Internal shell annuli yield inaccurate growth estimates in the freshwater mussels Elliptio complanata and Lampsilis radiata

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SUMMARY

1. The objective of this study was to compare, in freshwater bivalves, growth rates inferred from the spacing of internal annuli to those obtained directly by measuring annual changes in shell length.
2. The unionid mussels studied were Elliptio complanata and Lampsilis radiata from a pond in the north-eastern U.S.A. Age was inferred from internal lines of 157 E. complanata and twenty-five L. radiata. Actual length change was determined from comparisons of annual remeasurements of marked E. complanata (n = 520) in 1992–95 and L. radiata (n = 81) in 1993–95.
3. High retrieval rates of painted mussels demonstrated the efficacy of the marking method.
4. Annual length changes determined by remeasurement were significantly lower than annual length changes predicted by length-at-age data from internal annuli. Should this be a common occurrence, past estimates of annual growth based on annuli are probably too large, and unionid mussels may be much older than previously assumed.

Introduction

Shell annuli are the principal source of age and growth rate information in bivalve molluscs. Growth rings appear on the outside of shells (external annuli) and as layers in the cross-section of deposited shell material (internal annuli). Most molluscan ecologists assume that one of these growth annuli is formed each year (Lutz & Rhoads, 1980; El Moghraby & Adam, 1984), and the measurement of the distance between these rings has been assumed for four centuries (MacCurdy, 1954) to reflect the annual growth performance of individual bivalves. Microscopic shell rings have even been postulated to record the length of lunar, daily and tidal cycles (Lutz & Rhoads, 1980). The analysis of growth rings recorded in molluscan shells is a research tool of great importance to palaeontology (Lutz & Rhoads, 1980), resource management (Turekian et al., 1982), ecology (Arnold et al., 1991), ecotoxicology (Harmon & Joy, 1990), archaeology (Lutz & Rhoads, 1980), analyses of climate change (Jones, 1983) and geophysics (Hughes, 1985).

In spite of their long-standing application (see review by Downing, Shostell & Downing, 1992), the empirical justification for using external or internal annuli to measure bivalve growth is questionable. Few have checked the assumption that external shell annuli are laid down annually, and these few tests have yielded equivocal results for freshwater mussels (e.g. Negus, 1966; Ghent, Singer & Johnson-Singer, 1978; Haukoja & Hakala, 1978). Downing et al. (1992) recently used marking to test the assumption of annual external annulus formation in Lampsilis radiata and found that counts of shell annuli greatly underestimated age and overestimated growth rates. Further, they found that the median-length mussel formed only half of an external annulus each year, and that many mussels had formed no new external annuli at all, even several years after marking. Although some have suggested that internal annuli may yield more reliable age and growth estimates than external growth...
lines (Jones, 1983), no direct test of the annual formation of internal annuli has been made for freshwater mussels.

The objective of our study was to compare growth rates inferred from the spacing of internal annuli with those obtained directly by measuring annual changes in shell length. If bivalve shell annuli are formed once per year, then calculations of growth history based on internal shell annuli should agree with growth measurements made annually on living individuals. If internal annuli are formed more frequently than once per year, due for example to reproductive events or fluctuating conditions, then the growth histories inferred from internal annuli should underestimate the observed annual growth. Alternatively, if annuli are not formed consistently every year, or are often indiscernible, then estimates of yearly growth based on internal annuli might be greater than that seen in annual measurements of individual mussels.

Materials and methods

Growth of marked individuals was compared with that inferred from internal growth rings formed by Elliptio complanata (Lightfoot) and Lampsilis radiata (Barnes) in Worden Pond, Rhode Island, U.S.A. (41°26’N, 71°34’W). Identity was verified using Smith (1986), and voucher specimens are deposited in the Zoology Department, University of Rhode Island. Worden Pond is a shallow ($Z_{\text{max}} = 1.8$ m, $Z_{\text{mean}} = 1.2$ m) soft-water lake with a surface area of 430 ha. It is considered eutrophic (Green & Herron, 1994).

We collected shells of living animals for ageing in July 1991, and estimated age for E. complanata ($n = 157$) and L. radiata ($n = 25$) by microscopically examining internal growth lines as thin sections (Neves & Moyer, 1988). Internal growth annuli were defined as areas of discontinuity of the prismatic layer that extended from the nacreous layer to the periostracum (see Fig. 9 of Day, 1984). Thus, a group of animals of varying sizes were aged by counting internal annuli, allowing us to determine the average size of these two species at a variety of ages. We measured shell length using dial callipers according to Hinch, Kelly & Green (1989). The ‘size-at-age’ data were used to calculate von Bertalanffy growth curves using the program of Saille, Recksiek & Prager (1988). The von Bertalanffy equation (Ricker, 1975) is:

$$L_t = L_a(1 - e^{-K(t - t_0)})$$

where $L_t$ is the shell length at a given age ($t$, years) measured in mm, $t_0$ is the extrapolated age at which theoretical length is zero, $L_a$ is the theoretical shell length at infinite age, $K$ is a fitted constant reflecting the rate of approach of $L_t$ toward $L_a$, with increasing age, and $e$ is the base of natural logarithms. According to Gulland (1964), the relationship between the predicted rate of annual shell growth ($\Delta L_t$) and shell size ($L_t$) can be calculated:

$$\Delta L_t = L_a[e^{-K} + L_t(e^{-K} - 1)]$$

Expected incremental growth rates ($\Delta L_t$, mm year$^{-1}$) of animals of a given size ($L_t$) could thus be inferred from internal annuli. These annual growth rates can then be compared with actual, field-measured growth rates.

Actual rates of growth of mussels’ shells were determined by remeasuring marked individuals in subsequent years. During late July and early August 1991 we marked 402 E. complanata, from the same area of the pond as aged mussels, using the method of Ghent et al. (1978) by cleaning both valves and spraying with Krylon® paints (white primer and fluorescent orange). Animals were returned to the water in the same area within 30 min to minimize stress. Before animals were returned to the lake, we measured their lengths ($L_t$, mm) and numbered individuals with permanent marker. Because Kesler & Bailey (1993) demonstrated a difference in shell shape of E. complanata from different substratum types in Worden Pond, these marked and measured mussels were placed in two types of sediment: 189 were placed in sand and 213 were placed in silt. Silt substratum did not have a gritty feel when rolled between the fingers and was a dark colour. We also measured and marked thirty-six L. radiata in June 1992 and returned these to sand sediment.

To test the possibility that marking might reduce shell growth, we enclosed two size-matched groups ($n = 20$ for each) of E. complanata with wire fence (2 m diameter, square mesh size 2.5 cm) in July 1994. One group of mussels was marked and one was not. The lengths of all individuals in each treatment were recorded. All mussels (except two marked individuals) were retrieved and remeasured in June 1995. The mean (± SE) shell lengths of marked E. complanata in this enclosure in 1994 and 1995 were 48.66 (± 2.38)

mm and 49.30 (± 2.53) mm, respectively. The mean shell lengths of unmarked mussels in this same enclosure in 1994 and 1995 were 47.84 (± 2.94) mm and 48.75 (± 2.68) mm, respectively. Average length change was less than 1.0 mm in both marked and unmarked *E. complanata*, and there was no significant difference between any means (t-tests, *P* > 0.05). There was no significant difference in growth rates of marked and unmarked mussels, thus marking did not influence shell growth rates. Further, average growth rates of marked *Lampsilis* found using our marking method fell directly between average shell-length growth rate data found for *Lampsilis* in two populations in another lake (Downing & Downing, 1993). These results strongly suggest that growth in marked and unmarked mussels is not widely divergent and that our mark and recapture estimates reflect real, *in situ*, growth rates.

During June of 1992–95, we retrieved marked mussels using SCUBA and remeasured their lengths. Individuals were taken from the water, measured and immediately (< 1 min) returned to water. They were returned to approximately the same location from which they were taken. Actual annual rates of change in shell size (ΔL, mm year⁻¹) of individuals were determined as the difference in shell lengths in subsequent years. The relationship between ΔL and total shell length (L₀) was compared with the expected relationship derived from counting internal annuli (eqn 2) by a technique similar to that of Tsutukawa & Hewett (1977). If the internal annuli are good estimators of age, then actual estimates of ΔL at L₀ should be distributed evenly around the relationship described by eqn 2. Tests of goodness-of-fit of eqn 2 to real shell growth rates were performed by testing the null hypothesis that the distribution of (ΔL - ΔL₀) does not differ significantly from zero. This hypothesis was tested for each species of mussel from each sediment type using the Wilcoxon signed-rank test (Snedecor & Cochran, 1989).

**Results**

Marked mussels grew slowly but consistently over the years. The length-specific growth rates (ΔL) for *E. complanata* from sand (in 1992–95) and silt (in 1993) sediments are shown in Fig. 1. The slopes of these relationships differ significantly from zero (*P* < 0.05), with smallest mussels having greatest rates of change in length. Growth from August 1991 to June 1992 was not included because this interval was not a full year.

We tested the assumption of homogeneity of slopes of relationships between shell growth and initial length for individuals from sand and silt sediment, using the combined data for *E. complanata* remeasured during 1992 and 1993. Shell growth rates declined similarly with shell length in animals placed on sand and silt, demonstrated by the interaction of sediment–location and initial length not being statistically significant in a multiple regression analysis (df = 1,742, *P* = 0.097). Simple ANCOVA showed, however, that mussels living on silt and sand substrata grew at significantly different rates. Mussels in silty sediment grew significantly more rapidly than those living on sand (ANCOVA, df = 1,742, *P* < 0.00001).

The equations describing annual length change (ΔL, mm year⁻¹) vs shell length at the beginning of the growth year (L₀, mm) for *E. complanata* remeasured in 1993 from both substrata were:

- Sand: \( \Delta L = 1.73 - 0.027L₀ \) (n = 153, \( r² = 0.23 \))
- Silt: \( \Delta L = 3.86 - 0.063L₀ \) (n = 104, \( r² = 0.25 \))

The overall equation for *E. complanata* remeasured in sand from 1992 to 1995 was:

\( \Delta L = 2.20 - 0.036L₀ \) (n = 416, \( r² = 0.21 \))
The relationship of $\Delta L$ to $L_t$ for *L. radiata* collected in sand in 1993–95 is shown in Fig. 2. The equation describing annual length change ($\Delta L$, mm year$^{-1}$) vs initial length for individuals collected in 1993–95 was:

$$\Delta L = 1.81 - 0.03L_t \quad (n = 81, \ r^2 = 0.28)$$

Growth estimates based on measurement of internal annuli yielded much more rapid apparent increases in size with age than did remeasurements of marked individuals. The predicted length-at-age relationships for *E. complanata* and *L. radiata* were determined from von Bertalanffy equations. Von Bertalanffy growth equations (eqn 1) for *E. complanata* were:

- Sand: $L_t = 70.5(1 - e^{-0.163(t-2.52)})$
- Silt: $L_t = 83.6(1 - e^{-0.148(t-1.17)})$

The standard errors of $L_m$ for sand and silt were 2.4 and 3.6, respectively. The standard errors of $K$ for sand and silt were 0.027 and 0.021, respectively. Using the method of Chen, Jackson & Harvey (1992), these growth equations were found to be significantly different ($F = 28.78$, df = 3149, $P < 0.001$).

The von Bertalanffy growth equation describing length at age for *L. radiata* was:

$$L_t = 73.5(1 - e^{-0.163(t+1.13)})$$

The predicted lengths from these equations allowed determination of annual length changes vs initial length for comparison with direct measurements. These relationships are given for *E. complanata* from sand and silt and *L. radiata* from sand in Fig. 3. Wilcoxon tests of fits of relationships between $\Delta L_t$ (determined from internal annuli) to $\Delta L$ (determined from remeasurement of marked individuals) demonstrate that growth rates based on internal annulus measurements severely overestimate growth and therefore underestimate age (Table 1). Growth inferred from internal annuli can be nineteen times higher than the actual rate of growth, and errors are most severe for small individuals.

Marked mussels showed low mortality rates. Only a few mussels disappeared from the population each year. Annual retrieval of *E. complanata* in sand sediment was higher than in silt. Retrieval rates for *E. complanata* marked in 1991 from sand were 97% (1992), 84% (1993), 87% (1994) and 76% (1995). Retrieval rates for *E. complanata* from silt sediment were 81% (1992) and 49% (1993). Dense growth of the macrophyte *Najas* sp. on silty substrata in 1994 made retrieval impossible. While no macrophyte growth occurred in 1995, no marked individuals could be found in silt during 1995. Retrieval from sand of the thirty-six *L. radiata* marked in 1992 also declined over time: 86% (1993), 86% (1994) and 72% (1995).
Table 1 Comparisons of relationships between \( \Delta L \) (predicted length change at \( L_0 \) determined from internal annuli), to \( \Delta L \) (length change at \( L_e \) determined from remeasurement of marked individuals) using a Wilcoxon signed-rank test-statistic (Z). \( n \) is the number of growth rate comparisons, SD is the standard deviation of \( (\Delta L_0 - \Delta L) \), \( P \) is the probability that internal annuli yield the same annual growth rates as actual growth measured by measuring marked individuals in subsequent years, i.e. that average \( (\Delta L_0 - \Delta L) = 0 \)

<table>
<thead>
<tr>
<th>Species</th>
<th>Sediment</th>
<th>Average ( (\Delta L_0 - \Delta L) ) (n; SD)</th>
<th>Wilcoxon Z</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elliptio complanata</td>
<td>Sand</td>
<td>2.633 mm (416; 0.787)</td>
<td>17.67</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Elliptio complanata</td>
<td>Silt</td>
<td>3.888 mm (104; 0.840)</td>
<td>8.85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lampsis radiata</td>
<td>Sand</td>
<td>2.133 mm (81; 1.058)</td>
<td>7.813</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Discussion

It is likely that growth rates based on annual re measurement of individual mussels accurately reflect real in situ growth rates. The high rate of retrieval of living, marked mussels demonstrates the effectiveness of this marking technique. Numbers marked on the shells were clearly readable after 4 years. Shell material was deposited by many of the mussels after painting, and thus the marking technique does not appear to inhibit shell secretion. Further, direct comparison of the growth of size-matched mussels that were marked with growth of unmarked animals showed no significant differences. High retrieval rates of animals marked using this method suggest that mortality caused by painting was very low for these species.

Some length changes of large individuals were negative. Given the small amount of length change that we observed, especially of larger mussels, measurement errors may account for some of these negative values. Real negative shell growth has been reported by others (e.g. Downing et al., 1992). While some of our mussels may have actually shrunk, most of our mussels that decreased in size shrank less than the normal rates of remeasurement error given by Downing & Downing (1993) (± 1 mm, 95% of the time).

The amount of growth observed in the recapture remeasurement data was very low. We know of no lower reliable annual measurements of growth in shell length of E. complanata. One potential concern when interpreting these low growth rates might be that our marking method somehow inhibited growth. First, it seems very unlikely that growth inhibition due to short-term marking and handling would have continued for 4 years. There was no trend toward increased growth rates with time since marking. The similar changes in length of both marked and unmarked mussels in the enclosure experiment also demonstrated the lack of a marking effect.

Another potential explanation for the low growth in shell lengths that we observed might be that growth in the past (recorded in internal annuli) was greater than current growth (measured by marking) due to poor or degraded water quality. This is unlikely, however, because routine monitoring suggests no substantial changes in water quality in Worden Pond over at least the last 6 years (Green & Herron, 1979).

The greater growth of E. complanata in silt is inconsistent with the observations of several workers. Bailey & Green (1988) reported a positive relationship between growth rate and wave exposure, implying that sandy sediments should yield greater mussel growth than mud and silt. While slightly more exposed to wave action than the silt site, the sand habitat in Worden Pond is not an extremely high-energy environment that might inhibit mussel growth. Therefore, following Bailey & Green's (1988) results, one should expect faster growth in length at our sandy site. Likewise, Kat (1982) observed E. complanata to have reduced growth when found in muddy substrata. Without uniform definitions of substratum type, however, it is difficult to make comparisons of the influence of substratum on growth in different habitats. Hinch, Bailey & Green (1986) found fastest growth in shell length in L. radiata from sand sediment. Annual remeasurement of mussel shells indicates greater growth of E. complanata in silt, and this finding is consistent with the length-at-age data derived from internal annuli, but both of these sets of data contradict earlier studies.

To allow comparison with other studies we used the procedure of Metcalfe-Smith & Green (1992) and used our internal annulus-determined ages and lengths to calculate the annual growth in shell length between the ages of 5 and 9. These rates were 5.3–
they predicted much higher $L_\infty$ values for individuals in sand. While Day (1984) measured $L_\text{radiator}$ shell length from the umbo to the posterior edge, her growth equation coefficients were similar those of $L. \text{radiator}$ from Worden Pond.

Growth rates determined from the remeasurement data of this study differ greatly from those derived from length-at-age data estimated using internal annuli. The similarity of growth derived from internal annuli data to literature values, although lower in individuals from sand, suggests that Worden Pond mussels were ‘aged’ in a manner consistent with techniques used by other workers. The disparity between growth estimates from remeasurement and internal annuli data may be due to the inappropriate assumption that internal shell annuli of mature individuals indicate annual growth in Worden Pond. If mature individuals do not form shell rings every year, estimates of growth based on length-at-age data will overestimate real annual growth. This is consistent with the phenomenon described by Downing et al. (1992). Should this be a common occurrence, two significant points emerge: first, past estimates of annual growth based on annuli are probably overestimates, and second, unionid mussels may be much older than previously assumed. Both of these possibilities have significant consequences for the management and conservation of freshwater mussels.

References


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