INTERNATIONAL COUNCIL FOR
THE EXPLORATION OF THE SEA

C.M. 1989/H:44
Pelagic Fish Committee

THE TOTAL FECUNDITY OF WESTERN HORSE MACKEREL (TRACHURUS TRACHURUS L.)

by

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Errata

Line 9 on page 4 should be replaced by:

The parameter b was estimated as b = 3.274 (SE = 0.301) and b = 0.991 (SE = 0.100)

Table 2 on page 9 should be replaced by :

TABLE 2. Results of the analysis of variance of Log(Fecundity) on Log(Length) or Log(Weight) and Method (volumetric or histometric).

	SS	df	MS	F	
Log(Length)	7.192	1	7.192	117.30	**
Method	0.116	1	0.116	1.89	n.s.
Log(Length)*Meth.	0.037	1	0.037	0.60	n.s.
Error	3.311	54	0.061	-	
Total	10.66	57	-	-	
Log(Weight)	6.750	1	6.750	96.43	**
Method	0.116	1	0.116	1.66	n.s.
Log(Weight)*Meth.	0.035	1	0.035	0.50	n.s.
Error	3.755	54	0.070	-	
Total	10.66	57	-	-	

** $p < 0.01$

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Abstract

In accordance with the terms of a contract with the Commission of the European Communities, the Netherlands Institute for Fisheries Investigations at IJmuiden, started a study on the total fecundity of Western Horse Mackerel (*Trachurus trachurus* L.).
In these investigations the potential total fecundity of horse mackerel was estimated, using both a volumetric and a histometric method. The traditional standard volumetric method was used for estimating the total fecundity and ovaries in prespawning condition were digested in Gilson's fluid. The histometric method, which has been developed at the Fisheries Laboratory Lowestoft (UK), has a number of advantages. The use of Gilson's fluid, a persistent poison, is avoided and maturity stages can be confirmed by identifying post-ovulatory follicles. In addition, the size at which oocytes become vitellogenic can be measured and largely, because digestion in Gilson's fluid is unnecessary, the method is much quicker. The results of the histometric method were not significantly different from those of the volumetric method. The total fecundity of Western Horse Mackerel was estimated at 1478 eggs per gram female fish in maturity stage 4 for the volumetric method and 1655 eggs per gram female for the histometric method.

Résumé

Un contrat avec la Commission des Communautés Européennes a permis à l'Institut Néerlandais de Recherches Halieutiques d'IJmuiden de démarrer une étude de la fécondité totale du chinchard dit de l'Ouest (*Trachurus trachurus* L.).
Ces traveaux ont porté sur l'estimation de la fécondité potentielle totale du chinchard en utilisant à la fois une méthode volumétrique et une méthode histométrique. La méthode volumétrique standard traditionelle a été utilisée pour estimer la fécondité totale et les ovaires en préponte ont été traités dans le liquide de Gilson. La méthode histométrique s'était développé à Fisheries Laboratory Lowestoft (GB) et offre de nombreux avantages. On évite l'utilisation du liquide de Gilson, poison persistant, et les stades de maturité peuvent être confirmés par l'identification des follicules postovulatoires. De plus, la taille à laquelle les oocytes deviennent vitellogènes peut être mesurée et, en grande partie parce que la digestion dans le Gilson n'est pas nécessaire, la méthode est beaucoup plus rapide. Les resultats de la méthode histométrique ne diffèrent pas significativement de ceux de la méthode volumétrique. La fécondité totale du chinchard dit de l'ouest a été estimée à 1478 oeufs par gramme de femelle au stade 4 de maturité par la méthode volumétrique et à 1655 oeufs par gramme de femelle par la méthode histométrique.

Introduction

In accordance with the terms of a contract with the Commission of the European Communities, the Netherlands Institute for Fisheries Investigations at IJmuiden, started a study on the total fecundity of Western Horse Mackerel (*Trachurus trachurus* L.).

The spawning of Western Horse Mackerel occurs between April and July over an area close to the edge of the continental shelf between the southern Bay of Biscay and the west of Ireland. The total egg production of Western Mackerel and Western Horse Mackerel was estimated by egg surveys, which were carried out every three years for estimating the spawning stock biomass of both species (Anon., 1987). The western egg surveys in 1977, 1980, 1983 and 1986 were originally intended to sample mackerel eggs, but the results showed that that both the timing and the extend of the surveys made them suitable for estimating horse mackerel egg production (Anon., 1988). To convert the total horse mackerel egg production into a spawning stock biomass estimate, it was necessary to estimate the length distribution of the spawning horse mackerel together with the length-fecundity relationship and/or the weight-fecundity relationship. The fecundity of Western Horse Mackerel was last estimated by Nazarov (1977), this has been used to calculate the spawning stock biomass of horse mackerel from the western mackerel egg surveys in 1980, 1983 and 1986 (Anon., 1987: Table 8.1). However, a more up to date fecundity estimate was necessary.

The aim of this study was to measure the total potential fecundity and at the same time to investigate the possibility of replacing the fecundity estimate using the traditional volumetric method with Gilson's fluid with one using a histometric method. The total potential fecundity of horse mackerel was measured prior to spawning using both a volumetric and a histometric method and the results from the two methods were compared.

Material and Methods

Biological sampling of horse mackerel

In April 1987 maturity stage 4 horse mackerel were collected southeast of the Great Sole Bank at the edge of the continental shelf by the commercial freezer trawler KW74. Horse mackerel ovaries were assigned to a particular maturity stage by visual inspection using the nine stage maturity scale used by Macer (1974). For the total fecundity determination only ovaries at maturity stage 4 were used. This stage is characterized by having fully developed vitellogenic oocytes oocytes, but no hyaline oocytes. However, the preliminary results from the histological analysis of these ovaries showed that most ovaries were misclassified by visual classification and that most ovaries appeared by histological examination to be still in maturity stage 3. Out of 129 fish collected in 1987 only 19 (=15%) were in the maturity stage 4 and therefore suitable for the total fecundity estimate. In March-April 1988 another 135 ovaries were collected in the main spawning area on the Great Sole Bank by the commercial freezer trawler SCH33. About 70 % of these ovaries were correctly classified as maturity stage 4. The length, maturity stage, total weight, ovary weight and age were recorded from each fish sampled. One lobe of the ovary from each fish was placed in 4% formaline for 24 - 48 hours and then transferred to 70% ethanol. This lobe was transferred to Gilson's fluid for volumetric analysis on arrival at the laboratory. The other lobe was fixed on board in 4% buffered formalin for subsequent histological examination.

The increase in weight from maturity stage 4 (prespawning) to maturity stage 5 - 7 (spawning) was estimated from the biological market sampling program for Western

Horse Mackerel from 1982 - 1988. These samples were collected from the main spawning area by Dutch commercial freezer trawlers.

Volumetric method

On arrival at the laboratory one lobe of each ovary was weighed and transferred to Gilson's fluid. The ovaries were digested over a period of at least three months with frequent agitation either on an orbital shaker for the ovaries sampled in 1987 or by hand several times daily for the ovaries sampled in 1988. The Gilson's fluid was filtered off using a 60 µm sieve for the ovaries sampled in 1987 and a 80 µm sieve for ovaries sampled in 1988. The oocytes were then suspended in 50% sucrose solution to maintain neutral buoyancy. During sub-sampling the solution was stirred with a domestic mixer and a sub-sample was taken from the top and from the bottom of the beaker with a 0.5 ml Stempel pipette. Each sub-sample was counted using semi-dark field microscopy and an ocular micrometer. The diameter of the oocytes was measured to the nearest 19.15 µm. Seven pairs of sub-samples were counted and a comparison between the top and the bottom sub-samples was made using the Wilcoxon matched-pairs signed-rank test (Siegel, 1956). This test showed no significant difference between the numbers of vitellogenic eggs in the two sub-samples. It was decided to count a single sub-sample from the middle of the beaker for the remainder of the samples.

The egg size frequency distributions were then raised to total numbers within one ovary lobe using the counts of eggs > 96 µm and raised to the total for the whole ovary using ovary weights.

Histometric method

The histometric method, which was used, has been developed at the Fisheries Laboratory in Lowestoft (UK) (Emerson et al. in preparation).

Samples were dissected out of an ovary lobe preserved in buffered formalin and the relative volume was measured using a displacement technique (Scherle, 1970). On account of the relatively large connection between the two lobes of an ovary only two transverse slices, one from the middle of the ovary and one from the posterior end, were dehydrated in ethanol, embedded in resin and cut in 5 µm thick sections. In contrast to the mackerel the staining of the vacuoles in oocytes of horse mackerel ovaries could not be achieved successfully using P.A.S.-Mallory's or Harris haematoxylin and eosin. However, staining with Harris haematoxylin and eosin had an advantage in that the previtellogenic oocytes (without vacuoles), when compared with the vitellogenic oocytes (with vacuoles) gave a darker blue colour and these were therefore used for staining. Two transverse sections of the ovary were taken and from each slide 10 fields were randomly selected and photographed at a 25x magnification. The resulting slides were projected at a 10.5x magnification onto a screen of a microfilm viewer and a Weibel M168 multipurpose grid (d = 0.0091 cm) superimposed onto the image. A point and intersection count was carried out on each slide. The number of oocytes in the ovary was estimated using the method of Weibel and Gomez (1962).

The diameter at which 50% of the oocytes were vacuolated was determined and was used as a minimum size threshold for vitellogenic eggs. When 10 ovary samples were counted, the results from the middle and posterior end slices were compared using the Student t-test (Sokal and Rohlf, 1969). This test showed no significant difference between the two sub-samples for both the number and the area of the vitellogenic oocytes. It was decided therefore to count slices from the middle of the remaining ovaries.

Histological sections were examined to: (i) verify the shipboard macroscopic classification and ensure that spawning (indicated by the presence of post-ovulatory-follicles) had not commenced; (ii) determine the lower threshold of vitellogenic eggs; (iii) determine the total fecundity by raising the counts of vitellogenic eggs to the total volume of the ovary.

Statistical analysis

An analysis of the variance Log(Fecundity) was carried out on Log(Length) or Log(Weight) and Method (volumetric and histometric) based on the data listed in Table 1.

The two formulas below were tested, where F = fecundity, W = weight, L = length and Method = volumetric or histometric method.

$$Log(F) = a + bLog(L) + Method$$
$$Log(F) = a + bLog(W) + Method$$

The parameter b was estimated as b = 3.210 (SE = 0.325) and b = 0.938 (SE = 0.110) and did not differ significantly from 3 in the fecundity-length relationship and from 1 in the fecundity-weight relationship. Because Log(Length)*Method and Log(Weight)*Method interaction was not significant, the final estimates of fecundity-length and fecundity-weight were carried out according the formulas:

$$F = a + bL^3 \quad \text{and} \quad F = a + bW$$

Results

Misclassification of maturity stage.

After histological examination 85% of the ovaries collected in 1987 appeared to be misclassified by visual inspection. Most of the ovaries appeared to be still in maturity stage 3. Therefore in 1988 fish were collected with ovaries, judged bye eye, to be late maturity stage 4. In this instance histological examination showed that only 30% were misclassified.

Onset of vitellogenesis.

The onset of vitellogenesis was determined as the size at which 50% of the oocytes showed vacuolation (Figure 1). This lower size threshold of vitellogenic oocytes of 101 µm in the histological samples was equal to a threshold of 96 µm in the volumetric samples. The latter included an estimated adjustment of 5% for shrinkage in Gilson's fluid.

Increase in weight from maturity stage 4 to stages 5 - 7

An increase in weight of 5% was estimated from maturity stage 4 (prespawning) females to stage 5 - 7 (spawning) females from horse mackerel samples from 1982 - 1988 from the main spawning grounds caught by Dutch commercial freezer trawlers. The total egg production can be converted to a biomass of females at maturity stage 4 by using the fecundity estimate, but this biomass at maturity stage 4 should then be raised with a factor of 1.05 to obtain the spawning biomass.

Volumetric method

Table 1 shows the total fecundity estimates of 29 fish using the volumetric method. The fecundity-length relationship was estimated by using the lower vitellogenic threshold of 96 µm (corrected for shrinkage in Gilson's fluid):

$$\text{Fecundity} = 81{,}019 + 9.107\ L^3(\text{cm}) \qquad R^2 = 0.583$$

The fecundity-weight relationship for maturity stage 4 female horse mackerel was:

$$\text{Fecundity} = 131{,}510 + 1{,}094\ W(\text{g}) \qquad R^2 = 0.538$$

This fecundity-weight relationship is shown in Figure 3. The intercept was not significantly different from 0, therefore the fecundity was also calculated as 1478 eggs per gram female horse mackerel at maturity stage 4, which was calculated from the

mean fecundity / mean weight (Table 1). The results of the volumetric method were not significantly different from those of the histometric method (Table 2).

Histometric method

Table 1 shows the total fecundity estimates of the same 29 fish using the histometric method together with the confidence interval of 2*SE. The fecundity-length relationship was estimated as follows:

$$\text{Fecundity} = -72{,}302 + 13.688\ L^3(\text{cm}) \qquad R^2 = 0.680$$

The fecundity-weight relationship for maturity stage 4 female horse mackerel was:

$$\text{Fecundity} = -20{,}069 + 1{,}713\ W(\text{g}) \qquad R^2 = 0.682$$

This fecundity-weight relationship is shown in Figure 3. The intercept was not significantly different from 0, therefore the fecundity was also calculated as 1655 eggs per gram female horse mackerel at maturity stage 4, which was calculated from the *mean fecundity / mean weight* (Table 1). The results of the histometric method were not significantly different from those of the volumetric method (Table 2).

Discussion

The total potential fecundity has been estimated prior to spawning and it has been assumed that no 'de novo' vitellogenesis has occurred during spawning, although the oocyte size frequency distributions (Figure 2) show no clear distinction between previtellogenic and vitellogenic oocytes. Macer (1974) also showed a similar frequency distribution of oocytes in maturity stage 4 without a clear distinction between previtellogenic and vitellogenic modes. The present fecundity estimate has not been corrected for artretic losses. Macer (1974) presented percentages of atresia for maturity stage 4 (6%), 7 (21% and 48%) and 8 (100%) and 9 (100%). Quantifying atresia is difficult, because it is still not known for how long an atretic egg remains within the ovary and therefore a correction factor cannot be applied. The estimated total fecundity could be a conservative estimate, because the realized fecundity could be lower due to atresia, which would result in an underestimate of the spawning stock biomass. However, the fecundity could also be higher, if the horse mackerel was an indeterminate spawner, which would result in an overestimate of the spawning stock biomass. A correction for egg mortality would also increase the spawning stock biomass, because the estimated total egg production would be converted to a higher total egg production at the time of spawning.

Macer (1974) showed that in horse mackerel ovaries vacuolation of oocytes virtually ceased after maturity stage 4 and that, subsequently, the non-yolky, vacuolated oocytes disappeared because of either the development of yolk or resorption. Therefore, the onset of vitellogenesis was assumed to correspond to the size at which 50% of the oocytes showed vacuolation. Above this threshold all oocytes were counted for both the volumetric and the histometric method.

Nazarov (1977) calculated the diameters of previtellogenic oocytes, which become vitellogenic in the following season to vary between 45 and 175 µm. Developing oocytes were larger than 175 µm. Macer (1974) estimated from histological samples that vacuoles first appeared in significant numbers in oocytes with a diameter of 100 µm, and at a size of 160 µm all oocytes had vacuoles and the threshold at which 50% of the oocytes become vacuolated is 118 µm. The estimated threshold of 101 µm is below Macer's estimate of 118 µm, but this could be partly or entirely due to differences in shrinkage between the two histological techniques. Small changes in the size of the threshold will influence the fecundity estimate very much, because there are many oocytes in the size classes around this threshold as is shown in Figure 2.

The extremely abundant 1982 year class has a mean length at age of 27 cm or a mean

weight at age in the spawning stock of 141 gram in 1988 (Anon., 1989). Volumetric and histometric estimates of fecundity-length and fecundity-weight relationship give similar results for this mean length and mean weight of this year class. Unfortunately the fish smaller than 28 cm are not included in this fecundity estimate, because the sampling of the ovaries only took place in March-April, when the older and larger fish were in maturity stage 4, but the smaller fish were unsuitable as they were later maturing.

In the text table below the fecundity at different fish lengths is compared with other relevant estimates of fecundity in the western and North Sea area together with the threshold above which the oocytes were counted for the total fecundity estimate.

Area	Length of fish (cm)	Range Total Fecundity ($*10^{-3}$ eggs)	Eggs per g female in stage 4	50% vacuolation threshold (µm)
English Channel and North Sea. Macer, 1974.				
Volumetric method	25 - 38	168 - 860	1492*	118
Celtic Sea. Nazarov, 1977.				
Volumetric method	25 - 41	54 - 833	818**	175
Celtic Sea (this study)				
Volumetric method	29 - 42	156 - 950	1478	96
Histometric method	29 - 42	164 - 1272	1655	101

* Derived from Macer's (1974) conversion of total egg production to biomass: fish with a mean weight of 231 g and a mean length of 29.5 cm have a fecundity of 344700 eggs, which is equal to 1492 eggs per gram female.

** Nazarov's figure of 818 eggs per gram female is based on gutted females.

The estimated vitellogenic threshold of 96 µm for the volumetric method is relatively close to the threshold of 118 µm, as estimated from Macer's data (1974), while the high threshold of 175 µm as used by Nazarov (1977) is probably too high, because this threshold probably represents a size above which all oocytes have vacuoles and is not the threshold above which 50% of the oocytes are vacuolated. This results in a relatively low fecundity. Macer's (1974) estimate of about 1492 eggs per gram is similar to the estimate 1478 eggs per gram obtained using the volumetric method and is only slightly lower than the histometric estimate (1655 eggs per gram).

The intercepts of the fecundity-weight relationships of both the volumetric and histometric methods (Figure 3) did not differ significantly from 0. Therefore, the simple use of 1478 or 1655 eggs per gram female in maturity stage 4 is justified for the conversion of total egg production to biomass. This biomass for maturity stage 4 should then be raised to spawning biomass by a factor of 1.05.

The result from the volumetric and histometric methods showed no significant differences and for the following reasongs the histometric method is preferred. The advantages of the histometric method are: (i) The poisonous Gilson's fluid eliminated (ii) No digestion needed and therefore less time consuming (iii) Identification of the post-ovulatory-follicles (iv) Estimation of atresia in each ovary (v) Cytological identification of the onset of vitellogenesis (vi) Ability to count eggs at different maturity stages (throughout their development). The disadvantages of the histometric method are: (i) accurate volume measurements of ovary essential (ii) maintenance of constant density during processing of the tissue.

Clearly, there are still some problems before an accurate fecundity estimate can be given with complete confidence. The main problems still are: (i) Is horse mackerel a determinate spawner or not? (ii) How to correct the total fecundity estimate for atresia.

(iii) How to estimate an accurate size of the lower threshold, above which all oocytes should be counted for the total potential fecundity.

Investigations should be carried out in future to estimate the spawning stock biomass by applying the batch fecundity and the fraction of females spawning to the daily egg production at peak spawning time, this biomass estimate should then be compared to the estimate of the spawning stock biomass obtained using the traditional method in which the total egg production is converted to biomass by the total fecundity. Tank experiments might also be very useful for calculating differences between potential and realized fecundity. Investigations of this nature would help to calculate levels of atresia and establish whether or not the horse mackerel is a determinate spawner.

Acknowledgements

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TABLE 1. The length and weight of 29 Western Horse Mackerel sampled in 1987 and 1988 together with the volumetric and histometric fecundity estimate. The confidence intervals of 2 * SE are given for the histometric method. The fecundity estimates are given in thousands.

		Volumetric	Histometric Fecundity		
Length	Weight	Fecundity		+2*SE	-2*SE
28.9	168	231	256	361	151
28.9	179	207	164	212	114
30.1	192	282	400	522	277
30.9	233	330	383	478	288
31.4	217	412	519	585	453
31.5	213	156	227	276	178
32.7	277	513	448	638	258
32.8	279	344	284	376	192
33.2	274	508	444	570	319
33.5	320	382	527	634	421
33.9	300	409	454	517	391
34.4	273	502	534	696	372
35.1	268	396	452	547	356
35.1	212	485	600	798	402
35.4	320	568	348	433	263
36.0	318	432	384	457	311
36.6	343	717	663	854	473
37.0	378	950	540	748	332
37.2	428	623	957	1160	754
37.8	404	672	747	933	561
38.0	432	530	633	759	506
38.3	431	539	527	631	424
38.7	398	582	612	762	464
39.6	421	666	776	965	588
40.2	481	652	845	1054	636
40.5	494	624	796	966	625
41.4	564	610	1272	1612	932
41.6	540	708	947	1254	639
42.3	585	661	714	929	500

TABLE 2. Results of the analysis of variance of Log(Fecundity) on Log(Length) or Log(Weight) and Method (volumetric or histometric).

	SS	df	MS	F	
Log(Length)	6.92	1	6.92	96.65	**
Method	0.086	1	0.086	1.20	n.s.
Log(Length)*Meth.	0.020	1	0.020	0.28	n.s.
Error	3.864	54	0.0716	-	
Total	10.89	57	-	-	
Log(Weight)	6.18	1	6.18	86.31	**
Method	0.086	1	0.086	1.20	n.s.
Log(Weight)*Meth.	0.020	1	0.020	0.28	n.s.
Error	4.605	54	0.0716	-	
Total	10.89	57	-	-	

** $p < 0.01$

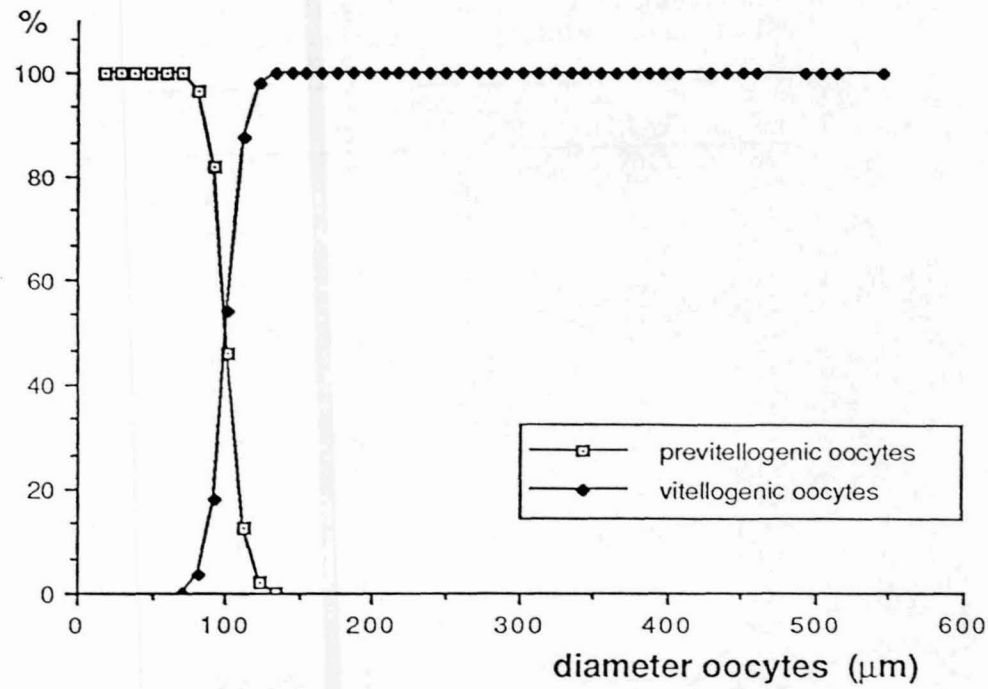

FIGURE 1. The percentages of previtellogenic and vitellogenic oocytes for the different oocyte diameters from 14 ovary samples of Western Horse Mackerel by the histometric method.

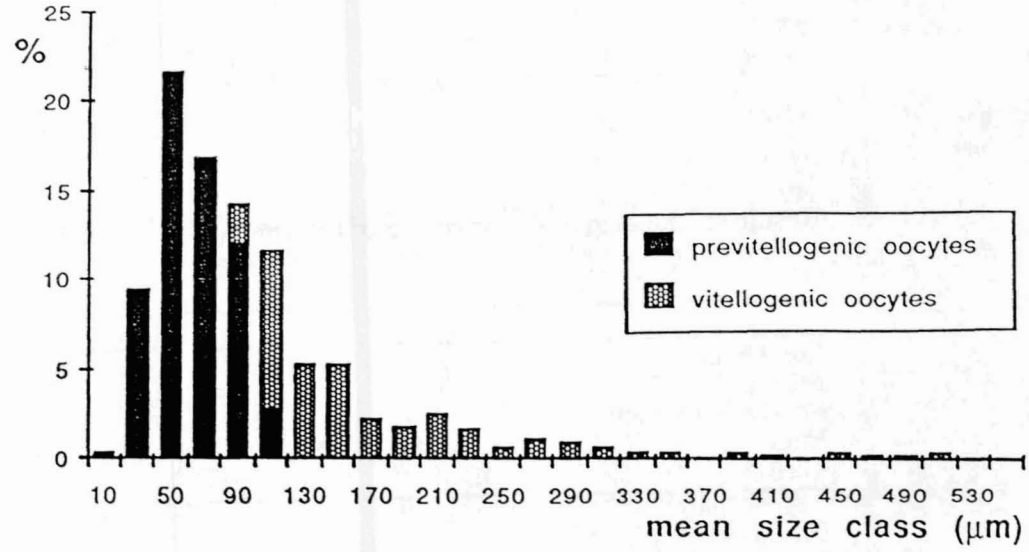

FIGURE 2. The percentage frequency distribution of previtellogenic and vitellogenic oocytes of 14 Western Horse Mackerel ovaries in maturity stage 4.

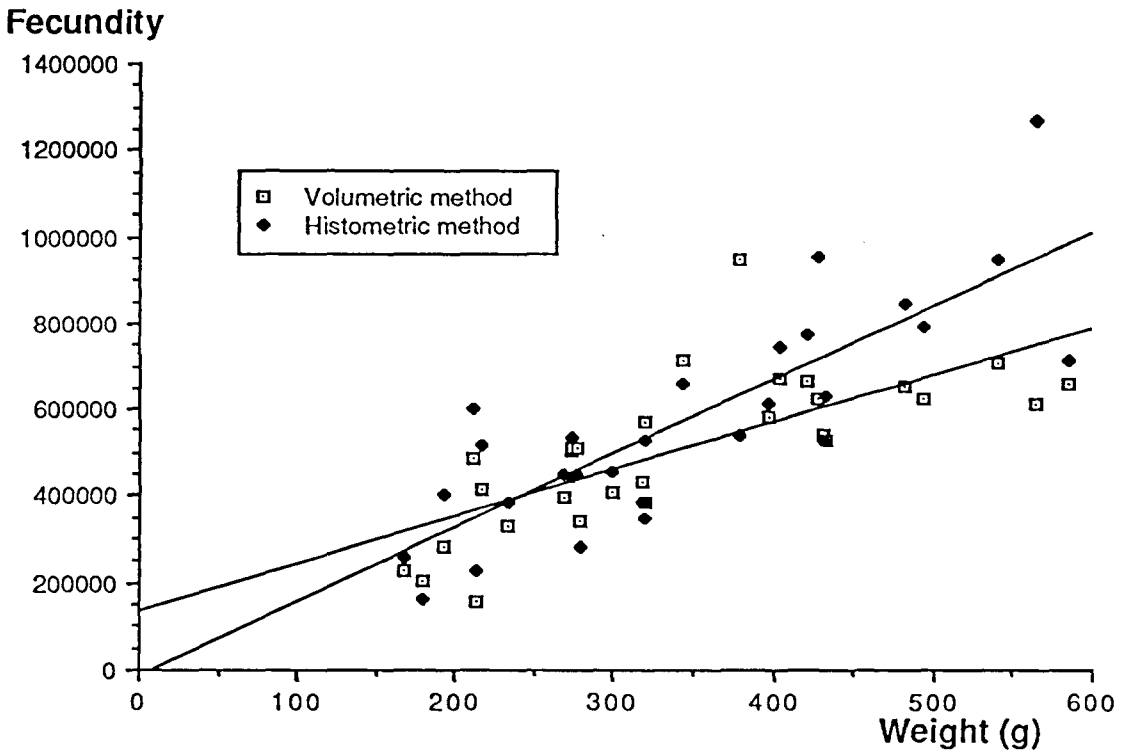

FIGURE 3. The relationship between weight (g) and fecundity of Western Horse Mackerel in maturity stage 4 as estimated by the volumetric and histometric method.