Unionid Growth Curves Derived from Annual Rings: A Baseline Model for Long Point Bay, Lake Erie

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Growth curves for *Lampsilis radiata* and *Anodonta grandis* collected in July 1981 at three stations in Long Point Bay, Lake Erie, were determined based on measured lengths at annual winter shell rings. A Walford Plot technique was used to convert annual growth-in-length increments to length vs. age growth models. For the two species, we used simulated baseline and environmental impact data to illustrate the use of baseline growth models in testing for long-term environmental deterioration.

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À partir des longueurs au moment de la formation d'anneaux annuels d'hiver sur la coquille, nous avons établi les courbes de croissance de *Lampsilis radiata* et d'*Anodonta grandis* recueillis en juillet 1981 à trois stations de la baie Longue-Pointe (lac Érié). Le tracé de Walford a été utilisé pour convertir les augmentations annuelles de croissance en longueur en modèles de croissance en longueur vs. âge. Nous avons fait appel, pour les deux espèces, à une simulation de données de référence fondamentale et d'impact sur l'environnement dans le but d'utiliser le modèle de croissance de base pour vérifier la détérioration à long terme de l'environnement.

IN temperate latitudes bivalve mollusks undergo seasonal variations in growth that are recorded in their shells as winter growth rings. Use of molluscan shells as records of past and present-day environments has been recently reviewed and thoroughly documented by Rhoads and Lutz (1980). Growth rate as an indicator of environmental impact has been largely neglected.

Bivalve growth rates are known to vary with environmental conditions. Oxygen supply, turbidity, substratum, temperature, light, depth, population density, food supply, and strong-water agitation all influence growth rates in bivalves (Hallam 1965; Stansbery 1967). Rapid temperature fluctuations are an important cause of pseudoannuli, or growth interruptions not related to normal winter growth cessation, and the number produced is proportional to the temperature instability of a body of water (Stansbery 1967). Pseudoannuli, however, are reliably identified in unionids as thin and incomplete bands, differing in color and texture, and lacking in the periodicity of yearly bands (Isely 1914; Coker et al. 1920; Stansbery 1961; Negus 1966). Recent methods for reading bands from sectioned shells (Rhoads and Lutz 1980) can replace, or validate, analysis of bands on the shell external surface.

Because growth history of bivalves can be determined from growth banding, change of growth rate caused either by short-term or long-term environmental change can be discerned. Kennish (1976) measured the effects of thermal effluent on the growth of the bivalve *Mercenaria mercenaria* and found a reduction in daily summer growth increments of 10 – 30% due to excessively high water temperatures. On the other hand, Negus (1966) observed increased growth of *Anodonta anatina* because of a heated effluent.

Unionids with wide geographic distribution such as *Lampsilis radiata* and *Anodonta grandis* are valuable research organisms because much taxonomic, zoogeographic, and ecological information on them already exists, and because growth rate differences related to natural environmental variability can be identified. Long Point Bay, which supports a large fish spawning community and important waterfowl populations, is a valuable study site. It is a popular commercial and sportfishing area, heavily cottaged, and potentially vulnerable to impact from the nearby Nanticoke industrial development area. Here we derive baseline growth rates from annual growth rings for *L. radiata* and *A. grandis*, to illustrate use in future tests of environmental change.

Methods and Materials

Sampling for live clams was done by SCUBA in July 1981 at four stations which are shown in Fig. 1. Clams were detected by sight and touch; all sizes were sampled. Abun-
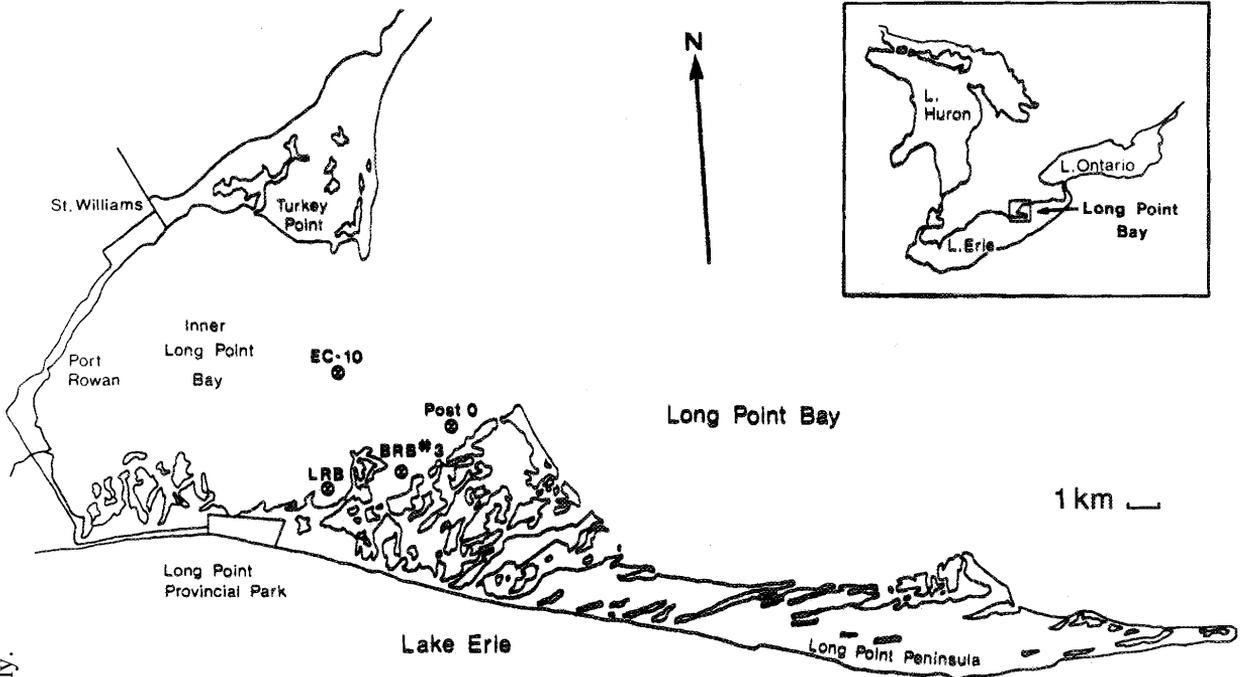
FIG. 1. Long Point Bay showing the four sampling stations. Inset shows location of bay in Lake Erie.

dances of the three most common species varied among the stations (Table 1). *Elliptio dilatata* was abundant at only one station; therefore, we restricted our study to *L. radiata* and *A. grandis*.

Lengths at annual winter rings were measured with vernier calipers and recorded separately for each species and station. The annularity of the measured rings is highly probable. We have not done marking studies at Long Point Bay, but such studies on *Anodonta* in a Manitoba lake (Green unpublished data), a Quebec lake (G. L. Mackie, University of Guelph, personal communication), and the River Thames in England (Negus 1966) indicate annual (winter growth cessation) rings. Unionid species differ in separation and clarity of annual rings on the shell external surface — for example, *Elliptio* rings are difficult to read (Strayer et al. 1981) whereas *Anodonta* rings are especially clear and *Lampsilis* almost as clear. On our specimens the spacing of the presumed annual rings on the external shell surface was very regular and consistent from individual to individual within each species.

Early workers were concerned with variable darkness of lines on the shell surface, and doubted the reliability of aging methods based on "annular rings" of unionids (e.g. Lefevre and Curtis 1912). However, subsequent workers (Coker et al. 1920; Negus 1966) found that annual rings on the shell surface, verified by examination of internal bands in shell sections, are easily distinguished from "disturbance rings" in that annual rings have a closely packed stop/start pattern (and are therefore darker) whereas disturbance rings are lighter and thinner single lines. There is, of course, greater difficulty when working with shallow-water marine bivalves, especially at lower latitudes; a less extreme seasonal temperature cycle combined with tidal cycles of various periodicities results in

weaker annual rings with many non-annual rings between them. However, for lentic unionids Chamberlain (1931) cites Isely (1914) as concluding "from his study of some 900 specimens of North American species of fresh-water mussels which he tagged and subsequently recovered ... that the winter rings ... are usually sufficiently regular and definite to be used as indicators of age." Chamberlain himself concludes, from his work with *Lampsilis* species in Minnesota and Wisconsin lakes, that "Considering all of the data obtained from over 1,100 specimens, the application of the annual-ring method to growth studies of fresh-water mussels seems both reliable and practical, judging from the uniformity of the growth curves and the ease with which these measurements may be made on the great majority of shells."

In any case the only necessary assumption for our objectives and for the statistical methods proposed and illustrated here is regularity, not annularity. Certainly the rings are regular, on the shell external surface and also in thin sections (after Clark 1980) as illustrated in Fig. 2. If, for example, the rings were semiannual rather than annual, then the statistical tests which follow would be unaffected. If there were also some added error due to disturbance rings being sometimes

TABLE 1. Number of clams collected at the four stations in Long Point Bay.

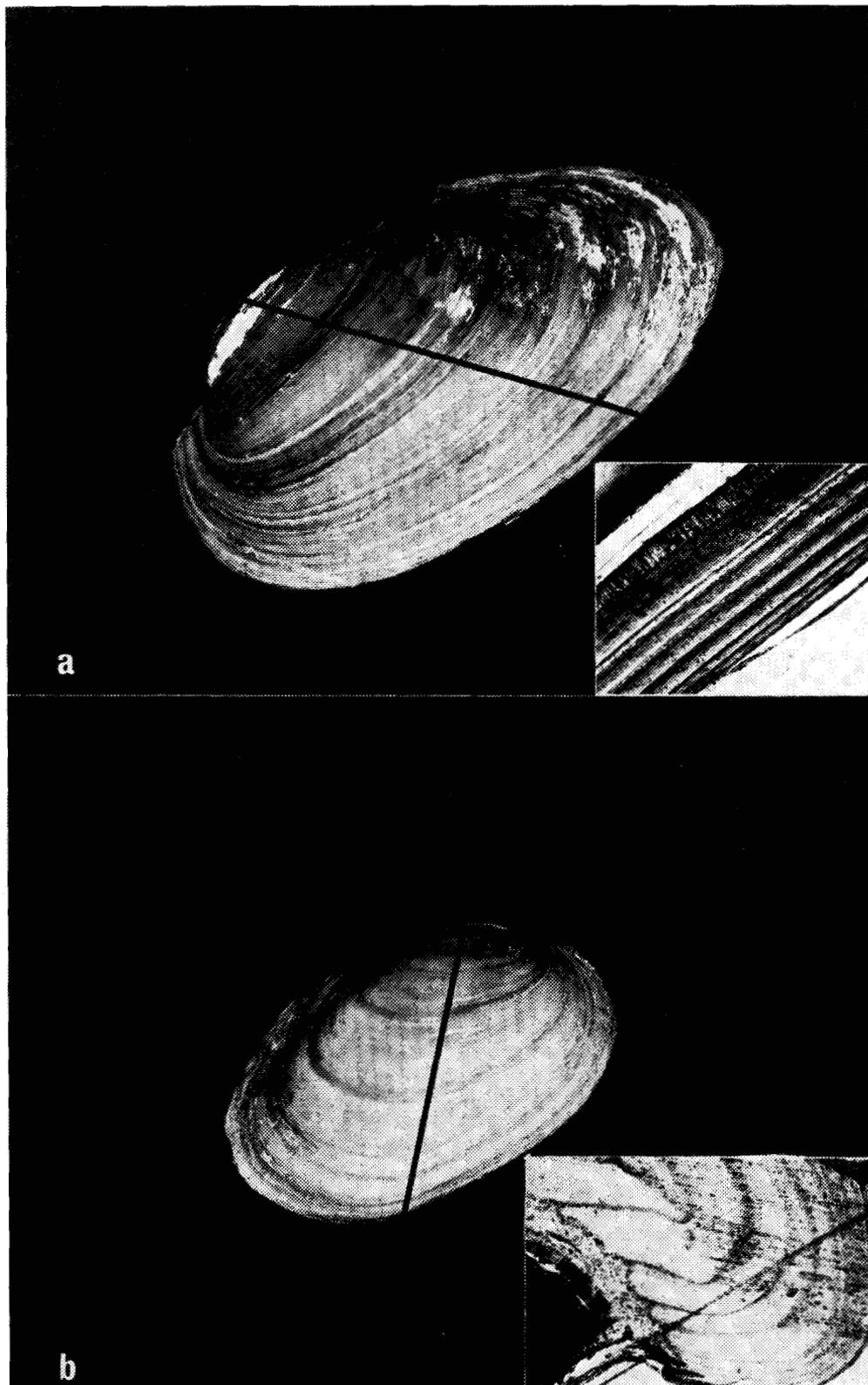| Species	Stations			
	Post 0	EC-10	BRB#3	LRB
Lampsilis radiata	24	17	7	17
Anodonta grandis	9	17	10	3
Elliptio dilatata	20	1	1	0

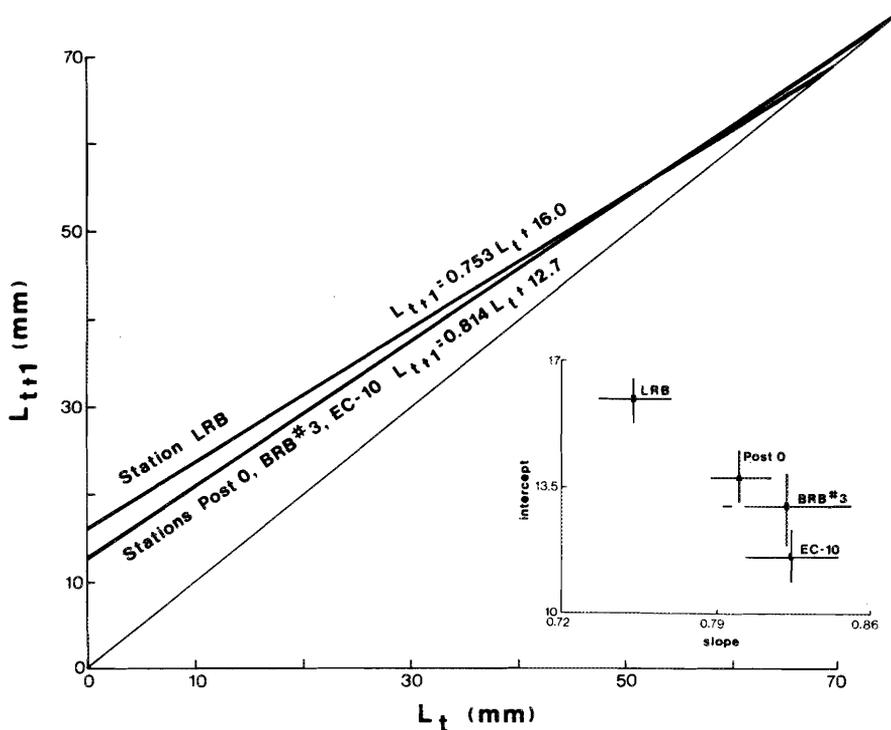FIG. 2.   Thin sections of (a) *Anodonta grandis* and (b) *Lampsilis radiata* verify age by annual growth rings.

FIG. 3. Walford Plots for *Lampsilis radiata* of lengths at consecutive winter rings, and inset of intercepts vs. slopes ± SE, showing differences between station LRB and stations Post 0, BRB#3, and EC-10.

treated as annual rings, then the tests would be biased but conservatively so (i.e. the error variance would be inflated and any real change in growth rates would be more difficult to detect).

We have used the "Walford Plot" method of Ford (1933) and Walford (1946) as applied by Green (1973), to describe the growth data and to test hypotheses. A Walford Plot is a difference equation representation of the data as a "length next year (L_{t+1}) versus length this year (L_t)" bivariate plot. A linear Walford Plot model $L_{t+1} = bL_t + a$ implies the Von Bertalanffy growth model $L_t = L_\infty(1 - e^{-Kt})$, assuming $L_0 = 0$. The parameters are related as $L_\infty = a/(1 - b)$ and $K = -\ln b$, where L_∞ = asymptotic final size, K = instantaneous growth rate at $t = 0$ expressed as a fraction of L_∞, b = fraction of total growth remaining after the 1st year, and a = 1st year's growth. A nonlinear Walford Plot would imply some other member of this family of growth curves which includes the Gompertz and logistic models (Richards 1959; Kaufman 1981). All of these models have corresponding Walford Plot models. However, the Von Bertalanffy model sufficed for the data considered here.

Our analysis and hypothesis-testing of growth rate parameters use classical linear additive model statistics (e.g. analysis of covariance) on the Walford Plot models rather than analysis based on index-type derived variables (e.g. Richardson et al. 1980; Kaufman 1981). Our approach applies a standard method (analysis of covariance) to a simple linear regression model $(L_{t+1} = bL_t + a)$ which relates the original measured variables (lengths at winter rings), rather than complex de-

rived variables, to each other. We believe that this approach creates fewer problems with underlying assumptions, and with ambiguities in interpretation of results. A possible extension of this approach is discussed later.

Our data base is of sufficient size for our purposes here. Each of the clams in Table 1 yields about 8 points on the Walford Plot, and the minimum error degrees of freedom in any test is 172. Rings can be thought of as nested within clams, and our statistical analysis understood as using an error df which pools among-clam and among-rings (within-clams) variation. Even if the more conservative approach of using only among-clam variation as the error term had been used, there would have been a more-than-adequate minimum of 69 error df, and similar results. In fact we would caution against overreplicating in such studies, which could lead to "statistical significance" of trivially small (re — biological importance) growth rate differences. Replication should be sufficient to provide robustness against failures of assumptions in the tests (see Green 1979), and then chosen to provide the Type I and Type II error rates wanted for the magnitude of growth rate change being detected.

Analysis of covariance indicated that the null hypothesis of similar slopes for the four stations should be rejected $(F(1, 416 \text{ df}) = 4.84, P < 0.05)$. *Lampsilis radiata* at station LRB had a higher growth rate (inset, Fig. 3). Station LRB was closest to shore and less than 1 m deep whereas stations Post 0, EC-10, and BRB#3 were 2–4 m deep. We also noted the reduced numbers of *A. grandis* at this site. Because of its biological and environmental differences from the other
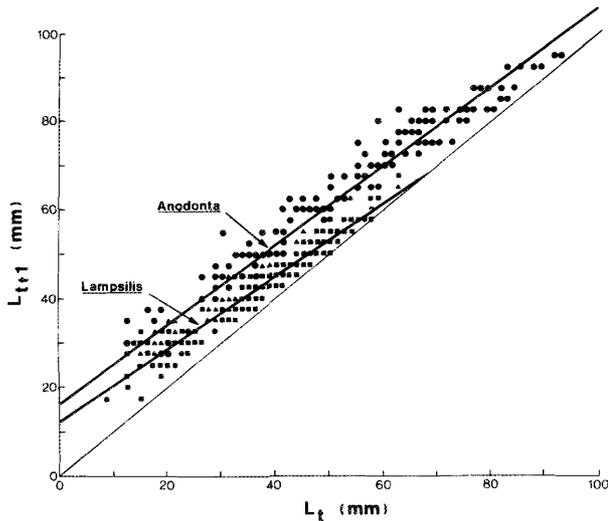
FIG. 4. Walford Plots of lengths at consecutive winter rings for the three stations pooled for *L. radiata* (■) and *A. grandis* (●). (▲ is overlap.)
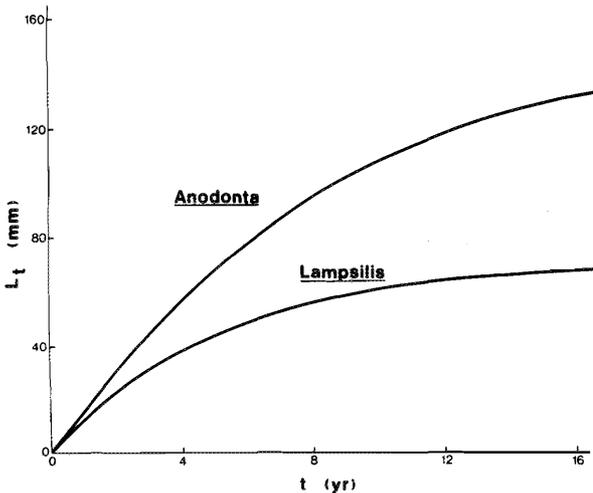

FIG. 6. Walford Plots of lengths at consecutive winter rings for *L. radiata* simulated H_O data (●) and simulated H_A data (■). (▲ is overlap.)

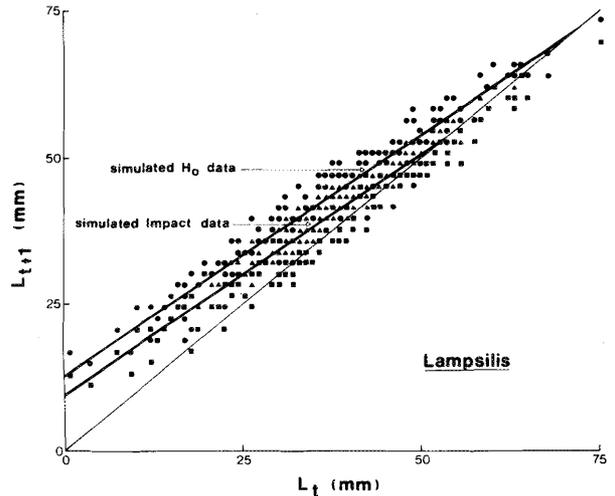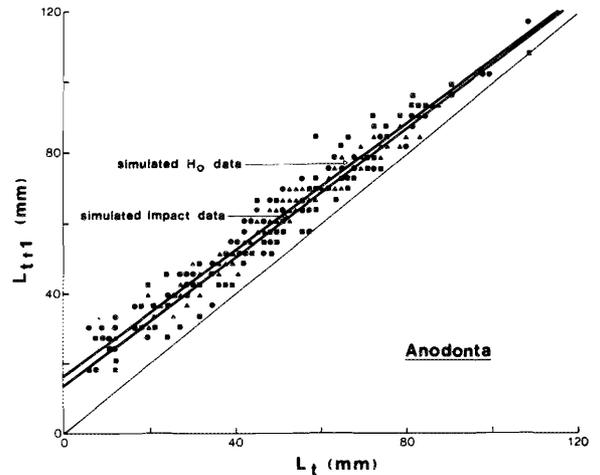FIG. 5. Baseline growth curves for *L. radiata* and *A. grandis*.

FIG. 7. Walford Plots of lengths at consecutive winter rings for *A. grandis* simulated H_O data (●) and simulated H_A data (■). (▲ is overlap.)

Simulation of H_O (Baseline) and H_A (Impact) Data

stations we dropped station LRB from the remaining analysis.

The Walford Plot growth equations for the three stations pooled (Fig. 4) are as follows:

L. radiata $L_{t+1} = 0.814L_t + 12.7$
A. grandis $L_{t+1} = 0.894L_t + 16.9$.

Analysis of covariance indicated that the slopes and intercepts do not differ ($P > 0.05$) among the three stations, for either species.

The Von Bertalanffy growth model was used to represent the baseline growth curves (Fig. 5) for the two species:

L. radiata $L_t = 68.6(1 - e^{-0.205t})$
A. grandis $L_t = 158.6(1 - e^{-0.113t})$.

For *L. radiata* the H_O data were simulated to have the same statistical properties as the Walford Plot data shown in Fig. 4, namely $L_{t+1} = 0.814L_t + 12.7 + \epsilon$ where ϵ has approximately the same normal distribution of errors about the regression line ($= \sqrt{\text{error mean square}} = \sqrt{5.49} = 2.34$). The H_A data were simulated to represent a hypothesized impact that lowered the amount of 1st-year growth (the intercept a) but did not change the fraction of growth remaining to be done (the slope b). Thus a different asymptotic size would be approached at the same rate, with growth rate lower at all ages. These data had the same properties as the H_O data except that the 1st year of growth was lowered by 3.5 mm to

$a = 9.2$ mm, yielding the Walford Plot growth equation $L_{t+1} = 0.814L_t + 9.2$.

Similarly for *A. grandis* the H_O data were simulated to have the same statistical properties as the Walford Plot data shown in Fig. 4, namely $L_{t+1} = 0.894L_t + 16.9 + \epsilon$ (with $\epsilon = \sqrt{\text{error mean square}} = \sqrt{13.35} = 3.65$). The H_A data were simulated to represent the effect of a hypothesized impact, but in this case the impact lowers the amount of 1st year growth by 3.5 mm and increases the fraction of growth remaining to be done so as to yield the same asymptotic size $L_\infty = 158.6$ mm (Fig. 5), which yields a Walford Plot growth equation of $L_{t+1} = 0.916L_t + 13.4$. Here the effect of the impact is to lower initial growth rate but to compensate for that decrease by retaining a higher fraction of that growth rate in older ages.

Testing H_A (Impact) Data Against H_O (Baseline) Data

We used analysis of covariance to test for differences between simulated data and the actual 1981 data. As expected, the simulated H_O *L. radiata* data did not differ from the actual 1981 data in either slope or intercept ($P > 0.05$). The simulated H_A data were tested against the simulated H_O data and, as expected, slopes were similar ($P > 0.05$) whereas intercepts differed ($P < 0.01$) as shown in Fig. 6.

Also as expected, the simulated *A. grandis* H_O data did not differ in slope or intercept from the actual 1981 data ($P > 0.05$), and against it the simulated H_A data were tested. The slopes of the two models differed ($P < 0.05$), thus rendering a test of intercepts meaningless, and demonstrating that the two growth equations are different as shown in Fig. 7.

Discussion

We have shown how growth rate parameters can be used to test hypotheses about change in environment, e.g. long-term increase or decrease in temperature, or presence of a pollutant. To do so we simulated a biological response to impact consisting of about 29% decrease in growth rate, in one case throughout the life span and in the other case a maximum effect at the beginning of life. Such a magnitude of decrease is consistent with observed variation in adult size in *L. radiata* (Green 1972) and in growth rates in *A. grandis* (Green unpublished data) over a wide range of environments. The error distribution was based on that observed for our Long Point Bay material. The results of the tests, using our recommended methods, agree with the properties of the simulated data.

If absolute age of a live clam can be estimated, then so can year of death and so can the year in which any particular band was formed. It is then possible to relate growth to yearly environmental variation (e.g. Negus 1966; Richardson et al. 1980; Jones 1981). Unionids can concentrate trace elements, and the shell is an exact chronology of chemical events (Tevesz and Carter 1980). Chemical concentrations in bivalve shell sections, separated at various growth bands, can be analyzed and related to specific years (i.e. specific events). This has not been our purpose here, but we plan such studies utilizing unionids and other bivalves, and feel that extensions

of the difference equation (i.e. Walford Plot) approach can provide a better statistical treatment of such data than that in published work to date. For example, the parameter a, the intercept, can be treated as a function of the yearly varying environmental parameter (call it X) that is hypothesized to modify growth rate. If a linear function of the deviation of X from its mean ($X' = X - \bar{X}$) during that time period is used, then we have the simple and statistically tractable model $L_{t+1} = a + bL_t + cX'$. A test of the null hypothesis H_O: "$c = 0$" corresponds to a test of H_O: "No linear model relationship between year-to-year variation in growth rate and year-to-year variation in environmental parameter X." The sequential yearly observations are of course not randomly allocated replicate observations (in time), as the simple covariance model implies, but this is equally true of the usual statistical treatment of such data. If this is a concern, then our proposed model could be analyzed as a time-series model which would take into account any autocorrelation between years.

Acknowledgments

CHAMBERLAIN, T. K. 1931. Annual growth of fresh-water mussels. Bull. U.S. Bur. Fish., Washington, DC. 46: 713–739.

CLARK, G. R. II. 1980. Study of molluscan shell structure and growth lines using thin sections, Appendix 1.2, p. 603–606. *In* D. C. Rhoads and R. A. Lutz [ed.] Skeletal growth of aquatic organisms. Biological records of environmental change. Plenum Press, New York, NY.

COKER, R. E., A. F. SHIRA, H. W. CLARK, AND A. D. HOWARD. 1920. Natural history and propagation of fresh-water mussels. Bull. U.S. Bur. Fish., Washington, DC. 37: 75–181.

FORD, E. 1933. An account of the herring investigations conducted at Plymouth during the years from 1924–1933. J. Mar. Biol. Assoc. U.K. 19: 305–384.

GREEN, R. H. 1972. Distribution and morphological variation of *Lampsilis radiata* (Pelecypoda, Unionidae) in some central Canadian lakes: a multivariate statistical approach. J. Fish. Res. Board Can. 29: 1565–1570.

1973. Growth and mortality in an Arctic intertidal population of *Macoma balthica* (Pelecypoda, Tellinidae). J. Fish. Res. Board Can. 30: 1345–1348.

1979. Sampling design and statistical methods for environmental biologists. John Wiley & Sons, Inc., New York, NY. 257 p.

HALLAM, A. 1965. Environmental causes of stunting in living and fossil marine benthonic invertebrates. Palaeontology 8: 132–155.

ISELY, F. B. 1914. Experimental study of the growth and migration of fresh-water mussels. Appendix III, Report U.S. Commissioner of Fisheries for 1913, Washington, DC. 24 p.

JONES, D. S. 1981. Annual growth increments in shells of *Spisula solidissima* record marine temperature variability. Science 211: 165–167.

KAUFMAN, K. W. 1981. Fitting and using growth curves. Oecologia 49: 293–299.

KENNISH, M. J. 1976. Monitoring thermal discharges: a natural method. Underwater Nat. 9: 8–11.

LEFEVRE, G., AND W. C. CURTIS. 1912. Studies on the reproduction and artificial propagation of fresh-water mussels. Bull. U.S. Bur. Fish. 30: 105–201.

NEGUS, C. L. 1966. A quantitative study of the growth and production of unionid mussels in the River Thames at Reading. J. Anim. Ecol. 35: 513–532.

RHOADS, D. C., AND R. A. LUTZ. [ed.] 1980. Skeletal growth of aquatic organisms. Biological records of environmental change. Plenum Press, New York, NY. 750 p.

RICHARDS, F. J. 1959. A flexible growth function for empirical use. J. Exp. Bot. 10: 290–300.

RICHARDSON, C. A., D. J. CRISP, N. W. RUNHAM, AND LL. D. GRUFFYDD. 1980. The use of tidal growth bands in the shell of Cerastoderma edule to measure seasonal growth rates under cool temperate and sub-arctic conditions. J. Mar. Biol. Assoc. U.K. 60: 977–989.

STANSBERY, D. H. 1961. The naiades (Mollusca, Pelecypoda, Union-iacea) of Fishery Bay, South Bass Island, Lake Erie. Sterkiana 5: 1–37.

1967. Growth and longevity of naiades from Fishery Bay in western Lake Erie. Abstr. Cond. Pap. Am. Malacol. Union Inc. 1967: 10–11.

STRAYER, D. L., J. J. COLE, G. E. LIKENS, AND D. C. BUSO. 1981. Biomass and annual production of the freshwater mussel Elliptio complanata in an oligotrophic soft water lake. Freshw. Biol. 11: 435–440.

TEVESZ, M. J. A., AND J. G. CARTER. 1980. Study of annual growth bands in unionacean bivalves, Appendix 1.4, p. 613–618. In D. C. Rhoads and R. A. Lutz [ed.] Skeletal growth of aquatic organisms. Biological records of environmental change. Plenum Press, New York, NY.

WALFORD, L. A. 1946. A new graphic method of describing the growth of animals. Biol. Bull. 90: 141–147.