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Measuring growth from shell rings in populations of Anodonta piscinalis (Pelecypoda, Unionidae)

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The following recommendations are given when shell rings are used in a growth study of *Anodonta piscinalis:* 1) The regularity of ring formation has to be checked, especially where stunted populations are studied. 2) When methods of measuring are standardized, it is possible to use measurements made by more than one person. 3) Sexes can be pooled unless the aim is to detect small differences in growth. 4) Account must be taken of the differential growth of mussels at different depths. 5) The most suitable single measure of growth is the length of the third annulus. 6) To eliminate interpopulation differences between good and bad years for growth and reproduction, it is best in comparisons to use the length (i.e. longest diameter) of the third annulus in the age class that has just passed its third winter. This also minimizes the effects of growth-rate-dependent mortality.

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Growth rings are formed in the shells of mussels and in the scales or ossified parts of fish when growth temporarily ceases or slows down. In temperate and cold climates this occurs in winter (TESCH 1968). In fish growth rings can be used to calculate the lengths of the fish at earlier stages in their lives. In mussels previous lengths can be read directly from the rings.

The reliability of such rings as a basis for studying growth depends mainly on the regularity of ring formation. Accuracy and repeatability in counting rings and in the measuring process are also important (e.g. WESTRHEIM 1973, LINFIELD 1974). Differences in growth among the various sectors of a population increase the variance of the results. The estimate of growth is also affected if different populations contain different proportions of such sectors. More difficulties arise if the earliest growth rings are estimated by back-calculation only, and if mortality operates in a growth-rate-selective way.

In the present paper we analyse the reliability and applicability of shell rings as the basis of a growth study in *Anodonta piscinalis* (Nilsson) (Unionidae), and recommend procedures for minimizing potential errors in such studies.

2. Material and methods

Mussels were collected in 1971—1974 by hand-picking from shallow water (0.5—1.0 m) from two river systems, Kokemäenjoki and Paimionjoki, in southwest Finland. Because of the method the smallest individuals (age classes 0 and 1, with 0 and 1 winter rings, respectively) were underrepresented in the samples (HAUKIOJA & HAKALA 1974).

The total length of the mussel and the longest diameter (= length) of each growth ring was measured to the nearest mm.

3. Interpretation of growth rings

When growth rings are checked the number of rings may be interpreted wrongly, and the length of a ring may be measured inaccurately.

A. Are growth rings formed annually?

Mussel growth does not always follow the rule one ring, one year. In *Dreissena polymorpha*, for example, growth slows down in summer at the time of reproduction, and two rings are formed per year (MORTON 1969). CROWLEY (1957) reported that environmental stress causes the formation of false rings in *Anodonta piscinalis*. These are further potential sources of error.

Practical work with *Anodonta piscinalis* soon gave the impression that in most populations rings are not difficult to interpret — especially if the mussels are growing rapidly and the colour of the shell is light. Fig. 1 shows an example of such a population (not very easy to measure, because growth was only moderate). This population had a dominant year class and its position in the age distribution moved one step to the right each year, indicating that growth rings were formed annually.

False rings are usually weak and can easily be distinguished from "true" winter rings. But in stunted populations with dark shells there may be difficulties. We made an experiment on one such population by marking 135 mussels in 1971 and 1972. A number was engraved on each shell with a scalpel. The mussels were released and some of them were recovered later. Table 1 summarizes the changes in the number of rings in recovered individuals. There are variations but in most cases the number of new rings coincided with the number of winters that had elapsed. Therefore, even in

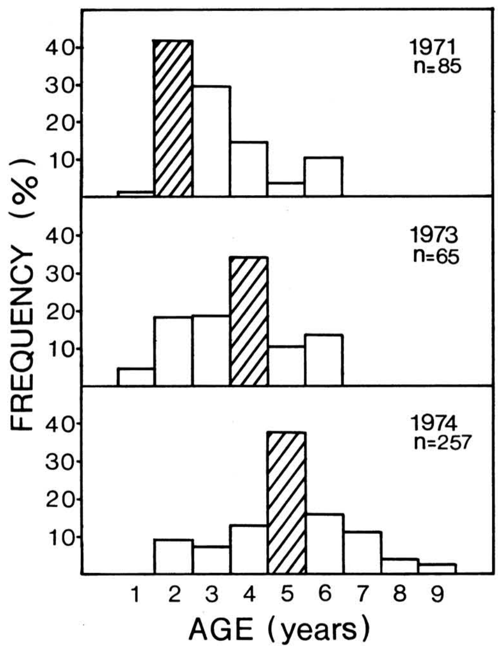

Fig. 1. Age distribution in a population (Kiikoinen, Mouhijoki) in different years. Hatching denotes the 1969 year class.

Table 1. Interpretation of growth rings in mussels marked individually.

Years elapsed	Number of new rings 0	1	2	3	4	5	mean
1	3	12	3	1	–	–	1.1
3	–	–	2	4	–	2	3.3

stunted populations growth rings could be determined correctly in most individuals.

At the beginning of the experiment the largest mussels were 7 years old. What was said above is relevant for mussels under this age. For mussels over 10 years old only approximate ages can be given. Growth may even cease completely at very great ages (unpublished).

B. Differences between measurers

Two persons measured the same 100 mussels marked individually with a number inside the shell. The mussels originated from a population with a moderate growth rate. Person *A* was accustomed to measuring while person *B* was a novice. *A* measured the same mussels again after 3 days.

Age was difficult to determine in 12 of the 100 mussels. In two cases the estimates differed by 2 years. There were fewer differences between the two series of age determinations made by *A* than between those made by *A* and *B* (Table 2).

These errors in age determination increased discrepancies caused by inaccuracies in measuring the lengths of the rings. The differences were greatest in the oldest year classes because errors in age determination were cumulative. When the measurements were compared in pairs, no significant differences were found (Table 3). In 13 out of 21 cases the differences were less than 1 mm, i.e. within the limits of accuracy in measuring.

Table 2. Differences in age determination of the same mussels. (A_1 = first determination by person A, A_2 = second determination by person A, B = determination by person B).

Difference in age (years)	A_1-A_2	A_1-B	A_2-B
−1	–	6	10
0	96	92	88
+1	4	1	1
+2	–	1	1

Table 3. Comparison of measurements of the same mussels. (A_1 = first measurement by person A, A_2 = second measurement by person A, B = measurement by person B).

	Ring	Difference of means (mm)	t	d.f.
$A_1 - A_2$	1	0.0	0.00	198
	2	0.2	0.25	198
	3	0.5	0.49	136
	4	0.4	0.27	79
	5	0.1	0.04	20
	6	0.1	0.04	16
	7	1.9	0.57	13
$A_1 - B$	1	−0.8	1.13	198
	2	−0.4	0.42	198
	3	1.0	0.88	135
	4	1.6	1.04	75
	5	2.2	0.72	21
	6	−0.1	0.04	16
	7	−1.9	0.86	10
$A_2 - B$	1	−0.8	1.14	198
	2	−0.6	0.63	198
	3	0.5	0.45	135
	4	1.2	0.78	78
	5	2.1	0.69	21
	6	−0.2	0.07	16
	7	−3.8	1.05	11

4. Sources of variation within a population

Differences between populations are easier to detect if variances are small. In the following we discuss the main factors causing variance in the mean length of a year ring within a population.

A. Differential growth of sexes

From the third year onwards females are significantly longer than males (Fig. 2), so if the sexes are not separated, variance in length increases in older year classes. If populations differ in sex ratios, the mean length is also affected. In nine populations the percentage of males varied between 39.0 and 55.5 (unpublished). But when the extreme sex ratios are used to calculate the mean length, e.g. at the 7th year ring, from the data shown in Fig. 2, the difference between the mean is still only 1.1 mm. This is practically the same as the error in measurement and in interpretation of the rings. Therefore, measurements for the two sexes can be pooled unless the aim is to detect tiny differences.

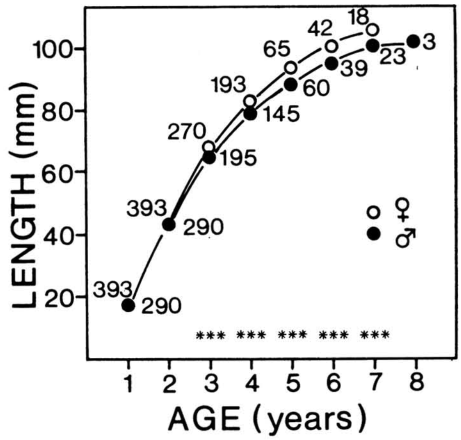

Fig. 2. Growth of sexes at Paimionjoki, Santio. Numbers of measurements are given. *** = mean lengths of sexes differ significantly ($P < 0.001$).

B. Good and bad years for growth and reproduction

During a warm summer *Anodonta piscinalis* may grow twice as much as during a cold summer (Negus 1966, Hakala & Haukioja, in prep.). Breeding success also varies annually (see Fig. 1) and consequently a year class may be almost entirely absent or may form up to 80 % of the whole population (unpublished). This may cause a large variation when comparisons are made, because adjacent populations seem to be entirely independent as regards annual reproductive success but strongly correlated in annual growth rates (unpublished). For instance, in a sample collected in 1973 at Paimionjoki, Mäntsälä, growth in length during the third summer varied as follows:

Age class	Growth (mm)	n
1967	18.5	30
1968	16.0	70
1969	8.0	53
1970	19.3	8

The unweighted mean for growth in the 3rd summer was 13.3 mm. In another population the age distribution might well have been 53, 8, 30, 70 for individuals of ages 3—6 years, respectively; the mean growth would then have been 17.8 mm. Thus the potential error is considerable.

C. Growth and probability of being caught

Movements of mussels need not be taken into account in growth studies unless they and the method of sampling lead to a sample biased in relation to growth. In Paimionjoki, Santio, we sampled at least 150 individuals each month (excluding months with an ice cover) in 1973 and 1974. The mean length of the third annulus and the coefficient of variation in a cohort showed seasonal fluctuation (Fig. 3). The mean length is greatest at mid-summer, when the sample is most homogeneous. The probable reason is that mussels which live in shallow water in summer have a warmer environment and grow more rapidly then than those living in deeper water. Causal relationships are not clear. Differential movements of sexes cannot explain the difference because the difference within a year is larger than could result from the differential growth of the sexes (Fig. 2).

5. Growth-selective mortality

Growth curves obtained by back-calculation describe only the earlier growth of the actual individuals measured. But what is of interest is usually the growth of a population, i.e. the average length of mussels of that population at a certain age. Growth curves obtained by back-calculation and population growth curves (obtained by annual sampling) are identical only if there is no growth-selective mortality in the population. But if mortality is higher in individuals that grow faster, back-calculation of the length of the first annulus from old individuals will give a smaller figure than the mean length of the age class at the time when the ring was formed. This is known as Lee's phenomenon (RICKER 1958).

In *Anodonta piscinalis* we found that selective mortality may operate in either direction or not at all. Samples from three populations, discussed below, were taken at the same time of the year (in autumn) by the same method (hand-picking) and measured by the same person.

In population A (Paimionjoki, Mäntsälä) the mean length of a certain annulus in a cohort was in most cases significantly smaller after a year (Table 4, Fig. 4). This means that selection operated against the individuals with the highest growth rates. In population C (Kiikoinen, Mouhijoki), sampled in 1971, 1973 and 1974, the mean length of a year ring in a cohort increased all the time (Table 4, Fig. 5). Here selection worked against the individuals with low growth rates. Males suffered higher mortality than females (unpublished), but the change in the mean length is larger than could have resulted from this alone. In Fig. 6 the population growth curve is from samples taken in different years. The curve for calculated growth is produced by back-calculation from the 1974 sample. The farther back we go, the more the two curves differ.

In population B (Paimionjoki, Santio) we have not found any clear tendency to growth-

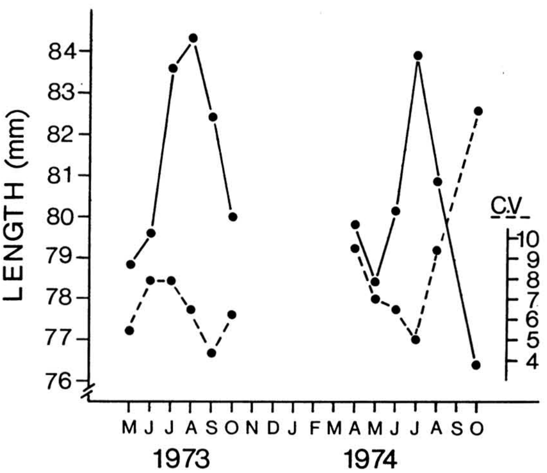

Fig. 3. Monthly estimates of the length (solid line) and the coefficient of variation (dotted line) of the third annulus in mussels hatched in 1969 at Paimionjoki, Santio.

Table 4. Trends in the mean length of annual rings in three populations (A = Paimionjoki, Mäntsälä, B = Paimionjoki, Santio, C = Kiikoinen, Mouhijoki) of *Anodonta piscinalis* as back-calculated from the same cohorts sampled in different years. The material comprises all year rings represented by more than 20 measurements. The statistical analysis used is Student's t test.

		Differences of means $P < 0.001$	$P < 0.05$	n.s.	Total
A	$\bar{x}$ greater	–	–	1	1
	$\bar{x}$ smaller	2	7	5	14
B	$\bar{x}$ greater	–	3	8	11
	$\bar{x}$ smaller	5	1	4	10
C	$\bar{x}$ greater	7	1	6	14
	$\bar{x}$ smaller	–	–	2	2

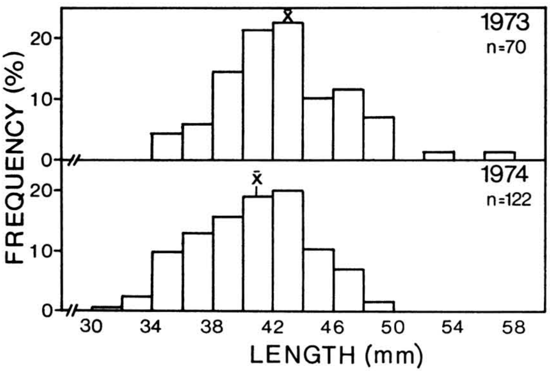

Fig. 4. Frequency distribution of lengths of the third annulus in the 1969 year class at Paimionjoki, Mäntsälä, as calculated from samples taken in 1973 and 1974.

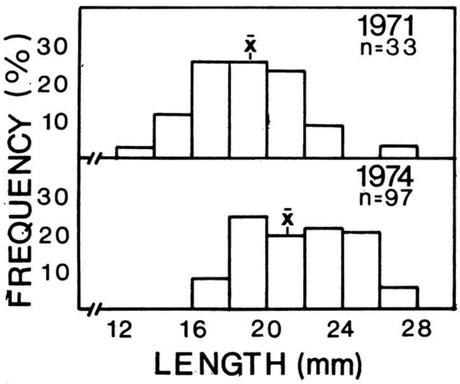

Fig. 5. Frequency distribution of lengths of the first annulus in the 1969 year class at Kiikoinen, Mouhijoki, as calculated from samples taken in 1971 and 1974.

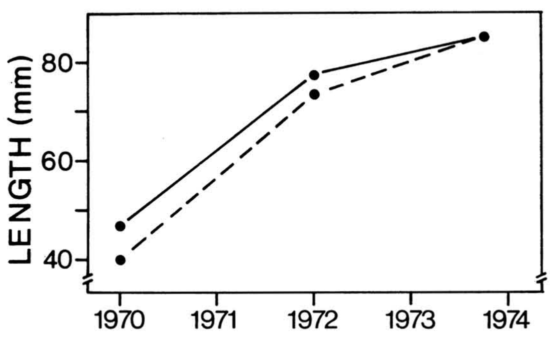

Fig. 6. Back-calculated growth (solid line) and measured growth (dotted line) of the 1969 age class (Kiikoinen, Mouhijoki).

dependent mortality (Table 4). Here the significant decreases in mean length are all due to the oldest year class, where the larger individuals tended to die before the smaller ones.

6. Conclusions

In the following we list recommendations for comparing growth in different populations of *Anodonta piscinalis*. With certain modifications they are also applicable to other species of molluscs and fishes.

1) Regularity of ring formation has to be checked in different populations, especially where growth is slow and consequently the rings are difficult to distinguish.

2) A good description is needed of the measuring procedure and of the practice to be adopted in interpreting doubtful cases. Once methods have been standardized, measurements by several persons can be pooled.

3) The sexes need not be separated in the samples unless the aim is to reveal very small differences.

4) The best sample is one comprising specimens from all depths at which the species occurs (up to 5 m in *Anodonta*, HAUKIOJA & HAKALA 1974). In practice, a useful alternative is to take samples from a given depth at the same time of year (preferably in autumn or in spring).

5) As a measure of growth in a population we prefer the mean length of the third annulus to parameters derived from growth equations. This is because parameters of asymptotic growth equations do not have a clearly biological interpretation (HAUKIOJA & HAKALA, in press). We recommend the third annulus in *Anodonta* because in populations where the average age is low it is difficult to find enough older individuals. Besides, all animals 3 years old or older are large enough to ensure that the sampling procedure is not size-selective.

6) For comparisons between populations we recommend the mean length of the third annulus in the age class that has just passed its third winter. When the third ring of one age class is used in each population instead of all third annuli, the error caused by d fferential annual growth is eliminated. This is especially valuable where the populations compared are geographi-

cally close enough to experience the same weather conditions, especially the mean temperature of the growing season. Choice of the youngest year class that is more than 3 winters old minimizes the effects of growth-selective mortality.

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