



Periodic growth and growth cessations in the federally endangered freshwater mussel Cumberlandian combshell using a hierarchical Bayesian approach

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ABSTRACT: Understanding and monitoring life history traits is often important in endangered species conservation. Populations of the endangered mussel Cumberlandian combshell *Epioblasma brevidens* have continued to decline in the Powell River, USA. Understanding and modeling mussel growth is critical for effective reintroduction of this endangered species. In this study, 2 yr old *E. brevidens* that were produced in our laboratory were released to the Powell River in 2009 to augment this declining population. A mark-recapture monitoring approach using passive integrated transponder (PIT) tags was used to assess the survival and growth of the released mussels. Hierarchical Bayesian growth models incorporating individual growth variations, periodic growth and growth cessations, along with multiple release occasions were developed and compared to the classic von Bertalanffy growth model. Our results showed that the hierarchical model that incorporated individual growth variation gave the best estimates of model parameters, yielding the lowest deviance information criterion value. Mussels exhibited different growth rates (K), including 0.015, 0.026, 0.110 and 0.050 (mo^{-1}), corresponding to the duration of laboratory culture (ages 2, 3 and 4 yr old) and a growth cessation (GC) for 5.98 mo, respectively. The other parameters of asymptotic length (L_{∞}) and age at zero length (t_0) were 51.36 mm and -0.648 mo. The flexible structure of Bayesian hierarchical models allowed us to examine growth characteristics of *E. brevidens* in a changing environment to better understand the details of its growth and lifespan, thus providing useful data for conservation management.

KEY WORDS: Freshwater mussel · Cumberlandian combshell · *Epioblasma brevidens* · Growth rate · Asymptotic length · Growth cessation · Periodic growth · von Bertalanffy growth model · Bayesian hierarchical model

INTRODUCTION

The freshwater mussel fauna (Unionoida) of North America contains the highest diversity in the world, with 281 species and 16 subspecies (Williams et al. 1993, Neves 2008, Graf 2013); however, it is the most

imperiled group of freshwater animals in North America (Ricciardi & Rasmussen 1999, Haag 2012). At least 29 species have gone extinct over the last 100 yr (Haag & Williams 2014); 75 species are currently listed in the USA as federally endangered, and an additional 13 species are threatened (Williams et

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al. 1993). To reduce the risk of species extinction, various conservation management strategies have been implemented over the last 30 yr, including reintroduction and augmentation of populations with hatchery propagated mussels (National Native Mussel Conservation Committee 1998, Hua et al. 2013) and translocation of adults to sites and populations in need of restoration (Cope & Waller 1995, Hamilton et al. 1997).

The Powell River, located in southwestern Virginia and northeastern Tennessee of the eastern USA, is part of the upper Tennessee River watershed. It supports a unique assemblage of >35 extant mussel species, 13 of which are listed as endangered species in the United States Endangered Species Act (ESA) of 1973. However, quantitative mussel surveys conducted in the Powell River from 1979 to 2004 documented a 63% decline in mean mussel density (mussels m^{-2}) (Ahlstedt et al. 2005). A more recent quantitative survey conducted from 2008 to 2010 revealed that mussel populations have continued to decline in the river, including the Cumberlandian combshell *Epioblasma brevidens* (Johnson et al. 2011). This species is listed as endangered under the ESA and is also considered globally Critically Endangered on the International Union for Conservation of Nature (IUCN) Red List. The recovery plan for *E. brevidens* recommends reintroduction of propagated mussels into previously occupied habitats (USFWS 2004). Hence, an effective approach for monitoring populations of this and other reintroduced mussel species is essential in order to evaluate population viability, shell and body growth, life history traits, habitat suitability, and the success of conservation efforts.

Body size, growth, and survival are important life history traits that have been monitored and analyzed by fisheries scientists for decades (Hilborn & Walters 1992, Haddon 2001). In fisheries, the most commonly used model to describe fish growth was developed by von Bertalanffy (1938), and this has since been applied to the analysis of growth in freshwater mussels (Neves & Moyer 1988, Hastie et al. 2000, Miguel et al. 2004, Jiao et al. 2008, Haag & Rypel 2011, Jones & Neves 2011). The von Bertalanffy growth model (VBGM) is expressed mathematically as a 3-parameter equation, $L_t = L_\infty[1 - e^{-K(t-t_0)}]$, where L_∞ is the theoretical maximum body length, K is the growth rate coefficient, and t_0 is the hypothetical age at zero length. The parameters in the equation are typically analyzed by combining data for all individuals in a population. Hence, the standard VBGM model assumes homogeneity of individuals in a population dynamic assessment, which can introduce bias into

model construction and parameter estimation (Pledger et al. 2003). A result of introducing bias is that estimates of model parameters are poorly characterized (Conner & White 1999, Cam et al. 2002). For example, growth rates of individuals can vary substantially with temperature, food availability, population density, and habitat location (Krohn et al. 1997, Swain et al. 2003, Kimura 2008, Jiao et al. 2010). Hence, growth models that incorporate individual variations are considered more realistic and appropriate to describe the growth pattern of a population (James 1991, Smith et al. 1997, Alós et al. 2010, Tang et al. 2014). These models incorporate residual variance into the modeling process, so growth heterogeneity of individuals can be assessed to better understand the species' population biology (Pledger et al. 2003). A Bayesian approach using Markov Chain Monte Carlo (MCMC) algorithms is considered the most efficient mathematical framework to incorporate and reduce model uncertainty (Wade 2000, Gimenez 2008).

The growth of aquatic animals often exhibits strong seasonal oscillations, mainly due to fluctuations in temperature, food supply (Shul'man 1974), body reserves (Bacon et al. 2005), and even social behavior (Alanara et al. 2001). Freshwater mussels experience growth cessation (GC) during the winter months in most North American geographic locations (Downing et al. 1992, Downing & Downing 1993, Anthony et al. 2001). Hence, the VBGM has been discussed and modified to allow for seasonal oscillations during specific growth periods. The seasonal growth model was originally proposed by Ursin (1963) and has been modified by including a sine-wave function into the traditional VBGM to allow for seasonal oscillations (Pitcher & MacDonald 1973, Cloern & Nichols 1978, Pauly & Gaschultz 1979, Somers 1988, Hoenig & Hanumara 1990, Allison 1994). However, earlier versions of the seasonal oscillating models may not have been well suited for characterizing mussel growth (Pauly et al. 1992). Further, another challenging issue in freshwater mussel conservation management is how to evaluate growth rates of populations that are exposed to a suite of environmental stressors, such as pollution and climate change, because changes in environmental conditions can cause variations in growth rates of successive cohorts or stocks of same species (Rizvi et al. 2012). Overall, estimates of growth parameters (K and L_∞) have not incorporated individual variations, seasonal variations, or growth cessations for freshwater mussels, primarily due to an absence of reliable data and advanced demographic modeling techniques.

Effective conservation and restoration management of endangered mussels will require a thorough understanding of the species' life history traits and population dynamics. Thus, the purpose of our study was to develop a new model incorporating individual variations and seasonal variations with GC to model the growth of *E. brevidens*. We also demonstrate applications of stochastic analyses with modifiable models through a Bayesian approach to meet the various realistic environmental circumstances.

MATERIALS AND METHODS

Laboratory propagation and tagging of mussels

Juveniles of *Epioblasma brevidens* were propagated and cultured at the Freshwater Mollusk Conservation Center (FMCC) of the Department of Fish and Wildlife Conservation, Virginia Tech, Blacksburg, VA, USA. All juveniles were from 1 propagation effort in 2007. Juveniles were reared in 2 culture systems utilizing pond water, one of which was an open-water recirculating system and the other one was a closed recirculating aquaculture system. In winter, a mixed algal diet (Shellfish Diet 1800 and Nanno 3600; Reed Mariculture) was used as supplemental food for juveniles, which were fed at a mean concentration of 35 000 cells ml⁻¹. Newly transformed juvenile mussels were cultured in tanks with fine sediment (<200 µm) at a depth of 1 to 2 mm, and were moved to a limestone gravel substrate (2 to 5 mm) for grow-out when they reached a length of 3 mm. Juvenile size (length) was measured and counted on 12 sampling occasions during the 2 yr culture period. Survival rates were calculated as percent of the number collected in the previous sampling period.

Once juvenile mussels were >20 mm long, they were tagged using Bulk PIT tags (TX1411SST; Biomark) to ensure high recapture rates of each individual. Glue-on shellfish tags (Type FPN; Hallprint) were also attached to the other valve. Double tagging on both valves of the mussels secured the identification information during the monitoring process. Tagged juvenile mussels were released at Brooks Bridge (36° 32' 05.5644" N, 83° 26' 30.3432" W), Powell River, Tennessee, USA, on multiple occasions, including 1 July 2009, 26 August

2009, 7 October 2009, 25 June 2010, and 11 October 2010 (Fig. 1). They were then relocated using the PIT tag detector for measurement of length, then returned to the release site. A total of 8 mark-recapture sampling events were implemented during this study.

Water quality measurement

Water temperature was determined using a YSI multi-probe water quality meter (model 55/12 FT). Organic matter content was measured through ash free dry weight (AFDW) following standard methods for the examination of water and wastewater (Method 10300 C, D; APHA 1998).

Models and equations

Two new growth models were developed and the VBGM was applied in this study with the following assumptions: (1) labeled juvenile mussels retained their tags throughout the study, and PIT tags were detectable without negative influence on their survival; (2) variation in mussel length followed a certain random distribution; and (3) sampling protocol and study area were constant.

In the formulation for Model 1, the VBGM was applied as:

$$L_t = L_{\infty}[1 - e^{-K(t-t_0)}] \quad (1)$$

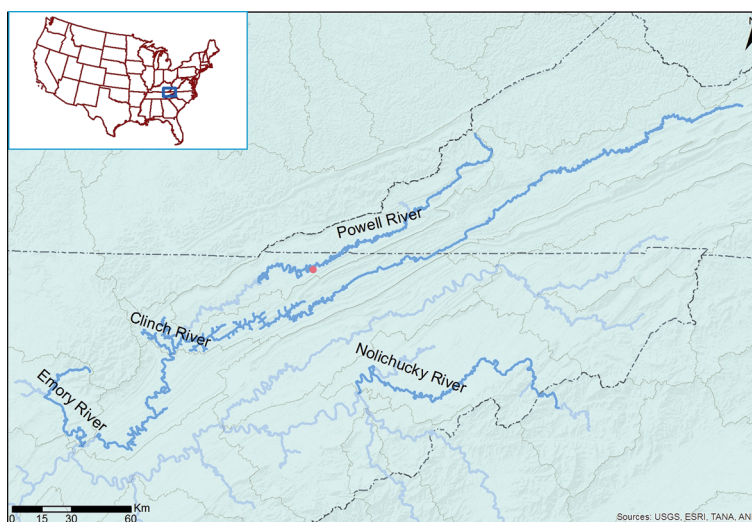


Fig. 1. Release location (red dot) of juvenile mussels of *Epioblasma brevidens* at Brooks Bridge (36° 32' 05.5644" N, 83° 26' 30.3432" W) in the Powell River, Tennessee, USA

where L_t represents the length of the mussel at time t (months), L_∞ (asymptotic length) denotes the theoretical average maximum body length, K represents growth rate coefficient that defines growth rate toward the maximum, and t_0 represents the hypothetical age when mussels were at zero length.

Applying the Bayesian approach to Model 1, a traditional and non-hierarchical VBGM was constructed as:

$$E(L_j) = L_\infty[1 - e^{-K(t_j - t_0)}] \quad (2)$$

where j represents the capture occasion (or sampling measurement, $j = 0, 1, 2, \dots, 8$; $j = 0$ denotes the time of mussel release); $E(L_j)$ denotes the expected mussel length on the j^{th} capture occasion; t_j represents the mussel age (months) on the j^{th} occasion; L_∞ , K and t_0 are corresponding parameters to those described above, and their prior probability distributions were $U(45, 100)$, $U(0, 1)$ and $U(-2, 2)$ separately. L_j was assumed to follow a normal distribution, $N(E(L_j), \sigma_L^2)$, and prior of σ_L^2 was assumed to be $U(0, 400)$.

Since released mussels exhibited uneven or abnormal growth with a potential non-growth season and different annual growth rates during the sampling observations (see Fig. 3), 2 new models were developed based on the above preliminary diagnostics to explore the growth of *E. brevidens* for the purposes of propagation and conservation. The newly developed models were then compared with the commonly used VBGM to test their goodness-of-fit.

The formulation for Model 2 included 2 scenarios. (1) Juvenile growth during the culture period in laboratory was analyzed using the VBGM to evaluate the parameters of K , t_0 and L_∞ , and (2) Model 2 was reconstructed from the VBGM based on the time-based length increment of the tagged mussels (Fabens 1965, Jiao et al. 2010) after they were released to the river:

$$E(L_t) = L_\infty[1 - e^{-K(t - t_0)}] \quad (3)$$

before mussel release (Model 2-1),

$$E(L_{t+\Delta t}) = L_t + (L_\infty - L_t)(1 - e^{-K\Delta t}) \quad (4)$$

after mussel release (Model 2-2),

where L_t represents mussel length at time t (months); $L_{t+\Delta t}$ denotes mussel length at time $t+\Delta t$; L_∞ , K , and t_0 are the same as in Eqs. (1) & (2). Δt is the duration between $t+\Delta t$ and t . The other parameters are the same as Eq. (1).

Applying the Bayesian approach, Model 2-2 incorporated variations in annual growth rates and considered the non-growth seasons, while Model 2-1 did not because all the mussels were cultured in the lab

environment during this time period. Model 2, the Bayesian non-hierarchical model was constructed as:

$$E(L_j) = L_\infty[1 - e^{-K_1(t_j - t_0)}] \text{ if } t_j < \text{the age at release,} \quad (5)$$

$$E(L_j) = L_{j-1} + (L_\infty - L_{j-1})[1 - e^{-K_g(\Delta t_{j-1} - \text{GC})}] \quad (6)$$

if $\Delta t_{j-1} > \text{GC}$ after 1 November,

$$E(L_j) = L_{j-1} \text{ if } \Delta t_{j-1} < \text{GC after 1 November} \quad (7)$$

where j represents the j^{th} capture occasion; $E(L_j)$ represents the expected mussel length at the j^{th} capture occasion; and L_j denotes the mussel length on the j^{th} occasion, and follows a normal distribution with mean $E(L_j)$ and variance σ_L^2 . K_1 denotes the growth rate of juvenile mussels cultured in laboratory; K_g denotes the growth rates of released mussels at various stages ($g = 2, 3, 4$ represents the time in 2009, 2010 and 2011 along with the ages of mussels, 2, 3 and 4 yr, respectively); Δt_{j-1} denotes the duration (months) between the recapture occasion of $(j-1)^{\text{th}}$ and j^{th} ; GC is the annual growth cessation (months), which begins on 1 November based on empirical growth data at the release site. Mussels were released on multiple occasions; therefore, this model was constructed on the combinations of separated analyses of each release occasion to minimize uncertainties. Priors of L_∞ , K , t_0 and σ_L^2 were assumed to be the same as in Model 1, and the prior of GC here was $U(2, 6)$.

Model 3 was generated from Model 2, additionally incorporating individual variations of released mussels since each mussel had an identical tag number at the release. Model 3, the Bayesian hierarchical model was constructed as:

$$E(L_j) = L_\infty[1 - e^{-K_1(t_j - t_0)}] \text{ if } t_j < \text{the age at release} \quad (8)$$

$$E(L_{i,j}) = L_{i,j-1} + (L_{\infty,i} - L_{i,j-1})[1 - e^{-K_{i,g}(\Delta t_{j-1} - \text{GC}_i)}] \quad (9)$$

if $\Delta t_{j-1} > \text{GC}_i$ after 1 November

$$E(L_{i,j}) = L_{i,j-1} \text{ if } \Delta t_{j-1} < \text{GC}_i \text{ after 1 November} \quad (10)$$

$$L_{\infty,i} \sim N(\bar{L}_\infty, \sigma_{L_\infty}^2)I(45, 100)$$

$$\bar{L}_\infty \sim U(45, 90)$$

$$\sigma_{L_\infty}^2 \sim U(0, 400)$$

$$K_{i,g} \sim N(\bar{K}_g, \sigma_{K_g}^2)I(0, 1)$$

$$\bar{K}_g \sim U(0.001, 0.3)$$

$$\sigma_{K_g}^2 \sim U(0.0001, 0.1)$$

$$\text{GC}_i \sim N(T, \sigma_T^2)I(2, 6)$$

$$T \sim U(2, 6)$$

$$\sigma_T^2 \sim U(0.0001, 5)$$

where i represents the i^{th} individual; j represents capture occasions; $E(L_{i,j})$ denotes the expected

length of the i^{th} mussel on the j^{th} occasion; K_1 denotes the growth rate of juvenile mussels cultured in the laboratory; $L_{\infty,i}$ and $K_{i,g}$ are corresponding parameters of mussel length and growth rate coefficient of individual i . They are assumed to follow normal distributions, described as $N(\bar{L}_{\infty,i}, \sigma_{L_{\infty,i}}^2)$ and $(\bar{K}_g, \sigma_{K_g}^2)$, in which $\bar{L}_{\infty,i}$ and \bar{K}_g (mean values of each parameter) are assumed to follow uniform distributions with the variance of $\sigma_{L_{\infty,i}}^2$ and $\sigma_{K_g}^2$, respectively. GC is assumed to follow normal distribution with mean T and variance σ_T^2 , in which T is further assumed to follow a uniform distribution. I denotes the limited boundary of the parameter distribution; the other parameters are the same as those in Model 2. Priors for hyperparameters were listed in Eq. (10) to help with clarification of the hierarchical model.

Bayesian theorem and WinBUGS program

The posterior distribution of the parameters was estimated using a Bayesian approach with the software WinBUGS version 14 (MRC Biostatistics Unit and Imperial College School of Medicine; Spiegelhalter et al. 1996). Three Markov chains were used in the analysis and Gelman Rubin statistics were used to determine the convergence of the posterior distribution (Spiegelhalter et al. 2004, Jiao et al. 2008, 2009). The burn-in iteration (50 000) and thinning interval (5) were determined based on the convergence criteria following Jiao et al. (2008). For each chain, the first 50 000 \times 5 runs were discarded, and another 5000 \times 5 runs were continued and saved. The Bayesian inference was generated from samples taken as random draws from the posterior distribution after the 3 chains reached convergence (i.e. 5000 of each chain were saved for the posterior distributions of the parameters in this case). The WinBUGS codes are available upon request or online at www.yanjiao.fishwild.vt.edu.

Goodness-of-fit

Deviance information criterion (DIC) was applied to the Bayesian model selection using MCMC simulation to determine posterior distributions of each model (Spiegelhalter et al. 2002). The DIC was used in this study to determine goodness-of-fit for the Bayesian models, and formulated as:

$$\begin{aligned} \text{DIC} &= 2\bar{D}(\theta) - \hat{D}(\theta) \text{ or } \bar{D}(\theta) + P_D \\ P_D &= \bar{D}(\theta) - \hat{D}(\theta) \end{aligned} \quad (11)$$

where D is deviance used to measure predicted goodness-of-fit for all 3 models, P_D is the effective number of parameters in the Bayesian model, \bar{D} is the posterior mean of the deviance, and \hat{D} is the deviance of the posterior mean. θ represents the stochastic nodes upon which the distribution of observation depends. Models were evaluated using rules of thumb (Burnham & Anderson 1998, Spiegelhalter et al. 2002), where a model with the smallest DIC value was defined as the best model; models within 1 to 2 DIC units of the 'best' model were also considered the best model, and models within 3 to 7 DIC units of the 'best' model were considered deficiently performed.

RESULTS

Juveniles of *Epioblasma brevidens* were successfully propagated and cultured to taggable sizes (>20 mm) for release to the Powell River in accordance with the species recovery plan (USFWS 2004). In total, 8310 juvenile mussels were produced and cultured at FMCC. As expected, juveniles grew faster in summer and slower in winter during the culture period in the laboratory, and experienced high mortality from Day 1 to Day 60, but survival rates stabilized after 2 mo (Fig. 2). Juveniles had a survival rate of 10% during the first month, 27% during the second month, and 100% thereafter on each sampling occasion.

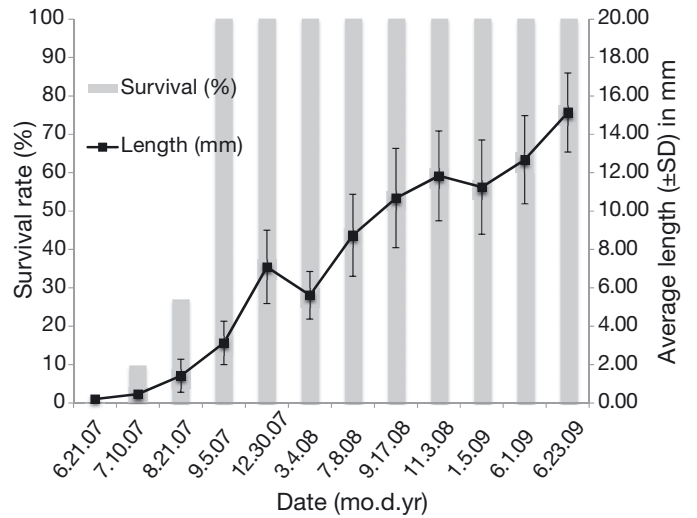


Fig. 2. Survival rate and growth of juveniles of *Epioblasma brevidens* from Day 1 to taggable size, June 2007 to June 2009

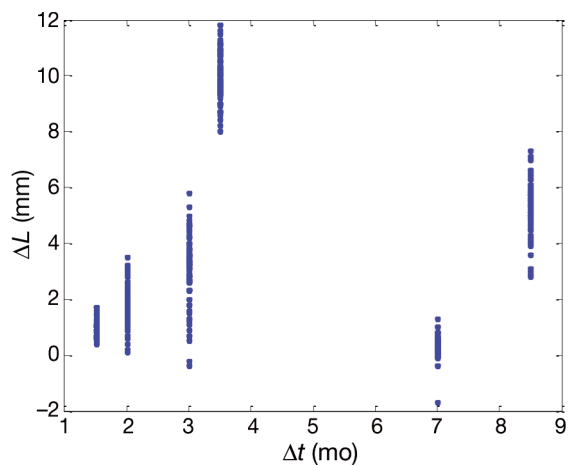


Fig. 3. Relative growth (incremental length ΔL sample duration Δt) of individual mussels at each recapture occasion

The increments of individual mussel length (Fig. 3) and their distribution (Fig. 4) of the adjacent sampling interval on each observation occasion exhibited fluctuation in the growth curve, with an annual GC or even negative growth starting from about 1 November to 1 May after mussels were released into the Powell River.

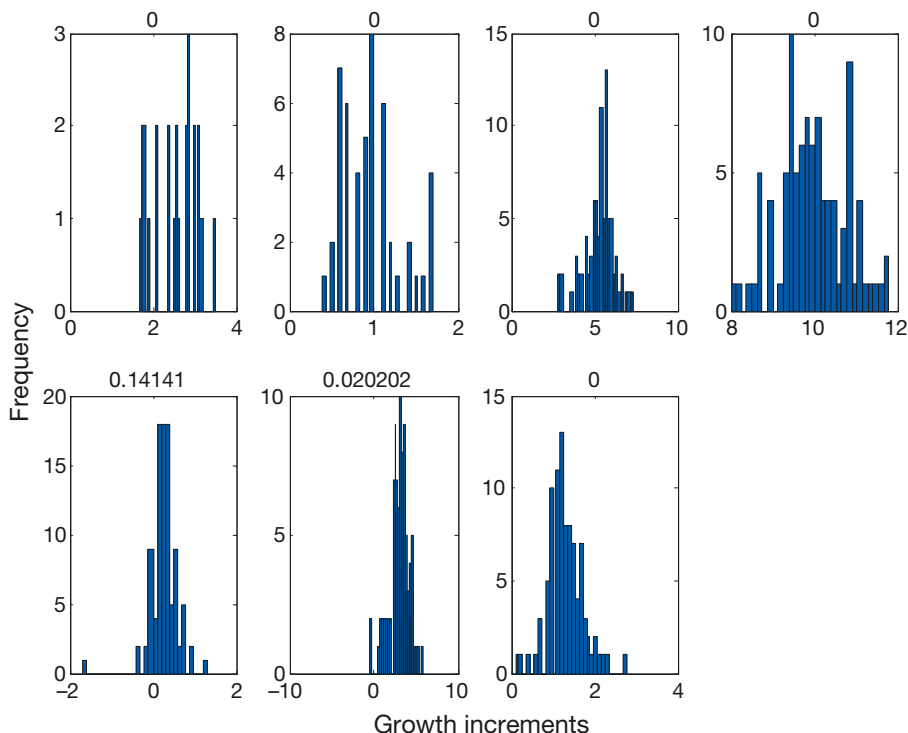


Fig. 4. Distribution of relative growth of individual mussels on each capture occasion on 26 August 2009, 7 October 2009, 25 June 2010, 11 October 2010, 10 May 2011, 17 August 2011, and 12 October 2011 (from top left to right and bottom left to right). Percentage of negative growth is shown above each histogram

Results from Model 1 showed that K , L_{∞} , and t_0 of released mussels were 0.009 mo^{-1} , 99.18 mm and 1.94 mo , respectively (Table 1). Model 2 provided 4 different growth rates of 0.015 , 0.031 , 0.105 and 0.050 mo^{-1} , respectively, which represented variations in mussel growth at 4 different stages. Obviously, 3 yr old mussels had the most rapid growth, followed by 4 yr old and 2 yr old individuals. Juvenile mussels grew slowly during their culture time in the laboratory and during the non-growth winter season, which lasted 5.975 mo . Further, L_{∞} and t_0 for *E. brevidens* estimated in Model 2 were 53.31 mm and -0.649 , respectively (Table 1). Mean growth rates of mussels at the 4 stages estimated from the hierarchical model were 0.015 , 0.026 , 0.110 and 0.050 mo^{-1} , respectively. The parameters of L_{∞} , T and t_0 agreed with the results from Model 2 as well, at 51.36 mm , 5.98 mo and -0.648 mo , respectively (Table 1). The goodness-of-fit of each model can be compared by both the DIC values and the plots of model fit (Table 1, Figs. 5–7). Model 1 (Fig. 5) had the lowest fit compared to Model 2 (Fig. 6) and Model 3 (Fig. 7), indicating that the classic VBGM is less suitable to predict mussel growth without incorporating non-growth seasons. In this study, juveniles of *E. brevidens* were released on multiple occasions; hence, cohorts released on the same occasion were grouped together for analyses, as indicated by the multiple lines presented in Figs. 6 & 7. Juveniles cultured in the laboratory were considered as 1 cohort, denoted by the light blue line in Model 2 (Fig. 6). The other parallel lines showed estimated average length of the mussel groups released during multiple time periods throughout the sampling occasions. Model 3 is the hierarchical model that incorporated individual variations. The multiple lines during the juvenile culture period in the laboratory indicated variations among the release groups in this model (Fig. 7). However, individual variations in length among mussels cultured in the laboratory or released to the river as cohort were not significant.

The results of our study indicated that the DIC values of the 3 models were substantially differ-

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Table 1. Estimated parameters of 3 models and their deviance information criterion (DIC) values. L_{∞} : theoretical average maximum body length; K : growth rate coefficient that defines growth rate toward the maximum; t_0 : hypothetical age when mussels were at 0 length. K_g : growth rates of released mussels at various stages ($g = 1$ represents the time in the laboratory; represents the time in 2009, 2010 and 2011 along with the mussel ages at 2, 3 and 4 yr, respectively); T : non-growth time (started on 1 November). L_{∞} and K_g in Model 3 represent the corresponding parameters of mussel length and growth rate coefficient of individuals following normal distributions of mean and variances (\bar{L}_{∞} , $\sigma_{L_{\infty}}^2$) and (\bar{K}_g , $\sigma_{K_g}^2$)

Model	DIC	Parameter	Mean	SD	95 % CI	
Model 1	3292.8	L_{∞} (mm)	99.180	0.806	97.080	99.980
		K (mo ⁻¹)	0.009	1.068×10^{-4}	0.009	0.010
		t_0 (mo)	1.939	0.061	1.773	1.998
Model 2	2546.0	L_{∞} (mm)	53.310	6.262	45.940	68.380
		K_1 (mo ⁻¹)	0.015	0.002	0.010	0.018
		K_2 (mo ⁻¹)	0.031	0.005	0.021	0.039
		K_3 (mo ⁻¹)	0.105	0.023	0.062	0.142
		K_4 (mo ⁻¹)	0.050	0.017	0.022	0.083
		GC (mo)	5.975	0.025	5.908	5.999
		t_0 (mo)	-0.649	0.977	-1.949	1.566
Model 3	1683.0	\bar{L}_{∞} (mm)	51.360	0.993	49.740	53.220
		$\sigma_{L_{\infty}}^2$	2.410	0.204	2.041	2.838
		\bar{K}_1 (mo ⁻¹)	0.015	5.758×10^{-4}	0.014	0.017
		\bar{K}_2 (mo ⁻¹)	0.026	0.002	0.022	0.029
		\bar{K}_3 (mo ⁻¹)	0.110	0.005	0.102	0.119
		\bar{K}_4 (mo ⁻¹)	0.050	0.004	0.044	0.057
		$\sigma_{K_g}^2$	8.299×10^{-4}	1.471×10^{-4}	5.416×10^{-4}	0.001
		T (mo)	5.980	0.013	5.950	5.998
		t_0 (mo)	-0.648	0.961	-1.948	1.525
		σ_t^2	0.004	0.004	1.732×10^{-4}	0.015

ent, with Model 3 having the lowest value of 1683.0, Model 1 the largest DIC value of 3292.8, followed by Model 2 with a DIC value of 2546.0. The difference in DIC units of Models 1 and 2 to Model 3 greatly exceeded 7 DIC units. Hence, Model 3 outperformed the other models and was determined to be the best model. Hierarchical Model 3, which considered the non-growth seasons and incorporated individual variations, optimally characterized growth of juvenile *E. brevidens* in the laboratory culture environment, and later when released to the river.

Water temperature and organic matter (represented by AFDW) were determined in this study from 28 October 2008 to 28 October 2009 (Table 2). The water temperature dramatically decreased at the end of October and rose at the end of April at the release site. Simultaneously, the AFDW declined about 3-fold during the low temperature period. These 2 parameters exhibited a very strong positive relationship (Pearson's $r = 0.72$). The duration of low temperature and AFDW during an annual cycle occurred during the period of mussel growth cessation.

DISCUSSION

The early life of juvenile mussels *Epioblasma brevidens* (from Day 1 to 2 mo) is a critical stage in the life cycle. The mussel culture methods used at FMCC have greatly improved the survival and growth of this endangered species (Hua et al. 2013). Rearing larger-sized (>20 mm) juvenile mussels for release is an important first step to restore imperiled populations. Mark-recapture techniques using PIT tags helps to ensure the success of evaluating mussel restoration efforts (Hua et al. 2015). Hence our study, covering the critical stages of juvenile propagation, culture, and release to monitoring provided a demonstration of conservation and restoration of an endangered mussel species.

The quantitative analyses directly influenced our understanding of mussel growth variation. Although the VBGM is perhaps the most commonly used growth model to characterize freshwater mussel length-at-age data (Hastie et al. 2000), this model needs to be evaluated on a case-by-case basis to avoid potential biases and erroneous parameter estimation.

For instance, the traditional VBGM was applied in our study and resulted in a poor goodness-of-fit. Most of the observations (blue dots) were obviously

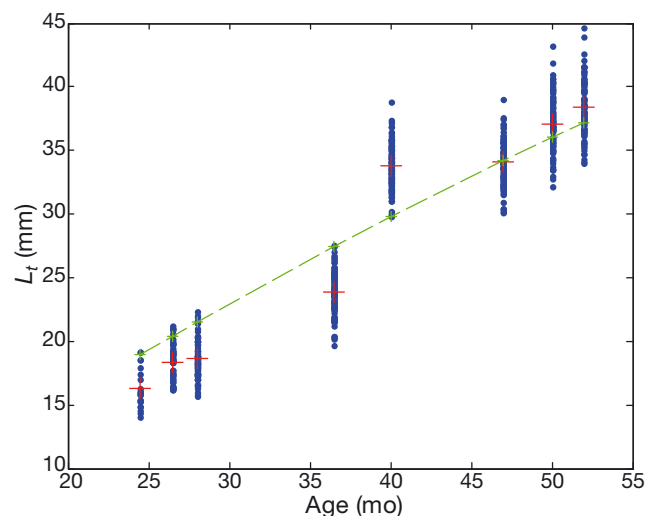


Fig. 5. Fits for Model 1 shown as green dashed line with estimated growth in length of released mussels of *Epioblasma brevidens* from 24.5 to 52 mo of age. ●: observation of an individual mussel; +: mean mussel size at each observation

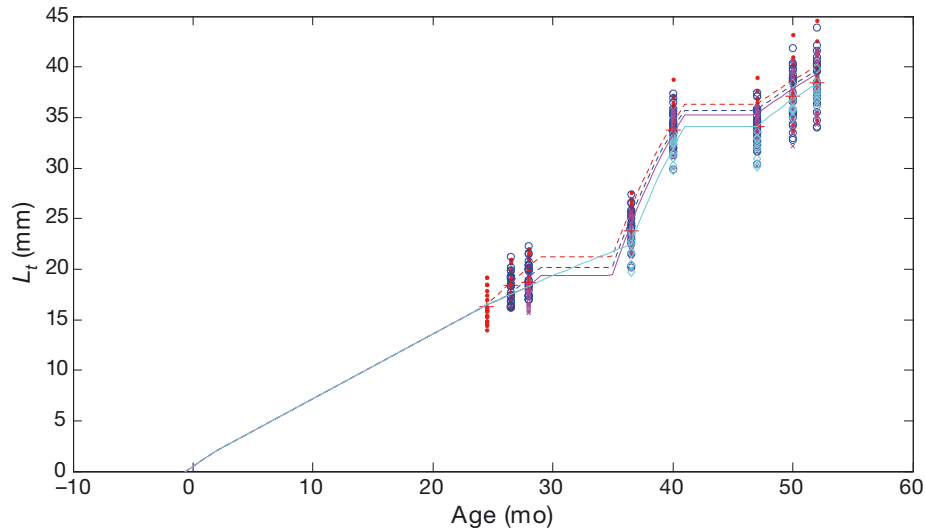


Fig. 6. Fits for Model 2 with estimated growth in length of juvenile mussels of *Epioblasma brevidens* from 0 to 52 mo of age (before and after release). Symbols: observed mussel sizes; lines: posterior model fit. Red filled circles and red dashed line: mussels released in July 2009; dark blue open circles and dark blue dashed line: mussels released in August 2009; maroon 'x' and line: mussels released in October 2009; light blue open diamonds and solid light blue line: mussels released in June 2010

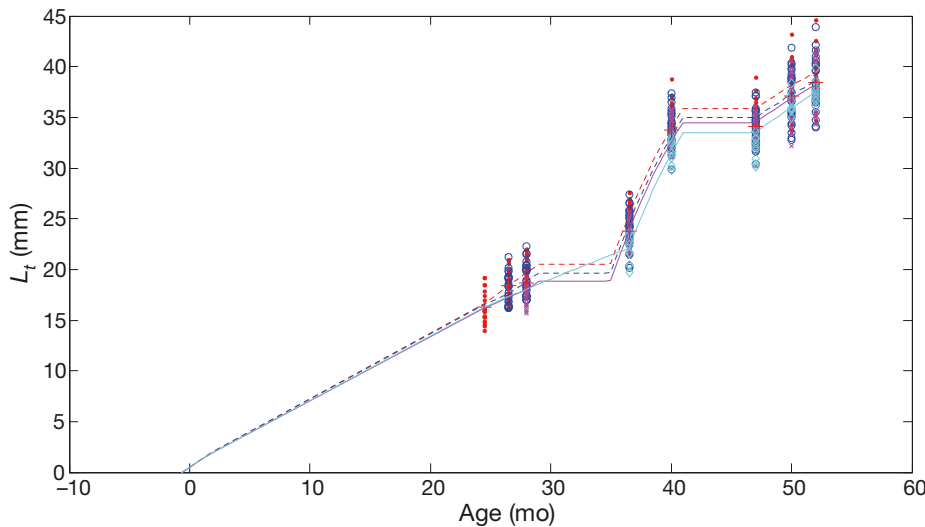


Fig. 7. Fits for Model 3 with estimated growth in length of juvenile mussels of *Epioblasma brevidens* from 0 to 52 mo of age (before and after release). See Fig. 6 for a description of symbols and lines

below the green dotted line (plot of VBGM growth curve) in Fig. 5, showing the overestimation. The estimated mean value of L_{∞} (99.18 mm) in Model 1 exceeded those of Model 2 and Model 3 by about 46 mm. Miguel et al. (2004) found that when the VBGM was applied to the youngest age classes (<6 yr) of freshwater mussels, it underestimated the value of L_{∞} . Poor fit of a model could be due to applying inappropriate models that are incapable of describing growth variation in individual animals.

In general, juvenile mussels grow rapidly during early life stages, peaking then decreasing in growth increments until achieving a near-constant size. Mussels also exhibit a seasonal growth pattern, with cessation caused by ambient environmental changes of low temperature, food restriction, minimal flow,

Table 2. Water temperature and organic matter values near the release site in Powell River, from October 2008 to October 2009. Organic matter content is indicated by ash free dry weight (AFDW)

Date (mm/dd/yy)	Water temperature (°C)	AFDW ($\mu\text{g ml}^{-1}$)
10/28/08	10.00	–
01/22/09	1.20	–
02/24/09	5.40	–
03/25/09	12.70	–
04/29/09	19.90	0.20
05/28/09	21.40	0.73
06/25/09	23.80	0.63
07/17/09	24.00	0.93
08/27/09	21.70	0.95
10/28/09	13.30	0.23

or inadequate energy storage for reproduction (Shul'man 1974, Adam 1990, Pauly et al. 1992, Downing & Downing 1993, Miguel et al. 2004). Our results indicated that the duration of GC for *E. brevidens* in the Powell River lasted approximately 6 mo, beginning in late October to early November when water temperatures decreased below 15°C, and ending in late April to early May when water temperatures increased above 15°C. During the winter, GC occurred during the laboratory culture period and after mussels were released to the Powell River. Loss in shell length was detected during their GC periods in the river, although the reduced length of individuals was inconsequential. This result corroborates a previous study reporting negative growth due to resorption in winter (Downing & Downing 1993). Regardless of GC or negative growth of mussels, we found that the duration of mussel GC correlated to the fluctuations in water temperature and food availability. Our determination of food availability was based on the AFDW of organic matter in sampled river water, which includes algae, bacteria, zooplankton, and other organic debris as suitable food (Silverman et al. 1997, Hua et al. 2013). The amount of AFDW declined to a low level from the end of October until the following May, while water temperatures declined to between 10 and 13°C at the end of October and remained lower until early April, then rose to 20°C in late April. Environmental changes directly influenced mussel growth, affecting GC and growth loss. However, Model 1 applied with the classic VBGM was not capable of simulating these seasonal growth patterns.

To determine seasonal growth patterns, a modified VBGM was applied by adding a sine wave with 2 more parameters to allow for seasonal oscillation (Pitcher & MacDonald 1973):

$$E(L_t) = L_{\infty}(1 - e^{-k_s}) \quad (12)$$

and

$$k_s = C \sin \left[\frac{2\pi(t-s)}{12} \right] + k(t-t_0) \quad (13)$$

where k_s is the function that describes seasonal perturbation through a sine wave, C donates the magnitude of the oscillations of sine wave, and s represents the starting point in time relative to t_0 . The other parameters, L_t , L_{∞} , t_0 , are defined as in Eqs. (2) & (3), and the value 12 equals sampling occasions in months.

Although this seasonal growth model was verified and applied to our data, it was incapable of accommodating growth during GC, which was similar to

that found in a previous study (Pauly et al. 1992). To solve this problem, Pauly et al. (1992) developed a better seasonal model by incorporating a no-growth time. We initially applied Pauly's improved mathematical function in our study, but it failed to simulate the growth pattern during the laboratory culture stage and post-release stage with multiple releases. Besides the above situation, the no-growth time in our study was much longer than the allowance of Pauly's model using the sine wave. Moreover, mussel growth exhibited obvious uneven oscillations that indicated different growth rates at various stages, which required the specification of different growth rates in the model to distinguish growth characteristics for each duration. Hence, 2 new models (Models 2 and 3) were developed to incorporate GC with a starting point of 1 November, associated with an upper boundary of 6 mo. According to the empirical data, we discarded the sine wave and generated 4 growth rates to fit the uneven oscillation growth pattern of *E. brevidens*. The estimated asymptotic length from Models 2 and 3 were 53.31 and 51.36 mm, respectively, much less than the value (99.18 mm) from Model 1. To investigate the reality of these results, we surveyed various museum collections to document the maximum length of *E. brevidens* collected from the Powell River, and found that the largest specimen was 71.9 mm (female) in the North Carolina State Museum of Natural Sciences, 62.4 mm (female) and 64.5 mm (male) in McClung Museum of Natural History and Culture, University of Tennessee, and 75 mm (female) in the Museum of Biological Diversity at Ohio State University. Parmalee & Bogan (1998) reported the average length of mature specimens of *E. brevidens* to be around 50 mm. Our results from Models 2 and 3 were seemingly plausible when compared to the museum records, because L_{∞} in this study was determined by laboratory-produced juveniles that experienced environmental changes in the early life stage.

It is known that growth parameters differ from species to species, but they also vary among individuals and among stocks within the same species, due to environmental responses and genetic differences. Our results indicated that growth rates differed at various stages, with the lowest K_1 in the culture period and highest K_3 at Age 3. Newly metamorphosed juvenile mussels experience environmental change after they excyst from host fish, which results in high mortality and slow growth. The second challenge to released juveniles is adaptation to the ambient environment and available food sources. Hence, K_2 did not exhibit rapid growth until acclima-

tion and Age 3 yr. Thereafter, mussels became sexually mature and gravid females were observed during the recapture events at Age 4, which was 1 yr earlier than predicted (Jones et al. 2012). The energy investment for reproduction influenced growth in length, resulting a smaller growth rate (K_4) at this stage. The other life history trait, t_0 , estimated from Models 2 and 3 were -0.649 and -0.648 mo, which likely indicated the duration of glochidial metamorphosis from parasitic glochidium (larvae of mussel) to juvenile mussel. These t_0 values agreed with empirical data, namely that glochidia of *E. brevidens* encyst on host fish for 15 to 20 d before becoming free-living juveniles. However, the estimated value (1.94 mo) from Model 1 was not reasonable. Hence, the life history traits of *E. brevidens* in the Powell River at different stages are best interpreted through Models 2 and 3.

To better understand the characteristics of population biology and ecology, individual variation is an important component to be considered in quantitative analyses (Pledger et al. 2003, Jiao et al. 2009, 2010), though it is challenging and requires advanced computing techniques. We developed a hierarchical model (Model 3) with multi-level priors to incorporate individual variation using a Bayesian approach, along with MCMC algorithms. Although the estimated parameters of life history traits of *E. brevidens* were very close to those of Model 2, Model 3 significantly out-performed Model 2 according to the rules of thumb (Spiegelhalter et al. 2002), the criterion of model selection. Thus, Model 3 was determined to be the best model due to its smallest DIC value (1683.0). Our results also showed that small variances of L_∞ and K values in all models were probably due to the similarity of the same cohorts.

In a previous study, the growth parameters of *E. brevidens* from the Clinch River, Tennessee, were estimated using classic VBGM, based on annuli for age-at-length (Jones & Neves 2011). However, using annular growth marks for delineating annual growth increments to estimate an age has been debated (Downing & Downing 1993, Strayer et al. 2004). Excluding measurement error and shell erosion, Downing & Downing (1993) reported negative growth in freshwater mussels, and indicated that growth rates based on annuli could be inaccurate since growth annuli were not reliable. Indeed, it is difficult to obtain lengths at defined ages of specimens from field records; hence, parameter estimation through use of annuli and corresponding lengths is still acceptable. We were able to produce juvenile mussels and release them at a precise age, thus re-

solving the problem of age determination. Information of juvenile growth at these early life stages was invaluable. Our individual growth model allowed for individual variation, duration of GC, and periodic growth rates (K_g), along with multiple release events (different starting points) to provide a flexible model framework adapted to alternative data structures and mussel growth variation. For example, it can be applied to growth patterns without GC by setting the $GC = 0$. The improvement of mark-recapture methodology using PIT technology greatly increased recapture rates and effectively reduced the uncertainties in parameter estimation.

It is important to have complete information for all age classes to determine the growth parameters of a species' lifespan (Haag 2009). Therefore with additional study, we can refine the results of our current estimation for this endangered species by incorporating middle and old age classes in the analytical modeling. Despite the advantage of this mark-recapture method, a bias derived from mussel handling was observed by Haag (2009). This can be resolved using unsampled mussels as controls in future studies.

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