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Thermochimica Acta 309 (1998) 181–192

thermochimica acta

Determination of instantaneous growth rates using a cubic spline approximation[1]

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Received 1 May 1997; received in revised form 18 September 1997; accepted 22 September 1997

Abstract

The well-known equation for the evaluation of relative growth rate (RGR) $RGR=\ln(w_2/w_1)/(t_2-t_1)$ was shown to be an average relative rate. Only in the case of exponential growth law is this equation valid for both average and instantaneous rates. It depends not only on the time interval of averaging $[t_1, t_2]$ but also on the error of measurements. Under certain conditions, real growth rate could not be seen among noise resulted from data errors. Other equations often used such as $RGR=[w_2-w_1]/[w_1((t_2-t_1)]$ or its modification $RGR=[w_2-w_1]/[0.5((w_1+w_2)((t_2-t_1)]$ and %increase=$\{(w_2/w_1)$^$[1/(t_2-t_1)]-1\}100\%$, do not describe rates and cannot be used for the purpose in mind. Such a situation impelled us to develop a new approach for determining instantaneous rates directly from the experimental data for any process. The idea of this method consists of an approximation of data by a cubic spline regression having first and second derivatives. The analytical differentiation of the spline regression permits the determination of instantaneous rate. The method of minimisation of the functional of average risk was used successfully to solve the problem. This method permits to obtain the instantaneous rate directly from the experimental data. The instantaneous rate is a highly sensitive characteristic for study of natural and anthropogenic influences on the biological and ecological processes. For illustration, we analysed heat production of microplankton and growth rate of red seaweed *Gracilaria verrucosa* versus temperature. The program is written in C++.

Keywords: Approximation; Cubic splines; *Gracillaria verrucosa*; Growth rates; Seaweeds; Sensitive criterion

1. Introduction

The concept 'rate' is widely used in the modern ecology and biology for the description of ecological and biological processes such as growth and productivity, photosynthesis and respiration, matter and energy fluxes, etc. As a rule, it is impossible to measure the instantaneous rate of a process studied in the course of an experiment. Usually, only the integral changes of a variable studied can be measured (eg. changes of weight or length in time) rather than its rate, provided that the measurements are conducted discretely at definite time moments. Under such conditions, one can obtain only the average rate of a process for the selected time interval. Assume, for example, the value w is measured in an experiment. By definition, the instantaneous absolute rate and the instantaneous relative rates are given,

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[1]Presented at the Tenth Conference of the International Society for Biological Calorimetry, Ascona, Switzerland, 27–30 April, 1997.

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0040-6031/98/$19.00
PII S0040-6031(97)00352-3

correspondingly, by

$$V = \frac{dw}{dt}, \quad V_{rel} = \frac{1}{w}\frac{dw}{dt}. \tag{1}$$

If at the instants of time t_1 and t_2 the measured values of w are equal to w_1 and w_2, respectively, then one can find the average absolute rate $\langle V \rangle$ and the average relative rate $\langle V_{rel} \rangle$ for the time interval $(t_2 - t_1)$. Operation $\langle \cdot \rangle$ of time averaging is defined as follows

$$\langle f \rangle = \frac{1}{t_2 - t_1}\int_{t_1}^{t_2} f(t)dt, \tag{2}$$

where $f(t)$ is an arbitrary function of time. Substituting dw/dt and $1/w(dw/dt)$ from (1) into (2) and integrating gives the following equations for average rates:

$$\langle V \rangle = \frac{1}{t_2 - t_1}\int_{t_1}^{t_2} \frac{dw}{dt}dt = \frac{w(t_2) - w(t_1)}{t_2 - t_1}, \tag{3}$$

$$\langle V_{rel} \rangle = \frac{1}{t_2 - t_1}\int_{t_1}^{t_2} \frac{1}{w}\frac{dw}{dt}dt = \frac{\ln w}{t_2 - t_1}\Big|_{t=t_1}^{t=t_2} = \frac{1}{t_2 - t_1}\ln\frac{w(t_2)}{w(t_1)}. \tag{4}$$

It is necessary to emphasise that Eqs. (3) and (4) were derived without any a priori assumptions of the dependence w on t and, hence, these equations are correct for an arbitrary dependence w on time. Authors very often omit the word 'average' in the expression 'average rate' and such a situation leads to some misunderstanding because the readers can mistakenly consider it to be a question of an instantaneous rate.

Eq. (4) was simultaneously introduced into biology in 1927 by the American and Russian scientists Brody and Schmalhausen [2,22]. They considered this equation to provide an instantaneous relative growth rate of organisms. Brody's arguments in support of this view were as follows. Let us write the expression for the instantaneous relative rate $k = 1/w(dw/dt)$ (Brody's notations) in the form of a differential equation

$$\frac{dw}{dt} = kw. \tag{5}$$

Solution of Eq. (5) gives the relationship of w on t

$$w = Ae^{kt}.$$

Brody wrote: "*The constant k has a perfectly definite meaning. It is the instantaneous relative rate of growth for a given unit of time*" (Brody, 1945, p. 508). Now, having the two values

$$w_1 = A\exp(kt_1), \quad w_2 = A\exp(kt_2)$$

at the instants of time t_1 and t_2, one can determine the instantaneous rate according to the following equation:

$$k = \frac{1}{t_2 - t_1}\ln\frac{w(t_2)}{w(t_1)}. \tag{6}$$

which is same as Eq. (4). Apparently, the discrepancy arises because Eq. (4) was derived as the average relative rate but Brody obtained the same expression for the instantaneous rate.

If the value k is constant and does not change with time, then Brody's reasoning is correct. Only in this particular case the expressions for the average relative rate and the instantaneous relative rate will coincide. But if k depends on time, then the differential Eq. (5) will not have such a simple solution and the expression for the instantaneous rate $k(t)$ will be more complicated. This results in a vicious circle! In order to find the instantaneous relative rate, according to Brody, it is necessary to know $k(t)$ or, in other words, the instantaneous relative rate.

An analogous situation also arises when Eq. (3) is closely examined. When w will depend on t linearly, the equations for the average and instantaneous rates will coincide. It should be emphasised once again that Eqs. (3) and (4) *are correct for any dependence $w(t)$* and represent *the average rates depending on the time interval over which averaging is taken.*

At present, a number of researchers [1,3,4,6,11,13,17–20] have been multiplying Eq. (6) by 100% without any reason and interpreting it as the rate expressed as a per cent of mass increase during the time interval but this operation is shown to be incorrect. The value k is a *dimensional* one, having the dimension T^{-1}.

Along with Eq. (4) some researchers use the following formula (7) for the study of growth and productivity of organisms [8,14,15,21,26] as well as for investigating the water movement and matter flux in ecosystems [7,9].

$$\langle V_{rel} \rangle = \frac{w(t_2) - w(t_1)}{w(t_1)(t_2 - t_1)}, \tag{7}$$

or its modification

$$\langle V_{\mathrm{rel}}\rangle = \frac{w(t_2)-w(t_1)}{\frac{1}{2}[w(t_1)+w(t_2)](t_2-t_1)}. \tag{8}$$

These formulae are incorrect. They do not describe an average relative rate, moreover, *they do not produce average* of any value for an arbitrary time interval $[t_1, t_2]$.

In order to complete the review of formulae used for calculating average rates, it is necessary to mention the formula which permits the calculation of the percentage increment (but not rate!) for a certain time interval $[t_1, t_2]$.

$$V_{\%} = \left[\left(\frac{w(t_2)}{w(t_1)}\right)^{1/(t_2-t_1)} - 1\right] \times 100\%. \tag{9}$$

Some investigators ([5,10,16,23] and others) mistakenly designate this value as the growth rate. The relationship between Eqs. (9) and (4) may be readily ascertained

$$\langle V_{\mathrm{rel}}\rangle = \ln\left(\frac{V\%}{100\%} + 1\right).$$

The reasons stated above have stimulated us to perform this investigation.

2. Methods

In Section 1, we have showed how the average rates can be obtained from observations. Now we are going to show that the average rates are poor parameters for the description of process dynamics. The question is that the variable quantities measured experimentally always have a certain scatter in data. Scattering of data can be affected by the error of instruments or random fluctuations of the process studied. At the moment, we are not interested in the cause of scattering; at present it is only essential that the conducting measurements provide the random variable which is characterised by the corresponding average and variance.

Presenting the average of observations, we have to present the error of the average also. It is clear that the error will depend on the interval of averaging. The larger the time interval is the lesser the error will be. Thus, we can calculate average rate over a large time interval with a small error but only one point is of little importance for the understanding of the dynamics of process studied within this large interval. One can proceed otherwise. Let us divide the whole of the time interval into many small ones and calculate the averages in every small interval. We shall receive as many points as small intervals contained in the entire time interval. Nevertheless, the errors in average rates will increase, as smaller time intervals are used for their determination. The errors can increase to such an extent that they may become comparable with average rates or even exceed them several-fold. In this case, we cannot plot a reliable curve in spite of having many points at our disposition.

Obtaining the instantaneous rate from observations represents a difficult task. Assume that some value $f(t)$ is to be measured in an experiment. As mentioned above, we do not measure the value $f(t)$ but

$$\tilde{f}(t) = f(t) + \varepsilon(t), \tag{10}$$

where $\varepsilon(t)$ represents noise. For the instantaneous rate to be determined, it is necessary to take a derivative of Eq. (10). Strictly speaking, the function $\varepsilon(t)$ *has no derivative* since it is discontinuous at every point. If, nevertheless, we try to differentiate numerically the right side of Eq. (10), then $\mathrm{d}f/\mathrm{d}t$ will disappear in the noise generated by the second term. Thus, in an attempt to find the instantaneous rate the following definition

$$f' \equiv \frac{\mathrm{d}f}{\mathrm{d}t} = \lim_{\Delta t\to 0}\frac{f(t+\Delta t)-f(t)}{\Delta t}, \tag{11}$$

will fail in advance.

There will be several ways to resolve such a difficult situation. One of them is the following: one can approximate the experimental data by a certain curve having the derivative and then just calculate it analytically. Thus, we get rid of the necessity to differentiate noise in the right-hand side of Eq. (10). How can we choose such an approximate function? Power, exponential, logarithmic and other functions, widely used by biologists, are not fit for such a role. They approximate the experimental data insufficiently because they have very few free parameters. Such a derivative appears to have nothing in common with the real one.

Polynomial regression cannot rescue the situation since there are functions which cannot be approximated uniformly as both the degree of polynomial and the sample size are large in magnitude. The Runge's

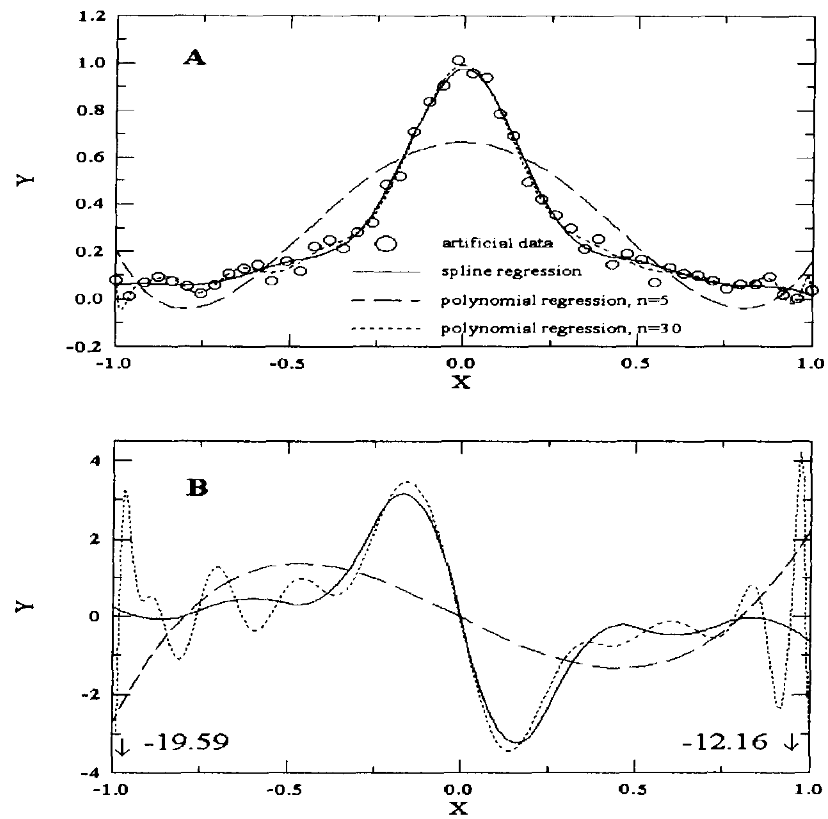

Fig. 1. (a) Approximation of artificial data (o) by means of both spline and polynomial regressions and (b) their derivatives. Data were simulated according to Runge's function $y(x)=1/(1+25x^2)$ and adding noise $\varepsilon(x)$ to it. Solid line: spline approximation, dash line: approximating polynomial for 5th power, dotted line: approximating polynomial for 30th power.

function $y(x)=1/(1\pm25x^2)$ on the interval $[-1,1]$ (Fig. 1(a)) is an example of such a function. Under a small degree, the polynomial approximates the Runge's function poorly over the entire interval. On increasing the polynomial degree, the quality of the approximation in the vicinity of zero is improved but an unstable behaviour of the polynomial near the limits of the interval becomes essential (Fig. 1(b)). The plot of the Runge's function is not shown here as it has a small difference from the approximating spline. The sliding polynomial does not ensure a sufficient smoothness of the derivative.

Nevertheless, at present, there are methods uniformly approximating the experimental curve, provided that the number of experimental points or sample size is increased. One such methods is the method of a spline regression. The spline is a piecewise polynomial function having continuous first and second derivatives. The first derivative obtained is smooth. Cubic polynomials are often chosen as such functions. The coefficients are matched in such a way that the functional of average risk $I(\alpha)$ is minimised. The reconstruction theory of dependences from the limited sample size on the basis of a functional minimisation is too complicated to be described here in detail. Only the basic features of the method will be presented in the present article, but details can be found in the original literature [24,25].

Experimenters have dealt very often with dependences when a random number y, obtained by a random trial with conditional probability density $P(y|x)$, is assigned to a number x. The problem of the reconstruction of conditional probability density $P(y|x)$ is excessively difficult. But, luckily, in practice it is usually needed to reconstruct not the density $P(y|x)$ but only its one characteristic, namely, the function of a conditional mathematical expectation $y(x)$, i.e. the function assigning for every number x the corresponding mathematical expectation $y(x)$ of the random variable y,

$$y(x) = \int yP(y|x)\mathrm{d}y. \tag{12}$$

The function $y(x)$ is called a regression.

Consider the problem of regression reconstruction on the basis of sample with limit size. Let the number x be generated randomly with a probability density $P(x)$. The random number y, obtained as a result of a random trial with the conditional probability density $P(y|x)$ is assigned for every number x. From the random restricted sample

$$x_1, y_1;\ x_2, y_2; \ldots; x_N, y_N \tag{13}$$

it is necessary to reconstruct the regression (12) in the function set $F(x,\alpha)$, i.e. to find the function $F(x,\alpha^*)$ which is closest to the regression $y(x)$. As a rule, neither $P(x)$ nor $P(y|x)$ is known. One can prove that the functional of the average risk

$$I(\alpha) = \int (y - F(x,\alpha))^2 P(y|x)P(x)\mathrm{d}x\mathrm{d}y \tag{14}$$

takes a minimum value on the regression, if $y(x) \in F(x,\alpha)$ or on the nearest function to this regression, if $y(x) \notin F(x,\alpha)$ [24]. The regression is sought in the class of cubic splines

$$y(x) = \sum_{j=1}^{n+4} \alpha_j G_j(x), \tag{15}$$

where G_j is the fundamental cubic spline, a_j the coefficients in question, and n the number of spline junctions over the interval. The functional of the empirical risk is built up for the restricted sample (13)

$$I_e = \frac{1}{N}\sum_{i=1}^{N}\frac{1}{\sigma_i^2}\left[y_i - \sum_{j=1}^{n+4}\alpha_j G_j(x_i)\right]^2, \tag{16}$$

where N is the sample size, y_i the vector of experimental data, and σ_i^2 the variance of y_i. Under certain conditions, the point α^* of the minimum of the functional $I_e(\alpha)$ provides the functional of average risk $I(\alpha)$ with a value which is close to minimum. To minimise (16), one can solve the normal system of linear equations

$$[S^T S]_{ij}\alpha_j = S^T_{ik}\frac{y_k}{\sigma_k}, \tag{17}$$

where α_j is a set of coefficients in question, $S_{ij} = G_j(x_i)/\sigma_i$ is a $N\times(n+4)$ matrix. Let α^* be a solution of the system of linear equations. The estimation of quality of constructed approximation is given by

$$J(n) = \frac{I_e(\alpha^*)}{1 - \sqrt{1/N[(n+4)(\ln(N/(n+4)) + 1) - \ln\eta]}} \tag{18}$$

where $(1-\eta)$ is a probability for the estimation to be true, and n the optimal number of junctions.

The outlined algorithms were realised as a program written in C++.

3. Results

3.1. Comparison of average and instantaneous rates

To illustrate the application of the method, we used data of heat production of microplankton (from Sevastopol Bay). These data were obtained by Lopukhin and kindly offered for analysis [12]. A seawater sample (500 ml) was taken at 9 a.m. from the surface. Microplankton organisms were divided into size fractions by filtration through a series of membrane filters (15, 2.5, 0.2, 0.05 μm). The ampoules with samples were installed into a Thermometrics-2277 thermal activity monitor. After thermal equilibration over 60 min, the heat signal was registered over a period of 20 h at 20°C (Fig. 2(a)). The rate of heat production is shown in Fig. 2(b). These curves were obtained by analytical differentiation of the approximating spline regression. Fig. 3 permits to compare results with the ordinary calculations of derivatives according to formula (3). We can see that the usual procedure of calculating derivatives is highly unstable.

The second example shows how to apply this method to obtain optimal temperature for maximal

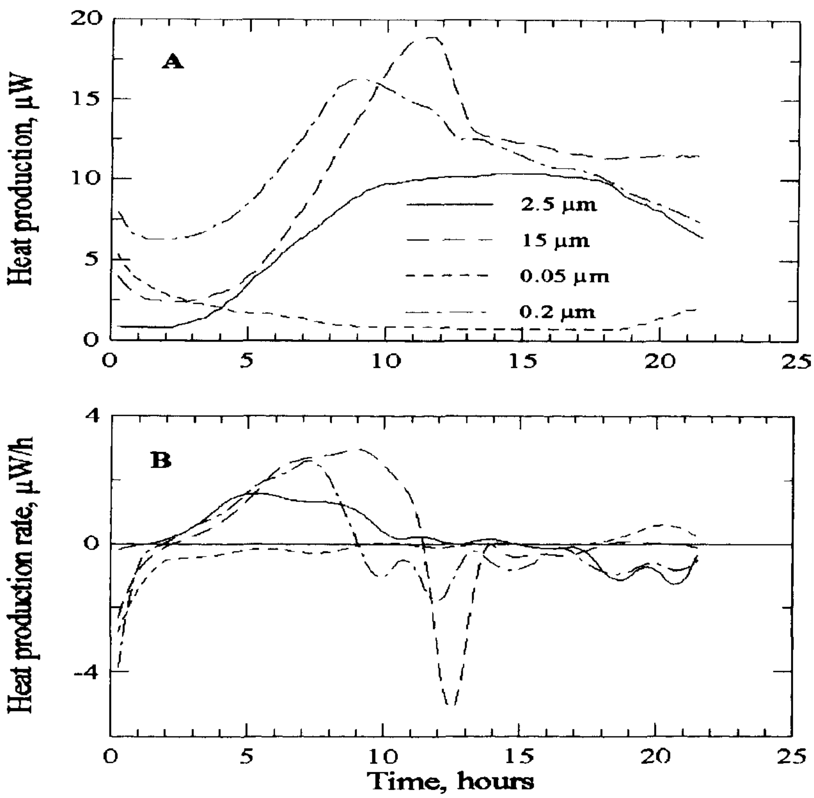

Fig. 2. (a) Heat production of microplankton from Sevastopol Bay. Micro-organisms were divided into size fractions by filtration through the system of membrane filters with mesh size 15 µm, 2.5 µm, 0.2 µm and 0.05 µm. (b) Heat production rates, calculated by analytical differentiation of the approximating spline regression.

growth rate. We used data of growth of the red seaweed *Gracilaria verrucosa* (from Kazach'ya Bay near Sevastopol). These data were obtained in Trinkenschu's laboratory. Growth experiment was conducted in the Plexiglas culture chamber from 21 May to 16 June 1992. The initial concentration of biomass was 3.12 g l^{-1}. Water was changed every day and at this time nutrients were added and the seaweed weight and temperature were recorded. The chamber was aerated at a constant rate with air to promote water mixing.

Experimental observations on *G. verrucosa* raw weight changes during 57 days and the approximating cubic-spline description of these data are presented in Fig. 4(a). The average relative rates of growth calculated according to Eq. (4) for Δt=1 day (saw-tooth curve) and the instantaneous relative rates of growth obtained by means of recommended method of spline regression (smooth line) are represented in Fig. 4(b). Here, the situation is even more 'dramatic' than in the first example. The saw-tooth curve cannot permit to draw any assumptions on the seaweed growth dynamics. Under an ordinary approach, there is nothing to do in this case but to average data and then to find the confidence interval for the average and, finally, to do nothing more than this. The suggested method permits to extract more detailed and principally new information from growth dynamics as it will be revealed further.

3.2. Optimal temperature for maximum growth rate

The instantaneous relative rate is a more sensitive parameter with respect to the external effects rather than the total biomass. The temperature changes during the experiment are presented in Fig. 5(b).

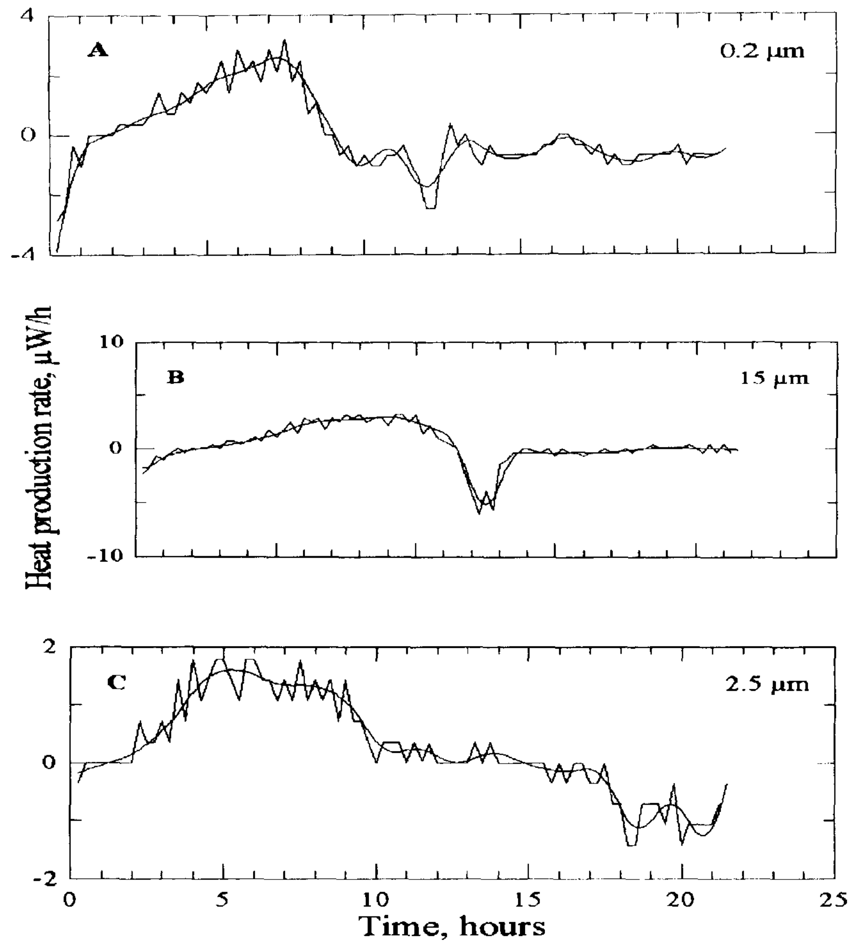

Fig. 3. (a) Heat production rates of microplankton from Sevastopol Bay. Comparison of the usual approach to calculation of derivatives (saw-tooth curve) and the method based on approximating splines (smooth curve).

Temperature in the experiment was not regulated and was subjected to the temperature of external air. On the 37th day from the beginning of the experiment, the temperature reduced abruptly due to a cold snap. The position of temperature jump on the plot is marked by a solid vertical line. There are two alternatives to approximate the discontinuous data.

(i) If there are many points near the vicinity of the discontinuity, one may ignore the presence of discontinuity and treat the data as a whole array. It is necessary to keep in mind that an approximating-spline curve will smooth the discontinuity, but the quality, however, of the approximation in the vicinity of the discontinuity will be deteriorated in this case. If there are a few points in the vicinity of the discontinuity, it causes unsatisfactory approximation over the entire interval. In such a case the second alternative is preferred.

(ii) Let us now postulate the presence and position of the discontinuity in the original data and independently treat the arrays of data from the left and right sides of the discontinuity. In this case, a better agreement will be obtained between the approximating curves and the original data. The disadvantage of such an approach is the subjective choice of the position of discontinuity (we must

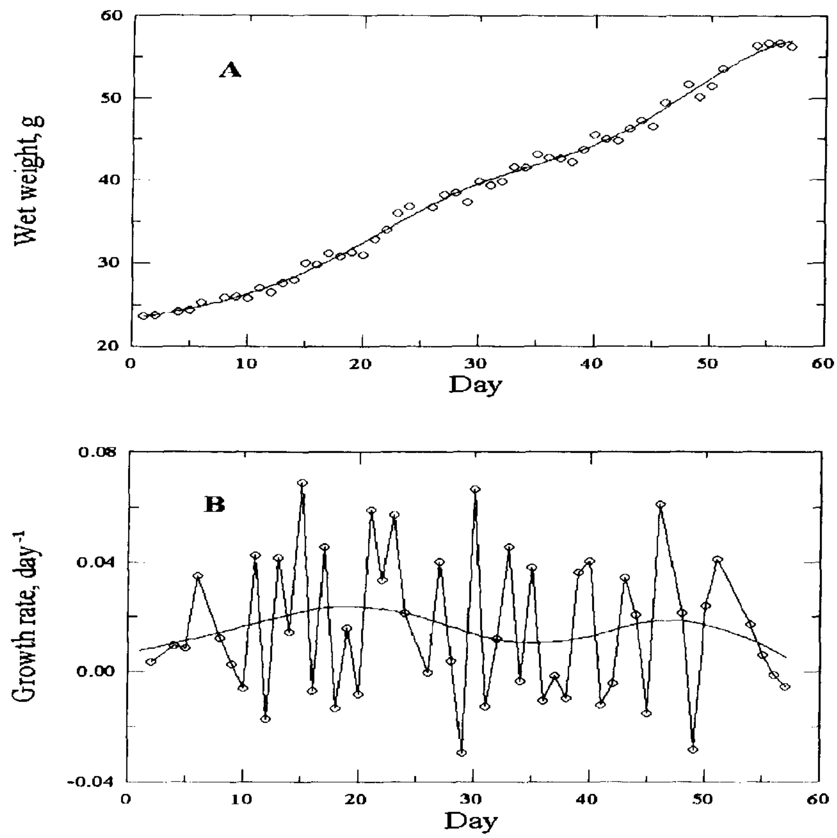

Fig. 4. *Gracilaria verrucosa*. (a) Approximation of experimental data of raw weight by means of cubic splines and (b) both average relative (solid line) and instantaneous relative (dash line) growth rates.

define it by hand) and decrease in points of every treated arrays.

We have used both the alternatives. The cubic spline approximation of the entire data array of temperature is shown by the dotted line in Fig. 5(b). The quality of the approximation is not sufficiently satisfactory. Especially, large differences are observed in the vicinity of the discontinuity, as was expected. In second alternative, the whole temperature data array was divided into two arrays of lesser length. The cubic spline approximation of data before the discontinuity is shown by the solid line in Fig. 5(b). The second data array had insufficient points for reliable spline approximation, so we have confined ourselves to plotting of linear regression (dash line) which is proved to be close to spline regression for the whole data array. Any discrepancy in the vicinity of discontinuity is the result of the impossibility of describing the discontinuous data by continuous spline function.

From 26 May (the 6th day of the experiment) till 26 June (the 37th day), the temperature was increased linearly as evidenced by the straight line of linear regression calculated for this range in Fig. 5(b). In the range 23.7–30.4°C, both the curves of the spline and linear regression were in close agreement. In Fig. 5(b), this temperature range is marked by the horizontal dash lines. Since we have the linear dependence, the temperature range (Y scale of Fig. 5(b)) can be readily transformed into an adequate time interval (X scale of Fig. 5(a)) which is marked by the vertical dash lines in Fig. 5(a). On this plot, the boundary values for temperature as well as optimal temperature of the maximum growth rate are plotted. In this

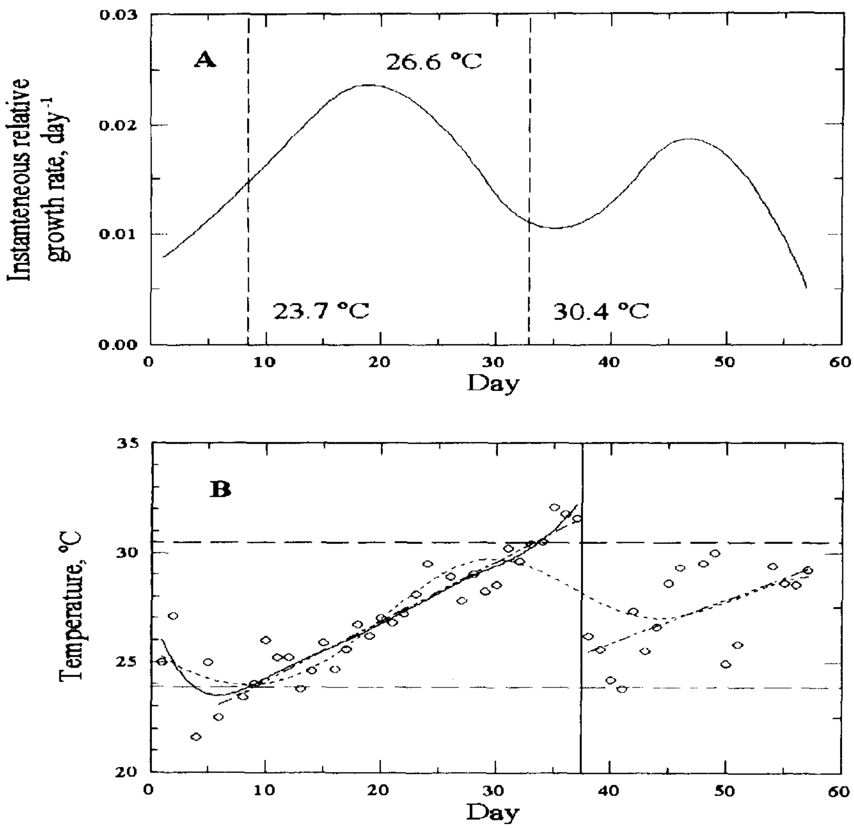

Fig. 5. *Gracilaria verrucosa.* (a) Changes of instantaneous relative growth rate and (b) temperature during the experiment. Dotted line: approximation of the whole data of temperature by means of cubic splines, solid line: approximation of temperature data before discontinuity by means of cubic splines, dash line: approximation of temperature data before and after discontinuity by means of linear regression.

marked range the curve of the instantaneous relative growth rate has approximately the symmetrical bell-shaped form and the peak of this curve corresponds to the maximum growth rate.

How can one explain the emergence of the bell-shaped curve? At first, the temperature was well below the optimal one. The increase of the temperature was followed by the increase of the seaweed growth rate to its maximum at t=26.6°C. Further increase of the temperature caused the growth rate to decrease and before the temperature jumped the rate was already minimal. The emergence of the second peak on the curve of the growth rate can also be easily explained. After the 37th day from the beginning of the experiment, the temperature has gradually returned to that for the vicinity of the maximal growth rate and the second peak appears on the curve. In spite of great variability of temperature data for this period, we have attempted, as before, to calculate the temperature which caused the second peak to appear. Again, at this time the temperature was 26.8°C, and it is practically the same as in the case of the first peak. So, the optimal temperatures coincide, in both the cases, before and after the temperature jump. But the absolute values of the growth rates, in the second case, were less than those in the first one, and the additional data for the explanation are needed.

4. Discussion

1. Because of vastly mathematical calculations, it is often fairly difficult to focus on the main problem around which all performances are displayed. So here,

we would like to pay attention to the reasons why the attempt to calculate the derivative in the form of $y'=\Delta y/\Delta x$ from a function specified by tabular data, is doomed to failure. At a glance it could be seen that the main obstacle for the correct calculation of derivative is the value of the step Δx of original tabular data because, by definition, the expression $\Delta y/\Delta x$ will tend to the derivative when Δx tends to zero. Indeed, the decrease in step, Δx, will not only improve the accuracy, but can also lead to a deterioration in the accuracy. An apparent contradiction appears with respect to the definition of the derivative. The point is that the original tabular data have certain errors although the definition of derivative is assumed to have an infinite accuracy. To illustrate this statement, we have analysed the artificial data simulated according to the function $y=\sin x+\varepsilon\chi(x)$ in the interval $[0,\pi]$, where $\chi(x)$ is a random value distributed uniformly in the interval $[-1,1]$ or the so-called artificial noise, where ε is an amplitude of noise. The results of calculations are shown in Fig. 6. It can be seen that the presence of even a slight invisible noise among original data results in a derivative with significant noise. But, it is most surprising that a decrease in the interval Δx (or increase in the number of points) deteriorates the situation. Such a behaviour of derivative can be explained as follows.

Consider more carefully what happens with the numerical differentiation of the second term. Consider an arbitrary value $\chi(x)$. For the next measurement (*it does not matter on which interval Δx this measurement is done*), the function $\chi(x)$ can again take the arbitrary value within the interval $[-1,1]$ with equal probability. As far as the function $\chi(x)$ 'does not remember' its previous value, at the next measurement it can again take the arbitrary value from the interval $[-1,1]$ with equal probability. Dividing the remainder of the obtained values by Δx, one can receive the parameter which is called the *numerical derivative* of the function $\chi(x)$. Supposing, we took half the value of Δx, then the remainder of the function would be the previous one, but the numerical value of the derivative would be larger by a factor of two. This leads to a greater scattering of values of the numerical derivative.

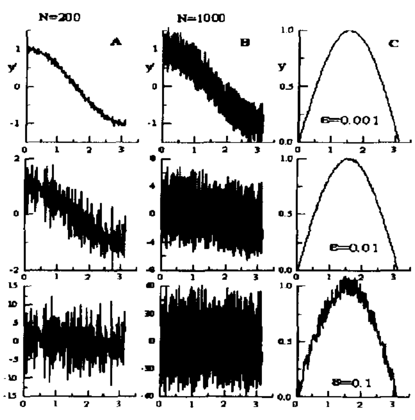

Fig. 6. Plot of function $y=\sin x+\varepsilon\chi(x)$ (c) and its numerical derivative (a,b) under different values of points N and noise amplitude ε.

From a mathematical point of view, the function $\chi(x)$ is discontinuous at each point and has no derivative, hence the entire function $y(x)$ has no derivative. However, such an absolute, precise solution of the problem cannot satisfy an experimentator. If the derivative does not exist in the usual meaning, then perhaps it is possible to find a function which could replace a non-existent derivative in any meaning? Different methods, used for numerical differentiation, choose this function in a different way. For this purpose, an additional function behaviour information is required (continuity, degree of smoothness, variance of noise, etc.). This a priori information is used by everyone who tries to reconstruct the derivative from the experimental data. Some authors correctly point out which additional requirements of a function is to be satisfied, but others omit this question. Nevertheless, all of them use this additional information in one way or another. In our case, such an additional information comprises in seeking a solution among the piecewise continuous polynomials of the third power, which have continuous first and second derivatives. These requirements do impose the restriction on the smoothness of the solution.

2. The selected mode of reconstructing the derivative was, as mentioned earlier, not unique. This problem can also be solved by means of different methods of regularization, but the parametric methods used are often expected to be more natural for biologists.

3. During the discussion of this approach, the following question arises very often: what is the minimal sample size which is necessary for obtaining the curve of spline regression having preassigned

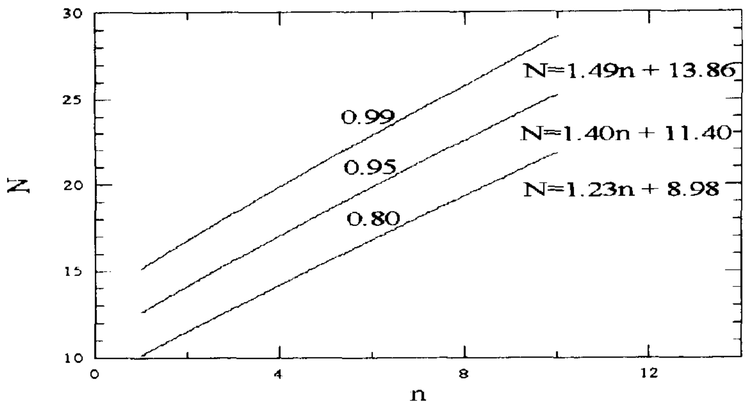

Fig. 7. Relationship between sample size N and minimal numbers of conjunctions n for probability 0.80, 0.95 and 0.99 with which the estimation of quality of constructed approximation is valid.

degree of reliability. The minimal value of the sample size can be obtained from the condition, provided that the denominator of Eq. (18) is positive. The relation between the minimal sample size, N, and the number of the conjunction, n, for different values of η are represented in Fig. 7.

5. Conclusion

It is shown that the method of data smoothing by means of an approximate cubic spline can be used successfully for calculating the instantaneous growth rate of organisms and for determining the rates of other biological processes. This approach makes it possible to take into account the entire course of dynamics of the process studied. The instantaneous rate is a very sensitive characteristic of biological processes. On the basis of this method, one can create new sensitive criteria for studying the influence of natural and man-made factors on biological and ecological processes. All this permits us to understand the mechanisms of biological processes more deeply.

Acknowledgements

The authors wish to thank Dr. R.P. Trenkenschu and Dr. A.S. Lopukhin for providing us with the data samples. We thank Prof. G.E. Schulman for the discussion of the article during his scientific seminar.

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