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The application of statistical verification in studies in fish variability

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Abstract — The method of analysis of time series of variability, developed on the basis of the values of variance, calculated according to one of the Wright formulae, is presented. The series of variability were approximated with polynomials and compared, using the method of covariance analysis for one-way classification using an arbitrary number of polynomial degrees. The method was applied in investigating the variability of body size of peled larvae (*Coregonus peled* Gmel.) reared in lake cages with the use of light for attracting zooplankton.

Key words: variability, fish body size, analysis of covariance, rearing in cages.

1. Introduction

For a long time the problems of individual variability have been among the basic interests of the biological sciences. The occurrence of intrapopulation differentiation and, above all, the differentiation of animals during their growth, have numerous practical and theoretical consequences. Again and again they prompt new attempts at investigating this phenomenon with the application of ever never techniques and on the basis of fresh theories. This is also true of the biology of fish. In recent years growing attention has been paid to problems connected with intraspecific variability.

The investigation of individual variability is burdened with a number of difficulties, among which methodical limitations play an important role. The most frequently applied measure of individual variability is the variance, i.e. the mean square of deviations of individual values from the arithmetic mean. Since the variance is always expressed in the same units as the value of the character measured, the relative measure of dispersion, i.e. the coefficient of variability (CV), is commonly used to characterize of the variability of different features of fishes. A serious drawback of this measure is the lack of statistical tests for comparing the value of different coefficients. Similar limitations are encountered in investigating the dynamics of differentiation of features in time, particularly in the case of comparing differently denominated time series. Different intermediate solutions are proposed for avoiding these difficulties. According to the suggestion of Lewontin (1966), the variances of logarithms of measurements can be used instead of CV. This measure is characterized by great stability and the type of applied unit is unimportant (metric or not metric ones).

2. Method

The analysis of variability was based on the variance of logarithmic values calculated according to one of the Wright formulae (Falconer 1974):

$$\sigma^2 \ (\log x) = 0.4343 \ \log \ (1 + C^2), \tag{1}$$

In the formula, C is the coefficient of variability expressed in the form of $\sigma/\bar{x}$ and calculated on the basis of arithmetic means.

In the given time interval, the series of variance values converted by the formula (1) results in a variability curve which can be described by the function

$$Y = a_0 + a_1x + a_2x^2 + \ldots + a_px^p \tag{2}$$

The function is a p-degree polynomial in relation to the independent variable x, where the individual values x_i are coded numbers of time units:

$$x_i = 1, \ 2, \ 3, \ \ldots, \ n.$$

The polynomial in the form expressed by the formula (2) can be replaced by a polynomial of the same degree in the form

$$Y^{(p)} = b_0\varphi_0 + b_1\varphi_1 + b_2\varphi_2 + \ldots + b_p\varphi_p, \tag{3}$$

where $\varphi_0 = 1$, $\varphi_1 = \varphi(x)$, $\varphi_2 = \varphi_2(x)$, ... $\varphi_p = \varphi_p(x)$ are orthogonal polynomials of the successive degrees zero, one, two ... p. This allows the calculation of each of the regression b coefficients, independently of the remaining ones, and the rapid verification of the significance of each of them. In the successive verification of hypotheses that the individual regressive components equal zero, an arbitrary exact approximation of the investigated curve of variability of the trait can be carried out with the use of a polynomial curve, if only a sufficiently high degree of the polynomial is applied (O k t a b a 1980). As the function of orthogonal polynomials the $Y^{(p)}$ function is reconverted to the form (2) in the final phase of the calculation, in order to facilitate its interpretation.

The curves of variability approximated in this way can be compared, using the method of covariance analysis for one-way classification using an arbitrary number p of polynomial degrees (P l a t t et al. 1975).

The method discussed above was applied in investigating the variability of the body dimensions of peled larvae (*Coregonus peled* G m e l.) reared in cages in Lake Legin (Jezioro Legińskie) in the years 1977—1979. The differentiation of the length and weight of fishes during their growth was determined on the basis of samples ($n = 50$ specimens) collected at 2—3-day intervals. The different days of rearing were coded, the day May 1 being taken as x_1 and, for the sake of simplification, all months being counted as having 30 days. On the basis of the measured values of the feature on the given day the distributive series were formed after the formula of Sterdgess (B a l i c k i, B i e l e c k i 1980) and then variance was calculated by converting it with the Wright formula (1) to the logarithmic form. The further procedure and the verification of successive statistical hypotheses were carried out according to the presented method. The calculations were made using an Odra 1204 computer in the centre of Mathematical Calculations at the Olsztyn Academy of Agriculture and Technology. The total number of peled larvae collected for calculations in May—June 1977—1979 was 2 300 specimens.

3. Results

In spite of similar tendencies in their pattern, empirical curves of variability of the body dimensions of the larvae were characterized by considerable random variations, this making their interpretation difficult (fig. 1). As the result of the polynomial approximation a high degree of "elucidation" of the different curves was obtained, on the assumption that the total percentage of the sum squares of deviations from regression (SS_p) should be higher than 90. For the equalization of curves at this level the highest degrees of the polynomial used were: eight for length and twelfth for weight (Table I).

Without random oscillations the curves of variability (fig. 2) were more legible. They were statistically verified using the covariance analysis. It was found in the comparison of the curves of the body length of larvae that they were parallel to each other (the accepted hypothesis

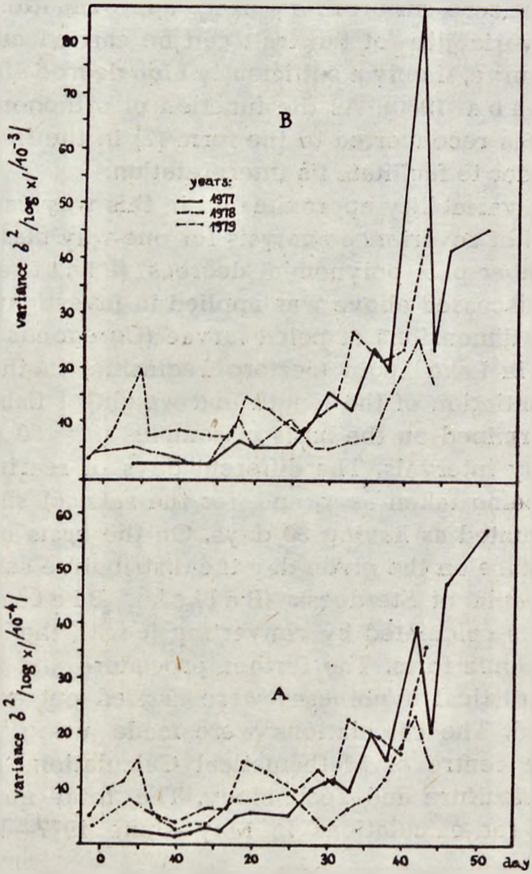

Fig. 1. Empirical curves of variability of body length (A) and weight (B) of peled larvae from fish rearing in lake cages in the years 1977—1979

$H_0^{(2)}$ at the level of $P(F) < 0.5201 = 0.088 > 0.05$). A similar result of the analysis was obtained in testing the variability of the body weight of peled larvae (the accepted hypothesis $H_0^{(2)}$ at the level of $P(F) < < 0.3120 = 0.008 > 0.05$).

However, the verification and rejection of the hypothesis $H_0^{(3)}$ made it possible to state that neither the curves of variability of body length nor those of body weight could be described by common equations. In this way complexes of improvement equations of regression were obtained (Table II). They differed in the different years by the value of a_0 coefficients. On the basis of the corrected equations, theoretical curves of variability of the traits were plotted (fig. 3). They characterized the variability of the size of the body of peled larvae in successive years.

Table 1. Parameters of polynomial approximated functions (equation 2) of variability of body size of peled larvae. i - degree of polynomial; a - parameters of polynomial in i-th degree; SS_i - percentage of total variance reducible by i-th degree of polynomial; SS_p - cumulated percentage of total variance

Feature	i	1977 a	1977 SS_i	1977 SS_p	1978 a	1978 SS_i	1978 SS_p	1979 a	1979 SS_i	1979 SS_p
Body length	0	$-1.405\ 894 \cdot 10^{-8}$	0.00	0.00	$2.271\ 322 \cdot 10^{-4}$	0.00	0.00	$5.373\ 337 \cdot 10^{-4}$	0.00	0.00
	1	$4.805\ 844 \cdot 10^{-5}$	72.71	72.71	$5.900\ 944 \cdot 10^{-4}$	45.40	45.40	$5.246\ 828 \cdot 10^{-4}$	48.98	48.98
	2	$-2.968\ 113 \cdot 10^{-6}$	17.28	89.99	$-1.613\ 290 \cdot 10^{-4}$	15.52	60.92	$-6.383\ 703 \cdot 10^{-5}$	18.34	67.32
	3	$8.399\ 580 \cdot 10^{-8}$	1.38	91.36	$1.608\ 017 \cdot 10^{-5}$	5.70	66.62	$-1.155\ 559 \cdot 10^{-5}$	1.44	68.76
	4				$-7.218\ 417 \cdot 10^{-7}$	0.43	67.05	$2.522\ 326 \cdot 10^{-6}$	1.17	69.93
	5				$1.488\ 801 \cdot 10^{-8}$	1.24	68.29	$-1.784\ 993 \cdot 10^{-7}$	7.28	77.21
	6				$-1.146\ 242 \cdot 10^{-10}$	27.78	96.07	$6.055\ 998 \cdot 10^{-9}$	0.55	77.76
	7							$-1.002\ 678 \cdot 10^{-10}$	9.83	87.59
	8							$6.508\ 661 \cdot 10^{-13}$	3.76	91.35
Body weight	0	$-4.789\ 129 \cdot 10$	0.00	0.00	$4.640\ 578 \cdot 10^{-3}$	0.00	0.00	$-7.386\ 377 \cdot 10^{-5}$	0.00	0.00
	1	$3.565\ 272 \cdot 10$	54.25	54.25	$4.054\ 286 \cdot 10^{-3}$	47.73	47.73	$1.437\ 240 \cdot 10^{-2}$	48.35	48.35
	2	$-1.107\ 324 \cdot 10$	5.55	59.80	$-1.280\ 426 \cdot 10^{-3}$	18.24	65.97	$-4.167\ 428 \cdot 10^{-3}$	20.35	68.70
	3	$1.940\ 630$	1.19	60.98	$1.358\ 095 \cdot 10^{-4}$	3.73	69.70	$5.088\ 473 \cdot 10^{-4}$	8.80	77.50
	4	$-2.164\ 602 \cdot 10^{-1}$	3.45	64.44	$-6.299\ 536 \cdot 10^{-6}$	1.42	71.12	$-3.149\ 755 \cdot 10^{-5}$	0.09	77.59
	5	$1.632\ 052 \cdot 10^{-2}$	0.34	64.78	$1.325\ 174 \cdot 10^{-7}$	2.33	73.45	$1.037\ 199 \cdot 10^{-6}$	5.96	83.54
	6	$-8.578\ 117 \cdot 10^{-4}$	1.11	65.89	$-1.033\ 160 \cdot 10^{-9}$	18.09	91.54	$-1.728\ 280 \cdot 10^{-8}$	0.06	83.60
	7	$3.180\ 810 \cdot 10^{-5}$	3.51	69.39				$1.145\ 390 \cdot 10^{-10}$	7.04	90.64
	8	$-8.287\ 671 \cdot 10^{-7}$	2.89	72.29						
	9	$1.484\ 165 \cdot 10^{-8}$	0.34	72.63						
	10	$-1.738\ 485 \cdot 10^{-10}$	3.38	76.01						
	11	$1.198\ 652 \cdot 10^{-12}$	10.41	86.42						
	12	$-3.686\ 276 \cdot 10^{-15}$	7.45	93.86						

In order to find out whether in the investigated period the pattern of the phenomenon of variability in the body length and body weight of peled larvae was similar, two groups of differently denominated curves were compared. The analysis of covariance showed that also in this case the curves were parallel (the accepted hypothesis $H_0^{(2)}$ at the level of $P(F) < 0.8852 = 0.6732 > 0.05$). Thus, it can be assumed that the pattern of variability of the body size of peled larvae was similar in the successive years of breeding in lake cages.

4. Discussion

In the ichthyological literature numerous works can be found which analyse the dynamics of variability during fish growth, using different kinds of curve (Sluckij 1978, Ryżkov 1980, Suchanov 1980). However, a common feature of these works is that the variability is presented in the CV form, this making the application of statistical tests impossible.

The analysis of variability based on the variance of logarithmicized values makes it possible to avoid these limitations. At a definite level of significance, by its use it could be found that, independently of density, the variability of the dimensions of the peled body was similar in the larval stages. In the successive years of fish rearing in lake cages, only the level of variability of traits changed. Thus, its variation can

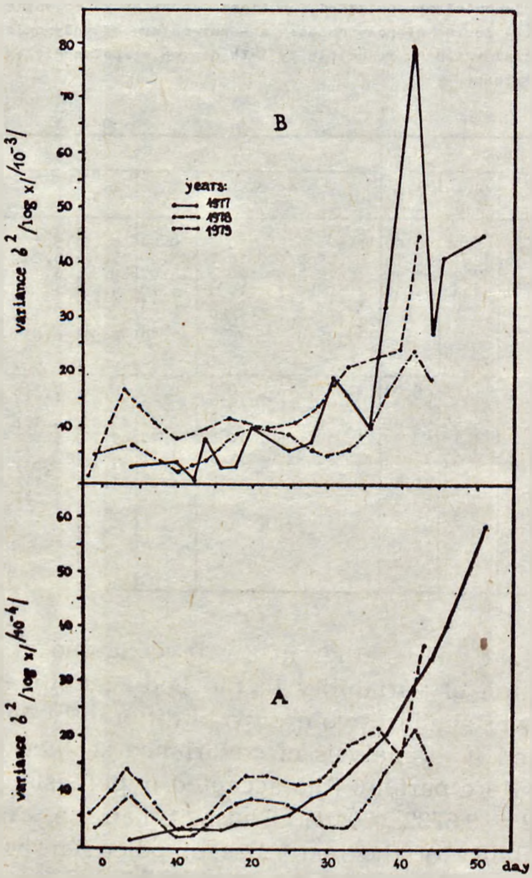

Fig. 2. Approximated curves of variability of body length (A) and weight (B) of peled larvae from fish rearing in lake cages in the years 1977—1979

be measured by the value of a_0 coefficients in corrected equations, this making it possible to assess rationally the level of variability in the different years. The observed differences have a definite biological expression. They can be explained by genetic conditions or by the diverse impact of the environment on larvae, this being supported by the literature (A y l e s, B e r s 1973, S y t i n a, T i m o f e j e v 1973, S l u c k i j 1978, L e b e d e v a 1980, R y ż k o v 1980). In the case of peled larvae from cage rearing, the highest level of variability noted in 1979 was, among other factors, brought about by the mass development of diatoms and their influence on the larvae (M a m c a r z, unpubl.).

In the given time interval the pattern of the empirical curve of variability is characterized by definite random variations of various amplitude in the particular stages of fish growth. Slight variations are

Table II. Parameters of corrected regression equations of variability of body size of peled larvae. i - degree of polynomial; a - parameters of polynomial in i-th degree

Year	i	Body length	Body weight
		a	a
1977	0	0.004	0.0060
	1	$3.077\ 282 \cdot 10^{-4}$	$4.618\ 381 \cdot 10^{-3}$
	2	$-6.186\ 348 \cdot 10$ [illegible]	$-1.260\ 916 \cdot 10^{-3}$
	3	$1.780\ 052 \cdot 10^{-6}$	$1.419\ 885 \cdot 10$ [illegible]
	4	$3.001\ 849 \cdot 10^{-7}$	$-1.439\ 794 \cdot 10^{-6}$
	5	$-2.201\ 578 \cdot 10^{-8}$	$2.824\ 907 \cdot 10^{-7}$
	6	$3.982\ 833 \cdot 10^{-10}$	$-5.011\ 593 \cdot 10^{-9}$
	7	$3.165\ 627 \cdot 10^{-12}$	$5.891\ 702 \cdot 10^{-11}$
	8	$2.364\ 145 \cdot 10^{-13}$	$-2.129\ 913 \cdot 10^{-12}$
	9	$-6.497\ 664 \cdot 10^{-15}$	$5.937\ 354 \cdot 10^{-14}$
	10	$8.898\ 301 \cdot 10^{-17}$	$-5.908\ 864 \cdot 10^{-15}$
	11	$6.623\ 112 \cdot 10^{-19}$	$-1.863\ 365 \cdot 10^{-17}$
	12	$-1.267\ 570 \cdot 10^{-20}$	$1.881\ 230 \cdot 10^{-19}$
1978	0	0.0001	-0.0009
	1	others as above	others as above
1979	0	0.0008	0.0071
	1	others as above	others as above

typical for the larval stages and for the growth of mature fish. The highest rapid changes in the variability coefficient were found by Sluckij (1978) in the initial period of fry growth. Therefore, the too close adjustment of the function to empirical data (a high degree of polynomial even in one equation) by way of approximation might render it impossible to test them using the method of covariance analysis. On the other hand, a too low degree of polynomial might insufficiently approximate the curve of variability. The best results are obtained by using this method to test the variability over periods with a small amplitude of random oscillations. This makes it possible to approach the phenomenon in its dynamic course without losing significant features in the differentiation of individuals (e.g., the passage from endogenous to exogenous food in larvae).

The method of analysis of variability presented here has a general character and allows the comparison of monomial series and those of different denominations in the same time interval. The method also has some limitations which need more comprehensive studies. It would be particularly important to determine the admissible share of the random factor in the pattern of the given function and, in this way, to find the optimum level of approximation of the trend. In the present work it was accepted that the share of the random factor should reach about 10%, this resulting in the introduction of high degrees of the polynomial. It seems that in order to standarize the investigations the time series should be described by the lowest degree of the polynomial at the given level of significance of the statistical verification (determined on the basis of the coefficient of the curvilinear regression).

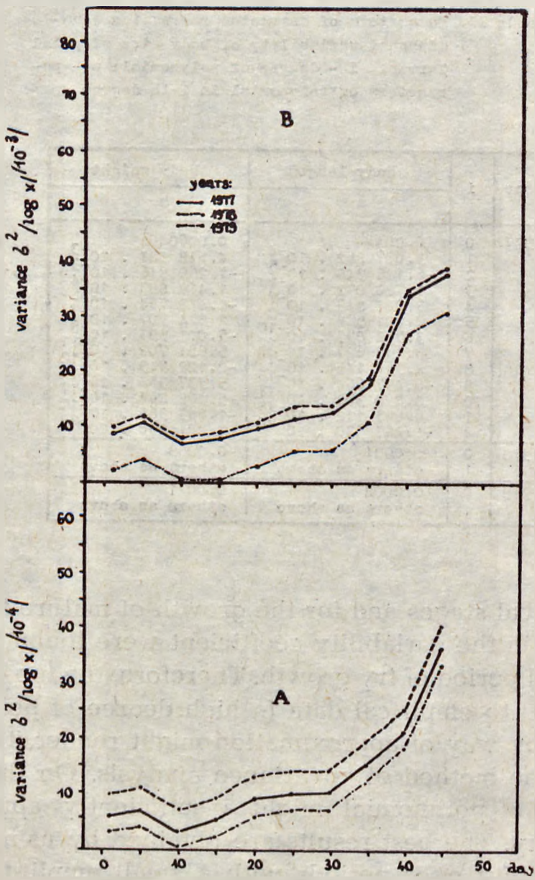

Fig. 3. Variability in body length (A) and weight (B) of peled larvae in the system of corrected equations in the years 1977—1979

Another limitation appears in comparing the variability series expressed by distant intervals of variance of logarithmicized values, e.g., the length of 10^{-4} and weight of 10^{-2}. Under these conditions, in spite of correct values of the covariance analysis, there appears a great deformation of corrected equations of the regression, formed in different intervals of the logarithmic scale.

5. Polish summary

Zastosowanie weryfikacji statystycznej w badaniach zmienności ryb

Przedstawiono metodę analizy zmienności w ujęciu dynamicznym, przyjmując za podstawę wartości wariancji, obliczonej jednym ze wzorów Wrighta. Stosując aproksymację wielomianową empirycznych szeregów zmienności (ryc. 1) uzyskano szeregi wyrównane (ryc. 2, tabela I), które porównywano metodą analizy kowariancji dla przypadku klasyfikacji pojedynczej z dowolną liczbą stopni wielomianu. W wyniku weryfikacji hipotezy o równoległości funkcji uzyskano teoretyczne krzywe zmienności (ryc. 3), wykreślone na podstawie poprawionych równań regresji (tabela II).

Metodę zastosowano do badania zróżnicowania rozmiarów ciała larw pelugi (*Coregonus peled* Gmel.) z podchowu sadzowego w Jeziorze Legińskim.

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