The effects of flow and stream characteristics on the variation in freshwater mussel growth in a Southeast US river basin


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The effects of flow and stream characteristics on the variation in freshwater mussel growth in a Southeast US river basin

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SUMMARY

1. The evaluation of the age and growth of animal populations is essential for understanding and predicting how populations will respond to changes in environmental conditions and anthropogenic stressors.
2. We used a novel, von Bertalanffy hierarchical modelling approach to quantify relationships between the growth of three freshwater mussel species and various site- and watershed-level factors including seasonal discharge, land cover and stream size in the lower Flint River Basin, Georgia, U.S.A.
3. Our modelling approach quantified the mussel-to-mussel variation in the von Bertalanffy parameters and accounted for biases associated with multiple measurements made on each mussel specimen, which are generally not accounted for as sources of bias in age and growth studies.
4. Modelling results suggested that maximum shell size parameter and the Brody growth coefficient varied across species, on average, 19 and 33%, respectively, among individual mussels within sample sites. The variation was related to short-term high streamflows during the spring season, stream size, channel geomorphology and land cover in the watershed.
5. This study provides insight to the factors affecting the growth of stream-dwelling freshwater mussels. Although hierarchical von Bertalanffy growth models are rarely used for freshwater mussel age and growth studies, this approach can provide important information regarding the ecology of freshwater mussels.

Keywords: annuli, hierarchical model, thin section

Introduction

Age and growth determination is an essential component of effectively managing animal populations. This is best exemplified in fisheries management, in which age and growth data are frequently used to investigate the influence of various environmental factors on fish population dynamics. For instance, fish growth reportedly varies in response to stream discharge (Paragamian & Wiley, 1987) and other physical habitat characteristics, such as substrate or structure (Quist & Guy, 2001).

Although rarely assessed in freshwater mussel (Unioniformes) studies, similar approaches to using age and growth metrics may be useful for identifying factors and mechanisms affecting these highly imperilled species.

Various methods have been used to determine age and growth of freshwater mussels including examination of external annuli counts (Negus, 1966; Hanson, Mackay & Prepas, 1988), repeated measurements from mark–recapture studies (Anthony et al., 2001; Tang, Jiao & Jones, 2013) and examination of internal annuli counts through thin sectioning (Neves & Moyer, 1988; Haag &
Comparisons of counts of external annuli versus internal annuli indicated that age often was underestimated when using external annuli counts, particularly in older individuals (Neves & Moyer, 1988). Further, Haag (2009) suggested that inferences drawn from repeated measurements during mark–recapture studies regarding age and growth of freshwater mussels are inaccurate due to handling effects that reduced growth, thus systematically overestimating age and underestimating growth. Therefore, internal annuli counts using mussel shell thin sections appear to provide a more accurate approach to ageing and measuring growth of these species. However, due to the sensitivity of freshwater mussels to disturbances, validation of internal shell annuli should be included in mussel age and growth studies utilising internal annuli counts (Haag & Commens-Carson, 2008).

Freshwater mussels are generally considered long-lived, slow-growing animals (Bauer, 1992; Ziuganov et al., 2000) based on limited information from few species throughout a relatively narrow distribution. However, studies suggest that growth and longevity of these animals varies with species (Haag & Commens-Carson, 2008; Haag & Rypel, 2011), sex (Haag & Rypel, 2011), population (Bauer, 1992; Haag & Rypel, 2011), climatic conditions (Black et al., 2010) and streamflow (Black et al., 2010). Therefore, examination of age and growth chronologies of freshwater mussels may provide valuable insight into the relative influence of environmental factors affecting these species because variation in annulus width may reflect responses of individual mussels to environmental conditions during the growing season (Rypel et al., 2008; Black et al., 2010).

The effects of natural and anthropogenic disturbances on freshwater mussel growth are largely unstudied. However, the range of threats to freshwater mussels posed by climatic and anthropogenic disturbances are typified in the Flint River Basin, Georgia. The upper portion of the basin is extensively altered by urbanisation, whereas lower portions of the basin are largely dominated by heavily irrigated row-crop agriculture, which has substantially altered stream flows (McDowell, 2006; Rugel et al., 2012). The basin was historically occupied by 33 freshwater mussel species with 29 species believed extant (Brim Box & Williams, 2000; Shea et al., 2013; Wisniewski et al., 2014). Previous studies in the Flint River Basin identified several mechanisms influencing the decline of mussel populations including severe drought, agricultural water use and impoundment (Golladay et al., 2004; Peterson et al., 2011; Shea et al., 2013). However, further understanding of the response of mussel populations to these disturbances may greatly aid the development of successful conservation strategies for these species. Therefore, we evaluated factors affecting the growth rate and maximum size of three freshwater mussel species in the lower Flint River Basin. We first validated internal shell annuli production and developed hierarchical von Bertalanffy growth models to estimate the variation of growth parameters among individual mussels. We then modelled variation in growth parameters as functions of individual and environmental covariates that included species, flow regime components, stream size, stream channel morphology and watershed land use.

Methods

Study area

We studied the age and growth of freshwater mussels in the lower Flint River Basin (LFRB) in southwest Georgia (Fig. 1). The LFRB contains the Fall Line Hills and Dougherty Plain districts of the Coastal Plain physiographic province. Streams located within the Fall Line Hills receive most of their water contribution from surface water runoff and are characterised by sandy-mud substrate with elevated turbidity levels. Streams within the Dougherty Plain receive substantial amounts of water input from the underlying Floridan aquifer and have greater amounts of coarse substrates and low turbidity (Mosner, 2002; Peterson et al., 2009). We grouped streams within the LFRB into strata based on stream size, physiographic province and the abundance of the three focal species (defined below) determined during previous research (Shea, 2011). To minimise the effects of mussel sampling on local populations, we randomly selected a minimum of four sample sites within each stratum that contained relatively large numbers of the three focal species, resulting in 20 sample sites (Fig. 1).

Focal species

We studied age and growth of three mussel species: Villosia vibex (Southern Rainbow), Villosia lienosa (Little Spectaclecase) and Elliptio crassidens (Elephantear) because their populations were relatively stable and locally abundant throughout the LFRB, and they each were relatively easy to identify (Williams, Bogan & Garner, 2008; Shea et al., 2011). Villosia vibex and V. lienosa are thin-shelled mussel species that occupy a variety of stream sizes from small creeks to large rivers and occur in sand and gravel substrates (Williams et al., 2008; Wisniewski et al., 2014). © 2014 John Wiley & Sons Ltd, Freshwater Biology, doi: 10.1111/fwb.12504
Both species are long-term brooders that are gravid from late summer or autumn until the following summer (Williams et al., 2008). *Elliptio crassidens* is a thick-shelled species found primarily in large streams with substrates composed of sand, gravel and cobble (Williams et al., 2008; Wisniewski et al., 2014). *Elliptio crassidens* is a short-term brooder, brooding from April until August, depending on geographic location (Williams et al., 2008).

**Mussel sampling and ageing**

To confirm that presumed annuli corresponded to annual growth checks in LFRB mussels, we collected individual mussels of two focal species *V. lienosa* and *V. vibex* during June and July 2009 and notched each along the ventral margin directly below the umbo using the triangular edge of a file to a depth of 1–2 mm. We tagged all notched mussels with 8 mm × 4 mm oval Hallprint shellfish tags (Hallprint Pty Ltd, Victor Harbor, SA, Australia), hand placed them in the stream substrate and did not disturb them for a minimum of 1 year. Tagged and recaptured mussels provided known age and growth since first capture, while notching demonstrated visual signs of both external and internal growth. Recapture of previously notched individuals provided visual evidence of external annual growth, and production of an internal annulus was evident after examining thin sections that included the notch.

Field crews sampled sites with no notched specimens between November 2009 and August 2010. Sites containing notched specimens were sampled between June and August 2010. Crews sampled each site for c. 1 h and then examined collected specimens. In sites where mussel populations appeared abundant, crews collected 20–30 individuals of each focal species that represented all size classes detected. In sites where insufficient numbers of focal species were collected, the crews conducted additional sampling to increase the number of focal species specimens. If the focal species’ population numbers appeared to be very low in a sample site (i.e. <10 mussels collected), mussel specimens were returned to the stream to prevent further population decline. All collected mussels were identified to species by field personnel and measured (i.e. length, width, height) to the nearest millimetre with dial calipers. We recorded tag numbers of recaptured mussels tagged during a previous study (Shea, 2011) and used these mussels to assess the potential effects of handling and tagging on growth (see Definitions and analysis, below). We euthanised specimens by separating the valves at the hingeline, assigned a unique identifier to the matching valves and placed specimens with labels in individual plastic bags.

We thin sectioned the right valve of each mussel using a Buehler Isomet low speed saw (Buehler Ltd., Evanston, IL, U.S.A.), with a Series 15HC diamond impregnated blade (Buehler Ltd.). Each thin section was sanded and polished using a five step polishing process.
detailed in Dycus (2011). Mounted thin sections were photographed using a Leica MZ6 modular stereomicroscope with 6.3 : 1 zoom lens equipped with a Leica DFC295 (Leica Microsystems Inc., Buffalo Grove Illinois, USA) digital microscope colour camera. Thin-section images were captured at 0.63× magnification and analysed using Image-Pro Plus (version 7.0; Media Cybernetics, Inc., Bethesda, MD, U.S.A.). Mussel thin sections too large for capture in a single image were captured using Image Pro’s tiling procedure that consisted of taking a series of photographs along a common axis and combining them into a single image based on spatially autocorrelated similarities (R. Bunn, Vashaw Scientific Inc., pers. comm.).

Personnel estimated mussel age by identifying and counting the number of annuli present in each thin-section image using a multiple-observer method. Before mussels were aged, all three observers were trained to identify internal annuli by two mussel-ageing experts. True annuli were those that originated at the umbo of the thin section and were traceable to the margin of the shell (Neves & Moyer, 1988; Haag & Commens-Carson, 2008). True annuli often were accompanied by a corresponding halo that surrounded the annulus as it joined the periostracum of the thin section which assisted classification of annuli exhibiting faint lines (Haag & Commens-Carson, 2008; Rypel et al., 2008).

We initially used an independent, multiple-observer method to evaluate the relative precision of annuli-based age estimates and found that initial observer agreement on mussel age was >50% for each focal species (Dycus, 2011). During the initial assessment, the average differences in estimated ages among observers were relatively low (i.e. <20% of final age). The final annuli used to estimate mussel age and growth were determined during a concert read in which thin-section images were viewed simultaneously by all three observers (Dycus, 2011). During the concert read, consensus annuli were identified and marked on the thin-section images, and mussel ages were estimated as the total number of consensus annuli. Specimens were removed from the study if observers failed to reach consensus on annuli location. During the concert read, observers collectively identified and marked reference points used to measure annual height-at-age for each thin section. The reference point was placed at the highest arch of the umbo (Fig. 2) and corresponded to the maximum umbo to ventral margin distance (i.e. mussel shell height) measured using dial calipers. The amount of shell erosion from the umbo to the ventral margin was visually estimated by consensus as the percentage of external shell of each thin section eroded (Fig. 2). Shell height-at-age was estimated by measuring the distance from the reference point to each annulus on the ventral margin (Fig. 2) to the nearest 0.00001 mm. To ensure accurate measurements, Image-Pro was calibrated using a DR-867 2-mm stage micrometer (Klarmann Ruling, Inc., Litchfield, NH, U.S.A.).

Verification of annuli

Previously notched V. lienosa and V. vibex aided in distinctions between true and pseudo-annulus production. Verification of annulus production required observers to agree that notched thin sections only had one true annulus post-notching. Notched thin-section photographs were mixed with non-notched thin sections and randomly placed in the ageing sets. The independent observers then aged all individuals. During the concert read, notched thin sections were identified and annuli placement was compared relative to the notch. We considered annuli production validated for a specimen if all three observers marked an annulus in the same location simultaneously.

![Fig. 2](https://example.com/fig2.png)

**Fig. 2** Thin section of an estimated 5-year-old *Villosa lienosa* collected from Kinchafoonee Creek showing the (a) shell wear present on the thin section estimated to be 20%, (b) the reference point used to measure annual growth and the (c) marks for each of the five annuli on the ventral margin of the shell. Measurement of height-at-age from the reference point to an annulus is shown with a broken line.
post-notching for a specimen. If observers marked multiple annuli or one annulus in multiple locations post-notching, observers discussed the discrepancy and attempted to reach a consensus. Annuli production of a specimen lacked validation when observers failed to reach a consensus or agreement on a single annulus post-notching. We assumed focal species had valid annuli production when more than 50% of notched specimens had validated annulus production and none (zero) had multiple annuli post-notching.

Definitions and analysis

We used average daily discharge data at each study site to identify flow regime components having the greatest influence on mussel growth. We downloaded daily discharge data for sample sites that were located immediately up or downstream (<5 km) of a U.S. Geological Survey stream gage (USGS, 2011). We estimated discharge for ungaged streams using published discharge models (McCargo & Peterson, 2010) and used linear regression (Sokal & Rohlf, 1995) to develop site-specific models relating measured discharge at ungaged sites to average daily discharge data where models were not available. Here, we used data from eight long-term USGS gaging stations in the LFRB (Fig. 1). The best approximating model for each study site was that with the largest coefficient of determination ($r^2$). We diagnosed linear regression residuals to ensure that regression assumptions regarding normality and homogeneity of variance were met for each model and log-transformed discharge measurements when necessary. Seasonal stream flow statistics were then calculated using daily discharge estimated for ungaged study sites for the entire record of mussel growth.

A primary objective of our study was to evaluate the relative influence of streamflows on freshwater mussel growth. Previous studies suggest that the influence of flow regime components on physical and ecological processes varies seasonally (Craven et al., 2010; McCargo & Peterson, 2010; Peterson et al., 2011). Thus, we characterised the flow regime for each season using three components: short-term low flows (10-day minimum discharge); short-term high flows (10-day maximum discharge); and long-term average flow conditions (median discharge for each season of recorded mussel growth). We only considered the effect of streamflows during growing seasons defined as spring (March–June) and summer (July–October) following Peterson et al. (2011). Remaining months were excluded because previous studies report that freshwater mussels become dormant in the winter months with little or no growth (Haag & Commens-Carson, 2008; Rypel et al., 2008; Versteegh et al., 2010), and our annulus validation (discussed below) indicated that winter was the period of slow growth and annuli deposition. All flow regime components were standardised by the contributing upstream watershed area of each site to allow comparisons of the effects of stream flows across different sized streams. We used predicted probability distributions for the model-estimated flow regime components (i.e. rather than single values) assuming a gamma distribution to incorporate prediction error.

We characterised each sample site based on physiographic province (i.e. Dougherty Plain or Fall Line Hills), stream size (link magnitude, following Shreve, 1966), gross channel morphology (confined or an unconfined channel, following Peterson et al., 2009) and land cover. We classified land cover as urban, agricultural and other by combining classes in the 2001 National Land Cover Dataset (USGS, 2001). Urban land cover was a combination of developed open space, and developed low, medium and high intensity urban space. Agriculture land cover was a combination of grasslands/herbaceous, pasture/hay and cultivated crops classes. The other category consisted of the remaining classes. Percentage land cover represented the percentage of urban and agricultural land cover in the contributing watershed upstream of a site.

We modelled growth rate of individual mussels using the von Bertalanffy (1938) equation:

$$l_t = l_\infty[1 - e^{-K(t - t_0)}]$$

where $l_t$ is the shell height at age $t$, $l_\infty$ is the asymptotic or maximum shell height, and $K$ is the Brody growth coefficient. We assumed that shell height at birth ($l_0$) was zero. Traditionally, the parameters $l_\infty$ and $K$ are estimated by rearranging the formula and regressing $l_{t+1}$ versus $l_t$ or using nonlinear estimation procedures (Hilborn & Walters, 1992). These approaches, however, generally assume no individual variability, which can result in biased estimates and standard errors (Hart & Chute, 2009). To incorporate individual variation, we modelled $l_\infty$ and $K$ hierarchically as:

$$l_\infty,i = \beta + u_{\infty,i}$$

$$K_i = \gamma + u_{K,i}$$

where $\beta$ and $\gamma$ are the mean asymptotic/maximum shell height (henceforth, maximum shell size) and mean Brody growth coefficient (henceforth, growth coefficient), respectively, and associated random effects ($u$) that were
assumed to be normally distributed among individual mussels \((i)\) with a mean of zero and variance \(\tau_e\) and \(\tau_w\), respectively. We also added a residual term \((e)\) to the von Bertalanffy (1938) equation:

\[
l_{t,i} = l_{\infty,i}[1 - e^{-K}] + e_{t,i}
\]

that was assumed normally distributed with a mean of zero and variance \(\sigma^2\). We initially fit the model to each species individually to estimate the predictable variation in \(l_{\infty}\) and \(K\) among individuals. We then evaluated the influence of seasonal flow regime components and site-specific stream characteristics on \(l_{\infty}\) and \(K\) by modelling each as a linear function of predictor variables in a single model. All models were fit using Markov Chain Monte Carlo (MCMC) as implemented in BUGS software, version 1.4 (Lunn et al., 2000) with 650 000 iterations, 20 000 burn in, thinning at 25 and diffuse priors. These values were determined by fitting the global (all parameters) model with 100 000 iterations and evaluating the output with the Raftery & Lewis (1995) diagnostic as implemented in the R package Coda (Plummer et al., 2006). The BUGS code used for this analysis can be obtained from the corresponding author.

Prior to constructing candidate models, we calculated Pearson correlation coefficients for all pairs of potential predictor variables. To avoid multicollinearity, we only included uncorrelated predictor variables \((r^2 < 0.45)\) together in candidate models. We also created binary indicator variables (i.e. 0 or 1) for the following categorical variables: species, with \(V.\ vibex\) coded as 1 when the species was \(V.\ vibex\) and 0 otherwise and \(E.\ crassidens\) coded as 1 when the species was \(E.\ crassidens\) and 0 otherwise (i.e. \(V.\ liensoa\) served as the statistical baseline species); channel confinement, with unconfined channels coded as 1 and 0 otherwise; tagged and notched specimens, coded as 1 for year following tagging and notch- ing and 0 otherwise; and physiographic province, with Dougherty Plain sites coded as 1 and 0 otherwise.

We used an information-theoretic approach, described by Burnham & Anderson (2002), to evaluate the relative plausibility of models relating seasonal streamflows, site-specific stream characteristics and species characteristics to the annual growth of the three focal mussel species. Our primary hypotheses of interest were to evaluate the relative influence of streamflow regime components on the growth coefficient \((K)\). Secondarily, we sought to determine the influence of stream characteristics and anthropogenic land use on mussel growth coefficient and maximum shell size \((l_{\infty})\). Thus, we contrasted four sets of growth coefficient models that included either 10-day seasonal low discharge, median seasonal discharge, 10-
the global model for each flow component using a simple discrepancy measure (Gelman, Meng & Stern, 1996).

Results

Fifty-three of the 830 total thin-sectioned mussels were omitted from the data set due to excessive erosion or failure to reach age consensus. The remaining 777 mussel thin sections were included in the analysis, although not all species were collected at all 20 sites. In total, field crews collected 402 *V. lienosa*, 282 *V. vibex* and 93 *E. crassidens* at 18, 17 and 3 sites, respectively. *Elliptio crassidens* was only found at the two mainstem Flint River sites and at one site in Chickasawhatchee Creek. *Villosa lienosa*, *V. vibex* and *E. crassidens* represented 52, 36 and 12% of the total number of mussels analysed, respectively. Fifty-six specimens were previously tagged, and 16 *V. lienosa* and 5 *V. vibex* were tagged and notched for annuli validation and age verification.

Annuli validation

Annulus production was present in 67% of notched *Villosa* species. Thin-section photographs of three notched mussels (i.e. 14% of total) failed to capture the notch, whereas four notched mussels (i.e. 19% of total) had little growth post-notching, presumably because they were older than other notched individuals. All observers agreed that 14 notched *V. lienosa* and *V. vibex* displayed a disturbance ring (i.e. response to notching) at the bottom of the notch followed by one true annulus post-notching (Fig. 3). No thin section had multiple annuli post-notching.

Of the focal species, *E. crassidens* had the oldest and largest individuals in the study that averaged 15 years old and 45 mm height, respectively. Twelve per cent of *E. crassidens* were estimated as older than 25 years, and the oldest specimen collected was estimated at 46 years. *Villosa lienosa* and *V. vibex* were younger and smaller than *E. crassidens* with average ages of six and seven, respectively. Shell heights for these species averaged 27 and 30 mm, respectively.

Site-specific stream characteristics

Five sample sites were located near a USGS gage and published models were available for estimating discharge at seven sites (Table 1). Discharge models created to estimate discharge at the remaining eight ungaged sites fit well ($r^2 > 0.95$). Seasonal discharges in the LFRB
varied substantially during the lifespan of mussels used in this study. During an average 6 year lifespan of *V. lienosa* and *V. vibex*, the flows experienced by mussels included above average flows during 3 years (2005, 2009–2010) and below average flows during 3 years (2006–2008; Fig. 4). Stream discharges at sampled sites also included observed flow cessation (zero discharge) events.

**Mussel growth models**

The hierarchical von Bertalanffy growth models fit with no predictor variables indicated substantial variation in growth among individuals and sites. On average, maximum shell size ($l_\infty$) varied among individual mussels from a low of 13% ($\sqrt{38.1/47.1}$) for *E. crassidens* to a high of 23% for *V. vibex* (Table 2). Mussel growth coefficients ($K$) were similarly variable among individuals and sites and ranged from a low of 24% for *V. lienosa* to a high of 45% for *E. crassidens*. The best approximating model for predicting the mussel growth parameters explained, on average, 75% of the variation in maximum shell size and 32% of the variation in the growth coefficient. The best approximating von Bertalanffy growth models included: maximum shell size modelled as a function of species, link magnitude, unconfined stream channel and link magnitude by species interaction; and the growth coefficient modelled as a function of species, tagging, percentage shell erosion, link magnitude, urban and agriculture land cover, spring 10-day high discharge and associated quadratic term and spring 10-day high discharge by link magnitude interaction (Table 3). Model weights ($w$) indicated that the best approximating model was 8.6 times more likely than the second-best approximating model, which contained the same predictor variables with agriculture land cover replacing unconfined channel morphology in the maximum shell size model. The three best approximating models represented our confidence set of models (Table 3).

Maximum shell size (i.e. shell height) differed among species and was related to stream size and stream channel confinement. Maximum shell size was greatest for *E. crassidens* and least for *V. lienosa*, with the difference in maximum shell size between the two species averaging 18.4 mm (Table 4). Maximum shell size was positively but weakly related to stream size for both *E. crassidens* and *V. vibex*, but not *V. lienosa* (Fig. 5).

**Table 1** A summary of flow regime components and the characteristics of the 20 study sites that were used to model the growth of mussels in the lower Flint River Basin, Georgia

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<th>Flow regime component</th>
<th>Mean (SD)</th>
<th>Minimum</th>
<th>Maximum</th>
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<tr>
<td>Spring Long-term average discharge</td>
<td>0.010 (0.014)</td>
<td>0</td>
<td>0.12</td>
</tr>
<tr>
<td>10-day low discharge</td>
<td>0.007 (0.007)</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>10-day high discharge</td>
<td>0.071 (0.112)</td>
<td>0.01</td>
<td>0.98</td>
</tr>
<tr>
<td>Summer Long-term average discharge</td>
<td>0.007 (0.007)</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>10-day low discharge</td>
<td>0.004 (0.004)</td>
<td>0</td>
<td>0.02</td>
</tr>
<tr>
<td>10-day high discharge</td>
<td>0.039 (0.113)</td>
<td>0</td>
<td>1.84</td>
</tr>
</tbody>
</table>

Site characteristics

| Watershed area (km$^2$)  | 1228 (2567.3) | 39      | 7989    |
| Link magnitude           | 964.1 (2385.43) | 11      | 8104    |
| Percentage agriculture land cover | 36.9 (17.01) | 3       | 58      |
| Percentage urban land cover | 8.5 (11.68)  | 2       | 45      |

Site-specific flow regime components were standardised (divided) by watershed area.

![Fig. 4 Estimated discharge for Ichawaynochaway Creek, Georgia, at U.S. Geological Survey gage number 02353500 for 1995 through 2011 (grey line) in comparison to mean estimated discharge based on 106 year of data (black line).](https://example.com/fig4.png)
Parameter estimates also indicated that maximum shell size was, on average, 2.6 mm smaller in streams with unconfined channels for all species (Table 4). The growth of freshwater mussels, as indexed by the growth coefficient (K), differed among species. Parameter estimates indicated that the growth coefficient of *E. crassidens* was substantially smaller than *V. vibex* and *V. lienosa*, respectively (Table 4). However, parameter estimates for *V. vibex* were small and imprecise in all models in the confidence set suggesting that, on average, the growth of *V. vibex* was not biologically different than *V. lienosa* (Fig. 5). Parameter estimates for tagging effect and percentage shell wear were small and relatively imprecise (Table 4).

The mussel growth parameter was strongly and positively related to spring 10-day high discharge (Table 3). However, the flow component quadratic terms revealed that growth decreased as the magnitude of these flows increased, suggesting a non-linear relationship between streamflow and growth (Fig. 6). Similarly, the interaction between flow and link magnitude suggested that the effect of flows decreased with stream size (Table 4). The mussel growth parameter also was related to stream characteristics and land use in the watershed. Parameter estimates indicated that mussel growth was positively associated with the amount of agricultural land cover and urban land cover within corresponding watersheds. Of these factors, agricultural land cover appeared to have the greatest effect on mussel growth (Fig. 6).

**Discussion**

Growth chronologies recorded in freshwater mussel shells can provide valuable insight into biotic and abiotic factors influencing these highly imperilled organisms. Despite their widely recognised conservation status, comprehensive mussel age and growth analyses are largely unpublished and have only recently gained attention in North American freshwater mussel research (Haag & Commens-Carson, 2008; Rypel *et al.*, 2008; Haag, 2009; Rypel, Haag & Findlay, 2009; Black *et al.*, 2010; Haag & Rypel, 2011; Tang *et al.*, 2013; Daniel & Brown, 2014). Nonetheless, validation of annulus formation during such studies is necessary to ensure accurate and meaningful results (Beamish & McFarlane, 1983). Shell notching of the ventral margin is among the most convenient and reliable annuli validation methods for freshwater mussel field studies (Neves & Moyer, 1988) and was therefore our method of choice. We validated the formation of a single annulus post-notching in 67% of notched *V. lienosa* and *V. vibex*, which falls between the 27% rate reported by Neves & Moyer (1988) and 92% by Haag & Commens-Carson (2008). The lack of multiple annuli post-notching in any thin section also verified annuli formation and assisted observers in distinguishing between true and pseudo-annuli. Annulus production was most evident in young individuals that

### Table 2

<table>
<thead>
<tr>
<th>Species</th>
<th>$l_∞$ (Mean)</th>
<th>$K$ (Mean)</th>
<th>τ (Mean)</th>
<th>$l_∞$ (SE)</th>
<th>$K$ (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Villosa lienosa</em></td>
<td>23.4 (0.158)</td>
<td>21.3 (1.00)</td>
<td>0.722 (0.012)</td>
<td>0.031 (0.0018)</td>
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<tr>
<td><em>Villosa vibex</em></td>
<td>26.6 (0.398)</td>
<td>38.9 (3.50)</td>
<td>0.749 (0.017)</td>
<td>0.051 (0.0032)</td>
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<tr>
<td><em>Elliptio crassidens</em></td>
<td>47.1 (0.679)</td>
<td>38.1 (1.76)</td>
<td>0.335 (0.011)</td>
<td>0.022 (0.00147)</td>
<td></td>
</tr>
</tbody>
</table>

$\tau$ is an estimate of the predictable variability of the parameter among individuals and sites.

### Table 3

<table>
<thead>
<tr>
<th>Candidate model</th>
<th>pD</th>
<th>DIC</th>
<th>ΔDIC</th>
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</tr>
</thead>
<tbody>
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<td>$l_∞$(<em>Elliptio crassidens</em>, <em>Villosa vibex</em>, Link magnitude, Unconfined, Link magnitude<em>E. crassidens</em>, Link magnitude<em>V. vibex</em>, K (<em>E. crassidens</em>, <em>V. vibex</em>, Tagging, Percentage shell wear, Link magnitude), Percentage Urban, Percentage Agriculture, Spring 10-day high discharge, Spring 10-day high discharge<em>2, Spring 10-day high discharge</em>link magnitude)</td>
<td>1101.74</td>
<td>23564.9</td>
<td>0</td>
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<tr>
<td>$l_∞$(<em>E. crassidens</em>, <em>V. vibex</em>, Link magnitude, Percentage agriculture, Link magnitude<em>E. crassidens</em>, Link magnitude<em>V. vibex</em>, K (<em>E. crassidens</em>, <em>V. vibex</em>, Tagging, Percentage shell wear, Link magnitude), Percentage Urban, Percentage Agriculture, Spring 10-day high discharge, Spring 10-day high discharge<em>2, Spring 10-day high discharge</em>link magnitude)</td>
<td>1101.48</td>
<td>23569.2</td>
<td>4.3</td>
<td>0.101</td>
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<td>$l_∞$(<em>E. crassidens</em>, <em>V. vibex</em>, Link magnitude, Unconfined, <em>E. crassidens</em> Link magnitude, <em>V. vibex</em> Link magnitude, K (<em>E. crassidens</em>, <em>V. vibex</em>, Tagged, Shell wear, Link magnitude, Percentage Urban, Percentage Agriculture, Spring 10-day low discharge, Spring 10-day low discharge*2)</td>
<td>1101.26</td>
<td>23571.6</td>
<td>6.7</td>
<td>0.030</td>
</tr>
</tbody>
</table>
had high annual growth. Previous mussel age and growth studies attributed inabilities to distinguish annuli post-notching to minimal annual growth (Neves & Moyer, 1988; Haag & Commens-Carson, 2008; Rypel et al., 2008). The lack of validated annuli production in 33% of notched thin sections in our study was likely due to our inability to distinguish annuli in slower growing and larger notched specimens. This, combined with the observed annuli production in 67% of notched specimens, suggests that freshwater mussels in our study consistently produced annuli. Therefore, we believe that thin sectioning was an appropriate and effective method for determining the age and annual growth of freshwater mussels in the LFRB.

Differences in growth and maximum size among species in our study are consistent with that of other North American mussel species (Haag & Rypel, 2011) in that E. crassidens attained greater size and age, but exhibited slower growth than V. lienosa and V. vibex which exhibited similar growth rates and lower maximum ages. We estimate that it takes, on average, 4 years for Villosa species to reach 95% of their maximum size and 8 years for E. crassidens, which is likely indicative of different life-history strategies of these species. Villosa lienosa and V. vibex are long-term brooders that mature early and use ubiquitous Centrarchid species as hosts (Keller & Ruessler, 1997; Haag 2012). In contrast, E. crassidens is a short-term brooder that presumably parasitises the genus Alosa (Howard, 1914), a group of fishes that is frequently characterised by variable year class strength. Hence, Villosa species have greater opportunities for successful reproduction over a short life, whereas E. crassidens may require more reproductive seasons as a compensatory response to annual variability in environmental conditions and host fish populations.

The majority of recent bivalve age and growth studies assume that maximum shell size and growth coefficients are constant among individuals within a population.
(Hart & Chute, 2009; Tang et al., 2013). However, Haag & Rypel (2011) and Haag (2012) reported variation in growth between sexes within populations of several mussel species in the Sipsey River, Alabama. Using the hierarchical Bertalanffy growth models, we found that mussel growth coefficients and maximum shell sizes varied substantially among individuals within and among populations (i.e. study sites). We also found that some of the variation was related to differences among species, site-specific characteristics and stream flows. However, significant variation in the growth coefficient and maximum shell size remained after accounting for these factors. To our knowledge, this study is among the first to explicitly model variation in these parameters making it difficult to assess whether the variation is typical of stream-dwelling mussels. The variation in these two parameters represents predictable differences among study sites and mussels that were unaccounted for by the predictors in the best approximating models. Given the relatively large number of study site characteristics examined, we believe that much of this variation reflects differences among individual mussels due to behavioural or physiological differences. Sex may also explain some differences in maximum size and growth among mussels within a site (Haag & Rypel, 2011), but we did not include sex as a predictor because only *V. lienosa* exhibited consistently recognisable sexual dimorphism among our study species. Regardless of the source of the variation, our study suggests that variation in growth among mussels is relatively large and needs to be explicitly incorporated into evaluations of mussel growth. Failure to do so can lead to biased model estimates, standard errors and estimates of model fit, such as coefficients of determination (Hart & Chute, 2009).

Variation in mussel growth was related to spring 10-day high discharge, which was among the most significant factors influencing mussel growth in our study. We also found a strong, quadratic relationship between spring 10-day high discharge and mussel growth, suggesting that the effect of flows on mussel growth is likely more complex than previously reported (Rypel et al., 2009; Black et al., 2010). Although the specific processes that caused this relationship are unclear, several hypotheses may explain this pattern of growth in response to stream flows. Because stream transport is dependent upon water velocity, particulate matter may settle out of the water column and become unobtainable to suspension-feeding mussels under very low flow conditions (Vaughn & Hakenkamp, 2001) until resuspended.

Fig. 6 Estimated relation between spring 10-day high discharge and the *Villosa lienosa* growth coefficient ($K$) for three stream sizes (top), three levels of urban cover (middle) and three levels of agricultural land cover (bottom). The growth coefficient was estimated with the best approximating model and assuming untagged mussels, an unconfined stream channel, and median values for link magnitude and land cover as default values. The three levels contrasted in each plot represent the median and upper and lower quantiles observed during the study. Standardised flows are expressed in discharge ($m^3 s^{-1}$) per km$^2$ watershed area.

during increasing stream flows. Rypel et al. (2009) and Black et al. (2010) speculated that increased total suspended solids (TSS) associated with high stream flows may increase processing cost, thus reducing growth of mussels at high flows. However, TSS does not appear to affect mussel growth (Gascho Landis, Haag & Stoeckel, 2013), suggesting that other mechanisms influence mussel growth at high stream flows. Alternatively, reduced growth at increased stream flows could represent interruptions in feeding due to behavioural responses to evade scouring during increasing stream discharges.

Mussel growth and maximum size also were related to several reach- and watershed-level variables measured in our study, presumably because mussel growth and size is a function of food quality and availability. Mussels occurring in eutrophic waterbodies frequently reach greater lengths than those occurring in less productive systems (Kesler & Van Tol, 2000; Anthony et al., 2001; Kesler, Newton & Green, 2007). Increased maximum size of mussels with increasing stream size in our study is consistent with the river continuum concept (Vannote et al., 1980). Under this concept, small streams contain greater ratios of allochthonous rather than autochthonous materials generally found in larger streams. Because clearance rates are functions of mussel gill-surface morphology (Silverman et al., 1995; Galbraith et al., 2009) and algal flux (Vanden Byllaardt & Ackerman, 2014), large allochthonous materials must undergo extensive processing before they are transported and ingested by mussels. However, large streams not only have high proportions of autochthonous materials, which are generally small and can be readily utilised by mussels, but also allochthonous input, which could increase the amount and diversity of food particles available for mussels. Hence, mussels occupying large waters likely have higher clearance rates than those in smaller waters because large waters have substantially higher flows but variable concentrations of food particles consumed by mussels which may also explain the decreasing influence of spring 10-day discharge on mussel growth in large streams.

The growth and maximum size of mussels occupying small streams and streams with unconfined channels were smaller that conspecifics occupying larger streams or similar sized confined channel streams. Small streams tend to experience more frequent and intense disturbances (Resh et al., 1988; Reice, Wissmar & Naiman, 1990). Similarly, diurnal fluctuations in temperatures and dissolved oxygen in unconfined streams are greater than similar sized confined channel streams and maximum temperatures tend to be higher and minimum dissolved oxygen levels lower during the summer (Li, 2006). Mussels that inhabited small streams and those with unconfined channels in the LFRB likely experienced harsher and more variable environmental conditions. Therefore, we hypothesise that smaller maximum size of mussels in small and unconfined streams may represent a life-history trade-off in which mussels mature at younger ages and attain maximum sizes more quickly than those in non-disturbed streams, increasing opportunities to reproduce in disturbed habitats.

The hierarchical von Bertalanffy growth models developed in our study broadened our understanding of freshwater mussel ecology by accounting for variability in growth and maximum size parameters among individual mussels. The model structure also allowed us to relate the values of parameters to various site and watershed characteristic in a single model, which is in sharp contrast to previous investigations (e.g. Rypel et al., 2009; Black et al., 2010; Haag & Rypel, 2011). Although our results are limited to three relatively common Flint River Basin species, this approach is directly applicable to other mussel species occurring across watersheds and has potential to increase our knowledge of ecological processes affecting this imperilled fauna. We were restricted to using only common species in our study because shell thin sectioning required sacrificing live animals in the Flint River basin as insufficient shell material is generally found in the basin. However, other basins having dense mussel populations with abundant middens may have greater opportunities to apply this analysis to additional and imperilled species, which will undoubtedly aid in their conservation and management.

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