Bull. Fac. Fish., Mie Univ.
No. 12 : 13−27
October 1. 1985

A Simple Method of Analysing the Polymodal Frequency Distributions of Fish Egg Dismeters and its Application to the Ovarian Eggs of Japanese Common Mackerel

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A computerized simple method of analysing the polymodal frequency distributions of fish egg diameters is presented. The basic algorithm to discover some significant peaks and troughs depends on increase or decrease of the frequency between two successive egg diameter classes. A peak is counted when two increases occur consecutively and followed by two consecutive decreases. A trough is recognized in a similar manner. For complicated form of distribuions, the basic algorithm is modifed and more detailed criteria are developed. The ovarian eggs of Japanese common mackerel were examined by this method. Of the ovaries 95% had from one to six peaks. In the frequency distribution of ovaries at the most advanced stage, there appeared a discontinuous space(DS) in which no egg existed. The DS divides the distribution into the developing egg group and the hydrated egg group. This simple method lacks mathematical strictness but is fairly useful and affords easy analyses of the distributions of fish egg diameters.

Key wards: polymodal distribution, ovarian eggs, common mackerel

Observing fish ovaries, a few modes are found in the frequency distribution of egg diameters. These modes provide very important information regarding the sexual maturation process of fish species. To analyse the frequency distribution of ovarian egg diameters, a microscopic observation has been emplyed. However, this method of measuring the distribution of egg diameters and counting the number of eggs in the ovary requires a great deal of work and time.

Recent studies, for example Asano and Tanaka(1984), which applied an image processor to measure the fish eggs, have made repid analysis possible. Once a frequency distribution is obtained, analysis of the characteristics of the distribution are made in many different ways, depending on each researcher, because no systematic methodology of analysis has

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yet been developed.

Only Imai and Tanaka(1984) have reported a method for analysing the frequency distributions of egg diameters. Their method uses computer graphic technics to separate the polymodal frequecy distributions of egg diameters into various components each of which forms an error curve. On the other hand, for the length distributions, a variety of methods has been developed to analyse the polymodal frequency curve into age groups(e.g. Cassie 1954, Tanaka 1956, Shimazu 1979, Matsuzaki *et al.* 1983, Akamine 1982, 1984, 1985). The basic algorithm commonly used is to fit some normal distributions to the objective frequency curve, though the procedure of curve fittings is different depending on each method. These methods would be useful for analysing the frequency distribution of egg diameters, provided that the egg diameters of each component are distributed normally. If this is not the case, serious error may be introduced. This paper presents a simple method for analysing the polymodal distribution, without assuming a normal distribution, by detecting peaks and troughs with a definite algorithm and estimating their positions and heights. This method is applied to the frequency distributions of ovarian egg diameters of Japanese common mackerel caught in the spawning season.

Materials and Methods

1. Materials and computation

Over a period of three years(1977−1979) a total of 1069 female mackerels were sampled from the fish landed at Kogawa fish market, Yaizu, Shizuoka Prefecture. These mackerels belong to the Pacific subpopulation and aggregate to the sea area around the Izu Islands during the spring spawning season. Overies were extracted from the female mackerels and preserved in 10% formalin. After more than one month, the frequency distributons of the ovarian egg diameters were estimated by the method of Asano and Tanaka(1984) to serve as fundamental data for this study.

A basic algorithm was prepared to detect significant peaks and troughs and was programmed by FORTRAN language. The analyses of the frequency distributions of egg diameters were done by the FACOM OSIV/X8 computer of the Ocean Research Institute, University of Tokyo, and by the FACOM 230−38S computer of the computer center, Mie University.

2. Basic rule for detecting peaks and troughs

The basic rule for recognizing peaks and troughs is as follows: In a frequency distribution of egg diameters, the frequency of diameter class I is expressed as $Y(I)$. Comparing $Y(I)$ with $Y(I+1)$, in sequence of increasing the class number I, if $Y(I+1)-Y(I)$ is positive or zero for two or more consecutive Is and then becomes negative or zero for two or more consecutive Is, a peak is recognized. Similarly, if $Y(I+1)-Y(I)$ shows consecutive negative or zero values and then consecutive positive or zero values, then a trough is recognized (Fig. 1). In practice, however, some distributions appear whose peaks and troughs can not be deteted by means of this basic criterion only. Thus, for the complex cases a generalized algorithm was developed by modifying the basic one. This algorithm is given later.

As small eggs with diameters less than 0.2 mm were not measured (some eggs were less than 0.15 mm), the class of the frequency distribution started with egg diameters of 0.2 mm(or 0.15 mm). A mackerel ovary contains a large amount of small yolkless oocytes. These non-matured oocytes always exist in abundance irrespective of the maturity stage of the gonad(Takemura 1957, Tateishi 1958, Usami 1966). So the first peak(peak 1) is fixed at 0.025 mm as a regular position and the following peaks are detected and numbered successively from two upward. The lowest frequency between peak 1 and peak 2 will be trough 1 and the lowest frequency between peak 2 and peaks 3 will be trough 2, and so forth. Because peak 1 is fixed at the start of the analysis, the first step in the analysis is to determine trough 1.

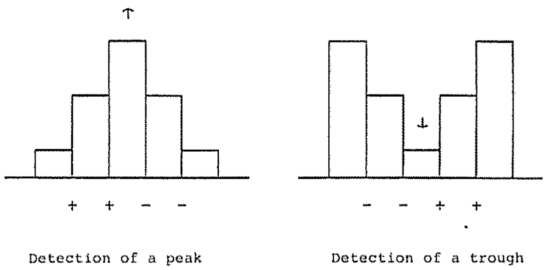

Fig. 1 Basic criterion to recognize a peak or trough.

3. Procedures for basic algorithm

A flowchart of analytical procedure is shown in Fig. 2. Let G=Y(I+1)−Y(I). The recognition of peaks and troughs is done by checking for the sign of G(we call this a "G decision"). In the flowchart the sign of G determines the branch direction: to M(positive), to E(zero) or to L(negative), respectively. If a sequential pattern of the sigh satisfies the criterion, a peak or a trough is admitted and then recorded. If not, I is increased by one and the G decisions are repeated. When G dicision No.34 result is negative or zero, then a peak is admitted. If it results in positive or zero at No.13, a trough is admitted. Besides, some other judgements and treatments are added in the program. They are explained below.

i) Zero detection and discontinuous space Some times frequency distribution of eggs is discontinuous, and the eggs are absent over a range of the diameter. Most of ovaries with this type of frequncy distribution are in the most advanced stage of maturation and possess hydrated eggs. This space separates the hydrated egg group from the egg group of less advanced stages. Therefore, this characteristic of egg distributions is important in studying the maturity stages of ovaries. We call this space: "discontinuous space(DS)".

If a G decision turns negative, the frequency of Y(I+1) could be zero. Whenever a G decision result is negative and at the same time Y(I+1) is zero, then without advancing to the next, it stays in the zero detection routine to observe how many zero frequencies occur consecutively. In the case of zero appearing only once, it returns to the normal routine. But if zero appears twice or more, midpoints of both the first and the final classes of zero frequency are recorded. When positive Y(I+1) is found in the zero detection routine, it escapes from the circuit and goes to decision No.30.

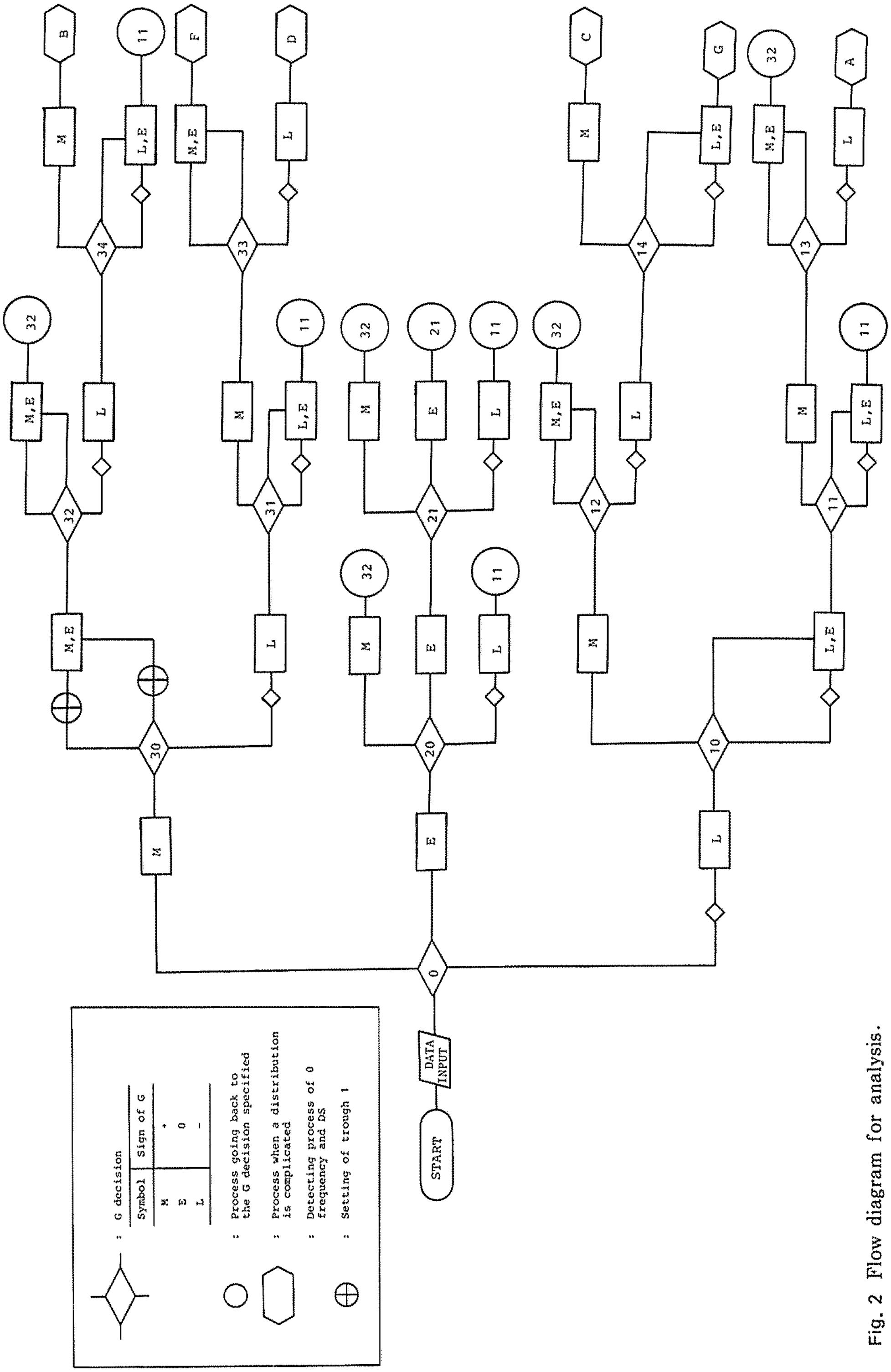

Fig. 2 Flow diagram for analysis.

ii) Setting of trough 1 If first two G decisions are all positive and no trough is recognized, then peak 2 will appear first without detecting trough 1. In order to avoid this situation in such a case, the position of trough 1 is set at the point of the left-hand margin of the smallest class and its height is defined by the frequency of this class. The procedure is formulated as follows:

1) When decision No.30 turn out M or E, if the number of troughs already counted is zero, let class No. 1 be trough 1 and go to decision No.32.

2) If the number of troughs already counted is not zero, simply proceed to decision No.32.

iii) Detection of date end In all G decisions a function of data end detection is provided. When it reaches the last class of frequency distribution after a series of decisions, the positions and numbers of observed peaks, troughs and DSs are recorded. This routine is executed just before each G decision.

4. Criteria when the distribution form of egg diameters is complex

In practice, even though some modes exist in a continuous frequency distribution, the frequency distributions around the modes do not always satisfy the fundamental criteria of peak detection. So that the operation with only basic criteria may ignore significant modes. Hence, a method for managing the complicated distributions where the basic algorithm can not be applied is considered below. Procedures are illustrated in the flowchart(Fig. 2). For example, if a series of G decisions becomes "LLML", it reaches result A, then a more detailed analysis is performed following the instructions given in Table 1. The recognition(or disapproval) of peaks and troughs is done depending on the form of frequency distribution, and the analysis proceeds to next step designated for each case. To better explain the decision algorithm, Fig. 3 shows various forms of frequency distributions corresponding to each of individual case in Table 1. Also for other complicated distributions pattern indicated by symbols B to G, treatments and their graphical explanatons are shown in Table 1~4 and in Fig. 4~6.

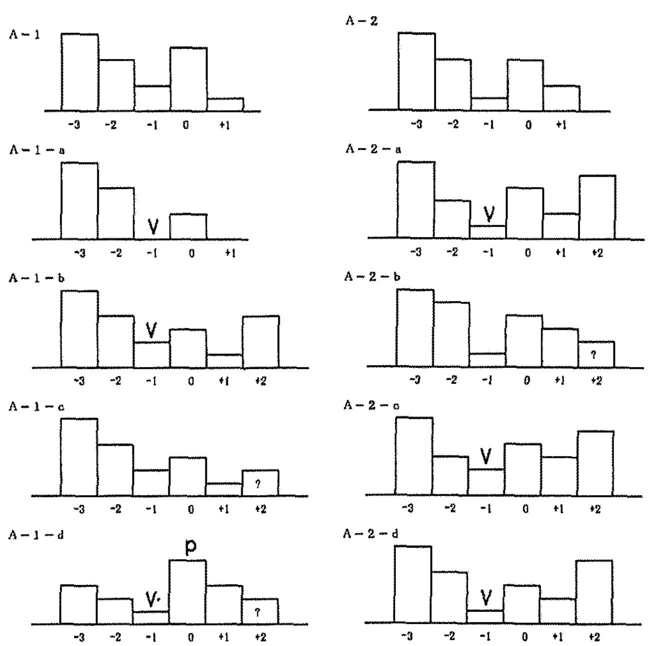

Fig. 3 Graphical explanation of criteria used in complicated distribution cases; Process A. P shows peak admitted and V trough admitted.

Table 1 Criteria for complicated distributions; Process A

Decision	Order of up and down*	Conditions	Treatment: Recognition: Trough	Peak	Destination to connect**
A	LLML				
1		Y(I+1)<=Y(I-1)			
a		Y(I+1)=0 AND Y(I-1)=0	I-1		GOTO 31
b		Y(I+2)>Y(I)	I-1		GOTO 32
c		Y(I+2)<=Y(I)			
1)		Y(I)<Y(I-3)			GOTO 11
2)		Y(I)>=Y(I-3)	I-1	I	GOTO 11
2		Y(I+1)>Y(I-1)			
a		Y(I+2)>Y(I)>=Y(I-2)	I-1		GOTO 32
b		Y(I+2)<=Y(I)<Y(I-2)			GOTO 11
c		Y(I+2)<=Y(I)			
1)		Y(I)>=Y(I-2)	I-1		GOTO 31
2)		Y(I)<Y(I-2)	I-1		GOTO 32
B	MMLM				
1		Y(I+1)>Y(I-1)			
a		NB $\neq$ 0			GOTO 32
b		NB = 0	I-3		GOTO 32
2		Y(I+1)<=Y(I-1)			
a		Y(I+2)<Y(I+1)			
1)		NB $\neq$ 0		I-1	GOTO 11
2)		NB = 0	I-3	I-1	GOTO 11
b		Y(I+2)>=Y(I+1)			
1)		NB $\neq$ 0	I	I-1	GOTO 30
2)		NB = 0	I-3, I	I-1	GOTO 30

NB = number of troughs
NP = number of peaks
* G decision: L --- G < 0
M --- G > 0
** number shows G decision in the diagram

Table 2 Criteria for complicated distributions; Process B and C

Decision	Order of up and down*	Conditions	Treatment: Recognition: Trough	Peak	Destination to connect**
C	LMLM				
1		Y(I-1)<Y(I+1) AND Y(I-1)<Y(I-3)	I-1		GOTO 32
2		Y(I-1)>=Y(I+1) AND Y(I-1)>=Y(I-3)			
a		NB=0	I-3	I-1	GOTO 11
b		NB≠0		I-1	GOTO 11
3		Y(I-1)>=Y(I+1) AND Y(I-1)<Y(I-3)			
a		NB=0			GOTO 11
b		NB≠0		I-1	GOTO 11
4		Y(I-1)<Y(I+1) AND Y(I-1)>=Y(I-3)			
a		NB=0	I-3		GOTO 32
b		NB≠0	I-2		GOTO 32
D	MLML				
1		Y(I-1)=Y(I+1)=0			GOTO 31
2		Y(I-1)<Y(I+1) AND Y(I-1)<Y(I-3)			
a		NB=0	I-1		GOTO 31
b		NB≠0			
1)		NB≠NP	I-1		GOTO 31
2)		NB=NP	I-1	I-2, I	GOTO 10
3		Y(I-1)>=Y(I+1) AND Y(I-1)<Y(I-3)			
a		NB=0			
1)		Y(I)>=Y(I-2)	I-3	I	GOTO 11
2)		Y(I)<Y(I-2)			GOTO 11

NB = number of troughs
NP = number of peaks

Table 3 Criteria for complicated distributions; Process D and F

Decision	Order of up and down*	Conditions	Treatment: Recognition: Trough	Treatment: Recognition: Peak	Treatment: Destination to connect**
D	MLML				
3					
b		NB≠0 AND NB≠NP			GOTO 11
c		NB≠0 AND NB=NP			
1)		Y(I)>=Y(I-2)			GOTO 34
2)		Y(I)<Y(I-2)		I-2	GOTO 11
4		Y(I-1)<Y(I+1) AND Y(I-1)>=Y(I-3)			
a		NB=0			
1)		Y(I-2)<Y(I)	I-3		GOTO 34
2)		Y(I-2)>=Y(I)			GOTO 11
b		NB≠0 AND NB≠NP			GOTO 34
c		NB≠0 AND NB=NP			
1)		Y(I)>=Y(I-2)			GOTO 34
2)		Y(I)<Y(I-2)		I-2	GOTO 11
F	MLMM				
1		Y(I-1)>=Y(I-3)			
a		NB=0	I-3		GOTO 32
b		NB≠0			
1)		NB=NP			GOTO 32
2)		NB≠NP	I-3		GOTO 32
2		Y(I-1)<Y(I-3)			
a		NB=0	I-1		GOTO 32
b		NB≠0 AND NB=NP			
1)		Y(I-2)<Y(I)			GOTO 32
2)		Y(I-2)>=Y(I)	I-1	I-2	GOTO 32

NB = number of troughs
NP = number of peaks

Table 4 Criteria for complicated distributions; Process F and G

Decision	Order of up and down*	Conditions	Treatment: Recognition: Trough	Treatment: Recognition: Peak	Treatment: Destination to connect**
F	MLMM				
2					
c		NB≠0 AND NB≠NP			
1)		Y(I-2) is higher than the nearest peak	I-1	replace the latest peak with I-2	GOTO 32
2)		Y(I-2) is lower than the nearest peak	I-1		GOTO 32
G	LMLL				
1		Y(I-1)<=Y(I-3)			GOTO 11
2		Y(I-1)>Y(I-3)			
a		NB=0	I-2	I-1	GOTO 11
b		NB≠0 AND NB≠NP			
1)		Y(I)<Y(I-2)			GOTO 11
2)		Y(I)>=Y(I-2)	I-2	I-1	GOTO 11
c		NB≠0 AND NB=NP			
1)		Y(I)<Y(I-3)		I-1	GOTO 11
2)		Y(I)>=Y(I-3)		I-1	GOTO 11

NB = number of troughs
NP = number of peaks

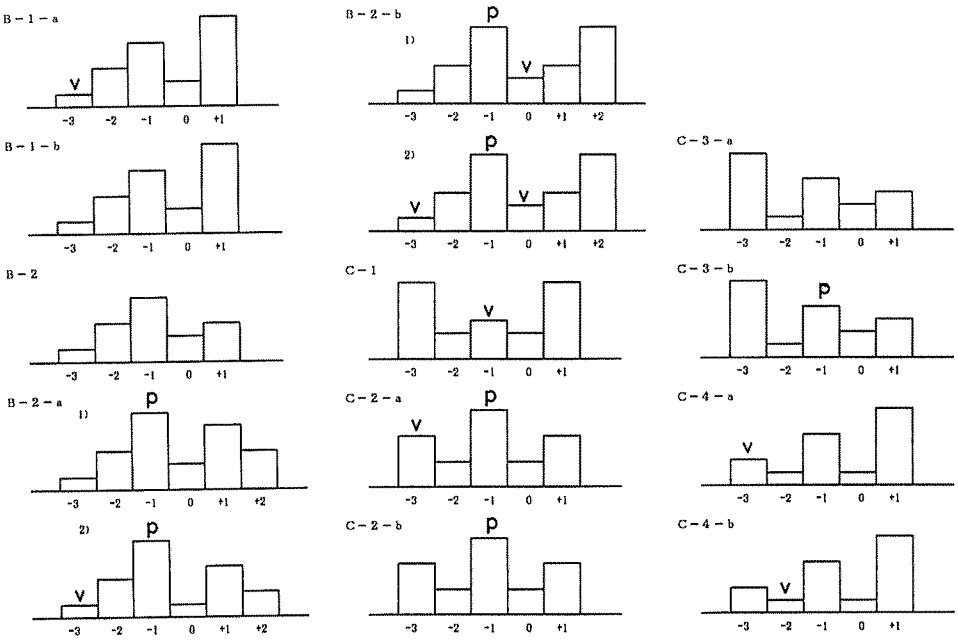

Fig. 4 Graphical explanation of criteria used in complicated distribution cases; Process B and C. P shows peak admitted and V trough admitted.

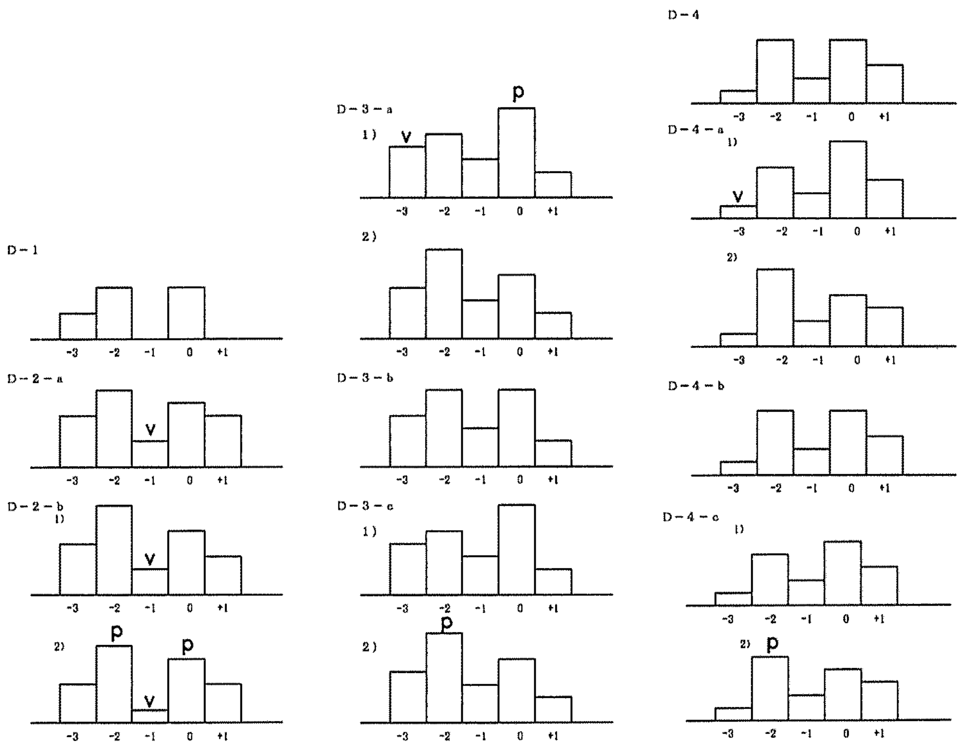

Fig. 5 Graphical explanation of criteria used in complicated distribution cases; Process D. P shows peak admitted and V trough admitted.

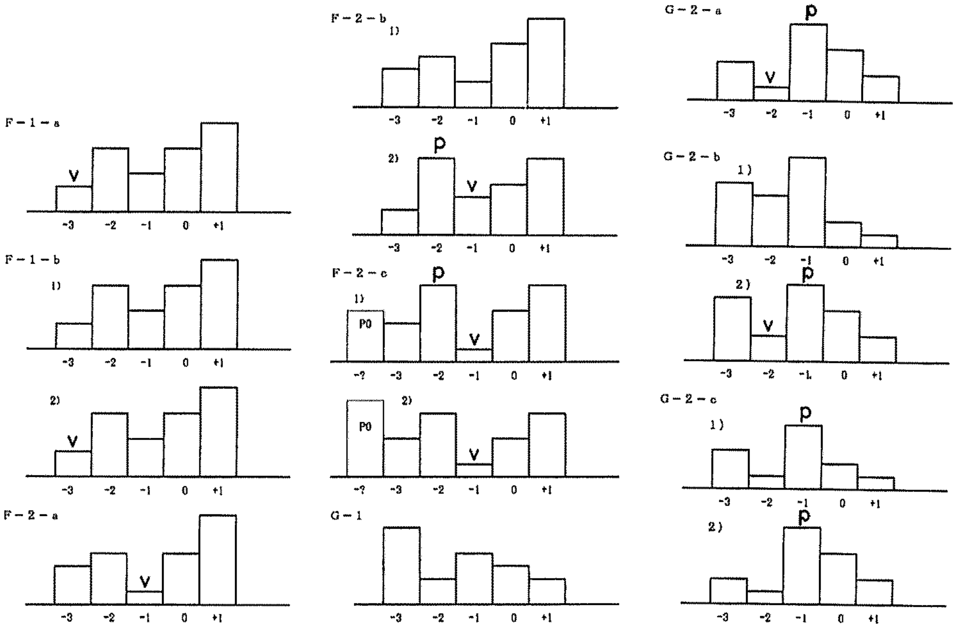

Fig. 6 Graphical explanation of criteria used in complicated distribution cases; Process F and G. P shows peak admitted and V trough admitted. PO indicates the nearest peak.

Results

1. Number of peaks, troughs and discontinuous spaces appearing in the frequency distribution

Tables 5～7 show the result obtained by the above algorithm. Ovaries are classified by the number of peaks and the numbers of ovaries are given for each sample in the table. The number of peaks varied from one to six. The single peak distribution type occupied a large part at the beginning of the spawning period before March. Then the double peak type increased, and with its decrease the 3 peak type became dominant. After that, the ovaries of the more than 3 peak distribution type appeared.

Examining the annual changes in Fig. 7, it was found that in 1977 the double peak distribution type was prevalent early in March. When this type decreased early in March, the dominant type changed to three peaks. The double peak type increased again in May in 1977. In 1979, the dominant type changed from the single peak to the double peak type early in February. Subsequently in March, the 3 peak type dominated in the composition of the peak types. In that year 4 and 5 peak types first appeared early in March, and the polymodel distributions of ovaries of four through six peak types continued to occur until early June, though the proportion was low.

The appearance of DS is summarized in Tables 5～7. In 1977, 21 ovaries from a total

Table 5 Number of peaks and discontinuous spaces(DSs) appearing in frequency distribution of egg diameters. Samples collected in year 1977

Date	N	Number of peaks						Number of ovaries which have the DS
		1	2	3	4	5	6	
1977. 3. 01	29	1	20(1)	8				1
3. 09	37	8	26(1)	3				1
3. 16	35	2	18	15(3)				2
3. 22	29	2	21	6				-
4. 04	24		16	8				-
4. 12	31		20	11				-
4. 18	40		10(1)	29(5)	1			6
4. 28	46	3(1)	21(2)	20(1)	2			4
5. 10	41		17(1)	23(3)	1			4
5. 18	42		23	17	2			-
6. 02	25		5(1)	20(2)				3
TOTAL	379	16	197	160	6			21

() indicates the number of ovaries which have the DS.

Table 6 Number of peaks and discontinuous spaces(DSs) appearing in frequency distribution of egg diameters. Samples collected in year 1978

Date	N	Number of peaks						Number of ovaries which have the DS
		1	2	3	4	5	6	
1978. 3. 07	24	2	15(1)	7				1
3. 16	27		4	23				-
4. 10	27		4	23				-
4. 17	26		6	20				-
4. 25	19		4	15				-
5. 02	20		1	15(3)	3(2)	1(1)		6
5. 05	20	1	8(2)	10(4)	1(1)			7
5. 11	34		7(1)	25(2)	1(1)	1(1)		5
5. 19	28	1	7(2)	14(8)	5(5)	1(1)		16
5. 24	28	2	10	14(1)	2(2)			3
6. 07	20		5(2)	14	1(1)			3
TOTAL	273	6	71	180	13	3		41

() indicates the number of ovaries which have the DS.

Table 7 Number of peaks and discontinuous spaces(DSs) appearing in frequency distribution of egg diameters. Samples collected in year 1979

Date	N	Number of peaks 1	2	3	4	5	6	Number of ovaries which have the DS
1979. 1.25	16	9	7					-
2.01	22	7	14(2)	1				2
2.06	38	7	25(1)	6				1
2.14	35	10	23	2				-
2.22	34	7	18	9				-
3.02	35		11(2)	22(1)	2			3
3.07	28		6(2)	13(3)	6(6)	3(3)		14
3.14	30		4	26				-
3.28	30		2	21(3)	6(4)	1(1)		8
4.20	31		10	18(3)	1(1)	1(1)	1(1)	6
4.26	19		1	15(2)	3(2)			4
5.07	50		7	38(1)	5(3)			4
5.16	28		4	22(3)	2(1)			4
6.01	21		1	15(4)	5(4)			8
TOTAL	417	40	133	208	30	5	1	54

() indicates the number of ovaries which have the DS.

of 379 individuals had the DS, 41 ovaries out of 273 fish in 1978 and 54 ovaries in 417 fish in 1979. The ratio of the ovaries which had the DS ranged from a few % to 57.1% depending on samples. DSs occurred in all of distribution types from one* to 6 peaks, but the majority of them belonged to the 2~4 peak type. Most of the ovaries of the 4~6 peak types had one or more DSs and particularly all the ovaries of the 5~6 peak types had DSs. When an ovary had a DS, the number of DSs ranged from one to three. Single DS occupied 84.5%, and 14.7% had a double DSs and only 0.9% had three DSs. Among the ovaries which had double DSs, the 4 peaks type was 47%, the 3 and 5 peak types were 23.5% respectively. Only one individual was found with three DSs, and this was identified as the six peak type.

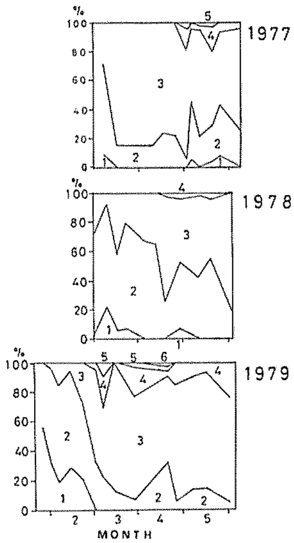

Fig. 7 Seasonal change in composition of the number of peaks in frequency distribution of egg diameters. Number in the figure show groups classified according to number of peaks.

2. Relationship between DS and distribution type From the positions of the DSs which appear in the frequency distributions of egg diameters of the ovaries of mackerel, we can classify the distribution types and grasp the dynamics of transitions from one type to another. The relationships between the position of the DS and the distribution types which are identi-

* The case is that a DS was detected and some number of eggs were found in larger egg size classes than the range of the DS, but their number was so small or the form of distribution was so irregular that they did not form peaks, and only peak No. 1 was admitted.

fied by the number of peaks and troughs are as follows.

Trough the DS has some length, the position of DS may be represented by the midpoint of the DS. The positions of the DS with regard to the different types of the frequency distributions of egg diameters are shown in Fig. 8. DSs appeared in a range from 0.472~1.012 mm. Of these, 6.0% were located in 0.6~0.7 mm, 44% in 0.7~0.8 mm, 31% in 0.8~0.9 mm, and 12.9% in 0.9~1.0 mm. Thus 75% of the DSs appeared in 0.7~0.9 mm. As to the relation between the number of peaks and troughs and the positions of the DSs, some of the 2 peak type tended to appear in the lower part of the range, but those of more than 2 peaks aggregated to appear in the range 0.7~0.9 mm or more. This suggests that there is a possibility that the ovaries of 3 peak—2 trough type or more contain the eggs of most advanced stage(0.7~0.9 mm and upward); most of them are hydrated. Because, in the ovaries of 2—2 type or less, the DS is located in the smaller part of the range comparatively, these ovaries contain many immature or developing eggs.

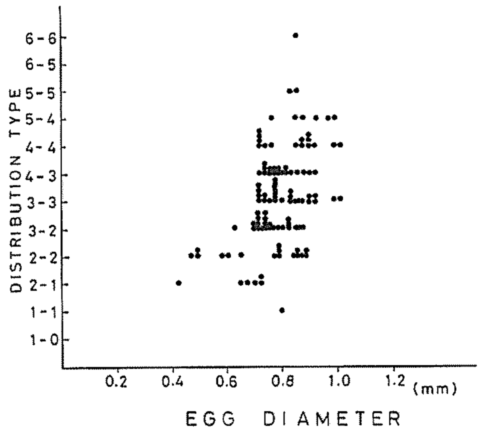

Fig. 8 Relationship between position of discontinuous spaces(DSs) and the distribution types shown by the number of peaks and troughs.

To examine the position of the DS corresponding to the peaks, the relationship between the positions of the DS and those of the peaks is shown in Table 8. In a total of 116 in-

Table 8 Relationship between peaks and positions of discontinuous spaces

Interval between peak	Frequency
1 — 2 *	8
2 — 3	29
3 — 4	31
4 — 5	6
1 + **	0
2 +	15
3 +	22
4 +	5
TOTAL	116

* X − Y means a DS is located between peak X and peak Y.
** X + means a DS is located at the right side of peak X (last peak).

dividuals in which the DSs appeared, 25% of them were located between peaks No.2 and No.3, 26.7% between No.3 and No.4. For those ovaries where the DSs appeared in the right side of the last peak, 12.9% appeared at the right of peak No.2, 19.0% at that of No.3. Summarizing the results, those which appeared at the right side of peak No.2 were 37.9% regardless of the presence of the peak No.3 or more, 45.7% at the right of the peak No.3, so totally 83.6% were found at the right hand of peak No.2 or No.3.

The process of development viewed from the appearance of the DSs in the ovaries of mackerel is summarized as follows.

1. With the progress in the maturation of the ovaries, eggs increase their size and the 2~3 peak types become dominant among the frequency distributions.
2. When the maximum diameters of eggs reach around 0.6~0.7 mm, some of eggs grow further beyond the DS. These eggs form peak No.4 and upward.

We may conclude that for the ovaries of mackerel, 2~3 peak types dominated in the frequency distributions of egg diameters. But most of the ovaries with the DSs are the type of 4 peaks or more and DSs are located in 0.7~0.95 mm and have eggs in larger sized than the DS.

Discussion

The method of analysing peaks and troughs presented in this paper is fundamentally different from other methods of curve fitting. Though there are some mathematical and theoretical problems, the method allows the analysis of the polymodel distributions into a few peaks and troughs by simple and systematic way. As the analysis is done by computers, there is no chance of subjective judgement by individual scientists once the criteria are set. The algorithms are easy to modify and change. While there is room for improving the criteria, still such a simple method as this is quite useful for this type of study.

To improve the criteria, it will be necessary for the method to introduce statistical concepts. Once the position and height of the peaks are determined, fitting of normal distribution curves will be performed easily. Extention of this method toward this direction would be next course of development.

Acknowledgement

We thank Dr. Takeo Ishii, Ocean Research Institude, University of Tokyo, for his useful suggestions which greatly assisted the study.

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