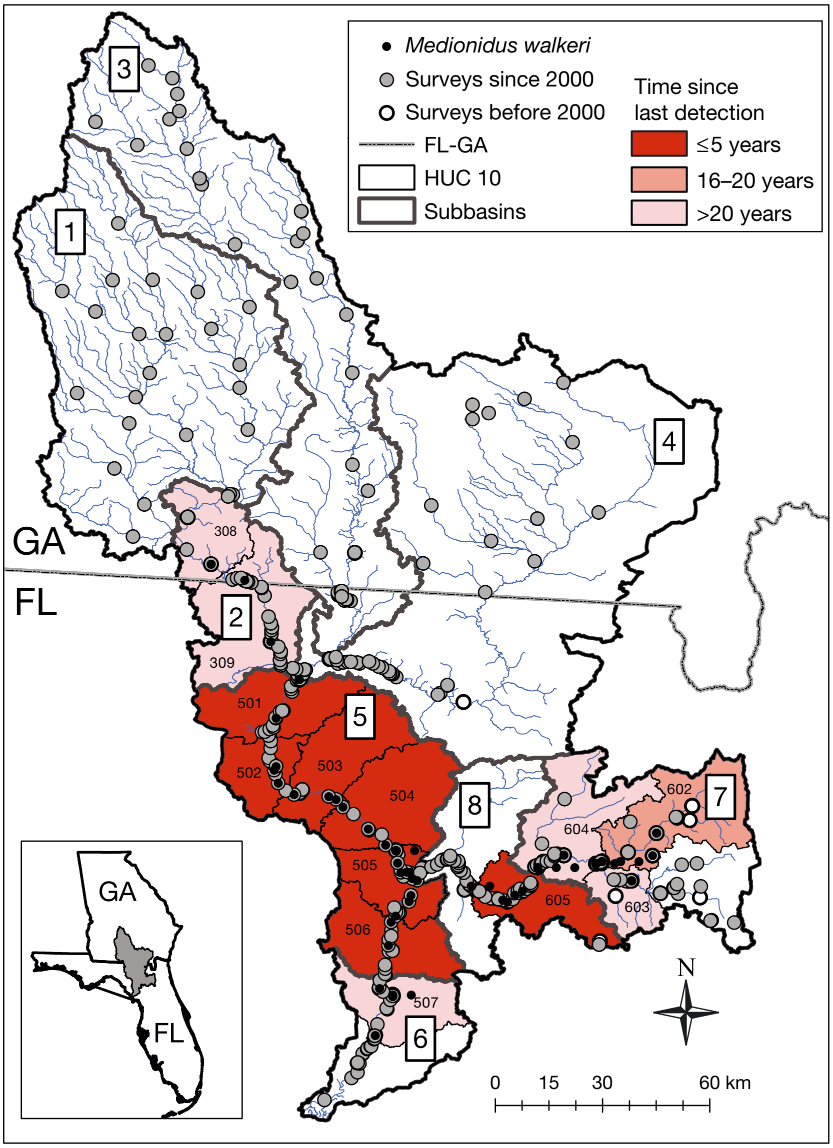

Fig. 1. Range and conservation status of the endemic freshwater mussel *Medionidus walkeri* in the Suwannee River basin, Florida and Georgia, southeast USA (inset map). Large numbers identify subbasins: (1) upper Withlacoochee, (2) lower Withlacoochee, (3) Alapaha, (4) upper Suwannee, (5) middle Suwannee, (6) lower Suwannee, (7) upper Santa Fe, and (8) lower Santa Fe. Level 10 hydrologic unit code (HUC 10) watersheds where *M. walkeri* has been recorded are outlined and identified by the last 3-digits of the corresponding HUC. Locations of mussel surveys (1980–2015) and sites where *M. walkeri* has been collected are also shown

changes every 24 h. The morphology and behavior of the mantle lure were documented using a dissecting microscope equipped with a digital camera. Water temperature was increased by 1°C every 3 d to accelerate development and release of glochidia. Tank bottoms were checked daily for eggs or glochidia. Three of the 4 gravid females released fully developed glochidia on Day 6 when tank temperatures reached 17°C. Viability of the released glochidia was low, presumably due to the extended time between release and viability assessment. We extracted fully developed glochidia from the fourth female (total length 36 mm) for fecundity estimation and host suitability trials using a 10 ml disposable syringe with a 20-gauge needle. Our fecundity estimation and glochidial viability assessment generally follow Fritts et al. (2014a) with a few modifications. Glochidia were suspended in a 1 l beaker containing 500 ml of well water before extracting ten 200 µl subsamples. Each subsample contained between 1 and 10 glochidia, and a total of 54 glochidia were tested for viability by adding 2 µl of a saturated NaCl solution to each subsample, creating a final concentration of approximately 1% NaCl. Total fecundity and total number of viable glochidia were calculated by extrapolation using the total number of glochidia and viable glochidia from the mean subsample counts, respectively.

We conducted our host suitability trial on 8 fish species in 6 families (see Table 2 below) using modified recirculating aquaculture systems (AHAB tanks; Aquatic Habitats) following standard laboratory inoculation methods (e.g. Fritts et al. 2012). All fishes used in the trial were collected from sites within the SRB using seine nets and were held in the laboratory for at least 2 wk prior to being inoculated with glochidia. A total of 40 host fishes (5 individuals per species) were simultaneously inoculated in a communal bath containing approximately 12 750 viable glochidia suspended by aeration in a 4 l glass beaker with approximately 3.2 l of well water for a final concentration of approximately 4000 viable glochidia per liter of water. After 15 min, each fish was removed from the bath and rinsed to remove unattached glochidia and placed in individual 1.5 or 3 l AHAB tanks. Tank outflows were continuously filtered through 153 µm mesh filter cups and checked for rejected glochidia or metamorphosed juveniles every 2 to 3 d. The number of rejected glochidia and juveniles recovered was counted using a dissecting microscope. The percent metamorphosis was calculated for each fish by dividing the number of recovered juveniles by the sum of glochidia and juveniles recovered from that same fish. The mean percent metamorphosis is reported for each fish species. Days to rejection is reported as the range of days post inoculation when rejected glochidia were observed.

RESULTS

Surveys and distribution

We created a 99-yr archive containing data for 312 verified *Medionidus walkeri* specimens (Table S1) collected during 64 out of 499 total survey events in the SRB (Table S2). The entire known range of *M. walkeri* appears restricted to the Suwannee River and its tributaries (e.g. New and Santa Fe Rivers in Florida) as far downstream as Manatee Springs, Florida, and as far upstream as the Withlacoochee River in southern Georgia (Fig. 1). The single museum record from the Hillsborough River (UMMZ 57470) reported by Williams et al. (2014) was found to be improperly labeled. Examination of associated specimens from the same date and locality (e.g. *Villosa lienosa*, UMMZ 57471), which have never been documented from the Hillsborough River drainage prior or subsequent to this survey, strongly suggests an error in labeling. This finding reinstates *M. walkeri* as an SRB endemic.

Our data indicate spatiotemporal changes in the overall distribution of *M. walkeri* over the past 99 yr. In surveys prior to 2000, *M. walkeri* was collected in 9 HUCs across 5 subbasins, whereas in surveys after 2000, it was found in 7 HUCs across 2 subbasins, only 3 of which were occupied both before and after 2000 (Fig. 1). Thus, since 2000, surveys have expanded the known distribution of *M. walkeri* to include 4 additional HUCs, all within the middle Suwannee subbasin (Table 1). Our evaluation of survey effort showed that over 84% of HUCs known to historically or currently support *M. walkeri* have received adequate survey effort, but 3 historically occupied HUCs still warrant additional sampling (Table 1). All HUCs in the middle Suwannee currently support *M. walkeri*; therefore, we consider these HUCs adequately surveyed. In the lower Suwannee, a single HUC historically supported *M. walkeri* and despite 38 surveys since 2000 and multiple surveys at both historically occupied locations, no *M. walkeri* have been found in this subbasin since the 1960s. Only 5 recent surveys have been conducted in a HUC in the lower Withlacoochee where *M. walkeri* was last detected in 1969. In the upper Santa Fe subbasin, HUC 602 has been surveyed 20 times since 2000 but still lacks recent surveys at 1 historical locality (Fig. 1).

Early life history investigations

Results from our laboratory trial show *M. walkeri* glochidia encysted and metamorphosed on all 5 *Percina nigrofasciata*. Glochidia metamorphosis occurred between 16 and 34 d post inoculation with an average metamorphosis success of 56.2 ± 8.9% (Table 2). Five *M. walkeri* glochidia metamorphosed 19 to 21 d post inoculation on 4 of 5 *Etheostoma edwini* tested with an average juvenile metamorphosis of 16.1 ± 7.9%. *Trinectes maculatus*, *Lepomis marginatus*, *Notropis texanus*, *Noturus leptacanthus*, *Etheostoma fusiforme*, and *Gambusia holbrooki* did not produce any metamorphosed juveniles. The number of days to rejection varied for each nonhost species and ranged between 3 and 19 d. Total fecundity for the single *M. walkeri* used for the host trial was estimated at 13500 glochidia with approximately 12750 (94%) considered viable prior to the inoculation.

We examined 90 *M. walkeri* specimens for gravidity (Table S1). A total of 21 specimens (23%) were observed gravid and the gill contents were examined and characterized for 13 of the gravid females (62%). Total length of the gravid females ranged from 23 to 38 mm. Immature glochidia were found in 4 individuals collected October 30, 2014. All remaining females examined were brooding fully developed glochidia, confirmed by exposure to saturated NaCl solution (on January 30, 2013 [n = 4], December 16, 2013 [n = 1], October 30, 2014 [n = 1], December 17, 2014 [n = 1], and May 28, 2015 [n = 2]) (Table S1). Fully developed glochidia ranged in size as follows: length 177 to 237 µm; height 226 to 300 µm; width 100 to 136 µm; dorsal margins 88 to 119 µm. All glochidia lacked a styliform hook and the shell outline was subspatulate with a straight dorsal margin, rounded ventral margin, and convex anterior and posterior margins (Fig. 2).

Observations of displaying *M. walkeri* revealed that the mussel uses the entire mantle fold to attract host fishes. The mantle lure consisted of an intricately developed papillate mantle fold located ante-

Table 2. Results of fish host tests for the parasitic larval stage of *Medionidus walkeri*. Nomenclature follows Page & Burr (2011). Five individuals of each fish species were tested. Data shows the range of days post-inoculation when rejected glochidia were observed; total numbers per species of rejected glochidia observed; range of days to metamorphosis where this occurred; number of individuals that metamorphosed into juvenile *M. walkeri*; and mean number of juveniles and percentage of metamorphosis for each host fish species where metamorphosis occurred

Fish families and species	Days to rejection	No. rejected	Days to metamorphosis	No. metamorphosed	Mean juveniles per fish	% metamorphosis (±95% CI)
Achiridae						
Trinectes maculatus	3–7	164	–	0	0	–
Centrarchidae						
Lepomis marginatus	3–7	127	–	0	0	–
Cyprinidae						
Notropis texanus	3–11	120	–	0	0	–
Ictaluridae						
Noturus leptacanthus	3–19	318	–	0	0	–
Percidae						
Percina nigrofasciata	3–21	183	16–34	280	56	56.2 ± 8.9
Etheostoma edwini	3–11	30	19–21	5	1	16.1 ± 7.9
Etheostoma fusiforme	3–7	24	–	0	0	–
Poeciliidae						
Gambusia holbrooki	3–11	7	–	0	0	–

Fig. 2. Morphology of *Medionidus walkeri* glochidia

rior of the incurrent aperture that was approximately 20 to 30% of the total shell length with 2 distinct segments. The first segment of the mantle margin occurred on the posterior 70% of the mantle fold and was orange with rusty-brown mottles externally and faded from orange to a vibrant blue grey to black internally (Fig. 3A). Papillae were small, circular, blunt, and orange to rusty in color with fine black banding and were irregularly spaced with crenulations between papillae (Fig. 3B). The second segment of the mantle margin occurred on the anterior 30% of the mantle fold and was thicker compared to the posterior section. It had strong crenulations forming 8 or 9 segments with single papillae per segment

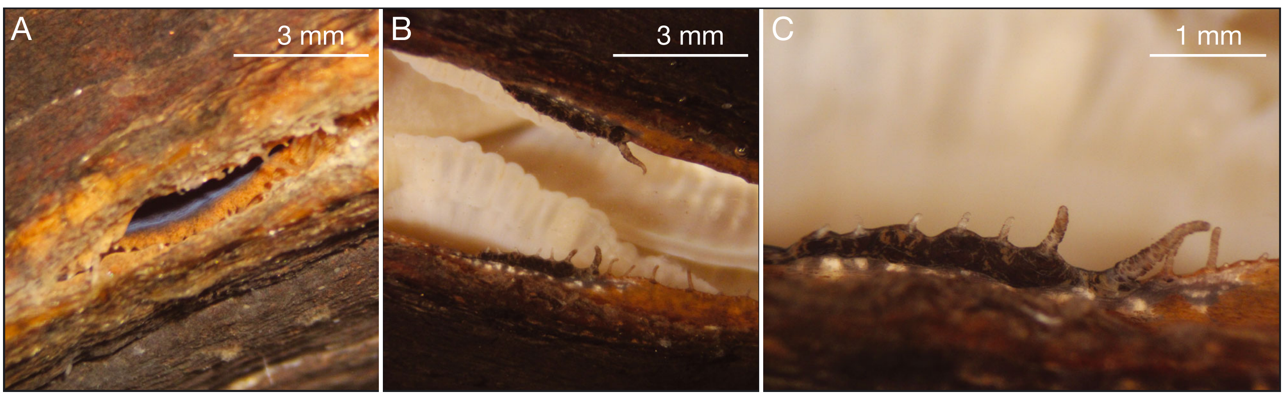

Fig. 3. Mantle displays of *Medionidus walkeri*: (A) papillate mantle fold ventral of the incurrent aperture with vibrantly colored interior of the fold; (B) papillate mantle fold found on the anterior portion of the mantle margin; (C) close-up view of the enlarged papillae occurring on the anterior of the mantle fold. All photos orientated with anterior to the left

(Fig. 3B). The interior and exterior of the anterior segment of the mantle fold appeared identical in color and shape. The papillae were circular, blunt, and translucent with occasional brown mottles. The most posterior papilla was at least twice the diameter and height of all others and had visible horizontal bands (Fig. 3C). When displaying its lure, the female mussel rapidly flicks the brilliant blue interior portion of the posterior mantle fold (Fig. 3A) open and closed while flexing and wiggling the larger papillae of the anterior portion of the mantle fold (Fig. 3B,C). The combination of actions provides both a color attractant and a motion lure that appears to imitate an aquatic insect larva.

DISCUSSION

Surveys and distribution

Our findings show the distribution of *Medionidus walkeri* has changed during the past century, with an overall reduction in range and fewer individuals found during recent surveys, displaying a common pattern observed for freshwater mussels across river systems worldwide (Cosgrove et al. 2000, Geist & Kuehn 2005, Haag & Warren 2010, Hinck et al. 2012, Jones et al. 2014, Lopes-Lima et al. 2016, Zipper et al. 2016). The observed spatiotemporal changes in distribution appear confined to the periphery of the species' range in the lower Withlacoochee, lower Suwannee, and upper Santa Fe subbasins (Fig. 1). For some species, the tendency exists for population densities to be lower and less stable along the periphery compared to the center of the species' geographic range (Brown 1984). Therefore, a decline in abundance could be expected to trigger a reduction in geographic range, first along the periphery and then towards the center of a species' historical range (Lawton et al. 1994). It was not possible to account for imperfect detection or estimate abundance due to the lack of information regarding effort expended and survey techniques used during each survey. However, the observed trend toward declining numbers of individuals found at repeatedly sampled sites indicates that *M. walkeri* collections have decreased over time, and the perceived range contraction might be indicative of declines in both distribution and abundance. We assume current surveys expend greater effort (e.g. snorkeling, SCUBA) than most historical surveys, but we recognize the potential confounding effects of variation in survey techniques in this study. Limitations aside, the repeated sampling, range-wide geographical coverage, and temporal span of our dataset reduce the extent of these problems and give us confidence to make inferences regarding spatiotemporal changes in *M. walkeri* occurrences at the subbasin and HUC 10 level. For example, several historical museum lots contain 20 or more *M. walkeri* specimens. These collections are substantial considering only 1 out of 384 surveys conducted in the SRB since 2000 has collected more than 7 *M. walkeri*. Although anecdotal, failure of modern collections to detect *M. walkeri* at historical localities formerly yielding large numbers of specimens strongly suggests the species was more abundant in the past.

In the past 20 yr, *M. walkeri* has been detected within 33% of historically occupied HUCs. *M. walkeri* has not been collected in the upper Santa Fe subbasin since 1996, indicating that the species occurs below detectable levels or is extirpated at this location. All HUCs in this subbasin have received adequate survey effort except HUC 602, which lacks surveys at 1 historical locality. *M. walkeri* is currently rare in the lower Santa Fe subbasin where surveys since 2000 have collected only 2 live specimens from a single HUC despite adequate survey efforts in 2 of 3 HUCs. The HUC lacking adequate survey effort in this subbasin is the Ichetucknee River (HUC 606), which historically has never supported *M. walkeri* or a diverse unionid fauna. In the lower Withlacoochee subbasin, *M. walkeri* has not been collected since 1969, but since only 50% of HUCs received adequate resurveying, the species' status in this subbasin remains uncertain. In the lower Suwannee subbasin, *M. walkeri* has not been collected since the 1960s despite adequate survey efforts. The only subbasin in which surveys since 2000 reliably found *M. walkeri* is the middle Suwannee. The majority of all *M. walkeri* collections (both historical and recent) occurred in this subbasin and researchers have found *M. walkeri* specimens during 49% of surveys conducted since 2000, averaging approximately 1 specimen per survey. The declines across most subbasins are likely the result of a combination of factors that are subbasin dependent.

Prior to the 1950s when most *M. walkeri* collections occurred, the upper Santa Fe subbasin was largely perennial (Scott et al. 2004), but USGS stream gauge data shows it has been dry multiple times since 2000 at one historical locality (Santa Fe River near Worthington Springs). This shift in hydrologic flow regime might explain detection failure by recent surveys as abnormally low flow conditions can result in high mussel mortality (Johnson 2001, Golladay et al.

2004). It is unclear why *M. walkeri* has never been common in the lower portion of the Santa Fe subbasin. Only 2 historical and 5 recent collections (3 are relict shell only) have been reported in the lower Santa Fe subbasin. Despite the lack of *M. walkeri*, most of this subbasin maintains good mussel diversity and habitat (FWC unpubl. data). The upper and lower Withlacoochee subbasins are affected by significant urban development and changes in land use, and releases of raw sewage effluent from the Valdosta Georgia Water Treatment Plant have been reported as recently as March 2016 (www.wctv.tv/home/headlines/Weekend-Storm-Causes-3-Sewage-Overflows-in-Valdosta-373784521.html). A lack of *M. walkeri* collections combined with evidence of deteriorated water and habitat quality suggests that *M. walkeri* occurs below detectable levels or has been extirpated from the lower Withlacoochee subbasin, but additional surveys are needed in HUC 309 to provide a more definitive determination of species occurrence. The Suwannee River in the lower Suwannee subbasin is tannic, deep, wide, and moderately swift, and thus more difficult to sample than other subbasins, which may explain the failure of recent surveys to detect *M. walkeri*, although a relic shell was found during a SCUBA survey in 2015. Additional surveys utilizing SCUBA or other more sophisticated technologies are necessary to further evaluate *M. walkeri* occurrence in the lower Suwannee subbasin. The continued existence and relatively high abundance of *M. walkeri* in the middle Suwannee subbasin might be a result of hydrologic stability and mediated water quality from groundwater inputs that are less prevalent in other SRB subbasins.

Early life history investigations

Many aspects of *M. walkeri* reproductive biology are similar to other congeners and members of the tribe Lampsilini, which are generally considered to be long-term brooders (bradytictic) (Barnhart et al. 2008). Collections of gravid female *Medionidus* spp. from other drainages, i.e. *M. acutissimus* (Haag & Warren 1997), *M. parvulus* (Williams et al. 2008), and *M. penicillatus* (Brim Box & Williams 2000, Fritts & Bringolf 2014b), indicate that *Medionidus* are gravid beginning in the fall through early summer the following year. These findings are in agreement with Zale & Neves (1982) who collected *M. conradicus* glochidia in stream drift during May to June and September to November and in greatest abundance from January to May. Our data support the idea that *M. walkeri* is bradytictic, brooding mature glochidia from October to May. Gravidity data for the months of February to April and June to July are unavailable for *M. walkeri*. These data gaps are significant considering that *M. walkeri* might spawn and be gravid during at least a portion of these months.

Concurrent with congeners and most other members of Lampsilini, *M. walkeri* is a lure-displaying host specialist that entices attacks from fish to parasitize potential glochidia hosts (Barnhart et al. 2008). This finding is important considering that the effectiveness of a visual lure is susceptible to both natural and anthropogenic impairments (e.g. turbidity from sedimentation, host fish abundance), making it important to understand how mussels parasitize their hosts. For example, studies have shown that even slight increases in turbidity can significantly impact the foraging behavior of darters (Becker et al. 2016). Additionally, a positive relationship between host abundance and both mussel recruitment and larval survival has been observed in experimental settings, with fecundity and host-attracting strategy also having an effect (Haag & Stoeckel 2015). Observations of *M. walkeri* host-infection strategy reveal close similarities in mantle structure, color, and movement to *M. conradicus* (www.youtube.com/watch?v=UK4ZM1BLO-E) and previous descriptions of *M. acutissimus* (Haag & Warren 1997, 2003). *M. walkeri* glochidia are also similar in both size and shape to its congeners. Glochidia are subspatulate, and size ranges overlap descriptions of *M. penicillatus* (O'Brien & Williams 2002), *M. parvulus* (Williams et al. 2008), and photographs of *M. conradicus* (Zale & Neves 1982).

Metamorphosis of *M. walkeri* was observed only on 2 of the 3 darter species tested (*Percina nigrofasciata* and *Etheostoma edwini*), which is consistent with findings on congeners with a few exceptions. Two previous studies of *Medionidus* spp. report *Percina* spp. and *Etheostoma* spp. as primary and secondary hosts, respectively (Zale & Neves 1982, Brim Box & Williams 2000). Two additional studies reported a total of 12 darters (*Ammocrypta beani*, *A. meridiana*, *E. artesiae*, *E. douglasi*, *E. nigrum*, *E. stigmaeum*, *E. swaini*, *E. whipplei*, *P. nigrofasciata*, *P.* sp. cf. *caprodes*, and *P. vigil*) served as glochidial hosts of *M. acutissimus* (Haag & Warren 1997, 2003). Similarly, Fritts & Bringolf (2014b) observed metamorphosis of *M. penicillatus* on all 4 darter species tested (*E. inscriptum*, *E. swaini*, *P. crypta*, and *P. nigrofasciata*) but reported high levels of variation among replicates for several fish species tested and trials that used glochidia from different females. Glochidia of *M. walkeri* failed to metamorphose on *E. fusiforme* in our study, indicating that not all species of darter may be suitable hosts. Primary fish hosts reported for *M. penicillatus* were darters (*P. nigrofasciata* and *E. edwini*) with *Gambusia holbrooki* and *Poecilia reticulata* as secondary hosts (O'Brien & Williams 2002), while *M. walkeri* glochidia did not metamorphose on *G. holbrooki* in our laboratory trials.

Host–parasite theory suggests low host fish abundance or high competition for host fishes could limit the reproductive success of certain mussel species (Smith 1985, Khym & Layzer 2000, Haag & Stoeckel 2015). A study by Fritts et al. (2012) shows that Flint River populations of the federally listed *Elliptoideus sloatianus* have limited recruitment and dispersal due to extirpation and blocked migration routes of the mussel's primary host fish, *Acipenser oxyrinchus desotoi*. The primary host fish for *M. walkeri*, *P. nigrofasciata*, appears continuously distributed throughout the SRB (Lee et al. 1980) suggesting insufficient numbers of host fishes might not be a factor in the perceived decline of *M. walkeri*. However, darters are sight feeders (Boschung & Mayden 2004) and the mussel's ability to attract and successfully inoculate the host could be limited during turbid conditions (Becker et al. 2016).

Evaluating *P. nigrofasciata* behavior and movement patterns during the *M. walkeri* parasitic larval stage is useful to characterize the mussel's dispersal and recruitment capabilities (Schwalb et al. 2011, Horký et al. 2014, Jones et al. 2015). *P. nigrofasciata* have been shown to be 'long-term residents' that generally live in small areas of about 30 m with only a few fish documented to move up to 420 m (Freeman 1995). This limited movement could restrict *M. walkeri* dispersal capabilities to several hundred meters. Limited dispersal capabilities are a concern for *M. walkeri* recovery and conservation considering the distance between known collection localities. A reduction in gene flow among mussel populations for species that utilize host fish with low dispersal capabilities, such as darters, has been documented (Jones et al. 2015); therefore, low host fish dispersal might restrict the ability of *M. walkeri* to recolonize formerly occupied habitats without human intervention. For example, natural recolonization of *M. walkeri* above the Santa Fe River Rise in O'Leno State Park is highly improbable considering that the river flows underground through solution channels for approximately 5 km. Transplanting adults or releasing cultured juveniles might be the only option to restore *M. walkeri* to the upper Santa Fe subbasin.

Management implications

The life cycle of *M. walkeri* complicates conservation strategies, particularly given the causes for declines are enigmatic and conserving the species requires managing the species and the watersheds simultaneously. Habitats historically occupied by *M. walkeri* included small headwater creeks and medium and large rivers, but today the species appears restricted to the lower Santa Fe and middle Suwannee subbasins downstream of the Cody escarpment. The geomorphology of the middle Suwannee and lower Santa Fe subbasins lacks surface streams, limiting *M. walkeri* to a linear distribution in the mainstem of these rivers. As a result, *M. walkeri* has limited refugia from catastrophic events such as train and tanker spills (Jones et al. 2001) and mine tailing pond failures (PAS & LES 2005, Galloway et al. 2013) in the middle Suwannee or lower Santa Fe subbasins. Because *M. walkeri* appears to be rare and declining in the lower Santa Fe subbasin (Table 1), the species' confinement to this single subbasin for refuge habitat is of great concern for the species' persistence. Protecting areas known to currently support *M. walkeri* and reestablishing animals in formerly occupied habitat in the lower Withlacoochee or upper Santa Fe subbasin are options that may alleviate this problem.

M. walkeri currently does not have critical habitat designated; however, the USFWS is currently preparing a proposed critical habitat rule which will be published in the Federal Register in fall 2016 (S. Pursifull pers. comm.). The mainstem of the Suwannee River to Big Shoals and the lowermost portion of the lower Withlacoochee subbasin are protected as critical habitat for Gulf sturgeon *Acipenser oxyrinchus desotoi* (USFWS 2003), and the majority of the upper Santa Fe subbasin is protected as critical habitat for oval pigtoe *Pleurobema pyriforme* (USFWS 2007a, USFWS 2007b). These river reaches may be important to consider when designating critical habitat for *M. walkeri.* Additional sections within the known range of *M. walkeri* that may be important to protect are the lower Santa Fe and portions of the lower Withlacoochee subbasins. Establishing critical habitat protection for the lower Santa Fe subbasin is logical considering that *M. walkeri* was recently (2015) collected in the subbasin and that this section of river is the only known refuge habitat protecting *M. walkeri* from extinction if the middle Suwannee population were to collapse. Additionally, the lower Santa Fe subbasin provides a critical link between portions of the upper Santa Fe, which historically supported *M. walkeri,* and middle Suwannee subbasins and might be essential for natural recolonization and gene flow. Okefenokee Swamp and Lower Suwannee National Wildlife Refuges, located in the headwaters and lowermost portions of the SRB respectively, are outside of the documented range of *M. walkeri* and appear to provide no habitat for the species.

Our approach of combining a comprehensive museum inventory, field surveys, and early life history information provides resources that are critical to assessing status and considering conservation and recovery efforts for *M. walkeri*. By carefully reviewing all *M. walkeri* specimens and data derived from museum collections and recent surveys, we were able to document changes in the spatiotemporal distribution of *M. walkeri*. This information coupled with new information regarding the early life history of the species fills critical knowledge gaps necessary to make more informed management decisions.

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