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On the accuracy of cod fecundity estimations

by

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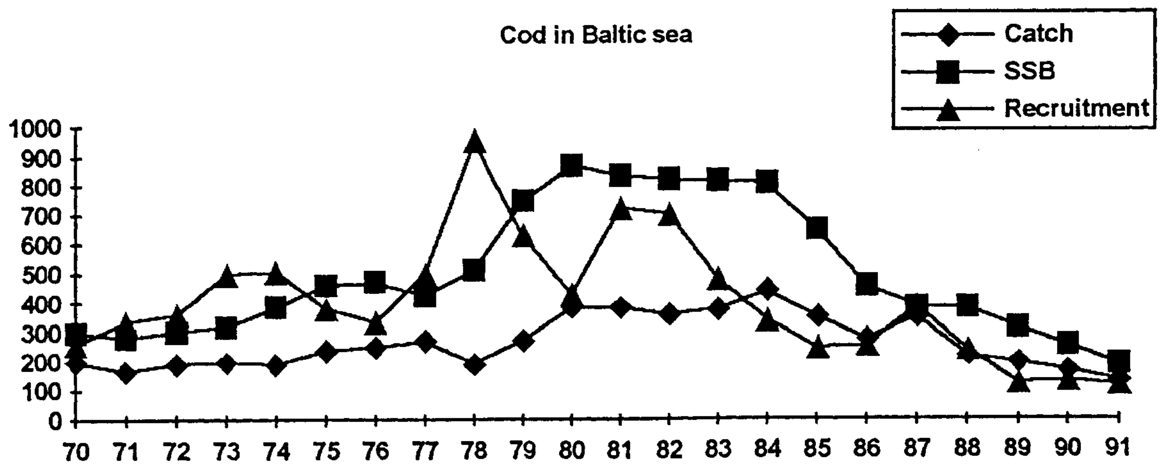
SUMMARY

This work solve the question : How many eggs or subsample of eggs of a cod gonad are to analyse in the lab, if the halve of the confidence interval D should be less then 10 % of the number of the eggs of the gonad with an error of first kind $\alpha = 0.1$?
Nine subsample of 50 eggs from each gonad should be weighted to reach the demand.

1. INTRODUCTION

In the Baltic sea in the last years strong changes appeared that affected the fishing resources. Today's a decrease of the cod stocks like it is not documented in this form can be observed.



The causes of this development are extremly complex and can be found in multiple factorial relationsships. Many references are given today that beside the fishing pressure the bad ecological condition play an essential role for the recruitment in the Baltic sea.

Quickly national and international calls for action can be seen in the intensification on the resarch of the recruitment mechanism of Baltic sea cod.

A part of this problem is the question concerning the quantity and quality of the spawning products, especially the fecundity, in dependence on
a) the individual factors of the femals and
b) the catch area and the stock identity.
It is to investigate also which relationships exist between the quantity and quality of the spawning products and the production of viable embryos.

Many publications are known regarding the estimation of the fecundity of different fish species (RAITT (1933), SIMPSON (1951), BOTROS (1959,1962), GÖTTING (1961), STRZYZEWSKA (1962),MAY (1967), SCHOPKA(1971), PINHORN (1984), KRENKEL (1990)et.al.).

It is noticeable that a general method does not exist for the estimation of the number of eggs. Generally two different methods of the estimation are known.
a) The direct counting of the number of eggs of a gonad with an automatic fish egg counter (BAXTER et.al. (1959), PARRISH et.al.(1960),DAVIS et.al. (1965), SCHULZ (1967), SCHOPKA et.al. (1973), PINHORN(1984),and WITTHAMES et.al.(1987)).
The advantage of this method is the possibility of counting a great number of eggs within a short time interval.

b) The estimation of the number of eggs of a gonad by manual counting of a defined number of subsamples using a binocular.RAITT(1933), KÄNDLER(1958), MAY(1967), BOTROS(1962), BAGENAL(1957), SIMPSON(1951) and PARMANNE (1991).

This method is time - consuming but it is also most accurate. We used this method for our work.

A main problem of the method of manual-counting is the estimation of the neccessary number of subsamples in order to achieve the demanded accuracy of the number of eggs of a gonad.

We decided to estimate the number of eggs with "dry weight-method" SIMPSON(1951), BAGENAL(1957),HODDER(1963) and PARMANNE (1991) of subsample using subsamples with constant number of eggs in contrast to the often used "volume-method" often used to take the subsamples POWELS(1958), THOMSON(1962), MAY(1967).

Additionally to the counting of eggs extensive resarch were done directed towards the estimation of the egg size in order to separate the reserve oocyten from the developed eggs.

In this connection investigations were carried out to estimate the influence of different mediums for preservation and fixing on the consistence and diameter of eggs.
The approach to this problem and the results will be published later.

2. Material and Methods

2.1 General handling of the gonads

2.1.1 Preparation, preservation and fixation

Only gonads not damaged and not containing transparent eggs were used for the estimation of the fecundity. The maturity stages were III and IV according to MAIER.

All countings and measurements were done manually using a binocular and graduated object sheets.

Before fixation the outer walls of the ovaries were broken up to enable a better attack of the fixing medium. The material were shaked to support the fixing process and a better separation of the eggs from the tissue. The times of fixing were chosen in dependence on the mass of the gonads and the fixing medium. Therefore they were variable.

2.1.2 Separation of the eggs

When the fixation process was finished, then the gonads were washed and pieces of the tissue were collected separatly using a binocular. Additional a cascade of different sieves with mesh sizes of 1.25 mm, 1.12 mm, 0.20 mm and 0.10 mm were used for the complete separation of eggs and tissue. The absolute separation of eggs from each other were achieved by pressing the eggs gently between fingers.

The investigations show that the main part of the eggs (70 - 80%) were hold back with the mesh size of 0.20 mm. The filtered fluid of each flushing was superwised on the existence of eggs in order to exclude losses.

All separated eggs were collected in a dish and thoroughly mixed. Then the subsamples of eggs of each gonad were taken for the estimation of the mean mass of one egg according to the principle of random sampling .

2.1.3 Drying and weighing

When the subsamples were counted out then the other eggs, the tissue and the subsamples were dryed separatly until a constant mass were obtained.

We found that a time interval of 24 hours and a temperature of 110°C is optimum. Attempts with lower temperature and shorter time intervals showed that the neccessary constancy of the mass were not obtainted.

After the drying process the eggs were transferred for cooling for several hours to a desiccator containing phosphorus pentoxide.. The mass of the materials were estimated with an accuracy of 0,00001 g.

3. Special investigations

Special investigations were done for the following problems.

3.1 Influence of different methods of preservations on the diameter of the eggs

3.1.1 Frozen gonads

The first preservation must be done with freezing at -18 °C because technical circumstances prevent a immediate preservation in fixation medium. In this case the questions of irreparable change for instance the damage or the change of the diameter of eggs are essential.

Three subsample containing 50 eggs from a fresh prepared gonad were counted out and the diameters were measured. Then samples were hermetically sealed and stored by -18°C. After 5, 14 day and also 3 month of storage the material was thawed and measured again.

3.1.2 Heating up gonads

Some authors (ROSENBOOM, 1985, THOMAS, 1988, and KRENKEL, 1990) showed that eggs can be also fixed by heating up. This method is not so expensive and can be done without toxic material. We investigated this method for cod eggs. In each case three subsamples containing 50 eggs from fresh prepared gonads were counted out. Then the eggs were put into
A) boiling water of 98°C for 5 minutes
B) water of 80 °C for 20 minutes and
C) water of 90°C for 20 minutes.
After this process the eggs were counting out again. Simultaneously samples were put in to the medium of ethanal (80%) or formol (4%, buffered) for 24 hours.

3.1.3 Fixation and maceration using different chemical solutions

Different mediums for instance ethanol (80%), formol (4%) and Gilson fluid (after SIMPSON 1951) were tested to estimate theire usefullness for fixing of eggs and maceration of tissue for different time intervalls of storage.
The mediums were tested to solve the following problems :
A) the separation of eggs and tissue and
b) the irreparable change of the eggs (shrinkage and destruction).

3.2 The determination of the necessary number of subsamples in order to achieve the demanded accuracy for the egg number of gonads

The planed method of dryed mass required the determination of the nescessary number of subsamples from each gonad in dependence on the demanded accuracy.

Two method were possible. The first method used a constant number of eggs in each subsample. The second method used a nearly constant volume having a mass of about 30 mg with a variable number of eggs in a subsample. 3, 5, 10 or 15 subsamples were investigated to assess the variance of the mean mass and the neccessary number of subsamples.

For the method of a constant number of eggs subsamples of 50, 100 and 300 eggs were taken. 3,5,10,15 or 20 subsamples were investigated.

In all cases the number of eggs in the subsample were counted out. Then the subsamples were dryed separetly. Afterwards the subsample were weighed.

4. RESULTS AND CONCLUSIONS

4.1.1 Frozen gonads

In four parallel experiments 96 - 100 % of the eggs were undamanged independent of the time of storage. Unfixed eggs were extrem sensitive. This was the cause that nearly 4 % of the eggs were destroyed. This form of storage of gonads is very usefull.

4.1.2 Heating up gonads

We use the following notations for the describtion of the results:

A boiled water (98 °C),
B water with 80 °C,
C water with 90 °C,
D ethanol (80 %) and
E formol (4%, buffered).

The following table illustrates the number of undamaged eggs in the different attempts.

Medium	A	B	C	D	E
No. of eggs counted out	100	100	100	100	100
Incub.-time	5 mins.	20 mins.	20 mins.	24 hours	24 hours
No. of eggs undamaged	61-90	91-95	90-95	100	100

Following conclusions can be given to the different kinds of preparation :

To method A: This method is very usefull to separate eggs and tissue. The eggs were tempered but up to 39 % of the eggs were destroyed or very strong compressed.

To methods B and C: Eggs and tissue were only incompletely tempered, the separation was bad, and the eggs were partially destroyed.

To method D: The eggs were well tempered but the shrinkage was extreme. The separation of eggs and tissue were manually possible but difficult because of the very brittle tissue.

To method E: The eggs were good tempered. The separation of eggs and tissue were well possible.

The results illustrate clearly that the fixation by high temperature is not usefull for cod eggs.

4.1.3 Fixation and maceration using different chemical solutions

4.1.3.1 Ethanol

After the storage for 24 hour the eggs were completly harded. No eggs were destroyed. The disadvantage of the medium was that eggs were strong shrinked and deformed. The determination of the diameter were not correctly possible. The separation of eggs and tissue was difficult because the tissue was brittle. It was difficult to pick up the small parts of tissue by hand and it was very time-consuming.

4.1.3.2 Formol (4%, buffered)

Formol (4%, buffered) is a usefull medium for fixing the cod eggs. Only few of the well harded eggs were deformed. The shrinkage of the eggs was small, measurable and regular for all eggs. The separation of eggs and tissue was completly possible.

If the mass of gonads was higher than 500 g, it was necessary to prolong the time interval of fixation to 48 hours. Eggs were not destroyed. The elasticity of the harded eggs permitted a good handling.

4.1.3.3 Gilson-fluid (according to SIMPSON)

The fixation with Gilson-fluid needed an incubation time of two to four month. This large intervall were not helpfull for the practical work. The eggs were not deformed but unusual sensitive. The shrinkage of the eggs was strong and irregular dependent on the diameter. The quantification of this process was very difficult. In Gilson-fluid the tissue disintegrated. The partial very small pieces could be separated only incomplete using the cascade of sieves.

The comparison of the results of all methods demanded that for routine work the fixation with formol (4%, buffered) should be used.

4.2 Estimation of the neccessary number of subsample

Here only the results of the determination of the number of subsample shall be described. The notations and the equations are described in Appendix A.

If the mass of the eggs of a gonad is normal distributed and independent with N[E(x), V(x)], and the autocorrelation of the mass of single eggs within a subsample can be neglected because the eggs are well mixed, then the mass of the subsamples $y_n(j)$ is also normal distributed with $N[n * E(x), n * V(x)]$ and the mean mass of one egg is normal distributed with N[E(x), V(x) / n].

Two possibilities were investigated for the determination of the number of eggs of a gonad.

4.2.1 M_n subsamples consisting of equal number of n eggs

M_n subsamples consisting of equal number of n eggs were counted out and then the mass of each subsample was determined after drying. Then the mass of the subsample $y_n(j)$ is a stochastic sample. This procedure was repeated using different number of eggs (n = 50, 100 and 300) and variable numbers of subsamples (M_n = 3,5,10,15 and 20). All subsample were taken from the same gonad.

Table 4.1. shows the means $E(y_n(j))$ and $E(z_n(j))$ and the standard deviations $S(y_n(j))$ and $S(z_n(j))$ for the mass of subsamples and the mass of eggs.

The experiments were repeated for different n and M_n. It should be pointed out that the standard deviation is very high for n=100, V=3 and M_n=15. The same results were fond for n=300, V=1 and M_n=5 as well as for V=2 and M_n=5.

In Figures 4.1, 4.2, and 4.3 the means $E(z_n(j))$ are shown for different n. Figure 4.4 shows a summary of all samples. Figures 4.5, 4.6, and 4.7 illustrate the values of $E(z_n(j))$. Figure 4.8 shows the summary of all data.

The results demonstrate that the mean $E(z_n(j))$ is approximativly normal distributed. Then the mean mass of an egg E(x) and its variance can be assessed by

$$E(x) = E(y_n(j)) / n$$
$$V(x) = V(y_n(j)) / n.$$

Table 4.2 shows the estimations of E(x), $V(y_n)$ and V(x) for different n. If the extrem values were eliminated for n = 100 and n = 300, we got values of E(x) and V(x) as presented in the lower part of the table

Making use of these results the estimation of the neccessary number of subsample M_n can be calculated for different n using

$$E(x) = 0.0275 \text{ and}$$
$$V(x) = 1.0E\text{-}3 \text{ and } 8.6E\text{-}4, \text{ respectively.}$$

Equation (10) can be used to estimate the number of M_n for any n according to the restriction

$$D(N) \leq 0.1 * N$$

and the errror of firt kind $\alpha = 0.1$.

Tables 5.3 and 5.4 give the results for different n and different variances.

The minimum number of eggs are to be counted out if it is worked with single eggs. But in this case the neccessary work increases for determine the mass of single eggs, and the accuracy of the scale can have a negative influence. The number of subsample M_n decrease with an increase of n but the number of eggs counted out increases.

For our work it is recommended to use values of $M_n = 9$ and $n = 50$

4.2.2 A constant subvolume of the gonade were used for subsamples.

In this case the number of eggs n(j) and the mass of subsamples $y_n(j)$ are random variables. Subsamples of a volume having a mass of about 30 mg were taken, and different repetitions of M in two series of tests were carried out. For the second series of tests the eggs were taken from another gonad. No informations of the fish like length, mass, stage of maturity were available for characterizing the gonads.

Table 4.5 shows the estimated mean and standard deviations for the variables were

n(j) number of eggs in a subsample

$y_{n(j)}(j)$ mass of a subsample

$z_{n(j)}(j)$ mean mass of an egg in subsample

for bothe series of tests and all M.

Figures 4.9 and 4.10 show the X-Y plotts of n(j) and $y_{n(j)}(j)$. Figure 4.11 shows a summary of all data. Strong differences can be observed between the two series of tests. The causes are not clear. The number of subsamples shown in Table 4.6 can be calculated with equation (14) using the demanded accuracy (A) and a mass of the subsample of about 30 mg.

The results show clearly that the varability of n(j) is the cause of the increase of total variance, especially, if a correlation exists between the number of eggs and the mass of subsamples as it occurred in the first series of tests.

The comparison of all results of both methods demand that for the routine work the method of constant number of eggs in the subsamples described in 4.2.1 should be used.

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Appendix A : Notations and equations

E	mean
V	variance
S	standard deviation
n	number of eggs in a subsample
n(j)	number of eggs in the j th subsample of a constant volume
$y_n(j)$	mass of the j th subsample with n eggs
$E(y_n(j))$	mean mass of a subsample of n eggs
$V(y_n(j))$	variance of mass of subsamples of n eggs
M_n	number of subsamples with n eggs
x(ij)	mass of the i th egg in the j th subsampl
E(x)	mean mass of one egg in a gonad
V(x)	variance of the mass of one egg in a gonad
W	mass of a gonad
N	number of eggs of a gonad
V(N)	variance of the number of eggs of a gonad
D(N)	halve confidence interval of the number of eggs in a gonad
$t(M_n\text{-}1,1\text{-}\alpha/2)$	quantil of the Student distribution
α	error of first kind
$z_n(j)$	mean egg mass from the j th subsample with n eggs

$$y_n(j) = \sum_{i=1}^{n} x(ij) \quad (1)$$

$$E(y_n(j)) = n * E(x) \quad (2)$$

$$V(x) = n * V(y_n(j)) \quad (3)$$

$$z_n(j) = y_n(j) / n \quad (4)$$

$$V(z_n(j)) = 1/n^2 * V(y_n(j)) \quad = n * V(x(ij)) / n^2 \quad = V(x(ij)) / n \quad (5)$$

If x(ij) is normal distributed with N[E(x), V(x)] and independent then

$y_n(j)$ is normal distributed with N[n * E(X), n * V(x)] and

$z_n(j)$ is normal distributed with N[E(x), V(x) / n].

$$E(N) = W * n / E(y_n(j)) = W / E(x) \quad (6)$$

$$V(N) = V(W * n / E(y_n(j))) = (W * n)^2 * V(1 / y_n(j)) \quad (7)$$

$$\approx (W * n)^2 * 1/E^4(y_n(j)) * V(y_n(j)) = W^2 / (n * E^4(y_n(j))) * V(x) \quad (8)$$

$$D(N) = t(M_n\text{-}1,1\text{-}\alpha/2) * \sqrt{\{V(N) / M_n\}} \quad (9)$$
$$= t(M_n\text{-}1,1\text{-}\alpha/2) * W / E^2(x) * \sqrt{\{V(x) / (M_n * n)\}}$$

The value of M_n can be estimated for all n with the restriction $D(N) \leq k * N$ with $k = 0.1$

$$Mn \geq t^2(M_n\text{-}1,1\text{-}\alpha/2) * V(y_n(j)) / (E(y_n(j)) * k)^2 \quad (10)$$
$$= t^2(M_n\text{-}1,1\text{-}\alpha/2) * V(x) / [n * (E(x) * k)^2]$$

We used for the sample pattern b)

$$E(N) = E(W / z_{n(j)}(j)) = W * E(n(j) / y_{n(j)}(j)) \approx W * E(n(j)) / E(y_{n(j)}(j)) \quad (11)$$

$$V(N) = V(W / z_{n(j)}(j)) = W^2 * V(n(j) / y_{n(j)}(j)) \quad (12)$$

$$\approx W^2 / E^2(y_{n(j)}(j)) * [V(n(j)) + (E(n(j)) / E(y_{n(j)}(j)))^2 * V(y_{n(j)}(j)) - COV(n(j), y_{n(j)}(j)))]$$

It can be assumed, that $COV(n(j), y_{n(j)}(j))) = 0$, if the eggs are mixed thoroughly.

$$\approx W^2 / E^4(y_{n(j)}(j)) * [E^2(y_{n(j)}(j)) * V(n(j)) + E^2(n(j)) * V(y_{n(j)}(j))] \quad (13)$$

The variance of the number of eggs is to consider addidtional to the variance of the mass.

Then follows

$$M \geq t^2(M-1,1-\alpha/2) * V(z_{n(j)}(j)) / (E(z_{n(j)}(j)) * k)^2 \quad (14)$$

Appendix B : Tables

Table 4.1: **Estimated mean and standard deviation for the mass of subsample and the mean mass of one egg in the subsample**

V	M_n	$E(y_n(j))$	$S(y_n(j))$	$E(z_n(j))$	$S(z_n(j))$
n = 50					
1	5	1.39	0.16	0.028	0.0031
	9	1.35	0.16	0.270	0.0032
	15	1.36	0.11	0.027	0.0022
	18	1.40	0.09	0.028	0.0018
2	5	1.16	0.24	0.023	0.0049
	10	0.99	0.10	0.020	0.0020
	15	1.15	0.26	0.023	0.0052
	20	1.15	0.22	0.023	0.0044
3	5	1.41	0.26	0.028	0.0052
	10	1.35	0.14	0.027	0.0029
	15	1.45	0.17	0.029	0.0034
	20	1.34	0.11	0.027	0.0023
	30	1.36	0.17	0.027	0.0033
Total	177	1.30	0.26	0.026	0.0041

V	M_n	$E(y_n(j))$	$S(y_n(j))$	$E(z_n(j))$	$S(z_n(j))$
n = 100					
1	3	2.697	0.15	0.027	0.0015
	5	2.70	0.10	0.027	0.0010
	10	2.87	0.44	0.029	0.0044
	15	2.64	0.15	0.026	0.0015
2	3	2.48	0.19	0.025	0.0019
	5	2.52	0.15	0.025	0.0016
	10	2.42	0.13	0.024	0.0013
	14	2.57	0.20	0.026	0.0022
3	3	3.01	0.04	0.030	0.0004
	5	2.84	0.07	0.028	0.0007
	11	3.35	0.19	0.033	0.0019
	15	2.79	0.78	0.028	0.0078
Total	99	2.71	0.40	0.027	0.0039

V	M_n	$E(y_n(j))$	$S(y_n(j))$	$E(z_n(j))$	$S(z_n(j))$
n = 300					
1	3	7.80	0.26	0.026	0.0010
	5	10.74	1.17	0.036	0.0056
2	3	7.33	0.056	0.024	0.0001
	5	7.30	0.56	0.024	0.0019
3	3	9.01	0.056	0.030	0.0002
	5	8.80	0.14	0.029	0.0005
Total	24	8.61	1.50	0.029	0.0050

Table 4.2 : The estimation of E(x) and V(x) for different M_n

n	50	100	300
M	177	99	24
E(x)	0.026	0.027	0.029
$V(y_n)$	0.043	0.1578	2.219
V(x)	8.537E-4	1.577E-3	7.397E-3

M		84	14
E(x)		0.027	0.028
$V(y_n)$		0.081	0.647
V(x)		8.108E-4	2.681E-3

Table 4.3 : The number of subsample (M_n) in dependence on n where E(x) = 0.0275 and V(x) = 1.0E-3

n	1	50	100	300
$V(y_n)$	1.0E-3	0.05	0.1	0.3
$E(y_n)$	0.0275	1.375	2.75	8.25
$(E(y_n) * k)^2$	7.56E-6	0.019	0.076	0.681
$V(y_n) / (E(y_n) * k)^2$	132.23	2.645	1.322	0.441
M_n ($\alpha = 0.1$)	363	9	6	3
Total number of eggs	363	450	600	900
M_n ($\alpha = 0.05$)	518	12	8	5
Total number of eggs	518	600	800	1500

Table 5.4 : The number of subsample (M_n) in dependence on n where E(x) = 0.0275 and V(x) = 8.5E-4

n	1	50	100	300
$V(y_n)$	8.5E-4	0.043	0.085	0.255
$E(y_n)$	0.0275	1.375	2.75	8.25
$(E(y_n) * k)^2$	7.56E-6	0.019	0.076	0.680
$V(y_n) / (E(y_n) * k)^2$	122.40	2.25	1.12	0.375
M_n ($\alpha = 0.1$)	309	8	5	3
Total number of eggs	309	400	500	900
M_n ($\alpha = 0.05$)	441	11	7	4
Total number of eggs	441	550	700	1200

Table 4.5 : **Estimated mean and standard deviation of the number of eggs in the subsample n(j), the mass of subsample $y_{n(j)}(j)$, and the mass of one egg of the subsample $z_{n(j)}(j)$**

V	$M_{n(j)}$	E(n(j))	S(n(j))	$E(y_{n(j)}(j))$	$S(y_{n(j)}(j))$	$E(z_{n(j)}(j))$	$S(z_{n(j)}(j))$
1	3	172.67	15.50	5.93	0.84	0.03	0.002
1	5	177.60	16.56	6.01	0.24	0.03	0.003
1	10	159.50	14.40	7.46	2.50	0.05	0.02
1	15	154.87	22.86	7.63	2.09	0.05	0.01
Total	33	161.33	20.26	7.18	2.05	0.05	0.01
2	3	194.67	6.81	6.70	0.60	0.03	0.00
2	5	205.00	20.43	7.07	1.00	0.03	0.00
2	10	205.50	27.85	7.33	1.83	0.04	0.01
2	15	195.13	16.07	6.39	1.79	0.03	0.01
Total	33	199.73	20.33	6.81	1.64	0.03	0.01
Summary	66	180.53	27.92	6.99	1.85	0.04	0.01

Table 4.6 : **The neccessary number of subsample M where k = 0.1**

V	1	2	total
$M_{n(j)}$	33	33	66
$V(z_{n(j)}(j))$	2.14E-4	5.49E-5	1.65E-4
$E(z_{n(j)}(j))$	0.04536	0.03405	0.03970
$V(z) / (E(z) * k)^2$	10.74	4.73	10.45
M ($\alpha = 0.1$)	30	15	30
Total	4800	3000	5400
M ($\alpha = 0.05$)	43	21	43
Ttotal	6880	4200	7740

Appendix C: Figures

Figure 4.1

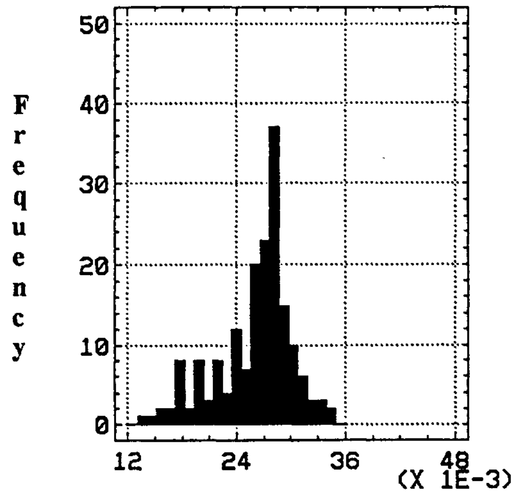


Figure 4.2

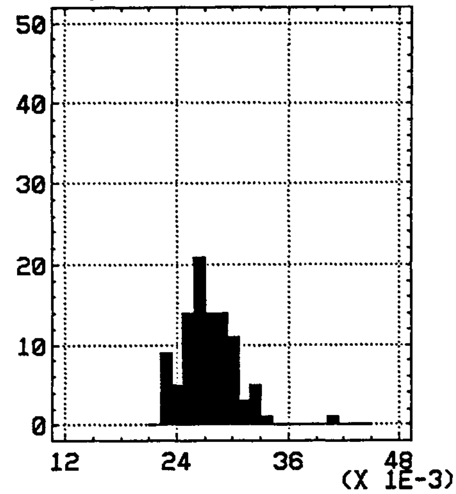


Figure 4.3

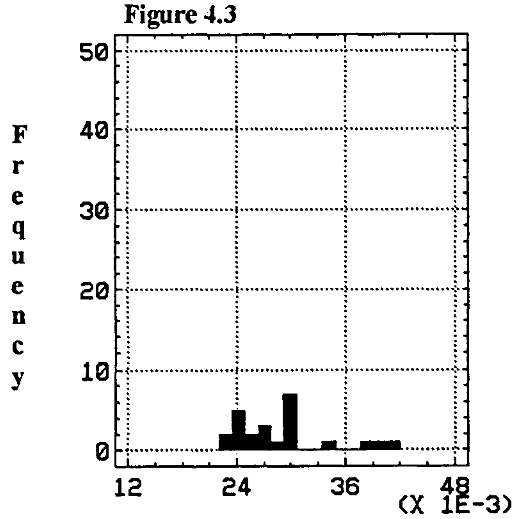
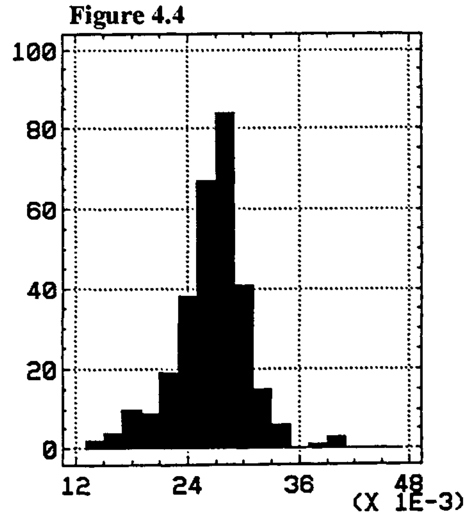


Figure 4.4



Frequency distributions for following data

Figure 4.1	**Mean egg mass for n = 50**
Figure 4.2	**Menn egg mass for n = 100**
Figure 4.3	**Mean egg mass for n = 300**
Figure 4.4	**Summary of mean egg mass for all data**

Figure 4.5

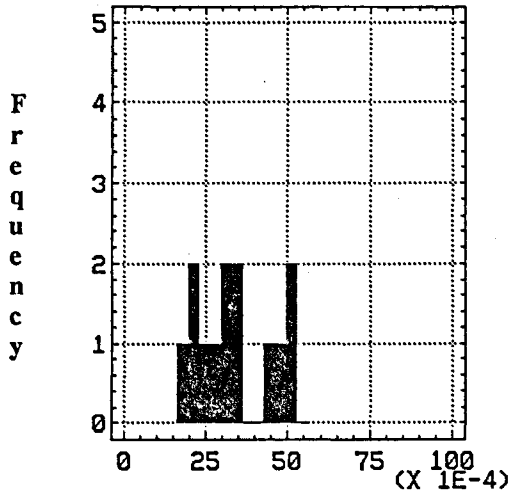


Figure 4.6

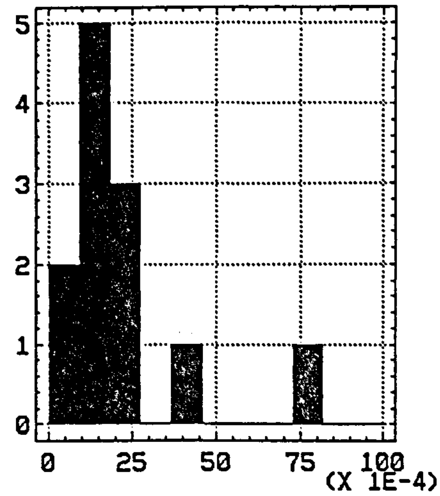


Figure 4.7

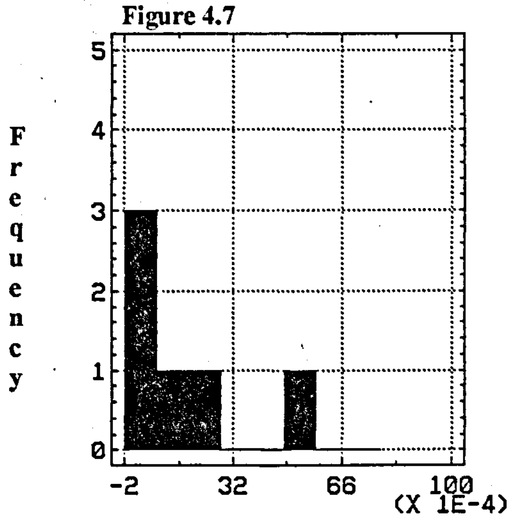
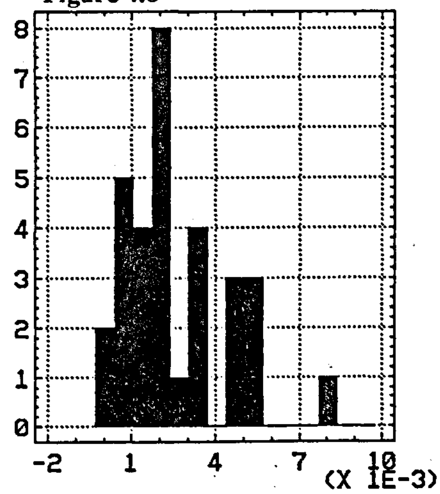


Figure 4.8



Frequency distributions for following data

Figure 4.5	**Distributiob of variance of mean egg mass for n = 50**
Figure 4.6	**Distribution of variance of menn egg mass for n = 100**
Figure 4.7	**Distribution of variance of mean egg mass for n = 300**
Figure 4.8	**Summary variance of mean egg mass for all data**

Figure 4.9

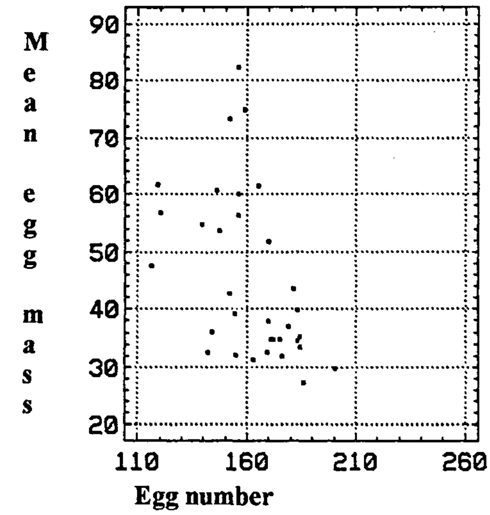


Figure 4.10

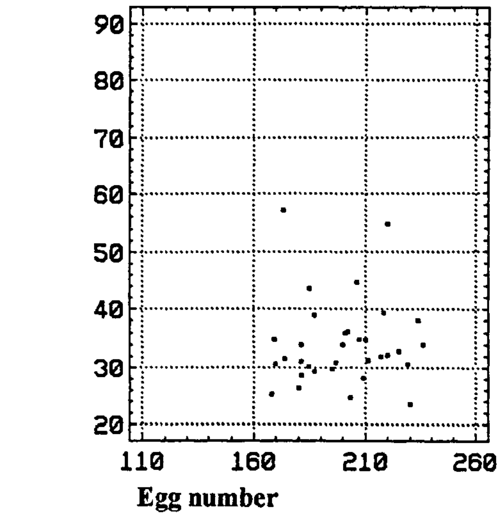
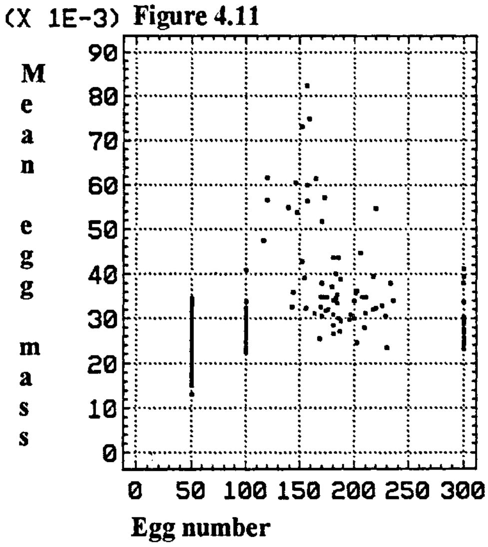


Figure 4.11



XY-plott for following data

Figure 4.9	**XY-plott of egg number (x) and mean egg mass (y) for n = 50**
Figure 4.10	**XY-plott of egg number (x) and mean egg mass (y) for n = 100**
Figure 4.11	**XY-plott of egg number (x) and mean egg mass (y) for n = 300**