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# DENSITY, DEMOGRAPHY, AND MICROHABITAT OF CAMPELOMA DECAMPI (GASTROPODA: VIVIPARIDAE)

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### ABSTRACT

*Campeloma decampi*, the Slender Campeloma, is a federally endangered snail endemic to the Tennessee River drainage in Alabama, U.S.A. We studied a population in Round Island Creek, Limestone County, in July, 2010, to obtain information about density, microhabitat, and demography. The overall mean density at the site was 49.2/m<sup>2</sup> ( $\pm$  14.4 SE), but the distribution was highly clumped. We used generalized linear models and multi-model inference to examine the response of snail density to seven microhabitat explanatory variables. The greatest densities were associated with shallow, low-flow areas with silt and clay near the stream margin. Shell heights ranged from 4.3–34.7 mm, and the size distribution appeared to be composed of three cohorts possibly representing age 0+ recruits, age 1+ individuals, and individuals  $\geq$ 2 years of age. The population was dominated by small individuals (4–12 mm; modal size class = 6 mm), and individuals >20 mm made up only 7% of the population. This size distribution suggests that parturition occurs over a protracted period from late winter to summer and that most individuals produce only one or two broods in their lifetime; however, additional sampling and information about life span are needed to more conclusively describe the reproductive strategy.

**KEY WORDS** *Campeloma decampi*, Freshwater Gastropod, Endangered Species, Microhabitat, Density, Demography, Slender Campeloma

### INTRODUCTION

Understanding the life history and ecological requirements of imperiled freshwater snails is a high priority for their conservation (Lysne et al., 2008; Strong et al., 2008; Johnson et al., 2013). *Campeloma decampi* (Binney, 1865) (Slender Campeloma, Viviparidae) is a freshwater snail endemic to a small portion of the Tennessee River drainage in northern Alabama (Haggerty & Garner, 2008; U.S. Fish and Wildlife Service, 2012). In 2000, *C. decampi* was listed as endangered under the U.S. Endangered Species Act (Federal Register, 2000; Johnson et al., 2013). Rapid urban and industrial growth within the species' range threatens its survival, and ecological data are needed to effectively monitor and manage remaining populations.

Little is known about the life history and ecology of *C. decampi*. It reaches about 35 mm in size (shell height), and like other members of the Viviparidae, it is ovoviparous and is most likely a detritivore (Garner, 2004; Haggerty & Garner, 2008). Preliminary observations suggest that *C. decampi* has a highly clumped spatial distribution and occurs primarily in shallow habitats with little current near stream margins and emergent vegetation, and it burrows into fine substrates or detritus (Garner, 2004; Haggerty & Garner, 2008). Other *Campeloma* species are found in similar habitats, and they give birth to live young in winter or spring, and in some cases into the summer (Allison, 1942; Bovbjerg, 1952; Vail, 1978; Imlay et al., 1981; Brown et al., 1989).

These specialized habitats appear necessary for feeding and reproduction.

The goals of this study were to 1) quantitatively describe the spatial distribution of *C. decampi* and the microhabitat characteristics associated with the species, and 2) provide information about demography and reproduction in our study population.

## METHODS

The study was conducted in Round Island Creek, Limestone Co., Alabama, U.S.A., which supports high densities of *C. decampi* (Haggerty & Garner, 2008). Round Island Creek is a third-order stream approximately 25 km long with a drainage area of 135 km<sup>2</sup>. It lies within the Tennessee Valley District of the Interior Low Plateau Physiographic Province (Sapp & Emplainscourt, 1975) and flows into Wheeler Reservoir at Tennessee River mile 298. The underlying geology of Round Island Creek is Fort Payne Chert and Tusculumbia Limestone (Osborne et al., 1988; Szabo et al., 1988). The drainage is primarily agricultural or forested, riparian zones are generally intact and banks are stable, and the stream is extensively canopied. Stream habitats include riffles, runs, and pools, and the substrate of the runs and riffles is mostly gravel with interstitial sand and silt. Pools and marginal areas often have deposits of mud and beds of Waterwillow, *Justicia americana* (Linnaeus) Vahl. Exposed bedrock occurs at some sites, but outcrops are generally not extensive. In June 2007, average physico-chemical measurements from three sites on Round Island Creek where *C. decampi* occurred were: temperature (27.4°C), dissolved oxygen (4.30 mg/l), dissolved oxygen percent saturation (51.6%), pH (8.4), specific

conductance (138.7  $\mu$ S/cm), total hardness (69.3 ppm), calcium hardness (45.3 ppm), and magnesium hardness (24 ppm) (Haggerty & Garner, 2007).

The study site was a 125 m stream reach at Ripley Road, Limestone County, Alabama (34.75290° N, 87.08437° W). Average channel width in the study reach was 10.6 m ( $\pm$  1.1 SE, n = 8 cross sections). We sampled at this site from July 16-22, 2010, a time when stream conditions were relatively constant and accurate sampling could be conducted. Data were collected along eight transects placed perpendicular to stream flow at approximately 10-15 m intervals. Transects were placed to encompass a range of suitable and unsuitable habitats for *C. decampi* based on previous qualitative observations (Haggerty & Garner, 2008). We sampled four 0.25 m<sup>2</sup> quadrats along each transect; one adjacent to each stream bank, and two at equidistant points between the banks. We excavated and removed the substrate within each quadrat to a depth of approximately 6 cm, washed the sediments with creek water across 10 and 2 mm mesh nested sieves, and then examined this material for *C. decampi*. We counted all individuals and measured shell height to the nearest 0.1 mm using digital calipers; the spires were not eroded, which allowed accurate shell height measurements of all individuals. No attempt was made to sex individuals, and all snails were returned to the area from which they were collected.

Seven environmental variables were measured at each quadrat location (Table 1): distance from stream bank (BD), distance to nearest emergent vegetation (DEV), water depth (WD), surface current velocity (CV), mean sediment grain size ( $\phi$ ), percentage of silt and clay (SC), and percentage of organic matter (OM).

**TABLE 1**

Average values ( $\pm$  SE) for microhabitat variables associated with quadrats having high density ( $>12$  individuals/m<sup>2</sup>) and low density ( $\leq 12$  individuals/m<sup>2</sup>) of *Campeloma decampi* in Round Island Creek, Limestone County, Alabama. Asterisks (P<0.05) and NS (not significant) report results of individual t-test or Wilcoxon test for each variable between high- and low-density quadrats.

Variables	High density (N = 12)	Low Density (N = 19)
Distance to bank (m)*	0.70 $\pm$ 0.21	2.73 $\pm$ 0.35
Distance to emergent vegetation (m) <sup>NS</sup>	2.71 $\pm$ 1.08	2.28 $\pm$ 0.56
Current velocity (cm/s)*	0.78 $\pm$ 0.58	3.46 $\pm$ 0.77
Water depth (cm)*	13.80 $\pm$ 2.50	31.51 $\pm$ 4.28
Sediment grain size ( $\phi$ ) <sup>NS</sup>	0.91 $\pm$ 0.16	0.64 $\pm$ 0.06
% Organic matter <sup>NS</sup>	5.76 $\pm$ 1.18	4.42 $\pm$ 0.21
% Silt and Clay <sup>NS</sup>	0.006 $\pm$ 0.001	0.003 $\pm$ 0.001

Distance to bank and emergent vegetation were measured with a measuring tape. Water depth was measured with a pole marked in 1 cm increments. Surface current velocity was measured with a measuring tape, standardized float, and stopwatch. Substrate characteristics ( $\phi$ , SC, OM) were estimated from sediment cores collected near the upstream edge of each quadrat with a 7.6 cm diameter, 1.2 m long galvanized metal pipe, which was forced into the substrate as far as possible and capped with a rubber stopper. A sturdy, flat piece of metal was positioned over the opening of the pipe as it was removed from the substrate. The pipe was then quickly raised and emptied into a 3.8 L zippered plastic bag. Samples were transported to the laboratory in a cooler of ice, and then frozen.

In the laboratory, frozen sediment samples were thawed, allowed to settle, decanted, and oven-dried for 24 hours at a minimum of 70°C. The dried samples were sieved across the following mesh sizes: 63mm, 8mm, 4mm, 2mm, 1mm, 500 $\mu$ m, 250 $\mu$ m, 125 $\mu$ m, and 63 $\mu$ m (Buchanan, 1984), and the fraction retained on each sieve was weighed. The program GRADISTAT and the Folk and Ward method were then used to obtain a logarithmic mean grain size ( $\phi$ ) for each sample (Blott & Pye, 2001). Silt and clay estimates were obtained from the percentage by weight of sediments that passed through the 63 $\mu$ m sieve. Prior to sieving, a subsample of approximately 20 ml of material was taken from each core sample and ashed for two hours at or above 550°C to estimate percent organic matter.

We computed the variance-to-mean ratio for snail densities across all quadrats to evaluate the spatial dispersion of the population (Ludwig & Reynolds, 1988). A size frequency histogram of the shell height measurements was used to depict population demography during the sample period.

An information-theoretic approach was used to examine associations among microhabitat variables and *Campeloma decampi* density (Burnham & Anderson, 2001, 2002; Burnham et al., 2011). We formulated 12 *a priori* candidate models based on previous observations of habitat (e.g., Haggerty & Garner, 2008) and published accounts of congeners (Medcof, 1940; Allison, 1942; Bovbjerg, 1952; Chamberlain, 1958; Imlay et al., 1981; Brown et al., 1989). Only models of interest and empirical support were included in the analysis (Table 2; Burnham & Anderson, 2001). Poisson regression in log linear models (i.e., generalized linear models), maximum likelihood estimations, and Akaike's information criterion corrected for small sample size (AIC<sub>c</sub>) were used to compare the fit and explanatory power of each model. Because of an error in the collection of sediment from one of the quadrats, all the data from that sample were excluded from analysis. When modeling count data, an important preliminary step is testing the fit of the global model including all variables ( $\phi + DB + DEV + CV + WD + SC + OM$ ). The global model provided a significantly greater fit to the snail density data than the null model (Whole Model Test:  $\chi^2 = 640.98$ ;  $P < 0.0001$ ), but it fit the data poorly (Goodness-of-Fit Test:  $\chi^2 = 254.95$ ;  $P < 0.0001$ ). Therefore, the calculated

**TABLE 2**

Ranked candidate models used to evaluate the influence of microhabitat variables on *Campeloma decampi* density at Round Island Creek, Limestone Co., Alabama. Models are ranked in ascending order by their QAIC<sub>c</sub> differences ( $\Delta$ QAIC<sub>c</sub>) relative to the best model in the set. Variables are distance to bank (DB), % silt and clay (SC), water depth (WD), current velocity (CV), % organic matter (OM), distance to emergent vegetation (DEV), and sediment grain size ( $\phi$ ).

Rank	Model	-Log Likelihood	K <sup>a</sup>	QAIC <sub>c</sub>	$\Delta$ QAIC <sub>c</sub>	$w_i$ <sup>b</sup>
1	DB + SC + WD + CV	166.50	6	45.54	0.00	0.56
2	DB + SC	209.08	4	47.26	1.72	0.24
3	DB + CV + SC	208.16	5	49.96	4.42	0.06
4	DB + OM	225.13	4	50.16	4.62	0.06
5	DB + $\phi$	225.22	4	50.17	4.63	0.06
6	SC + WD + CV	217.27	5	51.60	6.06	0.03
7	WD + SC	247.85	4	54.26	8.72	0.01
8	DEV + SC + CV	349.25	5	75.41	29.87	0.00
9	DEV + SC	384.78	4	78.96	33.42	0.00
10	CV + OM	388.69	4	79.67	34.13	0.00
11	CV + $\phi$	388.58	4	79.65	34.11	0.00
12	SC	410.79	3	81.00	35.46	0.00

<sup>a</sup> number of parameters in model

<sup>b</sup> probability that model is best in model set

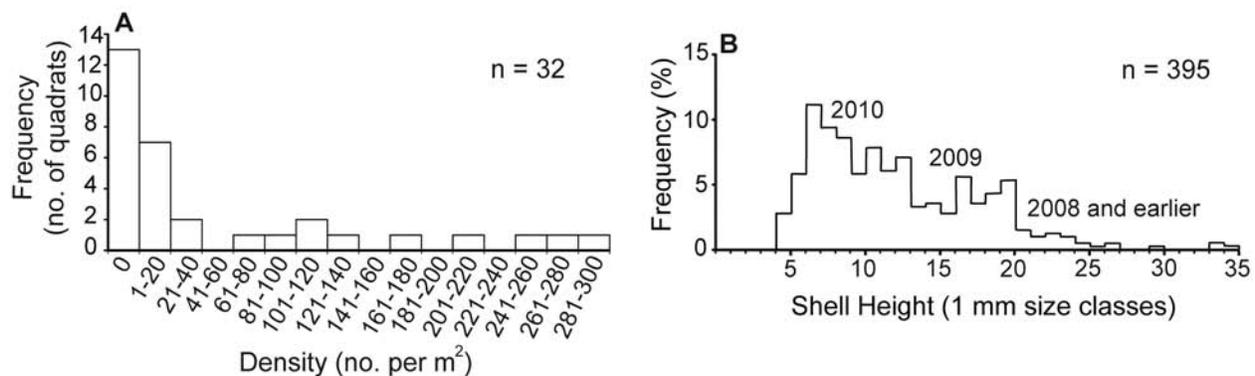
variance inflation factor for the global model ( $\hat{c} = 11.08$ ) was used for each candidate model to obtain a quasi-likelihood and a modified  $AIC_c$  (i.e.,  $QAIC_c$ ) (Burnham & Anderson, 2002). Variables based on percentages (SC, OM) were arcsine square root transformed before analysis. All analyses were conducted with JMP 9.02 (SAS Institute Inc., Cary, NC) and Microsoft Excel.

To provide a quantitative description of habitat that supported *C. decampi*, we categorized quadrats as high snail density ( $> 12$  individuals/m<sup>2</sup>) or low density ( $\leq 12$  individuals/m<sup>2</sup>), and calculated mean values of each habitat variable for both categories.

## RESULTS

A total of 395 *C. decampi* were captured from 19 (61%) of the 32 quadrats (Fig. 1). Most individuals were buried in the substrate. The overall mean density at the site was 49.2/m<sup>2</sup> ( $\pm 14.4$  SE), but the distribution was highly clumped (Fig. 1A; variance-to-mean ratio 33.7) and the highest recorded density was 284/m<sup>2</sup>.

The best supported model for explaining variation in snail density included four variables (DB, SC, WD, CV; Table 2), but other models had varying degrees of support. The difference in  $w_i$  between the best and the second best supported model (DB, SC) was small



**FIGURE 1**

A) Density-frequency distribution of *Campeloma decampi* in 0.25 m<sup>2</sup> quadrats in Round Island Creek, Limestone County, Alabama. B) Size-frequency distribution for *C. decampi* in July, 2010. Dates indicate suspected year of recruitment for apparent size cohorts.

(evidence ratio = 2.3) indicating that the second model was also plausible. Models 3-7 also had some empirical support ( $\Delta QAIC_c < 10$ ), but they all had low probabilities of being the best model; the remaining models were not supported ( $\Delta QAIC_c > 10$ ; Table 2). No variable occurred in all plausible models, but distance from the bank (DB) and % silt and clay (SC) occurred in over half of plausible models, including both of the top two models. Distance to emergent vegetation (DEV) did not appear in any plausible model.

Because our results indicated model uncertainty, a *post hoc* confidence set from the first five models ( $\sum w_i > 0.95$ ) was used to obtain model averages, unconditional SE values, 95% CI, and relative importance values for the variables shared among the models (Burnham & Anderson, 2002). Of the confidence set, distance from the bank (DB), % silt and clay (SC), and current velocity (CV) all had an effect on snail density (i.e., confidence interval excluded 0), but sediment grain size ( $\phi$ ) and % organic matter (OM) did not (Table 3); water depth had

a relatively high importance weight, but the 95% CI for this effect included zero. Parameter estimates indicated that density was inversely related to distance from bank and current velocity, but positively related to % silt and clay (Table 3). Among the confidence set, distance from the bank was the most important variable for explaining variation in snail density ( $\sum w_i = 1$ ), but % silt and clay and current velocity also had high relative importance weights (Table 3).

There were clear univariate differences in some microhabitat variables between quadrats with high and low densities of *C. decampi* (Table 1), and these patterns generally reflected results of the information-theoretic analysis. High density quadrats were closer to the bank and had significantly lower depths and current velocities than low density quadrats. There were no significant differences in sediment characteristics or proximity to emergent vegetation between high and low density quadrats.

**TABLE 3**

Model-averaged parameter estimates ( $\pm$  unconditional SE), 95% CI for estimates, and relative importance for variables explaining variation in *Campeloma decampi* density in Round Island Creek, Limestone County, Alabama.

Parameter	Model-averaged		Relative Importance ( $\Delta w_i$ )
	estimate $\pm$ unconditional SE	95% CI	
Distance to bank	-1.24 $\pm$ 0.32	- 1.88 to -0.59	1.00
% silt and clay	10.43 $\pm$ 1.80	6.74 to 14.11	0.89
Current velocity	-0.06 $\pm$ 0.02	-0.11 to -0.01	0.64
Water depth	-0.05 $\pm$ 0.03	-0.11 to 0.02	0.58
Sediment grain size ( $\phi$ )	-0.18 $\pm$ 1.96	-0.41 to 0.05	0.06
% organic matter	-1.27 $\pm$ 80.78	-2.86 to 0.032	0.06

The distribution of snail size was non-normal (Goodness-of-fit test,  $p < 0.0001$ ;  $n = 395$ ) and strongly right-skewed, and the population was dominated by small individuals (Fig. 1B). Shell height ranged from 4.3-34.7 mm, but the mean was  $12.0 \pm 0.3$ (SE), the modal size class was 6 mm, and over 75% of the population was  $< 17$  mm (Fig. 1B). The size frequency distribution showed evidence of at least three size cohorts, one centered on about 8 mm (about 4-12 mm), another centered on about 16 mm (about 13-19 mm), and another composed of individuals  $> 20$  mm; these largest individuals made up only 7% of the population.

## DISCUSSION

*Campeloma decampi* has a highly clumped distribution, which is apparently related to its specific microhabitat requirements. It primarily occupies shallow, slow-current areas along the stream margin where the substrate contains silt and clay. The highest densities of the species were found almost exclusively in this habitat type.

Observations from other sites in Round Island Creek and elsewhere in its range suggest that this habitat preference is a general characteristic of the species (Haggerty & Garner, 2008). Indeed, this type of habitat appears to be required by most species in the Viviparidae. Most viviparids feed on mud, detritus, and decaying organic matter, and high snail densities and growth are often associated with habitats rich in these materials (Allison, 1942; Chamberlain, 1958; Imlay et al., 1981; Richardson & Brown, 1989). Consequently, the low relative importance value for organic matter, sediment grain size, and the low ranking of the silt and clay

model in our study were surprising. We did not remove surface litter (e.g., sticks and intact leaves) from our substrate samples before processing, and inconsistency in the presence of these larger organic materials among samples may have obscured patterns related to finer, buried organic matter that serve as a food source for snails. Oxygen concentration in organic sediments also may influence the distribution of *C. decampi*. Some of our sample locations had relatively high percentages of silt, clay, and organic material but had the smell of hydrogen sulfide suggesting that they were hypoxic; such habitats can be inhabited by *Viviparus georgianus*, but they rarely contain *C. decampi*. It is also likely that concentrations of organic matter vary in these depositional habitats seasonally and among years.

Alternatively, the lack of strong relationships regarding potential food availability and fine substrates may indicate that other factors are equally important in determining habitat selection by *C. decampi*. The low flow, near-shore habitats that supported high densities of *C. decampi* may represent refuges from scouring flows (Bovbjerg, 1952); this may be especially important for large, globose species like *Campeloma* compared with more hydrodynamically streamlined species that occur in main channel habitats (e.g. pleurocerids). Shallow, near-shore areas also may be refuges from fish predation, which can be important in limiting snail density (Medcof, 1940). Regardless of the mechanism responsible for habitat selection, these shallow shoreline habitats clearly are critical for survival of this species and a better understanding of their characteristics and temporal stability is needed.

Our demographic data offer some insights into the life history of *C. decampi*. Assuming that *C. decampi* has a birth size (approximately 3–4 mm) and juvenile growth rate (1.7–2 mm/month during the first year) similar to other *Campeloma* (Van Cleave & Altringer, 1937; Chamberlain, 1958; Vail, 1978; Brown & Richardson, 1992), the smallest size cohort in our population (about 4–12 mm) may represent individuals that were born over a protracted period from late winter to mid-summer, 2010. This growth estimate also suggests that *C. decampi* reaches a minimum brood-bearing size during their first year (i.e., 15.0–21 mm; Van Cleave & Altringer, 1937; Medcof, 1940; Chamberlain, 1958; Brown & Richardson, 1992) and could give birth early the following year. This has been reported for some other species of *Campeloma* (Van Cleave & Altringer, 1937; Medcof, 1940; Chamberlain, 1958; Brown & Richardson, 1992).

The size cohort centered around 16 mm (about 13–19 mm) may have been made up of individuals born in 2009, while those larger than 20 mm represent individuals born in 2008 and earlier. Life span ranges from two to five years for some congeners (Van Cleave & Altringer, 1937; Medcof, 1940; Chamberlain, 1958; Brown & Richardson, 1992). Few of the *C. decampi* in our population appear to live three years or longer. The rarity of large individuals suggests that few produce more than one or two broods in their lifetime; this life cycle is more similar to subtropical populations than those in north temperate areas (Van Cleave & Altringer, 1937; Medcof, 1940; Brown & Richardson, 1992). It is unknown whether the Round Island Creek *C. decampi* population is sexual or parthenogenetic. Additional research is needed to better understand the life cycle of *C. decampi* and how it may be influenced by environmental conditions (see Crummett et al., 2013).

The high density of *C. decampi* and the preponderance of small individuals indicate that the population at our study site is healthy and reproducing. Qualitative observations from other Round Island Creek sites suggest that similarly robust populations exist throughout the lower and middle reaches of the stream (Haggerty & Garner, 2008). Nevertheless, the restricted distribution of this species makes it highly vulnerable and warrants additional research on its life history and demography.

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# **SIGNIFICANT RECENT RECORDS OF UNIONID MUSSELS IN NORTHEAST TEXAS RIVERS**

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### **ABSTRACT**

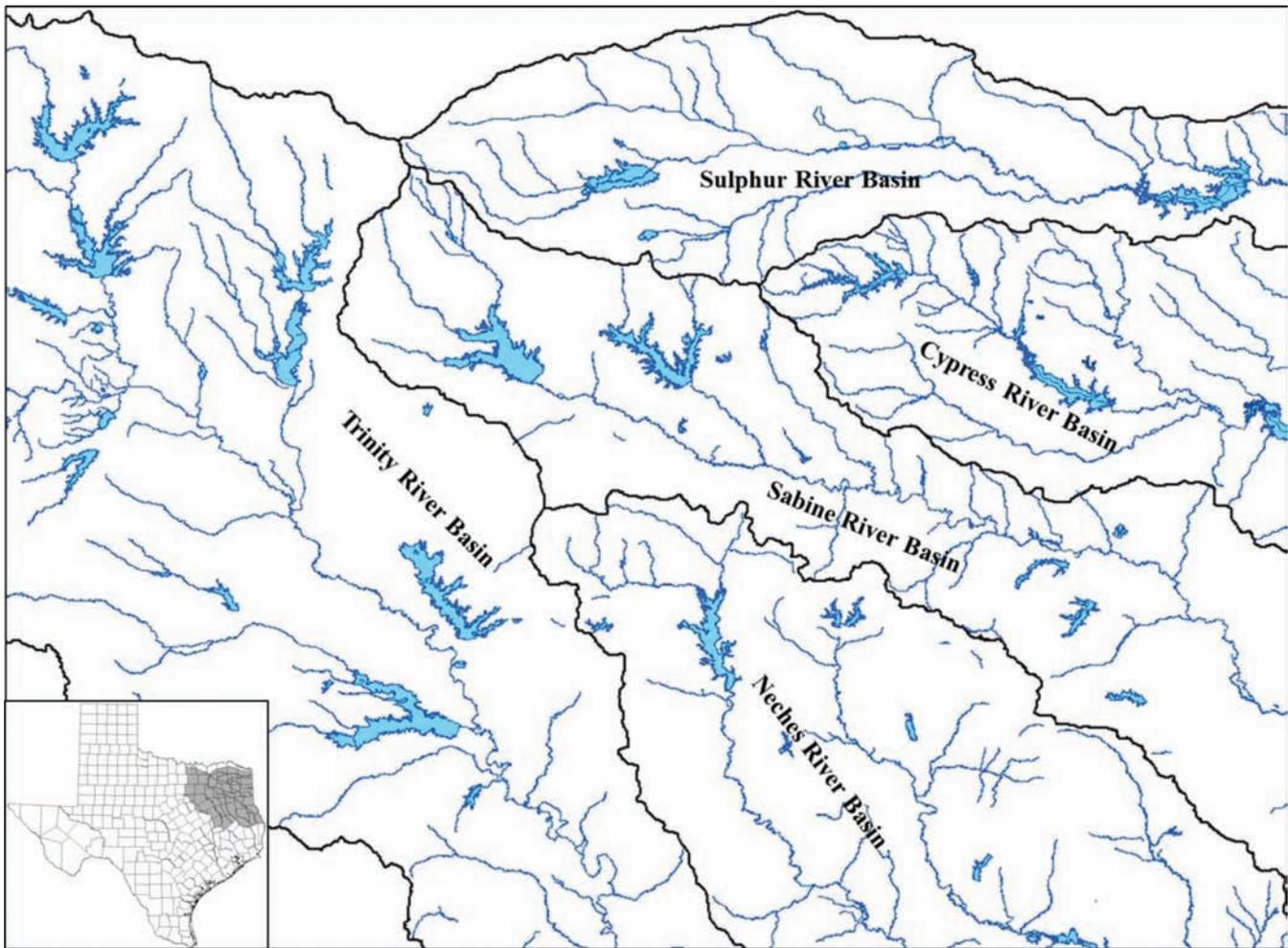
Five rivers in northeastern Texas, U.S.A. were surveyed for Unionid mussels from 2010 to 2012. We sampled 165 sites in the North and South Sulphur rivers, the Little Cypress Bayou, Black and Big Cypress creeks, the upper Sabine River, the Neches River, the Angelina River, the Attoyac Bayou, and the upper Trinity River. Each location was accessed by kayak and timed tactile surveys of 50 to 300 m of the river were conducted. We recorded a total of 20,134 mussels of 35 species, of which 16,714 were live. State listed species were found in all the rivers. The Neches River was the most speciose of all the large rivers of northeastern Texas and should be of prime conservation concern. The Sulphur River contained a few species that extended in from Oklahoma. The Trinity River, which runs through the Dallas/Fort Worth metroplex, surprisingly had two threatened species.

**KEY WORDS** Freshwater mussels, Unionidae, Northeastern Texas, Surveys, Conservation

### **INTRODUCTION**

Freshwater mussels of the family Unionidae have been impacted by anthropogenic factors for many decades and both the number of species and their abundances have declined throughout North America (Bogan, 2008; Downing et al., 2010). In the early 1990s, Texas Parks and Wildlife Department (TPWD) began systematic surveys in the state to determine the status of unionid mussels (Howells, 1997) and in 2009, 15 of the 51 species found in the state were listed as threatened: <http://www.tpwd.state.tx.us/newsmedia/releases/?req=20091105c> accessed June 5/2013.

This legal designation sparked interest in generating accurate current distribution records for those species. East Texas is a center for mussel diversity for the state because it contains both species exhibiting their westernmost distribution, other species that are either Texas endemics or only occur just eastward into western Louisiana or Arkansas (Neck, 1982; Howells et al., 1996; Burlakova et al., 2011) and some whose distribution extends south from Oklahoma. Northeast Texas has five drainage basins with rivers that start within the state and flow independently to either the Red River on the Louisiana border or to the Gulf of Mexico (Fig. 1).



**FIGURE 1**

Map of the Northeast Texas River basins containing the rivers where mussels were collected.

Because of these substantial water resources, northeast Texas has been a prime area for reservoir development and over 30 large dams have been constructed (Graf, 1999; [www.twdb.state.tx.us/waterplanning/swp/2012/](http://www.twdb.state.tx.us/waterplanning/swp/2012/) accessed June 5/2013). The rapid human population increases in the nearby Dallas/Fort Worth area have produced intense pressure to continue to build dams for their increased water needs. Northeast Texas also has a number of commercial interests that impact its water resources, including oil and gas drilling, intensive ranching, poultry operations and timber harvesting (Burlakova et al., 2011). These stressors led the writers of the 2005-2010 Texas Comprehensive Wildlife Conservation Strategy Plan (Bender et al., 2005) to identify “evaluating how instream flows and water quality impact rare and endangered species” as a high priority for northeast Texas.

Northeast Texas historically contained 42 species of unionid mussels (Park & Bachtal, 1940; Howells, 1997; Ford & Nicholson, 2006; Ford et al., 2009) and this represents the highest diversity in the state. Six of those species are listed as state threatened: Southern Hickorynut (*Obovaria jacksoniana*), Louisiana Pigtoe (*Pleurobema riddellii*), Texas Pigtoe (*Fusconaia askewi*), Triangle Pigtoe (*Fusconaia lananensis*), Sandbank Pocketbook (*Lampsilis satura*), and Texas Heelsplitter (*Potamilus amphichaenus*). Although *F. askewi* is considered to be a valid species, Burlakova et. (2012) considered *F. lananensis* to be a synonym of that species. Howells et al. (2012) disagreed and considered that *F. lananensis* should be recognized as distinct. In addition some north Texas *Fusconaia* have a similar morphology to *F. flava* (Howells, pers. comm.) such that all distributions of *Fusconaia* in Texas are of interest. Northeast

Texas also has records for other problematic species, such as Mapleleaf (*Quadrula quadrula*), Pimpleback (*Quadrula pustulosa*) and White Heelsplitter (*Lasmigona complanata*), which are at the southern or western extent of their distribution, and so are poorly known in the state (Table 1; Vidrine, 1993). Four other species,

**TABLE 1**

State threatened and rare unionid mussels recorded in the river basins during this study. Numbers of live and recent dead are shown (Live/Dead).

Species	Common name	Status in Texas	Trinity	Neches	Sabine	Cypress Creek	Sulphur
<i>Arcidens confragosus</i>	Rock Pocketbook	Species of concern	4/0	55/1	21/9	1/0	5/2
<i>Fusconaia askewi</i>	Texas Pigtoe	Threatened	15/16	886/80	282/214	2/3	2/10
<i>Fusconaia flava</i>	Wabash Pigtoe	No status	0/0	0/0	0/0	1/0	1/0
<i>Fusconaia lananensis</i>	Triangle Pigtoe	Threatened	0/0	148/16	0/0	0/0	0/0
<i>Lampsilis satura</i>	Sandbank Pocketbook	Threatened	0/1?	86/13	14/13	0/0	0/0
<i>Lasmigona complanata</i>	White Heelsplitter	Restricted distribution	0/0	0/0	0/0	0/0	1/0
<i>Obovaria jacksoniana</i>	Southern Hickorynut	Threatened;	0/0	24/2	0/0	0/0	0/0
<i>Pleurobema riddellii</i>	Louisiana Pigtoe	Threatened	0/0	437/29	1/1	18/4	0/0
<i>Potamilus amphichaenus</i>	Texas Heelsplitter	Threatened	0/0	6/5	7/16	0/0	0/0
<i>Quadrula pustulosa</i>	Pimpleback	Restricted distribution	0/0	0/0	0/0	0/0	3/0
<i>Quadrula quadrula</i>	Mapleleaf	Restricted distribution	0/0	0/0	0/0	1/0	24/9
<i>Strophitus undulatus</i>	Creeper	Species of concern	0/0	11/1	0/0	6/17	0/0
<i>Truncilla donaciformis</i>	Fawnsfoot	Species of concern	0/0	35/10	0/0	0/0	3/0
<i>Villosa lienosa</i>	Little Spectaclecase	Poorly known	0/0	10/5	0/0	0/0	0/0

Rock pocketbook (*Arcidens confragosus*), Creeper (*Strophitus undulatus*), Fawnsfoot (*Truncilla donaciformis*), and Little Spectaclecase (*Villosa lienosa*) are widely distributed in northeast Texas but rarely found (Howells et al., 1996) and are included in this report. The first three of these are listed as Species of Concern by TPWD. Internal TPWD surveys from 1992 to 1998 produced more recent distributional records for the mussels of east Texas (Howells et al., 1996; Howells, 1997) and surveys of reservoirs and bridge crossings on four rivers and stream segments produced new locality records for some of these species (Karatayev & Burlakova, 2007). However, long reaches between bridges on large rivers were not surveyed in these studies and so our understanding of the overall distribution of these mussels was limited. In 2010 we began mussel surveys of the larger rivers in all five river basins; this involved kayaking upstream and downstream of bridge access points in an attempt to clarify the distribution of unionids in northeast Texas. Here we report information on some of the least common of those species.

## METHODS

We surveyed 165 locations in the large rivers of five river drainages: the North and South Sulphur rivers in the Red River basin, the Little Cypress Bayou and the Black and Big Cypress Creeks in the Cypress River basin, the upper Sabine River in the Sabine River basin, the upper Neches River, the Angelina River and the Attoyac Bayou in the Neches River basin, and the upper Trinity in the Trinity River basin. The Trinity River was sampled using divers from Zara Environmental LLC and Half Associates Inc. In general, each river was surveyed from the upstream areas east of Dallas to downstream areas in northeast and eastern Texas. Surveys on the Sabine and Neches began in the summer of 2010 and the surveys on other rivers were started in 2011.

We traveled to each designated site by kayak and did initial reconnaissance of areas along the shore for shells. In appropriate locations we sampled using a timed hand search, or with SCUBA gear in deeper areas (Vaughn et al., 1997). Surveys were standardized on a per person-hour of searching (Strayer & Smith, 2003). Depending on the goals of the particular survey, multiple samples varying from 50 to 300 m of the river at that site were made.

All live unionids were collected, identified, counted and then returned to the river. Habitat data collected varied in association with goals of each particular survey but only general descriptions of the sites are used in this report. Vouchers were retained in the University of Texas at Tyler collection.

## RESULTS

From the five River basins, we recorded a total of 20,134 mussels of 35 species, of which 16,714 were live. In the Angelina River (including the Attoyac Bayou) we found 1,853 live mussels and 243 recent dead of 22 species. Of the 28 species recorded in the Neches River, 10,122 were live and 972 were recent dead. In the three branches of the Cypress River drainage we recorded 460 live and 292 dead of 21 species. From the Sulphur River basin we collected 22 species with 940 live and 95 dead. A total of 1,124 live and 679 dead of 16 species were recorded in the Trinity River in the

Dallas Fort Worth area. Fourteen threatened, rare or poorly known species were found in some or all the rivers during the survey (Table 1).

### State Threatened species

Five species listed as threatened by TPWD were found in this study (Table 1). Undetermined *Fusconaia* species were fairly common in many sites. The form matching the shell morphology of *F. askewi* was abundant in the Sabine, Neches and Angelina rivers, whereas *F. lananensis* morphs were identified in the Angelina River and Attoyac Bayou. *Fusconaia lananensis* was occasionally common with 56 individuals recorded at a single site in the Angelina River. *Fusconaia* species were rare in the Black and Little Cypress and also the Trinity and Sulphur rivers. In these water bodies very few live specimens were found but the morphology of the dead ones were usually similar to what is typically called the Wabash Pigtoe (*F. flava*) although some were comparable to *F. askewi*. Genetic analysis (mtDNA of the ND1



FIGURE 2

Wabash Pigtoe (*Fusconaia flava*) from the Sulphur River.

gene) of one individual from the Sulphur River (Fig. 2) that morphologically resembled *F. askewi* genetically matched *F. flava*, whereas one from Black Cypress Bayou (Fig. 3) that resembled *F. flava* genetically matched *F. askewi*, which suggested that both species may occur in these rivers. *Lampsilis satura* was uncommon in all

rivers with a maximum of six at a site, but a total of 100 live and 26 recently dead specimens were found in the Sabine, Neches and Angelina rivers. Recruitment was also evident as juveniles of this species were collected. One weathered dead specimen from the Trinity River was tentatively identified as *L. satura*. *Obovaria jack-*



*Fusconaia flava*  
Black Cypress Bayou, TX

### FIGURE 3

Wabash Pigtoe (*Fusconaia flava*) from the Black Cypress Creek.

*soniana* was very rare and only found in sites close to Texas Highway 84 on the Neches River. This area of the river is connected to the floodplain, which appears to be important for the species (Troia, 2010; Troia & Ford, 2010). *Pleurobema riddellii* was common in the Neches and Angelina rivers where most of the total 455 live and 33 dead were found. A few individuals of this species were found in the Big and Little Cypress rivers and one live and one dead were found in two sites on the Sabine River. Prior to this study no live *P. riddellii* had been recorded in the Sabine River in over 35 years. Recently live individuals have also been recorded in the upper Trinity River (J. Krejca, Zara Environmental LLC, pers. comm.). Additional surveys may reveal more localities for this species. *Potamilus amphichaenus* was one of the rarest overall species with only 13 live and 21 dead recorded in the Sabine and Neches rivers and only one or two individuals at a site. *Potamilus amphichaenus* was also one of the few mussels in which more dead individuals were found than live ones, which may indicate higher predation rates (Walters & Ford, in press).

### Species of Concern

*Arcidens confragosus* is widely distributed in east Texas but is typically uncommon at each site (Howells et al., 2000). In this survey we found 76 live and 10 recently dead specimens in the Sabine, Neches, Angelina, Little Cypress and Sulphur rivers (Table 1). They generally were dispersed and no more than nine individuals were found at a single site. One *Lasmigona complanata* was found in the Sulphur River just downstream of the Wright-Patman Reservoir (Heffentrager & Ford, 2012). *Strophitus undulatus* was rare with 17 live and 18 dead specimens recorded from all three branches of the Cypress River and in the Neches River and Attoyac Bayou. *Truncilla donaciformis* was common in the Neches River at several sites just above the B. A. Steinhagen Reservoir with 38 live and 10 dead in total. Three were found live in two sites on the Sulphur River.

### Rare or poorly known species

*Quadrula pustulosa* was confirmed (genetic analysis on two individuals) in the Sulphur River with three live

individuals collected (Fig. 4). *Quadrula quadrula* was also found at most sites on the Sulphur River (Fig. 5) and one was found in Big Cypress Bayou. *Villosa*

*lienosa* were found in several sites in the Neches River, one site on the Angelina River, often with two or three at a site although only 10 total live individuals were collected.



**FIGURE 4**  
Pimpleback (*Quadrula pustulosa*) from the Sulphur River.

## DISCUSSION

Our records suggest that many of the conclusions of Howell's 1997 report for east Texas mussels are still accurate. The Sulphur River basin has a few species that extend into Texas from Oklahoma (i.e., *L. complanata*, *Q. quadrula* and *Q. pustulosa*). The Cypress Creek Basin rivers have few rare species with the exception of *S. undulatus* and *P. riddellii*. *F. askewi* are still abundant in many sites in the upper Sabine River with evidence of abundant recruitment. That river also has the highest quantity of *P. amphichaenus* although individuals were scattered and most records were for recently dead individuals. *Potamilus amphichaenus* has a thin shell that

makes them vulnerable to terrestrial predators (Walters & Ford, in press). The occurrence of living *P. riddellii* in the Sabine River is important as it extends northward its known recent distribution. Even without its tributaries, the Angelina River and Attoyac Bayou, included the Neches River has the most speciose mussel fauna of the large rivers of northeast Texas. Besides an abundance of *Fusconaia* and *Pleurobema* species, *L. satura*, *T. donaciformis*, *O. jacksoniana*, *V. lienosa*, *P. amphichaenus*, *A. confragosus* and *S. undulatus*, are all found in the Neches River (Table 1). This river should be of prime conservation concern for mussels in the state.

**FIGURE 5**

Mapleleaf (*Quadrula quadrula*) from the Sulphur River.

Although this survey only included a very small portion of the Trinity River, results from there were significant in recording both *Fusconaia* species and *P. riddelli* in the upper reaches of the Elm Fork in Fort Worth. It had been expected that mussels were likely extirpated from the Trinity River in that highly populated area (Strecker, 1931) but other recent surveys in that metroplex found significant mussel populations (Krejca, pers. comm.). This may relate to improvements in water quality or just a paucity of data for that river. Additional surveys in that river are especially recommended.

Although most unionid mussel species are undoubtedly uncommon in northeast Texas it is important to note that these surveys still located live specimens of rare species and occasional significant mussel beds. This study suggests several avenues for additional work in northeast Texas on this fauna. First, continued research and additional protection is needed for all populations of riverine mussels but particularly the Neches River and areas in the middle reaches of the upper Sabine River where mussels were both diverse and abundant. We now have enough distributional data in these two rivers to begin to conduct population studies on some of the im-

portant species there, potentially protecting some of the largest populations of these threatened species. We also have sufficient information to indicate that some additional species, such as *O. jacksoniana* and *S. undulatus*, should be listed in the state. Second, improvement in current anthropogenic impacts is needed in all rivers but particularly in the Sulphur River, Cypress Creek basin and upper Trinity River. These rivers were more obviously modified by dams, agriculture and industries but have some important species, although in very low numbers. Additional distributional data are needed in these three river basins to help make appropriate decisions concerning protection of the rare species found in these drainages.

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# A COMPARISON OF TWO TIMED SEARCH METHODS FOR COLLECTING FRESHWATER MUSSELS IN GREAT LAKES COASTAL WETLANDS

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## ABSTRACT

Given the catastrophic losses of freshwater mussel diversity across the Laurentian Great Lakes, the identification and protection of remnant assemblages are priority conservation actions. In contrast to riverine mussels, there has been little evaluation of different sampling gear and strategies to support the design of coastal wetland inventory or monitoring programs. We compared timed-search (qualitative) collections from 21 Lake Ontario coastal wetlands using clam rake and visual/tactile methods. Live mussels were collected with visual/tactile searches from 90% of wetlands sampled, and from 71% with the clam-rake. A total of 756 live mussels (representing nine species) were collected. Collections included three mussel species at risk: *Ligumia nasuta*, *Quadrula quadrula*, and *Toxolasma parvum*. Compared to clam-raking, visual/tactile searches collected more than twice as many live individuals and fresh shells, a broader range of sizes and significantly more species (and at a faster rate). Estimates of live mussel abundance and species number associated with each method were imprecise (CV > 0.35). The concordance of variation in mussel assemblage structure among wetlands (as described by each method) was not consistent or in strong agreement. Based on our findings, we recommend visual/tactile searches for future coastal wetland sampling efforts.

**KEY WORDS** Unionid, Dreissenids, Clam rake, Visual/tactile, Wetlands, Monitoring

## INTRODUCTION

A third of freshwater mussel species in the province of Ontario (Canada) have been assessed as either federally threatened or endangered (COSEWIC 2012). Initial declines in native unionid populations have been related to the degradation of riverine habitats (Metcalf-Smith et al., 1998). More recent and rapid declines followed the invasion of North America by dreissenid mussels: Zebra Mussel (*Dreissena polymorpha*) and Quagga Mussel (*D. bugensis*) (Schloesser & Nalepa, 1994). By the early 1990s, native mussels were nearly extirpated from the offshore waters of Lakes Erie and St. Clair (McGoldrick et al., 2009). However, remnant mussel assemblages have persisted in near-shore and coastal wetland areas of Lakes Erie, Huron and St. Clair (Nichols & Amberg 1997; Zanatta et al., 2002; Bowers & Szalay, 2003; Crail et al., 2011;

Sherman et al., 2013). Compared to adjacent open water habitats, wetlands are less suitable for dreissenid colonization and survival (Sherman et al., 2013), thereby providing a refuge for native mussels. Given that dreissenid mussel removal may not be practical and brood-stock is required for reintroductions, recovery depends on identifying and protecting remnant native mussel assemblages.

Actions undertaken to protect and recover Ontario's mussels at risk include the identification of protected habitats, and ongoing assessment of species status. To meet these commitments, the following information is required: (1) the locations of individuals and populations, (2) descriptions of the biophysical attributes of habitat for different life-stages, (3) the state of populations (i.e. density, size and age structure, sex-ratio), and (4) the presence of invasive species (Cudmore et al.,

2006; DFO, 2011a). Outside of the Lake St. Clair delta, these activities have focused on populations in south-western Ontario rivers. Riverine mussel assemblages are sampled with standardized time-search (Metcalf et al., 2000) and quadrat methods (Metcalf-Smith et al., 2007). In contrast to riverine mussels, little research has been undertaken on the design of wetland inventory or monitoring programs. The evaluation of alternative gear and sampling strategies is required for species found in inland lakes and coastal wetlands (e.g. *Ligumia nasuta*).

In this study, we compared timed-search (qualitative) collections from Lake Ontario coastal wetlands using clam rake and visual/tactile methods. Both methods have been used in previous wetland mussel surveys (Bowers & Szalay, 2003; Sherman et al., 2013). Other sampling approaches have included snorkeling surveys and opportunistic surveys of temporarily dewatered habitats (Nichols & Amberg, 1999; McGoldrick et al., 2009; Crail et al., 2011). The two methods were chosen based on the logistics of sampling coastal wetlands (soft sediments, poor water clarity and dense aquatic vegetation), and because they are not time-intensive (Bowers & Szalay, 2003). Also, it was expected that the clam-rake would be able to sample habitats too deep for visual/tactile methods. Comparisons were based on the: (1) number of species detected, (2) number and sizes of individuals collected, and (3) precision of mussel species and abundance estimates. We also assessed the concordance of mussel assemblage patterns described using clam-rake and visual/tactile data.

## METHODS

### Field Sampling

During the summers of 2011 and 2012, 21 coastal wetlands along the Canadian (Ontario) shoreline of Lake Ontario were sampled. Sites were located between the cities of St. Catharines (43°11'14" N; 79°16'52" W) and Kingston (44°14'17" N; 76°32'55" W). Wetlands represented a mix of types (barrier beach, flooded river mouth and large embayments) and were 13 to 2093 (median: 86) hectares in size (Environment Canada and Ontario Ministry of Natural Resources, 2003). Aquatic macrophyte coverage ranged from absent to extensive, and water clarity (as measured with a transparency tube, Anderson & Davic, 2004) was poor (< 0.2 m) to excellent (> 1.2 m). Water depths sampled ranged 0.05 to 1.5 m.

Mussel collection methods were compared using a paired-sample approach. At each wetland, 12 sampling points were randomly selected. No *a priori* information on sediment characteristics, water depth or spatial variation in mussel densities was available to stratify each wetland before points were selected. At each sampling point, one hour of search effort with each method was

completed concurrently. Sampling was limited to within 50 m of the start point, and areas sampled by either method did not overlap. Assignment of a method to an area was ad hoc, but not based on any criteria. Visual/tactile searching involved either floating on air mattresses and hand searching the sediment for mussels (on the surface and probing through sediment for burrowed mussels), or searching for mussels with an underwater viewer (Plastimo® Round Underwater Viewer, 0.33 m diameter) or polarized lenses. In wetlands with clear water, mussels could be visually detected by spotting siphons or small clusters of dreissenids. It is estimated that tactile searches of soft sediments sampled up to a depth of 0.1 m. For the clam-rake method, an Eagle Claw® Clam Rake (0.84 m long handle, with a 0.26 x 0.15 m metal basket and ten 0.15 m long steel teeth) was dragged through the sediment and wetland vegetation. Spacing of wire mesh within the basket was 2.5 cm x 5 cm.

Live individuals and fresh shells were identified to species (Metcalf-Smith et al., 2005). Shell length (mm) of live individuals was measured with a dial caliper ( $\pm 0.1$  mm). Live mussels and the total mass of attached dreissenids were weighed separately ( $\pm 0.1$  g). After processing and removal of dreissenids, live mussels were returned to the sediment close to their area of collection.

### Data Analysis

Differences between the two methods were tested using the following data: (1) number of species detected, (2) number of individuals collected, (3) number of sampling points containing mussels, (4) precision of parameter estimates (mussel abundance and species number), and (5) shell length (minimum, mean and maximum). For datasets 1-3, separate comparisons were done for live individuals and fresh shells. Precision (calculated for each wetland) was based on the coefficient of variation ( $CV = \text{Standard Error}/\text{Mean}$ ) (Thompson, 2002). Except for precision and shell length, significant differences between the sampling methods were tested with the paired t-test. Due to differences in mussel data, an unequal number of CVs was calculated for each sampling method. Therefore, tests for significant differences were undertaken with the unpaired t-test. Differences in shell length were tested using the Sign-test (Zar, 1984). Species detection rates for each method were compared by calculating the mean (across wetlands): (1) time till the first species was detected at a wetland, and (2) cumulative number of species detected after each hour of searching.

Two approaches were applied to assess the concordance of variation in mussel assemblages among wetlands, as described using clam rake and tactile/visu-

al data on live individuals. To assess whether estimates of live mussel abundance and species richness across wetlands agreed, the Spearman Rank Correlation was calculated. Secondly, distance matrices were constructed from site-by-species matrices of species presence/absence (Jaccard) and log-transformed species abundance (Bray-Curtis) data (Legendre & Legendre, 1998). The relationship between matrices constructed from clam rake and tactile/visual data was evaluated using the Mantel test. Significance was assessed with a Monte

Carlo randomisation method, using 9999 permutations (Manly, 2007). Statistical analyses were completed using PAST version 1.94 (Hammer et al., 2001).

## RESULTS

A total of 756 live mussels (representing nine species) were collected from Lake Ontario coastal wetlands (Table 1). Between one and five species were detected as live individuals from each wetland. At least one live

**TABLE 1**

Comparison of relative abundance (% of total collection) and frequency of occurrence (% of wetlands sampled) of wetland mussel species collected using clam-rake and visual/tactile methods. Absolute numbers are provided in parentheses. Summary statistics are based on live individuals.

Species	Visual/Tactile		Clam Rake	
	Relative Abundance	Frequency of Occurrence	Relative Abundance	Frequency of Occurrence
<i>Elliptio complanata</i>	11.6 (65)	14.3 (3)	19.7 (38)	14.3 (3)
<i>Lampsilis cardium</i>	2.3 (13)	9.5 (2)	14.0 (27)	4.8 (1)
<i>Lampsilis siliquoidea</i>	0.5 (3)	9.5 (2)	5.2 (10)	14.3 (3)
<i>Leptodea fragilis</i>	0.7 (4)	4.8 (1)	2.0 (4)	4.8 (1)
<i>Ligumia nasuta</i>	3.2 (18)	23.8 (5)	5.2 (10)	14.3 (3)
<i>Pyganodon grandis</i>	65.2 (367)	81.0 (17)	40.4 (78)	47.6 (10)
<i>Quadrula quadrula</i>	13.0 (73)	4.8 (1)	11.4 (22)	4.8 (1)
<i>Toxolasma parvum</i>	1.2 (7)	4.8 (1)	1.0 (2)	4.8 (1)
<i>Utterbackia imbecillis</i>	2.3 (13)	19.1 (4)	1.0 (2)	9.5 (2)

mussel was collected from most wetlands (except Big Island Marsh, Bay of Quinte). *Pyganodon grandis* was the most widespread (collected from all wetlands where live mussels were found) and abundant species (65% of visual/tactile and 40% of clam rake collections). Other species were encountered at five or fewer wetlands, and typically represented <10% of the total collection. Three at-risk mussel species (COSEWIC 2012) were collected: *L. nasuta*, *Quadrula quadrula*, and *Toxolasma parvum*, of which *L. nasuta* was the most widespread. For both methods, live individuals were more often collected than fresh shells (Figure 1). In total, 870 shells (either halves or whole) were collected. Shells of all species except *T. parvum* were found. At half of the wetlands, some species (range: one to three species) were detected only as fresh shells (Table 1). At four wetlands, the presence of shells was the only indicator of the occurrence of *Elliptio complanata*, *Leptodea fragilis* and *Utterbackia imbecillis*. Shells were also the only evidence of *L. nasuta* within Presqu'île Bay.

### Abundance and Number of Mussel Species

Live mussels were collected with visual/tactile searches from 90% (19 of 21) of wetlands sampled, and from 71% (15 of 21) with the clam-rake. Visual/tactile searches collected three times as many live mussels ( $t = 2.35$ ;  $p = 0.02$ ) and twice as many fresh shells ( $t = 2.81$ ;  $p = 0.01$ ) as clam-raking (Figure 1). They also produced significantly more (>35%) sampling points at each wetland with live individuals ( $t = 4.1$ ;  $p < 0.001$ ), and more species from live individuals than clam-raking ( $t = 2.95$ ;  $p = 0.008$ ). Alternately, there was no difference between methods in the number of sampling points with shells at each wetland or species detected with shells ( $t$ -test;  $p > 0.10$ ). Clam-raking only detected species not present in visual/tactile collections at five wetlands. There were no significant differences between search methods in the precision of live mussel abundance and species number estimates ( $t$ -test,  $p > 0.25$ ). Overall, estimates were typically imprecise ( $CV > 0.35$ ).

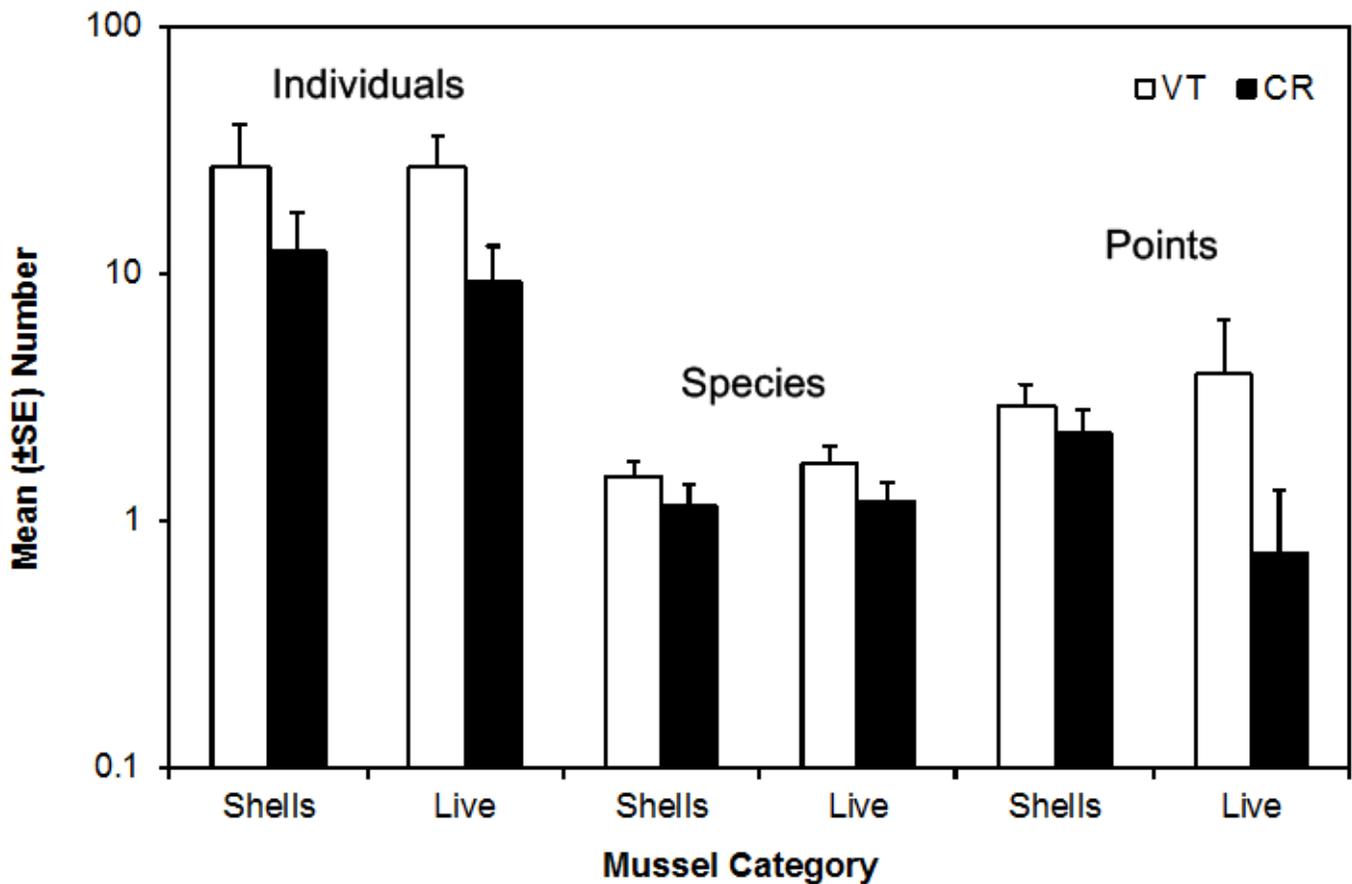


FIGURE 1

Comparison of mussel catch rates in Lake Ontario coastal wetlands using visual/tactile (VT) and clam-rake (CR) sampling methods. "Points" refers to the number of sampling points within a wetland where a fresh shell or live individual was collected.

Over the sampling period, detection of new species within wetlands was more rapid with visual/tactile searches than clam-raking (Figure 2). Although mean ( $\pm$ SE) time spent searching until the first mussel was collected at a wetland was slightly longer (VT: 3.5 hr  $\pm$  0.9. CR: 2.7 hr  $\pm$  0.7), there was little improvement in the detection of new species after 10 hours of visual/tactile searching or six hours of clam-raking. When compared to combined species lists (both methods) for each wetland, the mean percentage of mussel species detected using the visual/tactile method was greater than 80%. By contrast, clam-raking detected (on average) 23% fewer species known from each wetland (mean = 57.5%).

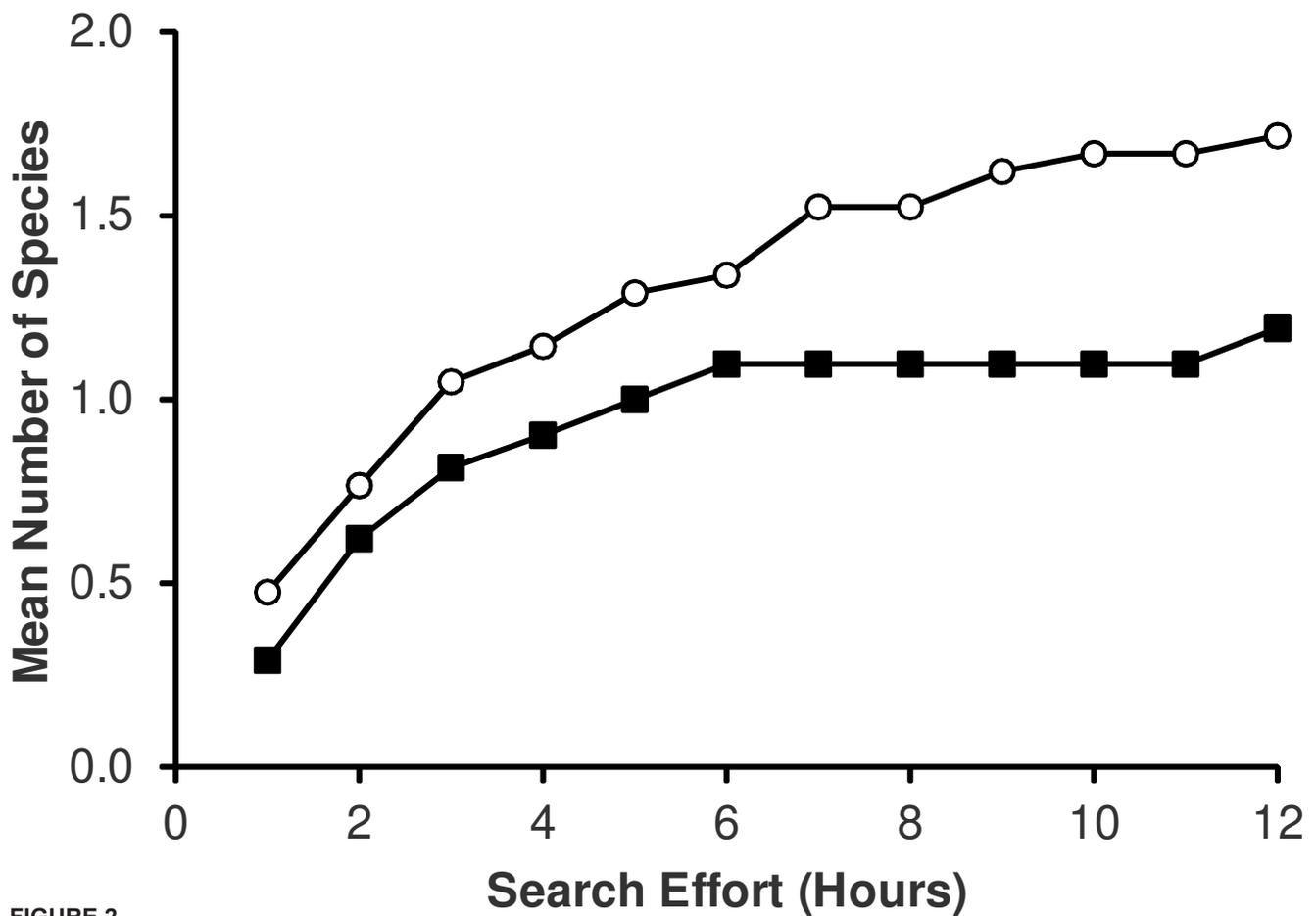
#### Shell Length

A greater range of shell lengths was associated with visual/tactile collections of live individuals than clam-rake samples (VT: standard deviation (SD) = 26.6, CR: SD = 24.9) (Figure 3). Compared to clam-rake, mean lengths of visual-tactile collections from each wetland were significantly greater (Sign Test:  $p < 0.05$ ). Dif-

ferences in mean length ranged from 1.0 and 39.6 mm (mean = 13.5). There were no significant differences between the lengths of the smallest or the largest individuals collected from each wetland (Sign Test:  $p > 0.18$ ).

#### Variation Among Coastal Wetland Mussel Assemblages

The number of live individuals ( $r_s = 0.81$ ,  $p < 0.001$ ) and species ( $r_s = 0.78$ ,  $p < 0.001$ ) collected by each method was strongly correlated across wetlands. However, distance matrices constructed from species presence-absence data (Jaccard) were not correlated ( $r = -0.04$ ,  $p = 0.60$ ). At 10 of the 21 wetlands, there was no overlap in the species composition of visual-tactile and clam-rake collections. Most of these cases reflect the failure of a sampling method to collect any mussels. Using species abundance data (Bray-Curtis), there was a weak correlation ( $r = -0.23$ ,  $p = 0.007$ ) between distance matrices associated with each method. The relative abundances of individual species were equal at only 10% (2 of 21) of wetlands sampled.



**FIGURE 2**

Comparison of increase in mussel species richness with increase in effort, during visual/tactile (○) and clam-rake (■) surveys of Lake Ontario coastal wetlands. Mean species richness represents the average calculated across all wetlands sampled.

## DISCUSSION

Sampling gear and strategy evaluations for North American freshwater mussels have largely focused on riverine habitats. Given the catastrophic losses of freshwater mussel diversity across the Great Lakes, the inventory and population monitoring of remnant populations in coastal wetlands are priority recovery actions. Our study shows that visual/tactile surveys are more efficient at collecting mussels and detecting species than clam-raking. This result is consistent with Sherman et al. (2013) who reported that visual searches of a Lake St. Clair site collected four times more mussels than clam-raking. We also found a broader range of shell lengths to be associated with visual/tactile collections. It is not known whether this result reflects differences in the likelihood of capture between methods, or that the probability of detecting the smallest and largest sizes increases as one collects more mussels.

Compared to visual/tactile sampling, the clam-rake permits sampling at deeper water depths and further into soft sediments. However, it was less effective at collecting mussels and often labour-intensive. When sampling heavily vegetated habitats or soft sediments, the basket required continuous cleaning to remove plant and/or organic material and careful searching to find mussels. Unionids in Lake Erie wetlands have been observed to burrow 2-40 cm into the substrate for at least part of the day (Nichols & Wilcox, 1997). In rivers, the excavation of bed material during quadrat sampling improves the likelihood of collecting juveniles and small-bodied species (Obermeyer, 1998). In our study, there was no evidence that dragging the clam-rake through sediments improved the detection of small individuals. This may reflect the loss of small individuals through the basket mesh, or that tactile sampling was effective at detecting burrowed individuals by probing the sediments. Certain wetland characteristics (deep water or dense aquatic

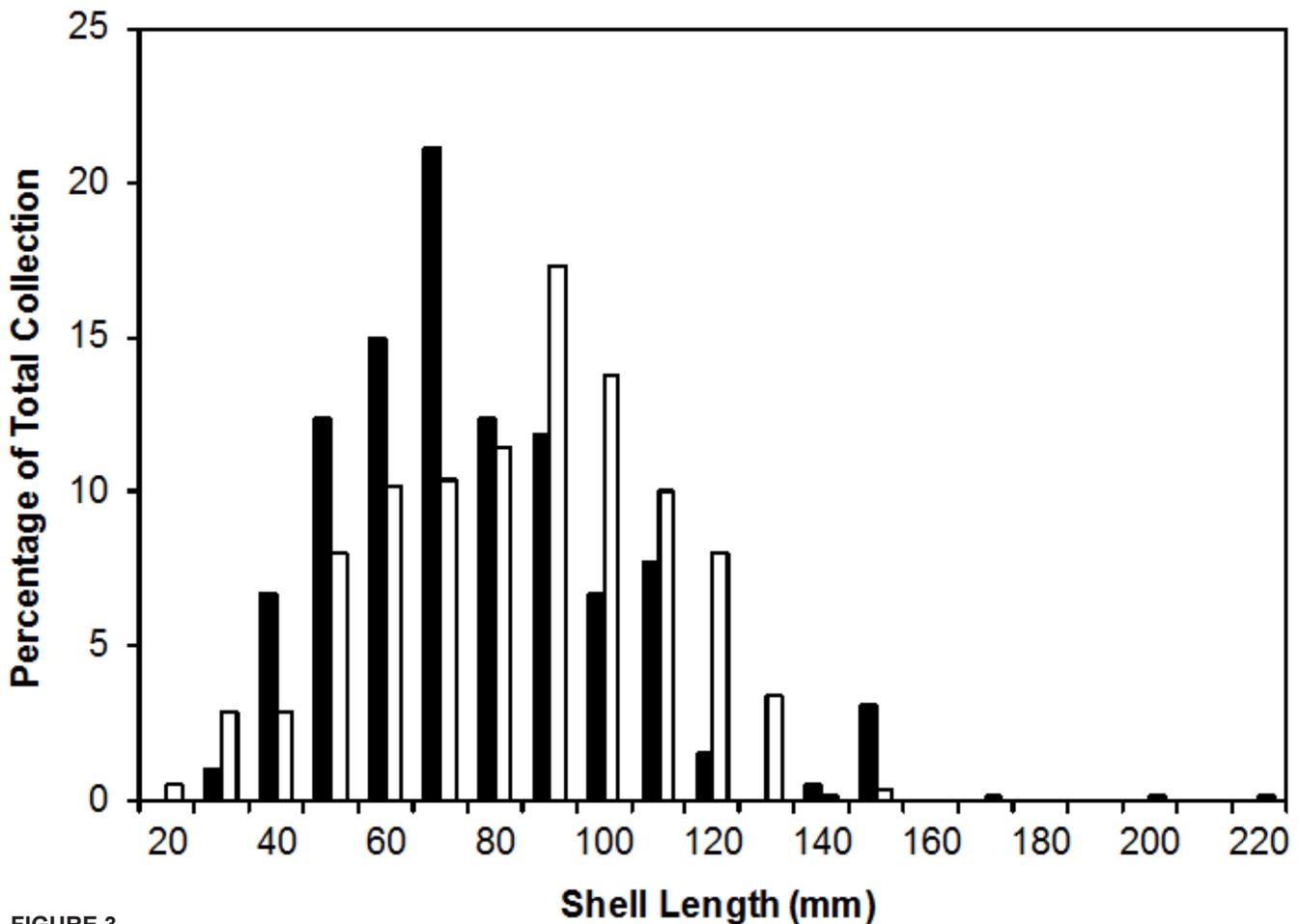


FIGURE 3

Length-frequency distributions of mussels collected from Lake Ontario coastal wetlands using visual/tactile (white) and clam-rake sampling methods (black).

vegetation) may prevent visual/tactile searches. In these cases, the completeness of species lists may be improved by increasing the time spent clam-raking and/or shoreline searches for fresh shells.

While the qualitative sampling approach applied in this study was appropriate for gear comparison and species inventory, quantitative sampling strategies are recommended for population monitoring (Strayer & Smith, 2003). Both methods evaluated in this study provided imprecise parameter estimates and were unsuitable for long-term monitoring. Future research could test whether stratified sampling designs, and/or large increases in search effort would improve precision. Alternatively, the ability of quantitative approaches developed for riverine mussel populations (e.g. systematic quadrat sampling with random starts) to provide abundance estimates could be evaluated for these low-density populations. If it is not necessary to track the number of individuals (or it is deemed impractical), repeat survey designs

could be implemented across lower Great Lakes coastal wetlands to monitor species distributions instead (MacKenzie et al., 2012).

The overall objective of our study was to inform the design of native freshwater mussel collections in Great Lakes coastal wetlands. However, recovery plans for mussels at risk also identify the need to monitor dreissenid distribution and abundance. This information is used to interpret threat risks for individual populations (DFO 2011b). Live dreissenids were collected from nine of the wetlands we sampled. At these sites, dreissenid shells were present at 30% of sampling points. Across a variety of European and North American waterbodies, zebra mussel infestation rates (number or mass attached to native unionids) are correlated with zebra mussel densities (Lucy et al., 2013). We found the mass ratio of attached dreissenids to live unionids ranged from 0.0006 to 2.0 (mean = 0.15). Counts or weights of zebra mussels (and presence of byssal threads) on live mussels may

therefore provide a surrogate abundance index for monitoring and risk assessments.

Great Lakes coastal wetlands are important habitat for amphibians and reptiles, birds, fishes and mammals (Sierzen et al., 2012). Over the past 15 years, an increasing number of studies have demonstrated that, as refuge habitats, coastal wetlands are also important for unionid conservation throughout the Great Lakes basin. We found Lake Ontario wetland mussel assemblages to be less diverse than Lake Erie and Lake St. Clair wetland assemblages (Bowers & Szalay, 2003; Zanatta et al., 2002; Crail et al., 2011) but more diverse than those recently sampled in Lake Huron and Lake Michigan (Sherman et al., 2013). *Ligumia nasuta* (formerly considered one of the most common species of the lower Great Lakes) was believed extirpated from the Canadian waters of Lake Ontario (COSEWIC 2007). We detected small remnant populations of this endangered species at five wetlands. Additionally, undocumented populations of *Q. quadrula* (Threatened) and *T. parvum* (Endangered) were identified at another (Jordan Harbour). These findings highlight the need for additional inventories of coastal wetlands in the lower Great Lakes and upper St. Lawrence River to properly delineate critical habitats and identify provincially significant wetlands (OMNR 2013). We recommend that these surveys be implemented using visual/tactile methods.

## ACKNOWLEDGEMENTS

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# USE OF OCCUPANCY MODELING TO ASSESS THE STATUS AND HABITAT RELATIONSHIPS OF FRESHWATER MUSSELS IN THE LOWER FLINT RIVER, GEORGIA, USA

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## ABSTRACT

The Flint River in southwestern Georgia is known for its historically diverse mussel fauna, but the current status of the fauna is poorly known. The rediscovery of two presumed extirpated and extinct species in 2006 and 2008 exemplifies the need for a large-scale survey of the river. We used an occupancy modeling approach to estimate the presence of mussel species at 39 locations along a 119 km reach of the lower Flint River between Lake Seminole and Albany Dam. Twenty species were collected and evidence of recent reproduction was documented for 8 species. *Elliptio crassidens*, *E. fumata/pullata*, and *E. nigella* were the most abundant species and accounted for 43%, 40%, and 8% of the total mussels collected, respectively. Among species, mean detection probabilities averaged 0.25 and ranged from 0.01 to 0.69, whereas occupancy averaged 0.56 and ranged from 0.03 to 1. We fitted models relating site-level and sample-level habitat characteristics and site location to detection and occupancy for nine species. Detection probabilities varied among species, substrate, searcher experience, and distance from Albany Dam. Estimated occupancy varied by species and substrate composition indicating different substrate use by different species. Our modeling approach indicated that our sampling design was efficient for detecting most species with the exception of rare species. The Lower Flint River continues to harbor a widely distributed and diverse assemblage of freshwater mussels. The occupancy modeling approach used in our study was a useful and efficient method to assess the status, distribution, and habitat use of freshwater mussels in the Flint River while also providing a measure of sampling efficiency. Similar model-based study designs may be effective in other streams, particularly when sampling resources are limited.

**KEY WORDS** Occupancy, Detection, Flint River, Freshwater Mussels, *Elliptoideus sloatianus*, *Elliptio nigella*

## INTRODUCTION

The Apalachicola-Chattahoochee-Flint (ACF) River basin originates in the Blue Ridge and Piedmont physiographic provinces of Georgia and flows south into Florida before emptying into Apalachicola Bay in the eastern Gulf of Mexico. The basin is known for its unique and imperiled mussel fauna (family Unionidae), which historically included about 33 species (Brim Box & Williams, 2000). Mussel populations have declined or been extirpated from much of the basin due to impoundment,

pollution, and sedimentation, and 15 species in the basin are now extirpated or imperiled. The Flint River basin supports some of the most important remnants of the ACF fauna including at least 27 species (Brim Box & Williams, 2000). However, most recent sampling efforts in the Flint River basin have focused on tributaries (e.g., Brim Box & Williams, 2000; Golladay et al., 2004; Gagnon et al., 2006; Shea et al., 2013), and the mainstem remains poorly sampled. Furthermore, most previous surveys focused on documenting species presence/

absence and distribution, which provides limited data for assessing temporal changes in populations. The recent rediscovery of the presumed extirpated *Amblema neislerii* and the presumed extinct *Elliptio nigella* in the Flint River suggests that the mainstem is an important conservation refuge in need of intensive survey and monitoring. Because of the large size of the river, sampling methods and analyses are needed that can maximize efficiency while also providing useful inferences about the status of the fauna.

Occupancy estimation is a model-based approach to estimate the probability of species presence in an area while accounting for the imperfect detection probabilities that are inherent in most sampling methods (MacKenzie et al., 2002). Detection probability, which may vary across species, time, and space (McKelvey & Pearson, 2001; MacKenzie et al., 2002), is the probability of detecting a species at a site and is conditional upon the species being present and collected when present. Presence/absence (hereafter referred to as detection/nondetection) data are used to jointly model species presence and detection in a hierarchical logistic regression model. Occupancy models are based on Capture-Mark-Recapture models and use replicate samples collected at a site to construct a binary capture history based on the detection (1) or non-detection (0) of target species. Replicate samples may be collected temporally through repeated visits to a site or spatially by taking replicate samples on a single occasion. The capture history is used to estimate the probability of detecting a target species in a single replicate sample when the species is present and available for capture. Occupancy is defined as the probability that a species is present at a site, but imperfect species detection can cause occupancy to be underestimated (MacKenzie et al., 2006; Wisniewski et al., 2013a). Occupancy models use detection probabilities to correct naïve occupancy (the proportion of sites observed occupied), which reduces bias due to imperfect sampling. Occupancy models can be scaled to large areas such as watersheds or species' ranges, and the influence of site-level or sample-level factors on detection and occupancy can be estimated, which provides insight into the factors influencing species distribution and abundance. Because freshwater mussels are often difficult to sample due to their burrowing habits and variable sampling conditions, occupancy modeling may provide more accurate depictions of species' status and a better understanding of the factors that affect them (McKelvey & Pearson, 2001; Tyre et al., 2003; Wisniewski et al., 2013a).

We used occupancy models to examine the status and distribution of freshwater mussels in the lower Flint River. First, we conducted detection/nondetection surveys throughout the study reach, and we modeled

average detection and occupancy for all species found during our surveys. Second, we incorporated several site- and sample-specific habitat covariates in our models to examine the effects of these factors on occupancy and detection and how they varied among species. Specifically, we examined relationships between substrate composition, flow, and depth and mussel occurrence and detection. We also assessed how distance from a large hydropower dam was related to mussel occurrence because mussel species richness and abundance may increase with increasing distance from dams (Vaughn & Taylor, 1999). Lastly, we used estimated detection probabilities to assess the efficiency of our sampling design for detecting species

## METHODS

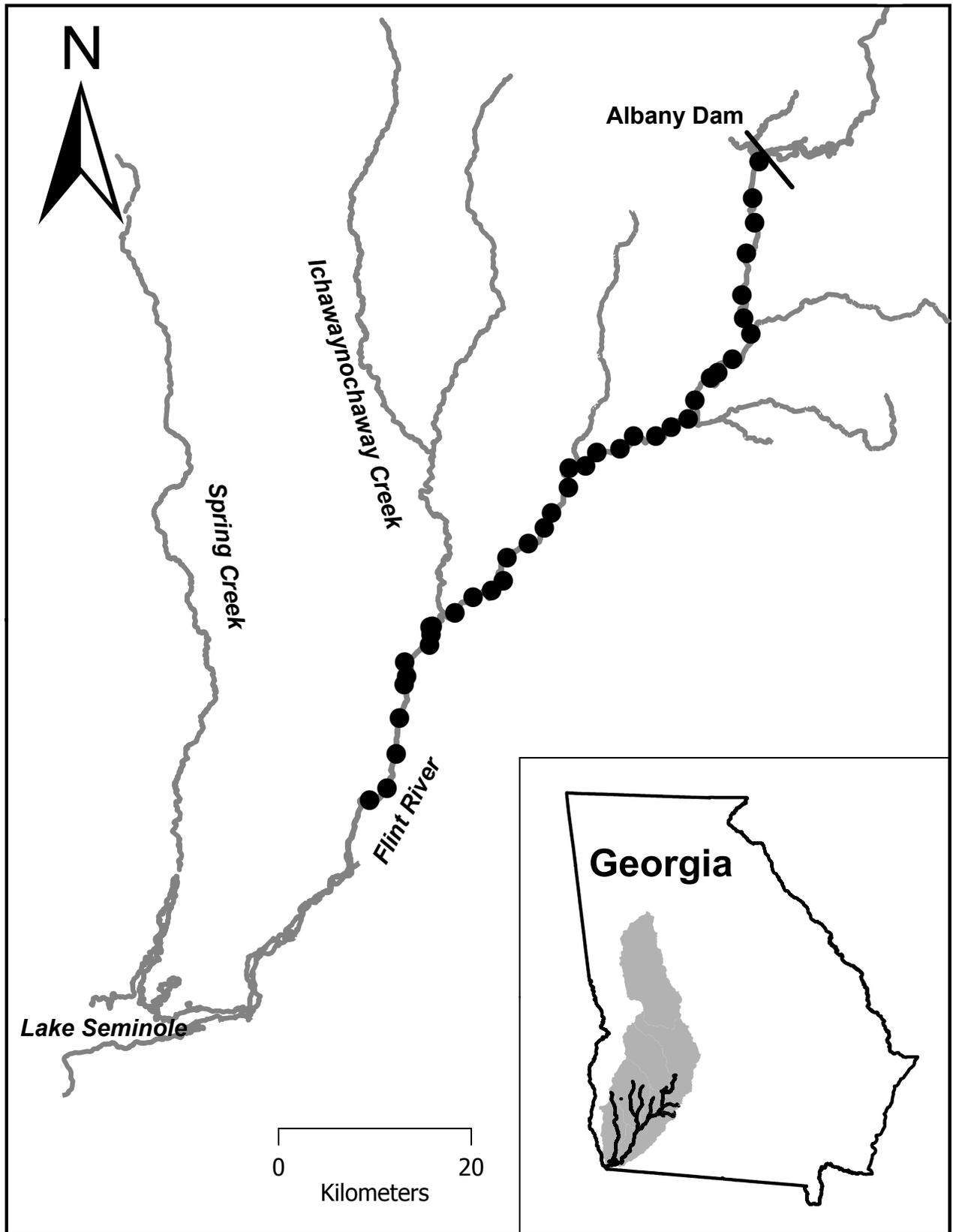
### *Study area*

We focused on a 119 km reach of the lower Flint River between Albany Dam in Albany, GA, downstream to the backwaters of Lake Seminole at river kilometer (rkm) 48, near Bainbridge, GA (Fig. 1). Albany Dam was constructed as a hydropower facility in 1919 and is currently operated by Georgia Power as a hydro-peaking facility but minimally increases river discharge during periods of operation (Couch et al., 1996). The study area is entirely within the Dougherty Plain physiographic district, which is underlain by karst, and the river receives substantial groundwater inputs from the Floridan aquifer via tributaries and in-channel springs. Substrates range from silt and sand to limestone boulders and bedrock. The river has a mean daily discharge of 113 m<sup>3</sup>/s at the USGS gage station located in Newton, GA (Couch et al., 1996). Ichawaynochaway Creek is the only large tributary flowing into this reach of the river, entering the Flint River at approximately rkm 84. The river channel is deeply entrenched, often with vertical limestone bluffs. Woody debris is relatively abundant in the stream channel.

### *Mussel sampling*

We sampled 39 sites in the study reach over 15 days between 23 May 2011 and 30 August 2011 (Fig. 1; Wisniewski et al., 2013a). Prior to sampling, a sequence of site characteristics was randomly selected according to two factors: (1) left ascending bank or right ascending bank and (2) dominant site macrohabitat (i.e., edgewater/stream margin, riffle, run, glide, and pool). We then travelled upstream on the river and sampled at the first location that met the characteristics of the first site on our sequence. After sampling this site, we moved upstream at least 2 km to the next specified site characteristic.

We randomly placed ten 10-m-long transect lines perpendicular to flow at each site. Searchers collected



**FIGURE 1**  
The lower Flint River, Georgia, with locations of the 39 sample sites.

all mussels within 0.5 m of each side of transects using tactile and visual survey methods with mask and snorkel in shallow water or surface-air-supply system in waters  $\geq 1.5$  m in depth. Crevices among and under coarse substrates were also searched using tactile searches. All mussels were identified to species, counted, and a maximum of 10 individuals per species per transect were measured along the longest axis parallel to the hinge-line. The smallest and largest individuals were measured when  $>10$  individuals of a species were collected in a transect. We pooled *Elliptio fumata* and *Elliptio pullata* for all analyses due to the difficulty in separat-

ing these species reliably. After processing, all mussels were returned to the river. Sampling time per site ranged from 0.35 person-hours to 5.65 person-hours with a mean time per site of 1.40 person-hours. *Utterbackia peggyae* and *Villosa villosa* were collected only during resampling of a subset of sites used for an additional analysis included in a previously published study (Wisniewski et al., 2013a). These species are included here in overall estimates of species richness and cumulative detection (Tables 1 and 2), but they were not included in occupancy and detection models.

**TABLE 1**

Freshwater mussel species collected in the lower Flint River, Georgia. # of sites is the number of sites at which a species occurred; % of sites represents naïve occupancy.

Tribe	Species	Common Name	# of Sites	% of Sites	Total number	% of total
<b>Amblemini</b>						
	<i>Amblema neislerii</i> (Lea, 1858)	Fat Threeridge	1	3	5	<1
<b>Lampsilini</b>						
	<i>Hamiota subangulata</i> (Lea, 1840)	Shinyrayed Pocketbook	3	8	3	<1
	<i>Lampsilis floridensis</i> (Lea, 1852)	Florida Sandshell	28	72	85	1
	<i>Lampsilis straminea</i> (Conrad, 1834)	Southern Fatmucket	2	5	2	<1
	<i>Toxolasma paulum</i> (Lea, 1840)	Iridescent Lilliput	15	38	105	1
	<i>Villosa lienosa</i> (Conrad, 1834)	Little Spectaclecase	13	33	85	1
	<i>Villosa vibex</i> (Conrad, 1834)	Southern Rainbow	23	59	50	1
	<i>Villosa villosa</i> (Wright, 1898)	Downy Rainbow	1	3	1	<1
<b>Pleurobemini</b>						
	<i>Elliptio arctata</i> (Conrad, 1834)	Delicate Spike	9	23	70	1
	<i>Elliptio crassidens</i> (Lamarck, 1819)	Elephantear	28	72	3,139	44
	<i>Elliptio fumata/pullata</i>	<i>E. fumata/pullata</i>	34	87	2,817	39
	<i>Elliptio nigella</i> (Lea, 1852)	Winged Spike	15	38	539	8
	<i>Elliptio purpurella</i> (Lea, 1857)	Inflated Spike	1	3	2	<1
	<i>Elliptoideus sloatianus</i> (Lea, 1840)	Purple Bankclimber	17	44	98	1
<b>Quadrulini</b>						
	<i>Megaloniaias nervosa</i> (Rafinesque, 1820)	Washboard	5	13	13	<1
	<i>Quadrula infucata</i> (Conrad, 1834)	Sculptured Pigtoe	21	54	143	2
	<i>Unio merus columbensis</i> (Lea, 1857)	Apalachicola Pondhorn	3	8	3	<1
<b>Anodontini</b>						
	<i>Alasmidonta triangulata</i> (Lea, 1858)	Southern Elktoe	2	5	4	<1
	<i>Utterbackia peggyae</i> (Johnson, 1965)	Florida Floater	1	3	2	<1
<b>TOTAL</b>			39		7,166	

**TABLE 2**

Cumulative detection probabilities of freshwater mussel species collected in the lower Flint River, Georgia. Values indicate the probability of detecting a species when the given number of 10 m X 1 m transects are searched at a site under the condition that the species is present.

Tribe	Species	Transects Searched									
		1	2	3	4	5	6	7	8	9	10
<b>Amblemini</b>											
	<i>Amblema neislerii</i>	0.40	0.64	0.78	0.87	0.92	0.95	0.97	0.98	0.99	0.99
<b>Lampsilini</b>											
	<i>Hamiota subangulata</i>	0.01	0.02	0.02	0.03	0.04	0.05	0.05	0.06	0.07	0.07
	<i>Lampsilis floridensis</i>	0.21	0.38	0.52	0.62	0.70	0.77	0.82	0.86	0.89	0.91
	<i>Lampsilis straminea</i>	0.01	0.01	0.02	0.02	0.03	0.03	0.04	0.04	0.05	0.05
	<i>Toxolasma paulum</i>	0.30	0.51	0.65	0.76	0.83	0.88	0.92	0.94	0.96	0.97
	<i>Villosa lienosa</i>	0.26	0.46	0.60	0.71	0.78	0.84	0.88	0.91	0.94	0.95
	<i>Villosa vibex</i>	0.16	0.30	0.41	0.50	0.58	0.65	0.71	0.75	0.79	0.83
	<i>Villosa villosa</i>	0.01	0.02	0.02	0.03	0.04	0.05	0.05	0.06	0.07	0.07
<b>Pleurobemini</b>											
	<i>Elliptio arcata</i>	0.28	0.48	0.62	0.73	0.80	0.86	0.90	0.93	0.95	0.96
	<i>Elliptio crassidens</i>	0.57	0.82	0.92	0.97	0.99	0.99	1.00	1.00	1.00	1.00
	<i>Elliptio fumata/pullata</i>	0.69	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00
	<i>Elliptio nigella</i>	0.39	0.63	0.77	0.86	0.92	0.95	0.97	0.98	0.99	0.99
	<i>Elliptio purpurella</i>	0.00	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.03
	<i>Elliptioideus sloatianus</i>	0.28	0.49	0.63	0.74	0.81	0.86	0.90	0.93	0.95	0.96
<b>Quadrulini</b>											
	<i>Megaloniaias nervosa</i>	0.17	0.31	0.42	0.52	0.60	0.67	0.72	0.77	0.81	0.84
	<i>Quadrula infucata</i>	0.34	0.57	0.72	0.81	0.88	0.92	0.95	0.97	0.98	0.98
	<i>Unio merus columbensis</i>	0.01	0.02	0.02	0.03	0.04	0.05	0.05	0.06	0.07	0.07
<b>Anodontini</b>											
	<i>Alasmidonta triangulata</i>	0.17	0.31	0.42	0.52	0.60	0.67	0.72	0.77	0.81	0.84
	<i>Utterbackia peggyae</i>	0.01	0.02	0.02	0.03	0.04	0.05	0.05	0.06	0.07	0.07

#### Covariate measurements

Searcher experience (years/searcher), substrate roughness, mean depth, mean velocity, and substrate

composition were recorded or measured at each transect to model variability in detection probabilities related to these factors. Percent woody debris and substrate

composition at the site level were measured as a composite from all transects at each site. Substrate composition categories were: clay (<0.06 mm and cohesive), silt (<0.06 mm), sand (0.06-2 mm), gravel (2-64 mm), cobble (64-256 mm), boulder (>256 mm), and bedrock (>256 mm, unbroken). Sites were visually characterized as swiftwater (riffles/runs/glides) or slackwater (pool/edgewater) because macrohabitat types defined when selecting sites are dependent on river stage. The distance from Albany Dam to each site was measured using the National Hydrography Database at a scale of 1:24,000 in ArcGIS 9.3 (ESRI, Redlands, CA). Site-level covariates were used to model variability in occupancy (Wisniewski et al., 2013a).

### Data analysis

Single-season occupancy models were generated for all species (MacKenzie et al., 2006) to estimate mean occupancy and mean transect-level detection probabilities throughout the 119 km study area (Wisniewski et al., 2013a). We estimated occupancy and detection probabilities in relation to site- and transect-level characteristics only for species occurring at  $\geq 10$  sites to ensure sufficient power to estimate influences of covariates on parameters (Wisniewski et al., 2013a). We assessed the ability of our sampling design to detect a species at a site by calculating cumulative detection probability ( $p^*$ ):

$$p^* = 1 - (1 - p)^K$$

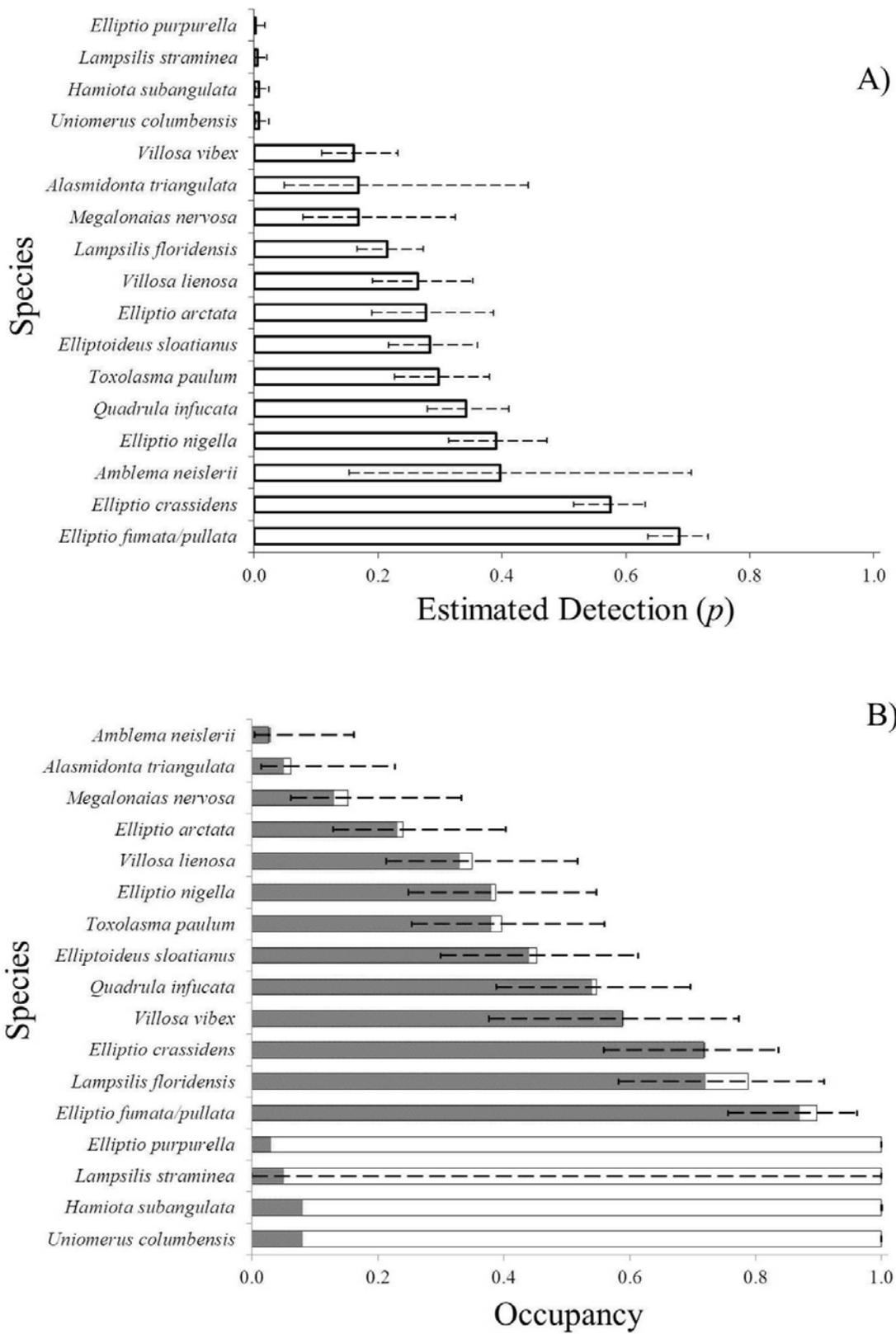
where  $p$  is the estimated detection for a single transect and  $K$  is the total number of transects (Bayley & Peterson, 2001; Hagler et al., 2011). Occupancy models were fit for each species in Program MARK (White & Burnham, 1999). We also developed an *a priori* set of 65 candidate models representing hypothesized relations between habitat variables and freshwater mussel occupancy and detection (see Wisniewski et al., 2013a; Table 1). To facilitate comparisons among models, we calculated Akaike weights, which range from zero to one with the best approximating candidate model having the highest weight (Burnham & Anderson, 2002). The most plausible models (confidence set) were those with Akaike weights that were at least 10% of that of the best-approximating model, which is similar to Royall's general rule-of-thumb of 1/8 or 12% for evaluating strength of evidence (Royall, 1997). To ease interpretation of parameter estimates, we calculated odds ratios (Hosmer & Lemeshow, 2000). The precision of each parameter estimate was evaluated by examining 95% confidence intervals. Parameter estimates with confidence intervals that contained zero were considered imprecise. Model structures and parameter estimates for *E. nigella*, *Elliptio sloatianus*, *Quadrula infucata* were previously reported (see Wisniewski et al., 2013a) and are not included in this study.

Single season occupancy models have four main assumptions in terms of our study: (1) the occupancy state of a site is closed during sampling, (2) sites are independent of one another, (3) probability of occupancy is equal across sites, and (4) detection probabilities are equal across all sites and transects given that a species is present (MacKenzie et al., 2006). Our sites were located a minimum of 2 km apart and sampling at a site was completed within 3 hours; therefore the occupancy state of freshwater mussels at a site is unlikely to change or influence occupancy states at other sites. Assumptions 3 and 4 likely are not met due to differences in physical habitat characteristics among sites, but the covariates (e.g., current, substrate) in our model structures account for these differences.

### RESULTS

Twenty mussel species were collected across all 39 sites (Table 1), and observed site species richness ranged from 0-13 (mean = 6) but only one site yielded no mussels. A total of 7,166 individuals were collected, and two sites accounted for 48% of total individuals. The fauna was dominated by *Elliptio crassidens* and *E. fumata/pullata*, which together made up 83% of the total catch, and they were found at 72% and 87% of sites, respectively. Noteworthy was the collection of 539 individuals (8% of total catch) of *E. nigella*, which was previously considered extinct (see Discussion); all other species individually composed  $\leq 2\%$  of the fauna. Despite the relative rarity of most species, many were widely distributed in the river. In addition to *E. crassidens* and *E. fumata/pullata*, *Lampsilis floridensis*, *Q. infucata*, and *Villosa vibex* were found at >50% of sites, and four other species (including *E. nigella*) were found at >30% of sites.

For most species, results of occupancy and detection modeling closely reflected patterns of species distribution and assemblage composition based on naïve occupancy (Fig. 2). Cumulative detection probability based on 10 transects exceeded 0.80 for most species, indicating that our sampling design was adequate for detecting most species when present (Table 2). Cumulative detection probabilities were <0.07 for *Hamiota subangulata*, *Lampsilis straminea*, *Elliptio purpurella*, *Unio columbensis*, *U. peggyae*, and *V. villosa*. Rankings of estimated detection probabilities at the transect level were roughly similar to rankings based on total catch, with the highest values for *E. fumata/pullata* and *E. crassidens* (Fig. 2, Table 1). Similar to cumulative detection, transect-level detection was low for *H. subangulata*, *L. straminea*, *E. purpurella*, and *U. columbensis* and reflected the rarity of these species in our samples. Estimated occupancy was nearly identical to naïve occupancy for all species except *H. subangulata*, *L. straminea*, and *V. villosa*.



**FIGURE 2**

(A) Estimated detection probabilities and (B) occupancy for freshwater mussel species collected at 39 sites in the lower Flint River, Georgia. Error bars are 95% confidence intervals. In panel B, naïve occupancy is indicated by shading. Data from Wisniewski et al. (2013a).

*minea*, *E. purpurella*, and *U. columbensis*. Estimated occupancy was 1.0 for all four of these species in contrast to their extreme rarity and limited distribution in our samples. However, 95% confidence intervals for these estimates were either unrealistically narrow (0.99-1.00 or 1.00-1.00) or extremely wide (0.00-1.00), suggesting that these estimates were biased by the low detection probability for these species.

**Factors influencing species occupancy and detection**

Thirteen models were included in the confidence set for *E. crassidens*. All models in the confidence set included detection varying by percent cobble and boulder substrates and water depth, and each of these covariates accounted for 94% of the model AIC<sub>c</sub> weight. Occupancy

modeled as a function of percent gravel and distance from Albany Dam accounted for 50% and 33% of the model AIC<sub>c</sub> weight, respectively. The best approximating model had 2.11 times more support than the next best model and odds ratios indicated that detection of *E. crassidens* decreased by 2.18 times for every meter increase in depth (Tables 3 & 4). Detection greatly increased with increasing amount of cobble substrates. Estimated occupancy of *E. crassidens* increased by 1.02 times for every 1-km increase in distance from Albany Dam. Confidence intervals for detection varying by percent boulder substrate and occupancy varying by percent gravel substrate contained zero and we were unable to conclude if these effects were negative or positive.

**TABLE 3**

Akaike Information Criterion (AIC<sub>c</sub>), number of parameters (K), ΔAIC<sub>c</sub>, and AIC<sub>c</sub> weights (w<sub>i</sub>) for the three best approximating models estimating detection probability (p) and occupancy (ψ) for nine freshwater mussel species in the lower Flint River, Georgia. Distance corresponds to distance downstream from Albany Dam.

Candidate model	AIC <sub>c</sub>	K	ΔAIC <sub>c</sub>	w <sub>i</sub>
<i>Elliptio crassidens</i>				
p(Cobble + Boulder + Depth) ψ(Gravel + Distance)	393.59	7	0	0.26
p( Cobble + Boulder + Depth ) ψ(Gravel)	395.09	6	1.50	0.13
p( Cobble + Boulder + Depth ) ψ(Distance)	396.32	6	2.73	0.07
<i>Elliptio fumata/pullata</i>				
p(Boulder + Bedrock + Depth) ψ(Clay + Distance)	440.29	7	0	0.23
p( Boulder + Bedrock + Depth ) ψ(Clay)	441.19	6	0.90	0.15
p( Boulder + Bedrock + Depth ) ψ(Distance)	441.36	6	1.06	0.14
<i>Lampsilis floridensis</i>				
p(Bedrock) ψ(Silt)	340.87	4	0	0.25
p(Sand) ψ(Silt)	342.06	4	1.19	0.14
p(Searcher) ψ(Silt)	342.06	4	2.19	0.08

**TABLE 3**  
(cont.)

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*Toxolasma paulum*

$p(\text{Depth} + \text{Sand}) \psi(\text{Gravel} + \text{Boulder})$	221.22	6	0	0.41
$p(\text{Depth} + \text{Sand}) \psi(\text{Gravel})$	222.45	5	1.23	0.22
$p(\text{Depth} + \text{Sand}) \psi(\text{Boulder})$	223.03	5	1.81	0.17

*Villosa lienosa*

$p(\text{Clay} + \text{Bedrock}) \psi(\text{Boulder} + \text{Bedrock})$	199.28	6	0	0.43
$p(\text{Clay} + \text{Bedrock}) \psi(\text{Boulder})$	201.32	5	2.04	0.16
$p(\text{Clay} + \text{Bedrock}) \psi(\text{Boulder} + \text{Gravel})$	202.58	6	3.30	0.08

*Villosa vibex*

$p(\text{Searcher}) \psi(\text{Cobble})$	226.42	4	0	0.82
$p(\text{Searcher}) \psi(\text{Woody debris} + \text{Cobble})$	230.28	5	3.86	0.12
$p(\text{Searcher}) \psi(\text{Woody debris})$	235.59	4	9.17	0.01

---

**TABLE 4**

Parameter estimates (standard errors), lower and upper 95% confidence limits, and scaled odds ratios for the best approximating models for occupancy ( $\psi$ ), and detection ( $p$ ) of six freshwater mussel species in the lower Flint River, Georgia.

Parameter	Estimate (SE)	Lower	Upper	Odds Ratio
<i>Elliptio crassidens</i>				
Detection ( $p$ )				
Intercept	0.506 (0.316)	-0.113	-1.126	
Cobble	4.556 (1.194)	2.216	6.896	95.19
Boulder	-6.675 (2.123)	-2.514	10.835	792.15
Depth	-0.781 (0.329)	-1.426	-0.136	2.18
Occupancy ( $\psi$ )				
Intercept	-1.294 (0.980)	-1.483	0.722	
Gravel	8.916 (4.728)	-0.351	18.184	> 1000
Distance	0.027 (0.013)	0.001	0.053	1.03
<i>Elliptio fumata/pullata</i>				
Detection ( $p$ )				
Intercept	1.767 (0.363)	1.056	2.478	
Depth	-0.884 (0.339)	-1.549	-0.220	2.42
Bedrock	-1.152 (0.366)	-1.869	-0.435	3.16
Boulder	6.781 (2.412)	-2.053	11.510	881.34
Occupancy ( $\psi$ )				
Intercept	-5.117 (2.512)	0.193	10.041	
Clay	2734.841 (0)	2734.841	2734.841	> 1000
Distance	-0.042 (0.029)	-0.099	0.014	1.04
<i>Lampsilis floridensis</i>				
Detection ( $p$ )				
Intercept	-1.130 (0.166)	-1.456	-0.805	
Bedrock	-1.111 (0.538)	-2.165	-0.057	3.04
Occupancy ( $\psi$ )				
Intercept	-0.008 (0.616)	-1.216	1.199	
Silt	1666.523 (0)	1666.523	1666.523	> 1000
<i>Toxolasma paulum</i>				
Detection ( $p$ )				
Intercept	-5.591 (1.021)	-7.593	-3.589	
Depth	3.049 (0.803)	1.476	4.622	21.10
Sand	3.270 (0.738)	1.823	4.717	26.31

**TABLE 4**  
(cont.)

Occupancy ( $\psi$ )				
Intercept	-0.076 (0.555)	-1.163	1.012	
Boulder	68.301 (51.226)	-32.101	168.704	> 1000
Gravel	-9.020 (5.661)	-20.117	2.076	> 1000
<i>Villosa lienosa</i>				
Detection ( $p$ )				
Intercept	-0.905 (0.244)	-1.384	-0.427	
Bedrock	-0.872 (1.105)	-3.038	-1.293	2.39
Clay	-346.08 (0)	-346.084	-346.084	> 1000
Occupancy ( $\psi$ )				
Intercept	-0.440 (0.519)	-1.458	0.578	
Bedrock	-4.403 (2.474)	-9.251	0.446	81.68
Boulder	21.570 (11.423)	-0.819	43.959	> 1000
<i>Villosa vibex</i>				
Detection ( $p$ )				
Intercept	-2.869 (0.517)	-3.876	-1.862	
Searcher	0.156 (0.060)	0.038	-0.274	42.93
Occupancy ( $\psi$ )				
Intercept	-0.659 (0.551)	-1.739	0.4214	
Cobble	193.366 (239.382)	-275.820	662.550	> 1000

Thirteen models were included in the confidence set for the combined group of *E. fumata/pullata*. All models in the confidence set included detection varying by percent boulder, percent bedrock, and water depth, and these covariates each accounted for 99% of the model AIC<sub>c</sub> weight. Occupancy modeled as a function of clay and distance from Albany Dam accounted for 48% and 47% of the model AIC<sub>c</sub> weight, respectively. The best approximating model had 1.57 times more support than the next best model and indicated that detection decreased by 2.42 times for every 1-m increase in depth (Tables 3 & 4). Detection decreased by 3.16 times for every one percent increase in bedrock substrate. Detection also increased by 881.34 times for every one percent increase in boulder substrate but the confidence interval was imprecise and included zero. Occupancy of *E. fumata/pullata* was strongly and positively associated with clay substrate as this species was found at all sites having clay substrate. Confidence intervals for distance from Albany Dam contained zero and we were unable to conclude if occupancy of *E. fumata/pullata* was nega-

tively or positively influenced.

Fourteen models were included in the confidence set for *L. floridensis*. Detection varying by percent bedrock, percent sand, and searcher experience, accounted for 36%, 14%, and 8% of the model AIC<sub>c</sub> weight in the confidence set of models, respectively. All models in the confidence set included occupancy varying by percent silt at a site, which accounted for 92% of the model AIC<sub>c</sub> weight (Table 3). The best approximating model had 1.81 times more support than the next best model and indicated that detection was 3.04 times less likely with each one percent increase bedrock substrate (Tables 3 & 4). Occupancy was strongly associated with the proportion of silt at a site as this species was rarely found in sites without silt (Table 4).

Six models were included in the confidence set for *Toxolasma paulum*. All models in the confidence set included occupancy varying by percent gravel substrate which accounted for 99% of the model AIC<sub>c</sub> weight. Detection varying by percent sand substrate and per-

cent bedrock substrate accounted for 94% and 5% of the model  $AIC_c$  weight in the confidence set of models, respectively. Occupancy varying by percent gravel, percent boulder, and distance from Albany Dam accounted for 82%, 57%, and 16% of the model  $AIC_c$  weight in the confidence set, respectively. The best approximating model had 1.85 times more support than the next best model and indicated that detection of *T. paulum* increased by 21.10 times for every 1-m increase in depth and increased by 26.30 times for every one percent increase in sand substrate (Tables 3 & 4). Confidence intervals for boulder and cobble substrate affecting occupancy of *T. paulum* contained zero and we were unable to conclude if this relationship was negative or positive.

Three models were included in the confidence set for *Villosa lienosa*. All models in the confidence set included occupancy varying by percent boulder and detection varying by percent clay and bedrock substrate, and these covariates accounted for 67% of the model  $AIC_c$  weight. Occupancy varying by percent bedrock accounted for 43% of the  $AIC_c$  model weight. The best approximating model had 2.77 times more support than the next best model (Table 3). Detection was strongly and negatively related to clay substrate as *V. lienosa* was rarely collected in this substrate (Table 4). Confidence intervals for bedrock and boulder substrate affecting occupancy and/or detection contained zero and we were unable to make inferences regarding these relationships.

Two models were included in the confidence set for *V. vibex*. Both models in the confidence set included occupancy varying by percent cobble substrate and detection varying by searcher experience, and these covariates each accounted for 94% of the model  $AIC_c$  weight. The best approximating model had 6.88 times more support than the next best model and indicated that detection increased by 1.17 times for each year of searcher experience (Tables 3 & 4). Confidence intervals for percent cobble substrate included zero and we were unable to conclude if this relationship was negative or positive.

#### Population size structure

Populations of most species included individuals from a wide range of sizes (Table 5). Of the ten species for which we had robust estimates of size distribution (i.e.,  $n > 30$ ), all but *Elliptio arctata* had individuals  $\leq 27$  mm length, and for most, minimum size was  $< 30\%$  of maximum size indicating a wide range of sizes and ages in the population. For some species (e.g., *E. fumata/pullata*, *E. nigella*, *Q. infucata*, *T. paulum*), the smallest individuals we found were probably near the minimum size detectable by visual or tactile sampling ( $\leq 15$  mm).

## DISCUSSION

### Status of lower Flint River mussels

High estimated occupancy of several species indicates that they are widely distributed throughout the lower Flint River, but low ( $\leq 0.30$ ) detection probabilities suggest that about half of the fauna are not collected when they occur at a site. Species detection may vary in response to numerous factors including life-history characteristics, behavior, habitat complexity, environmental conditions, and sampling methodology (MacKenzie et al., 2002), but local abundance may also influence heterogeneity in detection (Bayley & Peterson, 2001; Royle & Nichols, 2003; Royle et al., 2005). Abundance-induced heterogeneity in detection is more influential when local populations are small and this effect decreases with increasing population size (MacKenzie et al., 2006). Hence, abundance-induced heterogeneity likely influenced estimated detection probabilities for *H. subangulata*, *L. straminea*, *E. purplella*, and *U. columbensis*, which had exceptionally low detection. Consequently, the high estimated occupancies for these species are probably unrealistic because the models were unable to distinguish between true absence and nondetection (MacKenzie et al., 2002). We have no evidence to support that detection of these species was low because of behavioral or other ecological attributes, and our survey results indicate that these species are rare throughout the river. Estimated detection of other species exceeded 0.15 and provided relatively precise estimates of occupancy across the lower Flint River.

The Flint River continues to harbor a diverse and relatively abundant freshwater mussel assemblage. Additionally, evidence of recent reproduction ( $<$  about 25 mm shell length; Haag & Warren, 2007; Negishi & Kayaba, 2010) was apparent for most species with large sample sizes, and for several, the smallest individuals we found likely were 1-2 years old. These findings show the importance of the Flint River mainstem as a conservation refuge.

Most notably, *E. nigella* was considered a rare species even historically, and it was presumed extinct, with the last collection in 1958 (Brim Box & Williams, 2000; Williams et al., 2008). In our study, *E. nigella* was the third most abundant species (539 individuals) and our models predicted that it occupied nearly 40% of sites. Three individuals  $\leq 25$  mm were collected and several individuals were observed brooding embryos or glochidia. *Elliptio nigella* appears to be a large river species and it is most abundant in swift water in crevices among large boulders and cobble (Wisniewski et al., 2013a). The rarity and presumed extinction of this species may be due to the low amount of effort previously expended in the mainstem Flint River and the difficulty of sampling its spe-

**TABLE 5**

Population size structure of freshwater mussels collected in the lower Flint River, Georgia

Tribe	Species	Length (mm)			
		Number Measured	Minimum	Mean	Maximum
<b>Amblemini</b>					
	<i>Amblema neislerii</i>	5	48	54	64
<b>Lampsilini</b>					
	<i>Hamiota subangulata</i>	3	30	57	58
	<i>Lampsilis floridensis</i>	81	27	70	100
	<i>Lampsilis straminea</i>	2	53	73	92
	<i>Toxolasma paulum</i>	94	11	24	36
	<i>Villosa lienosa</i>	55	24	43	55
	<i>Villosa vibex</i>	37	14	58	75
	<i>Villosa villosa</i>	1	48	48	48
<b>Pleurobemini</b>					
	<i>Elliptio arctata</i>	58	45	61	85
	<i>Elliptio crassidens</i>	595	17	71	111
	<i>Elliptio fumata/pullata</i>	968	9	56	106
	<i>Elliptio nigella</i>	130	15	53	106
	<i>Elliptio purpurella</i>	2	56	57	58
	<i>Elliptoideus sloatianus</i>	98	23	127	204
<b>Quadrulini</b>					
	<i>Megaloniaias nervosa</i>	13	98	121	140
	<i>Quadrula infucata</i>	137	13	37	59
	<i>Unio merus columbensis</i>	3	56	59	61
<b>Anodontini</b>					
	<i>Alasmidonta triangulata</i>	4	48	54	64
	<i>Utterbackia peggyae</i>	1	30	30	30

cialized habitat. It is also possible that misidentifications may have contributed to its perceived rarity because the species has been synonymized by several previous authors (Frierson, 1927; Johnson, 1968; Brim Box & Williams, 2000) and the genus *Elliptio* provides particular identification challenges (e.g., Shea et al., 2011).

The rediscovery of *A. neislerii* in the Flint River in 2006 is important because it was previously known to survive only in the Apalachicola and Chipola rivers (Brim

Box & Williams, 2000). High estimated detection of *A. neislerii* provided precise estimates of occupancy, which indicate that this species is rare and narrowly distributed in the Flint River. However, lengths of *A. neislerii* ranged from 41-70 mm suggesting the presence of several year classes and relatively recent reproduction; a previous age and growth study in the Apalachicola River found that a 42-mm *A. neislerii* was 3 years old (USFWS 2006). Although we were unable to evaluate

habitat relationships of *A. neislerii*, we speculate that its rarity in the Flint River, both currently and historically, is a result of insufficient availability of suitable habitat. In the Apalachicola River, *A. neislerii* is found most frequently on gently sloping banks in stable, depositional habitats consisting of sandy silt (Brim Box & Williams, 2000), and the single site in the Flint River where we found the species strongly resembled these conditions. These habitats are exceptionally rare in the lower Flint River because much of the river is bordered by limestone bluffs. Furthermore, only 6 of 21 historical records of *A. neislerii* in the ACF are from the Flint River with 3 of these records specifically collected within our study reach (Brim Box & Williams, 2000); these observations suggest that the species has always been of restricted distribution in the river. Nevertheless, the presence of an additional, apparently viable population of this species lessens its extinction risk.

Other notable species include *Alasmidonta triangulata*, *E. sloatianus*, and *H. subangulata*. *Alasmidonta triangulata* was widely distributed historically in the ACF but has been found recently only at one site each in the Chattahoochee and Flint river systems (Brim Box & Williams, 2000). Although we collected only four live individuals at two sites and a recently dead individual at a third site, subsequent sampling in 2012 yielded 26 live individuals including several individuals  $\leq 30$  mm in length (J.M. Wisniewski, unpublished data). These records significantly increase the known distribution of *A. triangulata*, but our low occupancy estimates and the restriction of these populations to the extreme southern portion of Flint River near the backwaters of Lake Seminole suggest that total population size in the river is low. Although *E. sloatianus* composed only 1% of mussels collected in our study, it occurred at nearly 50% of our sites and its widespread distribution throughout the lower Flint River (see Brim Box & Williams, 2000) suggests that the overall population size is large and the Flint River population may be the largest remaining on Earth. We collected several apparent age classes including 23 mm and 39 mm individuals, which was unanticipated because access to the Flint River for the reported primary host fish, the migratory Gulf Sturgeon, has been blocked by Jim Woodruff Dam since 1957 (Fritts et al., 2012). This suggests that reported secondary hosts, the Blackbanded and Halloween darters, can facilitate recruitment to some extent in the absence of Gulf Sturgeon. *Hamiota subangulata* is rare in the mainstem Flint River and the largest remaining populations of this species are in tributaries (Brim Box & Williams, 2000; Peterson et al., 2011; Shea et al., 2013; Wisniewski et al., 2013b). However, the continued occurrence of this species in the mainstem, as well as other small stream species or stream size generalists, is vitally important because it shows

that the river has the potential to serve as a migration corridor between tributary populations.

#### *Factors associated with mussel detection or occupancy*

Although many previous attempts to find quantifiable differences in habitat use among mussel species have been unsuccessful (e.g., Brim Box et al., 2002; Strayer & Ralley, 2003; reviewed in Haag, 2012), we found strong differences in habitat use among several species. The most marked and consistent differences among species were in the substrate types with which they were associated. *Elliptio crassidens* showed an affinity for coarse, cobble and boulder substrates, but *L. floridensis* and *T. paulus* were strongly associated with silt and sand, respectively. Similarly, *E. fumata/pullata* was strongly associated with clay, but *V. lienosa* was nearly absent from this substrate type; a similar dichotomy in use of clay was seen in the Flint River for *Q. infucata*, which was found predominantly in clay, and *E. sloatianus*, which avoided clay substrates (Wisniewski et al., 2013a). Parameter estimates for some of these associations (e.g., *E. fumata/pullata*, *V. lienosa*) were large with no variance, which indicates near perfect separation of species detection or occupancy based on these variables (Webb et al., 2004). These patterns suggest strong ecological differences among species that are expected to have important bearing on community assembly and vulnerability to human impacts.

Other model factors had only limited effects on mussel occupancy or detection. Distance from Albany Dam appeared in the best models for two species, but the magnitude of this effect was low for *E. crassidens* and imprecise for *E. fumata/pullata*. Similarly, searcher experience was an important factor only for *V. vibex*, and the effect of experience was modest. Although occupancy of *E. nigella* was strongly influenced by the presence of swiftwater habitat (Wisniewski et al., 2013a), we found little support for preference of swiftwater habitat among the six species in this study.

#### *Application for freshwater mussel surveys and monitoring*

Occupancy modeling was useful in our study because it allowed us to quantitatively estimate the status of mussels across a large study reach and assess habitat relationships with considerably less effort than required for other commonly employed sampling approaches. Our study was conducted during a period of record low flows in the lower Flint River Basin (USGS, 2012), and our detection probabilities may be higher than those estimated during higher flow years because conditions were conducive to sampling mussels. Nevertheless, an important benefit of occupancy modeling is that incorporation of detection probability provides an objective measure of sampling efficiency. Cumula-

tive detection probabilities indicated that our sampling design was adequate to detect most species, with the exception of the rarest species. As in other mussel sampling approaches, consistent detection of very rare species requires an impractically large number of samples. In the context of occupancy modelling, sampling additional sites to increase precision of occupancy estimates would be a more efficient use of effort than increasing replication at a site in an attempt to increase detection.

Despite the routine use of occupancy modeling for other organisms (e.g., Bailey et al., in press), this method is used infrequently for freshwater mussels; rather, freshwater mussel studies often use presence/absence and species richness as response variables to examine factors affecting mussel populations (Spooner & Vaughn, 2009; Gangloff et al., 2011; Vaughn, 2012; Randklev et al., 2013). These approaches must assume that detection is a constant or random process rather than a systematic process related to population size, environmental variables, or search efficiency. However, heterogeneity in detection probabilities of freshwater mussels in response to various factors shows that this assumption is likely violated often (Meador, 2008; Shea et al., 2013; Wisniewski et al., 2013a). Consequently, species presence or richness frequently may be underestimated due to imperfect detection, and a failure to account for this bias can have serious effects on our understanding of mussel ecology (Wisniewski et al., 2013a).

We recommend the use of occupancy modeling for freshwater mussels for the following reasons: 1) it provides a level of confidence in sampling data, which accounts for false-absences that contribute bias into our understanding of factors affecting mussel occupancy; 2) it can be easily incorporated into many currently used freshwater mussel sampling designs with no or minimal modification to these designs; 3) sampling and analysis are practical to implement with limited resources; 4) analyses can be conducted with open-source software with extensive on-line documentation; and 5) similar models are available to estimate various demographic parameters of interest for freshwater mussel conservation (see Haag and Williams in press), including abundance (Royle, 2004; Nichols et al., 2007), species richness (Kéry & Royle, 2008), colonization/extinction (MacKenzie et al., 2003), recruitment and population growth (Pradel, 1996), and emigration (Kendall & Nichols, 1995). These analytical approaches can considerably advance our understanding of the processes affecting freshwater mussel populations, which ultimately will improve our ability to conserve these imperiled species.

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# **WALKERANA** The Journal of the Freshwater Mollusk Conservation Society

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## **OUR PURPOSE**

The Freshwater Mollusk Conservation Society (FMCS) is dedicated to the conservation of and advocacy of freshwater mollusks, North America's most imperiled animals. Membership in the society is open to anyone interested in freshwater mollusks who supports the stated purposes of the Society which are as follows:

- 1) Advocate conservation of freshwater molluscan resources;
- 2) Serve as a conduit for information about freshwater mollusks;
- 3) Promote science-based management of freshwater mollusks;
- 4) Promote and facilitate education and awareness about freshwater mollusks and their function in freshwater ecosystems;
- 5) Assist with the facilitation of the National Strategy for the Conservation of Native Freshwater Mussels (Journal of Shellfish Research, 1999, Volume 17, Number 5), and a similar strategy under development for freshwater gastropods.

## **OUR HISTORY**

The FMCS traces its origins to 1992 when a symposium sponsored by the Upper Mississippi River Conservation Committee, USFWS, Mussel Mitigation Trust, and Tennessee Shell Company brought concerned people to St. Louis, Missouri to discuss the status, conservation, and management of freshwater mussels. This meeting resulted in the formation of a working group to develop the National Strategy for the Conservation of Native Freshwater Mussels and set the ground work for another freshwater mussel symposium. In 1995, the next symposium was also held in St. Louis, and both the 1992 and 1995 symposia had published proceedings. Then in March 1996, the Mississippi Interstate Cooperative Research Association (MICRA) formed a mussel committee. It was this committee (National Native Mussel Conservation Committee) whose function it was to implement the National Strategy for the Conservation of Native Freshwater Mussels by organizing a group of state, federal, and academic biologists, along with individuals from the commercial mussel industry. In March 1998, the NNMCC and attendees of the Conservation, Captive Care and Propagation of Freshwater Mussels Symposium held in Columbus, OH, voted to form the Freshwater Mollusk Conservation Society. In November 1998, the executive board drafted a society constitution and voted to incorporate the FMCS as a not-for-profit society. In March 1999, the FMCS held its first symposium "Musseling in on Biodiversity" in Chattanooga, Tennessee. The symposium attracted 280 attendees; proceedings from that meeting are available for purchase. The second symposium was held in March 2001 in Pittsburgh, Pennsylvania, the third in March 2003 in Raleigh, North Carolina, the fourth in St. Paul, Minnesota in May 2005, the fifth in Little Rock, Arkansas in March 2007, the sixth in Baltimore, Maryland in April 2009, the seventh in Louisville, Kentucky in 2011, and the eighth in Guntersville, Alabama in 2013. The society also holds workshops on alternating years, and produces a newsletter four times a year.

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