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Extreme longevity in freshwater mussels revisited: sources of bias in age estimates derived from mark–recapture experiments

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SUMMARY

1. There may be bias associated with mark–recapture experiments used to estimate age and growth of freshwater mussels. Using subsets of a mark–recapture dataset for *Quadrula pustulosa*, I examined how age and growth parameter estimates are affected by (i) the range and skew of the data and (ii) growth reduction due to handling. I compared predictions from von Bertalanffy growth models based on mark–recapture data with direct observation of mussel age and growth inferred from validated shell rings.

2. Growth models based on a dataset that included observations from a wide range of length classes (spanning ≥ the upper 50% of the population length range) produced only slightly biased age estimates for small and medium-sized individuals (overestimated by 1–2 years relative to estimates from validated shell rings) but estimates became increasingly biased for larger individuals. Growth models using data that included only observations of larger animals (< the upper 50% of length range) overestimated age for all length classes, and estimated maximum age was two to six times greater than the maximum age observed in the population (47 years). Similarly, growth models using a left-skewed dataset overestimated age.

3. Reductions of growth due to repeated handling also resulted in overestimates of age. The estimated age of mussels that were handled in two consecutive years was as much as twice that of mussels that were handled only once over the same period. Assuming a constant reduction in the annual rate of growth, handling an individual for five consecutive years could result in an estimated age that is five times too high.

4. These findings show that mark–recapture methods have serious limitations for estimating mussel age and growth. A previous paper (*Freshwater Biology*, 46, 2001, 1349) presented longevity estimates for three mussel species that were an order of magnitude higher than estimates inferred from shell rings. Because those estimates of extreme longevity were based on mark–recapture methods and subject to multiple, additive sources of bias, they cannot be considered accurate representations of life span and cannot be used to conclude that traditional methods of bivalve ageing by interpretation of shell rings are flawed.

Keywords: age, longevity, mark-recapture, Unionidae, von Bertalanffy

Introduction

The effective management and conservation of freshwater mussels (Order Unionoida) depends on accurate information on age and growth. Freshwater mussels deposit conspicuous rings in their shells,

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similar to growth rings in trees, fish scales and otoliths, and hard structures in a wide variety of other organisms. It has long been assumed that shell rings are produced annually and interpretation of these rings forms the basis of our current understanding of mussel age and growth. Although the assumption that rings are formed annually has been validated for few mussel species, interpretation of shell rings generally yields robust age and growth models that make consistent predictions (e.g. Bruenderman & Neves, 1993; Christian *et al*., 2000; Jones *et al*., 2004).

Despite the widespread use of shell rings for estimating age and growth, this method has been controversial because of the lack of validation of the assumption of annual ring production. A series of papers examined the periodicity of shell ring production using mark–recapture experiments, in which the growth of marked individuals was monitored annually over several years (Downing, Shostell & Downing, 1992; Downing & Downing, 1993; Kesler & Downing, 1997). The growth of marked individuals was less than that predicted by shell rings, leading these authors to conclude that rings were deposited less than annually and, therefore, that mussels were much older than previously estimated. However, because even brief handling of mussels results in shell abnormalities and significant reductions in growth, a recent paper (Haag & Commens-Carson, 2008) concluded that the discrepancy between growth estimated from mark–recapture experiments and growth predicted by shell rings reported by Downing *et al.* (1992) and Kesler & Downing (1997) may be better explained by chronic disruption of normal growth patterns due to repeated handling rather than non-annual production of shell rings.

Recent work has demonstrated the annual production of shell rings in a large number of species, across multiple years and in a variety of habitats. In Europe, annual ring production by *Margaritifera margaritifera* (Linnaeus) is firmly supported and annual rings are now used widely to reconstruct past growth histories and environmental conditions (Schöne *et al*., 2004; Helama *et al*., 2006; Helama & Valovirta, 2008). In North America, annual ring production has been validated in >20 species using mark–recapture methods (Neves & Moyer, 1988; Howard & Cuffey, 2006; Haag & Commens-Carson, 2008); for several of these species, annual rings were validated independently using cross dating and correlation with annual hydrological variables (Rypel, Haag & Findlay, 2008). Finally, in Australia, Europe and North America, seasonal patterns of shell chemistry correlate closely with observed shell ring patterns (Nyström *et al*., 1995; Veinott & Cornett, 1996; Siegele *et al*., 2001).

In an influential paper, Anthony *et al.* (2001) extended the conclusions of Downing *et al.* (1992) and Kesler & Downing (1997) by using mark–recapture growth data to estimate mussel age. Anthony *et al.* (2001) estimated parameters of the von Bertalanffy growth equations using a common fisheries approach whereby:

$$L_\infty = [a/(1-\beta)] \tag{1}$$

and

$$K = -\ln\beta \tag{2}$$

where L_∞ is the theoretical maximum length at infinite age, K is a growth constant that describes the rate at which L_∞ is attained, and a is the y intercept and β is the slope of the linear regression of the Ford-Walford relationship based on mark recapture data (length at time $t + 1$ regressed on length at time t; Ricker, 1975). The estimated mean maximum ages of *Elliptio complanata* (Lightfoot), *Lampsilis siliquoidea* (Barnes) and *Pygandon grandis* (Say) were 149, 167 and 95 years, respectively, although the upper confidence limits of these estimates were as high as 237, 173 and 250 years. The mean estimates are an order of magnitude higher than estimates of longevity for these species based on shell rings (Ghent, Singer & Johnson-Singer, 1978; Nalepa & Gauvin, 1988). Based on these results, Anthony *et al.* (2001) concluded that ''…our current understanding of mussel age and growth dynamics may be severely flawed'' and ''… [mussels] may be among the most ancient living animals on Earth''. Their conclusions have been cited widely including prominent, recent reviews of mussel ecology (Strayer *et al*., 2004; Strayer, 2008), resulting in considerable confusion about the age and growth of freshwater mussels.

There may be a number of problems with the conclusions of Anthony *et al.* (2001). First, the study used an inversion of the von-Bertalanffy growth equation whereby a standard form of the equation:

$$L_t = L_\infty - (L_\infty - L_0)e^{-Kt} \tag{3}$$

where L_t is mussel length at time t (age in years), and L_0 is length at time = 0, was rearranged algebraically to solve for t as:

$$t = ln[(L_t - L_\infty)/(L_0 + L_\infty)]/-K \quad (4)$$

Solving the equation for t (age) is an intuitively appealing form of the equation but the version presented by Anthony *et al.* (2001) is in error. Because L_∞ is, by definition, $\geq L_t$, the quantity $(L_t - L_\infty)$ yields either a negative number or 0, neither of which has a logarithm, and the equation cannot be solved. An additional error is associated with the sign of the term L_0, which properly should be negative. The algebraically solvable and correct rearrangement of the equation is:

$$t = \ln[(L_\infty - L_t)/(L_\infty - L_0)]/-K \quad (5)$$

Second, for all four species examined, the Anthony *et al.* (2001) datasets included mainly large individuals and few or no small individuals, or were mostly from the middle of the size range. Estimates of growth, mortality and longevity are often greatly influenced by whether observations are made on small or large individuals (Campana, 2001). As a result, when mark–recapture data are not representative of the full size range of the population, estimated parameters can be severely biased and may not allow generalisation beyond the scope of the data (Haddon, 2001).

Third, the mark–recapture data of Anthony *et al.* (2001) were obtained from repeated annual measurements of marked individuals. Therefore, observed growth rates used to predict age may have been subject to the cumulative effects of repeated handling similar to those that might have affected the results of Downing *et al.* (1992) and Kesler & Downing (1997) (see Haag & Commens-Carson, 2008). Consequently, the growth constant K may be underestimated, resulting in inflated estimates of age.

Finally, although Anthony *et al.* (2001) attempted to validate their age estimates using a method based on observed mortality rates, this method was not independent because mortality rates were extrapolated and added to age estimates derived previously from von-Bertalanffy predictions, hence conflating these two approaches. Furthermore, their calculation of mortality rates did not use models appropriate for mark–recapture data (e.g. Hart *et al.*, 2001; Villella, Smith & Lemarié, 2004) and, because mortality is age-specific and varies throughout the life of a cohort (Haddon, 2001), single point estimates of mortality cannot readily be extrapolated to estimate longevity.

Because of the need for accurate growth models for mussels and the current confusion surrounding this topic, the conclusions of Anthony *et al.* (2001) need to be evaluated critically. Here, a mark–recapture dataset on mussel growth was used to evaluate potential sources of bias and error when using the inversion of the von-Bertalanffy growth equation to estimate mussel age. First, I evaluated how the range and skew of observed values in a mark–recapture dataset can affect estimates of growth parameters and, ultimately, estimates of age. Second, I evaluated how growth reduction due to handling can affect age estimates. Finally, I compared estimates of growth inferred from validated shell rings with predictions from mark–recapture data. The overall objective was to assess the general utility of using mark–recapture data to construct accurate, robust growth models for mussels.

Methods

I examined the performance of models of mussel growth using data on *Quadrula pustulosa* (I. Lea) from a previously published mark–recapture study conducted in the Little Tallahatchie River, Mississippi from 2003 to 2005 (Haag & Commens-Carson, 2008). The Little Tallahatchie River at the study site is regulated by a major storage reservoir just upstream and is impounded by a low-head dam just downstream. Substratum at the site was mostly sand and mean water depth was *c.* 3 m. In the previous study, mussels were collected in August 2003 ($n = 253$), measured (nearest 0.1 mm), marked by gluing numbered tags to the shell, and returned to the substratum. Mussels were relocated in August 2004 ($n = 193$ recaptures) and measured; 137 mussels were returned to the substratum and the remaining mussels were taken to the laboratory. Mussels were again relocated in August 2005 ($n = 45$ recaptures: 13 marked in 2003 but not found in 2004, and 32 measured and released in 2004). To reduce handling stress, mussels were kept submerged in mesh bags except when affixing tags to the shells; animals were returned to the substratum within 3 h on all collection dates. This dataset contains observations on the growth of mussels of a wide range of sizes and ages (12.2–69.1 mm length, 1–40 years age). See Haag & Commens-Carson (2008) for additional details about the study.

Range and dispersion of observed values

Using mark–recapture data from 2003 to 2004, simulated datasets were constructed that encompassed different percentages of the potential full length range for *Quadrula pustulosa* at the study site. The full dataset included individuals of 12.2–69.1 mm (2003 lengths), representing the upper 82% of the full length range in the population (i.e. only excluding individuals <12.2 mm). I then truncated the lower end of the dataset at increasingly larger values to create data subsets that were progressively dominated by large individuals. These truncated datasets represented the upper 70% of the length range (containing observations only on individuals >20.7 mm, $n = 191$), upper 50% (>34.6 mm, $n = 179$), upper 40% (>41.5 mm, $n = 133$) and the upper 30% (>48.4 mm, $n = 110$) of the entire population. I constructed Ford-Walford plots (2004 length regressed on 2003 length) and calculated L_∞ and K for each dataset from eqns 1 & 2. In this and all other analyses, the height of mature glochidia of *Quadrula pustulosa* (0.290 mm; Barnhart, Haag & Roston, 2008) was used for the value of L_0, following Anthony *et al.* (2001). Using these parameter estimates in eqn 5, I then estimated the age of mussels from 15 mm-L_∞ for each dataset.

Data from 2003 to 2004 were also used to construct simulated datasets representing varying degrees of skew. In this analysis, I simulated four datasets, each with 50 observations and encompassing the full range of sizes observed in the full dataset (12.2–69.1 mm), but representing four different distributions: symmetrical, right-skewed, moderately left-skewed and severely left-skewed. To create these distributions, I assigned each pair of observations (2003 length and 2004 length) to 5-mm size classes, based on 2003 length. The number of paired observations from each size class necessary to create each distribution was then randomly selected (e.g. the severely left-skewed dataset had a preponderance of observations in large size classes). From the resulting datasets, I calculated L_∞ and K from eqns 1 & 2 based on the slope and y-intercept of the Ford-Walford plots. For each distribution, this procedure was repeated 1000 times and the mean values of L_∞ and K were calculated. All simulations were performed using Resampling Stats Add-in for Excel (Resampling Stats, Arlington, VA, U.S.A.). Due to constraints of the number of small individuals in the dataset, I was able to construct only one level of right-skew; however, left-skew is of principal interest here because it is a common feature of mussel datasets due to the difficulty of sampling small mussels or their absence in many populations.

Handling effects

I examined the effect of reduced growth due to repeated handling on estimates of mussel age using mark–recapture data from 2003 to 2005. Separate Ford-Walford plots of length in 2005 against length in 2003 were constructed for mussels that were handled once (marked in 2003, not collected in 2004 and measured in 2005, $n = 13$) and mussels that were handled twice (marked in 2003, measured and released in 2004 and measured in 2005, $n = 32$). Both datasets had similar ranges and means of initial observed length and low skew (handled once: 18.9–66.2 mm, mean = 41.0, skew = 0.026; handled twice: 27.8–60.3 mm, mean = 44.7 mm, skew = −0.074). Mussels handled twice had significantly lower growth during 2003–05 than mussels handled only once (Haag & Commens-Carson, 2008). From Ford-Walford plots, L_∞ and K were calculated as described previously. I then used eqn 5 to estimate age for both handling groups but multiplied estimates by two to compensate for the 2-year interval of the observations.

Comparison of observed growth with predictions from mark–recapture data

Independent estimates of age and growth of *Quadrula pustulosa* in the Little Tallahatchie River were made by interpreting internal shell rings. The assumption of annual shell ring production has been validated for this species at this site (Haag & Commens-Carson, 2008). I measured shell length (nearest 0.1 mm) and estimated the age of 172 individuals collected between 2000 and 2005. For specimens with severe erosion of the umbo, the number of missing rings was estimated using mean length at age of young age classes (Hastie, Young & Boon, 2000); the maximum number of missing rings in a specimen was nine, but most eroded specimens were missing only one to three annual rings. Radial thin-sections (*c.* 300 μm) were prepared from one valve of each specimen following Haag & Commens-Carson (2008) and based on standard methods for bivalves (Clark, 1980; Neves & Moyer, 1988). Two experienced observers interpreted each thin-section independently; specimens about which the readers could not reach a consensus age were discarded. From these direct observations of individual mussel length at age, I estimated von-Bertalanffy growth parameters using nonlinear least squares regression (SAS 2002–2003). I then compared the mean observed age of mussels (based on validated shell rings) within 10 mm size classes with the mean age predicted by eqn 5 using parameters estimated from mark–recapture data.

Results

Range and distribution of observed values

The range and distribution of observed values in the dataset had major effects on estimates of mussel age. Estimates of age became increasingly inflated as simulated datasets contained fewer observations on the growth of small individuals. Datasets containing observations on ≥the upper 50% of the population length range resulted in age estimates that were similar to each other and to estimates from validated shell rings, but only for smaller individuals < about 45 mm (Fig. 1). Age estimates began to depart from shell ring estimates (overestimating age) for individuals > about 45 mm length. Estimates of maximum age (age at $L_\infty - 0.1$ mm) increased as the range of observations decreased [upper 82% (full dataset) = 47 years, 70% = 52 years, 50% = 66 years]. Datasets encompassing only the upper 30–40% of the population length range resulted in age estimates that were greatly inflated relative to more demographically complete mark–recapture datasets or to estimates from validated shell rings. Age estimates from the 40% dataset were higher throughout the observed length range but began to depart substantially (overestimating age) for individuals > about 30 mm (Fig. 1). Age estimates for the 30% dataset were substantially higher than estimates from all other datasets throughout the observed length range. Both the 30% and 40% datasets resulted in greatly inflated estimates of maximum age relative to estimates from more demographically complete datasets or from shell rings (40% = 91 years, 30% = 289 years).

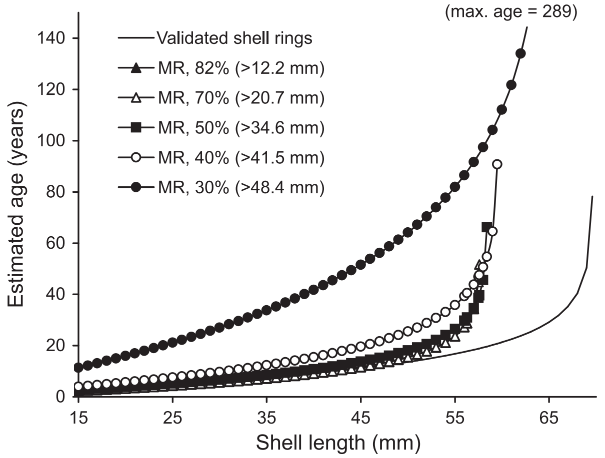

Fig. 1 Age estimates of *Quadrula pustulosa* derived from mark–recapture data from 2003–04 and from growth data inferred from validated shell rings. Curves for mark–recapture data (MR) were constructed for subsets of the data encompassing varying portions of the total observed length range for the population. For example, the curve labelled 82% was derived from a dataset including only individuals in the upper 82% of the length range (>12.2 mm, maximum observed length = 69.1 mm). Curves for 82% and 70% overlay each other. Ages were estimated using the inversion of the von-Bertalanffy growth equation as described by Anthony *et al.* (2001). Note that the y-axis is truncated for the dataset including 30% of length values.

Across all simulated mark–recapture datasets, estimates of the growth rate constant, K, decreased with a decrease in the range of observed values (82% = 0.131, 70% = 0.130, 50% = 0.107, 40% = 0.071, 30% = 0.023). Estimates of L_∞ increased with a decrease in the range of observed values (82% = 57.6 mm, 70% = 57.7 mm, 50% = 58.5 mm, 40% = 59.6, 30% = 65.1), but were all lower than predicted by shell rings (69.7 mm).

Skew also resulted in biased estimates of growth parameters and maximum age (Table 1). Both left-skewed datasets (a preponderance of observations on large individuals) resulted in greatly underestimated values of K and overestimates of maximum age relative to a symmetrical dataset, and the degree of bias increased with increasing skew. Right-skewed data resulted in estimates of growth parameters and maximum age that were similar to symmetrical data.

Handling effects

Growth reductions caused by handling resulted in biased estimates of age. Mussels that were handled twice had lower estimates of L_∞ (52.9 mm) and K (0.379, based on 2-year growth interval) than mussels that were handled only once ($L_\infty = 56.5$ mm,

Table 1 Estimates of von-Bertalanffy growth coefficients and maximum age for *Quadrula pustulosa* derived from simulated mark–recapture datasets with different distributional properties

Length range (mm)	n	Midpoint (mm)	Mean (mm)	Skew	L_∞	K	Estimated maximum age (years)
Symmetrical							
12.2–69.1	50	40.7	44.2	−0.182	58.7	0.131	49
Right-skewed							
12.2–69.1	50	40.7	34.9	1.124	59.0	0.134	48
Left-skewed							
12.2–69.1	50	40.7	52.6	−1.111	59.7	0.112	57
12.2–69.1	50	40.7	55.6	−2.179	60.0	0.107	60

Values of the mean length, skew, L_∞ and K for each dataset are the means from 1000 randomly generated simulations for each distributional type. Maximum age is the predicted age of an individual with length $L_\infty - 0.1$ mm, based on mean values of L_∞ and K.

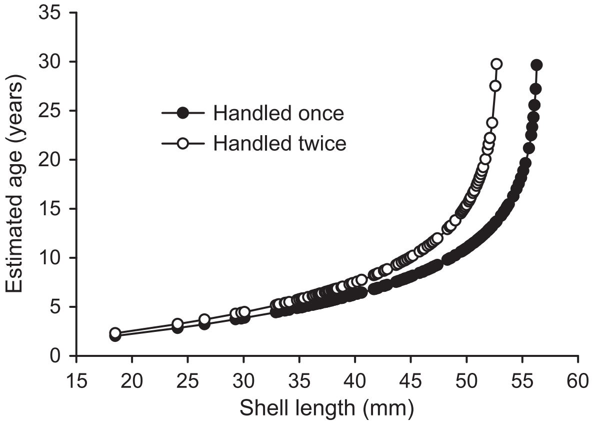

Fig. 2 Influence of growth disturbance due to handling on age estimates for *Quadrula pustulosa* derived from mark–recapture data from 2003–05. Ages were estimated using the inversion of the von-Bertalanffy growth equation as described by Anthony *et al.* (2001).

Table 2 Comparison of age estimates for freshwater mussels using three different analytical approaches. Both columns labelled 'Predicted' represent values obtained from von-Bertalanffy growth equations developed from length at age data (as inferred from validated shell rings) and mark–recapture data, respectively. Predicted maximum ages were estimated as the age of an individual with length $L_\infty - 0.1$ mm. The column labelled 'Observed' represents values obtained from direct age estimates (based on counts of shell rings) of 172 individuals

	Mean age (years)		
	Validated shell rings		Mark–recapture
Length range (mm)	Observed	Predicted	Predicted
10–20	1.5	1.5	2.3
21–30	3.4	3.5	4.5
31–40	5.5	6.0	7.3
41–50	10.2	10.0	12.1
51–57.6	17.4	15.0	28.3
>57.6	28.8	47.5	–
L_∞	79.8	69.7	57.6
K	–	0.093	0.131
Maximum age	48	78	47

Values of L_∞ and maximum age in the column 'Observed' are the maximum size and age observed in the population.

$K = 0.396$). Consequently, age estimates for mussels that were handled twice were higher throughout the entire range of observed lengths but were greatly overestimated for large individuals (Fig. 2). For example, the estimated age of a 52.6 mm individual handled twice was double that of a mussel handled only once (28 years versus 14 years). Assuming a constant reduction in the annual rate of growth due to handling, a 52.6 mm individual, handled for five consecutive years, would have an estimated age of 69 years, or five times the estimated age of a similarly sized animal handled only once (14 years).

Comparison of observed growth with predictions from mark–recapture data

Age estimates obtained from the rearrangement of the von-Bertalanffy growth equation based on parameters estimated from a demographically inclusive mark–recapture dataset (upper 82% of size range) were consistently inflated compared to ages determined from shell rings (Table 2). For size classes <50 mm length, age was overestimated by only *c.* 1–2 years. However, the age of individuals >50 mm was seriously overestimated using mark–recapture data (Table 2). Predicted maximum age (47 years) was similar to maximum observed age (48 years), but this was an artifact of the greatly underestimated value of L_∞, which made age estimation for larger individuals (observed length > L_∞) impossible (see Discussion).

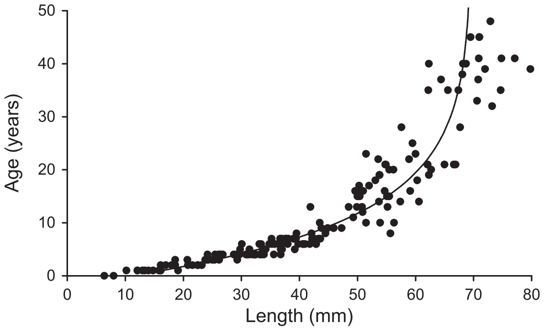

Fig. 3 Growth of *Quadrula pustulosa* in the Little Tallahatchie River, Mississippi as depicted by inversion of the von-Bertalanffy growth equation showing predicted (line) and observed (points) age at length (n = 172).

Ages predicted by the von-Bertalanffy growth equation based on shell ring data showed a similar bias for large individuals relative to direct estimates of mussel age. For mussels <57.6 mm length, predicted age was very similar to observed age but age of mussels >57.6 mm was overestimated by a wide margin (Fig. 3, Table 2). Maximum age predicted by the von-Bertalanffy model (78 years) was 62% higher than the maximum age observed in the population by direct count of validated shell rings (48 years). The estimate of maximum size predicted by the von-Bertalanffy model (L_∞, 69.7 mm) was 15% smaller than the largest individual observed in the population in this study (79.8 mm). For individuals >30 years of age, predicted length was less than observed length for 16 out of 20 individuals, indicating an asymmetrical distribution of residuals and a systematic bias in this portion of the relationship (Fig. 3).

Discussion

The use of mark–recapture data to derive growth models for freshwater mussels is subject to several sources of serious bias. First, estimates of growth parameters are highly sensitive to the distributional properties of mark–recapture data (Sainsbury, 1980; Campana, 2001; Haddon, 2001). Because of the difficulty of finding small mussels, or their absence in many populations, mark–recapture datasets are often limited to observations on larger individuals. Therefore, mark–recapture data should be expected to produce inaccurate estimates of age and growth to an extent that is dependent on the degree of size bias present in the dataset. My results show that datasets encompassing at least the upper 50% of the population size range can result in only slightly inflated estimates of age for small and intermediate size classes. However, the degree to which ages are overestimated increases sharply for large size classes, even in datasets that are relatively demographically complete. Furthermore, L_∞ predicted from mark–recapture datasets was lower than the maximum observed size in the population (as much as 28% lower) severely limiting the size range over which age can be estimated. Datasets strongly biased against small size classes will overestimate age throughout the length range of the population and can produce estimates of maximum age that are inflated by nearly an order of magnitude.

Even in datasets that include observations from a wide range of sizes, mussel populations are often dominated by intermediate to large size classes (Miller & Payne, 1988; Strayer *et al.*, 1994; Hornbach & Deneka, 1996; Haag & Warren, 2007). Samples taken at random from such populations will often be left-skewed, resulting in underestimates of growth rate and inflated age estimates, but datasets may also suffer from a paucity of very large individuals. The mark–recapture dataset which forms the basis of this paper (Haag & Commens-Carson, 2008) was taken from a population experiencing strong recruitment and having representation of individuals in all size classes (Haag & Warren, 2007). Nevertheless, the majority of individuals in the population were between *c.* 42 and 65 mm, resulting in a slightly left-skewed dataset (skewness = −0.47), but also having few observations of growth of individuals >65 mm.

A second reason that mark–recapture methods perform poorly for estimating age and growth is the negative effect of handling on growth. Even brief handling results in small but significant decreases in growth over a 1-year time period, but repeated handling can result in an accrual of growth impacts (Haag & Commens-Carson, 2008). Mark–recapture data will therefore underestimate growth and result in overestimates of age concordant with the extent of handling effects. Age estimates based on the growth rates of mussels that were handled in two successive years were as much as twice those estimated from growth of mussels that were handled only once in 2 years; by extension, chronic growth reduction due to repeated handling is likely to result in increasingly inflated age estimates. In bivalves, the deposition of new shell growth at the shell margin-mantle edge, and the unavoidable disruption of this process upon removal of the animal from the substratum, renders them unusually vulnerable (relative to fish and other organisms) to growth disruptions due to even brief, careful handling (Richardson, 1989; Mutvei & Westermark, 2001; Haag & Commens-Carson, 2008). Mark–recapture studies involving repeated handling of mussels over several years might well result in highly distorted estimates of age and growth.

Apart from the sources of bias associated with mark–recapture data, the von-Bertalanffy growth model itself has shortcomings for describing mussel growth. Despite its wide application and usefulness in many situations, the von-Bertalanffy growth model has been widely criticised. One of the main criticisms of this and similar models (e.g. Gompertz, Richards) is that, because they assume the existence of an asymptotic maximum length (L_∞), an asymptotic relationship may be forced to the data when it does not really exist, and the model often results in inaccurate or biologically unrealistic estimates of maximum size (Knight, 1968; Roff, 1980; Francis, 1988; Colbert *et al.*, 2004). In addition to simply mischaracterising maximum size, distorted values of L_∞ can also result in distorted values of the growth constant, K, which represents the rate at which animals approach L_∞. Further, as noted earlier, estimates of model parameters can be greatly influenced by an absence or paucity of observations on small and large individuals. The result of these weaknesses is that, while the von-Bertalanffy growth model may perform well for describing growth within the middle portion of the size range, it often performs poorly within the tails of the length distribution, especially for very large individuals that are approaching L_∞ (Sainsbury, 1980). Consequently, predictions about the age of very large animals based on von-Bertalanffy models probably bear little resemblance to reality in many situations.

These problems are readily apparent in the von-Bertalanffy growth model based on shell rings for *Quadrula pustulosa*. The model accurately estimates growth of individuals <30 years of age, and within this range has an even scatter of residuals above and below the predicted line (see Fig. 3). For animals >30 years old, however, the observed length of most individuals was considerably greater than that predicted by the growth model, which is constrained by the unrealistically low estimated value of L_∞. Because L_∞ represents the mean maximum length for the population, observed values greater than L_∞ are expected due to individual variability in growth. However, a value of L_∞ that departs substantially from the observed maximum size and, more importantly, is biased relative to the distribution of observed values, can be an indication that the model is inappropriate (Francis, 1988). This problem becomes of practical significance when using the inversion of the growth equation to estimate age (see Fig. 3). Due to the substantial underestimate of L_∞, as animals approach this theoretical maximum length, their ages are overestimated by an increasingly large factor and the age of individuals above this value cannot be estimated.

Several authors have warned against using the inversion of the von-Bertalanffy equation to estimate age of individuals and using these predictions to make comparisons between ageing methods (Kirkwood, 1983; Francis, 1988). Although solving for age is intuitively appealing, due to the presence of individual variation in growth, relationships of length at age and age at length are strictly equivalent only when applied to individuals but not to populations (Sainsbury, 1980). The reason for this is that, within a population, growth estimates obtained from observations of length at age (as obtained by interpreting shell rings) are associated with a fundamentally different source of error (variation in growth rate at age) that is not equivalent to error associated with observations of age at length (variation in growth rate at length, as obtained from mark–recapture studies to estimate annual growth increments). These two approaches can result in considerably different estimates of growth parameters and, ultimately, different predictions about age at length, especially for large individuals (Kirkwood, 1983). Consequently, Francis (1988) advised ''…researchers should refrain from using a comparison of growth parameters derived from the two types of data [age at length and growth increment data] as a means of validation of one or the other''.

Measurements of annual growth increments obtained from mark–recapture experiments seem poorly suited as a method for parameterising growth models. The von-Bertalanffy growth equation was derived originally to describe growth of an individual based on observations of its size at different ages (von Bertalanffy, 1957). In general, when extended to represent growth of a population, variability among individuals results in biased estimates of growth parameters, most notably an underestimate of the mean K for the population; this bias is small when parameters are estimated from length at age data (such as those inferred from shell rings) but can be large when estimated from growth increment data (such as obtained from mark–recapture methods) (Sainsbury, 1980). With regard to using growth increment data, Sainsbury (1980) stated ''In some populations (e.g. those with low levels of individual variation of growth parameters and an unchanging age composition) the problems may be minor, but in others the potential for error and confusion is great. *It would appear that many molluscs fall into the latter category* (italics mine).'' Although Sainsbury (1980) did not elaborate on why molluscs should be particularly prone to these problems, most freshwater mussels show high individual variability in length at age (this study; Rogers, Watson & Neves, 2001; Jones *et al*., 2004). Furthermore, in mussel populations with infrequent or periodic recruitment, age distributions will change markedly over time (e.g. Payne & Miller, 2000). These features of mussel biology imply that growth models constructed from mark–recapture data (even without handling effects) may provide at best coarse approximations of mussel growth.

The extreme age estimates for freshwater mussels presented by Anthony *et al.* (2001) were subject to all of the sources of bias discussed above. Although it is unclear how these authors solved eqn 4, this error probably had little or no effect on biasing their results. For example, by taking the absolute value of $[(L_t - \mathrm{L}_\infty)/(\mathrm{L}_0 + L_\infty)]$ in eqn 4 in order to obtain the natural logarithm of this quantity, I obtained results very similar to those obtained from the correct rearrangement of the growth equation (eqn 5).

The most obvious source of bias in their age estimates is due to handling effects. Repeated handling of mussels causes substantial decreases in growth over time (e.g. Haag & Commens-Carson, 2008) that will result in greatly inflated estimates of mussel age. Notably, the two populations with the highest predicted ages were those in which animals were handled for the greatest number of consecutive years (*Elliptio complanata*, Worden Pond, 8 years and *Lampsilis siliquoidea*, Wabana Lake, 6 years). More subtle, but equally important, sources of bias stem from the ways in which the von-Bertalanffy growth equation was used to make predictions about mussel age. These problems are complex and interrelated but can be placed into two categories. (*i*) *Non-representative data* – In all populations, datasets contained few or no observations on growth of small individuals and most observations fell within < the upper 50% of the size range for the population, resulting in highly skewed and non-representative data; in my simulations, this type of data resulted in seriously biased growth parameters and inflated age estimates. (*ii*) *Inherent limitations of the von-Bertalanffy growth equation* – As discussed previously, using mark–recapture data to parameterise von-Bertalanffy models is problematic, even without the confounding effect of handling, because of bias introduced by individual variation in growth which is unaccounted for in the model. Although Anthony *et al.* (2001) present confidence limits around their age estimates these represent variation among years and do not account for individual variation in growth at length. Furthermore, von-Bertalanffy models generally perform poorly when the equation is inverted to estimate age as the dependent variable, especially for large individuals. These multiple, additive sources of bias probably resulted in grossly inflated estimates of age.

The extreme ages of *Elliptio complanata*, *Lampsilis siliquoidea* and *Pyganodon grandis* reported by Anthony *et al.* (2001) could possibly be explained by the northerly location of their study sites. In two species of European freshwater mussels, maximum age increased with decreasing mean water temperature and increasing latitude, varying among populations from 30 to 132 years for *Margaritifera margaritifera* (Bauer, 1992) and from 8 to 23 years for *Unio crassus* Philipsson (Hochwald, 2001); a similar phenomenon is seen in marine bivalves (Bachelet, 1980; Nichols & Thompson, 1982). However, previous estimates of longevity for *E. complanata*, *L. siliquoidea* and *P. grandis* based on shell rings ranged from only 11 to 19 years and were obtained from populations at similar latitudes (e.g. southern Ontario, Ghent *et al*., 1978; Lake St. Clair, Nalepa & Gauvin, 1988) or even far to the north (boreal Alberta, Hanson, Mackay & Prepas, 1988) of the study sites of Anthony *et al.* (2001) (Rhode Island and Minnesota). Furthermore, Anthony *et al.* (2001) explain the observed poor agreement of shell ring patterns with mark–recapture growth rates by asserting that shell rings were produced irregularly or less than annually. In marine bivalves, fishes and trees, annual rings are most pronounced and regular in northerly latitudes with distinct seasons and become less distinct and regular with decreasing latitude (Tesch, 1971; Nichols & Thompson, 1982; Kruse, Guy & Willis, 1993; Biondi, 1999; Brienen & Zuidema, 2005). For these reasons, slow growth in northerly climates cannot account for the supposition of inordinately long life spans and does not outweigh the many sources of methodological bias associated with estimates of extreme age in mussels.

Many of the potential sources of bias in mark–recapture studies of freshwater mussels are difficult to avoid, making this approach of limited usefulness in describing mussel growth. Even when handling effects were minimised by conducting a mark–recapture study over a single year, mark–recapture data for *Quadrula pustulosa* provided reasonably accurate, though biased, depictions of growth for only a segment of the population and did not accurately depict growth of larger and older animals. As Anthony *et al.* (2001) point out, this approach has the additional disadvantage of estimating growth for only a single year and not incorporating annual variation in growth which is substantial in some populations (Rypel *et al.*, 2008). Finally, mark–recapture studies are laborious and subject to low return rates, especially in dynamic river environments (Haag & Commens-Carson, 2008), and a representative size range of animals necessary to make reasonably accurate estimates of growth is simply not available in many populations. Although mark–recapture studies are of limited use for estimating growth or corroborating growth estimates made by other methods (see Campana, 2001), this approach will remain important for mussels as an effective method to validate production of annual shell rings (e.g. Neves & Moyer, 1988; Howard & Cuffey, 2006; Haag & Commens-Carson, 2008).

The most accurate estimates of mussel age and growth are obtained by examination of validated, internal shell growth rings (Haag & Commens-Carson, 2008). In addition to avoiding sources of bias and other disadvantages inherent in mark–recapture methods, analysis of shell rings can be performed using dead shells from field or museum collections, avoiding problems of low sample size and disturbance of living animals, both of which are particularly problematic for studies of rare species (e.g. Helama & Valovirta, 2008). Examination of shell rings is subject to errors of interpretation including failure to count annual rings (resulting in overestimates of growth rate and underestimates of age) and counting non-annual rings as annual (resulting in underestimates of growth and overestimates of age). Rypel *et al.* (2008) used cross dating as a quality control technique and identified potential errors in 16% of the specimens they examined (this number included both interpretative errors and errors in measurement of growth increments used to build growth chronologies, so interpretive errors of rings were <16%). Errors in the number of annual rings identified for an individual ranged from −5 (i.e. five non-annual rings initially identified as annual) to +2 (e.g. two annual rings missed), but most errors involved a discrepancy of only one or two rings. Because this error was not systematic (i.e. resulting in either a consistent underestimate or overestimate of age) and because it is of low magnitude, interpretive errors of validated shell rings cannot be proposed as a source of serious bias in age estimates for mussels.

At this time, there is no evidence to suggest that freshwater mussels deviate from the nearly universal phenomenon of annual growth ring production exhibited by a wide variety of organisms. With regard to marine species, ''…the formation of annual shell increments is pervasive among bivalve mollusks, crossing lines of geography and taxonomy, and is fundamental in sclerochronological or paleoclimate analyses…'' (Jones & Quitmyer, 1996). Nevertheless, much additional work is needed on growth of freshwater mussels. Although the freshwater pearl mussel (Margaritiferidae) *Margaritifera margaritifera*, is reliably reported to live for >130 years (Bauer, 1992), most species appear to have more modest life spans. Existing data on the growth of unionids (based on validated and unvalidated shell rings) suggest that longevity varies widely among species. Some species appear to be short-lived (<10 years, Rogers *et al.*, 2001; Hanlon & Levine, 2004), others reach ages of at least 50 years (Rypel *et al.*, 2008), and still other species have intermediate life spans of 20–35 years (Jones & Neves, 2002; Haag & Staton, 2003; Jones *et al.*, 2004). Studies based on validated shell rings from a variety of species and habitats are needed to describe accurately patterns of age, growth and longevity in freshwater mussels.

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