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Spawning Process of the Yellowtail Flounder (Limanda ferruginea)

by

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SUMMARY.

The spawning of Limanda ferruginea (Pleuronectidae) is analysed from 1892 mature females of 35 different samples.

Batch fecundity is calculated using the "hydrated oocytes" method obtaining a value of 200.000±20.000 eggs

Hystological anaysis of 103 ovaries showed the maturation process being continuous. Once a batch of eggs is spawned the next one is ending the nuclear migration.

Based on the percentages of maturity stages found through the hours of the day and assuming hydratation process lasting the same that in other species (12 h), a daily spawning frequency is proposed for yellowtail flounder.

INTRODUCTION

The yellowtail flounder (Limanda ferruginea) has its distribution area through the Atlantic coast of North America, from the Chesapeake Bay (U.S.A) to the Strait of Belle Isle (Labrador). Its highest abundance is found between 20 and 40 fathoms (Leim and Scott 1966).

The spawning season varies with the latitude, being earlier in the southern part of its distribution area. In Grand Banks it seems to be in May-July (Pitt 1970).

Fecundity in this species has being studied in relation with the size, age and ovary weight (Pitt 1971, Howell and Kesler 1977).

The total amount of eggs to be spawned in one season keeps determined before the spawning process starts, and they are spawned in batches (Howell 1983).

Several groups of oocytes begun their maturation at the same time. This is distinguished by the nuclear migration to the animal pole, followed by the hydratation and ovulation. The eggs remain for a while in the ovary lumen before its expulsion.

The subject of this paper is to analyse the spawning process in yellowtail flounder following criteria already developed in other species such as Engraulis mordax (Hunter and Goldberg, 1980), Engraulis ringens (Santander et al., 1984), Sprattus sprattus (Alheit, 1988), Sardina plichardus (Perez y Cal, 1988), Katsuwonus pelamis (Hunter et al., 1986), Scomber scombrus (Alheit et al., 1987), Merluccius gayi (Alheit, 1986) and Seriphus politus (De Martini and Fountain, 1981).

MATERIAL AND METHODS

The samples analysed have being collected on board of a commercial freezing trawler, in the Southern part of the Grand Bank of Newfoundland (division 3N of NAFO). Each trawl lasted four hours and so a reference time for the samples needed to be established, choosing that of two hours before the turned.

1892 mature females (37-53cm of length) from 35 different samples (Table I) have been analysed. For every individual, total lenght was taken and a maturity stage assigned according to table II.

A sample of 103 ovaries was preserved in 4% buffered formaline (Hunter, 1985) for hystological analysis. In those individuals, total length and weight without ovary have being recorded. The weight of the preserved ovaries was taken later in the laboratory. Afterwards two pieces of ovary coming from the extreme parts were embedded in Paraplast, sectioned at 6 ym, and stained using Harris'hematoxylin and Eosine-Floxine B.

The "hydrated oocyte" method (Hunter, 1985) was used in order to calculate the batch fecundity (number of oocites spawned each time). In those ovaries were recent post-ovulatory folicles (type A) have not been observed, three subsamples of ovarian tissue of between 400 to 500 mg were removed from the anterior central and posterior positions of each ovary. They were placed on microscope slides in a drop of glycerine and the number of hydrted oocytes in each subsample was determined. Hydrated oocytes are easily separated from the others oocytes by their much larger size and their translucent appearance. The batch fecundity was calculated based on the average of the three subsamples and relative fecundity dividing batch fecundity by ovary-free female weight.

HYSTOLOGICAL CLASSIFICATION

To estimate the reproductive estate of the different macroscopic stages of maturity the most developed oocytes and the degree of degeneration of the postovulatoy follicles are identifyed.

Hunter y Goldberg (1980) developed criteria of ageing postovulatory follicles in Engraulis ringens. Further observations in other species indicate that this is a process occuring in most of the teleosts with small variations in the form and duration of each step. Following the criteria refered to above it has been made a classification of the postovulatory follicles into three degrees of reabsorption.

The recognized structures have been the following:

- Yolked Oocytes. They are in the last phase of vitellogenesis. The cytoplasm is completely full of yolk globules and the nucleus is central (Fig. 1)

- Oocytes Ending the Nuclear Migration. Nucleus is irregularly shaped and located in the animal pole and yolk globules have begun to fuse forming yolk plates (Fig. 2)

- Hydrated Oocytes. The nucleus is not visible. In early stages the yolk globules are fused into plates. At conclusion of this stage, interior of the oocyte shows a pinkish homogeneus aspect (Fig. 3).

Among the ovaries in stage IV, 22 showed no postovulatory follicles and the hydratation was high enough as to be used to calculate the batch fecundity. The mean value obtained was 200.000±20.000 eggs by spawn.

- Postovulatory Follicles A. they are follicles with no signs of follicle degeneration. The lumen is spacious. the granulosa epithelial cell layer of the follicle appears as an irregularly looped cord of cuboidal cells with prominent healthy nuclei linearly arranged. the granulosa appears only loosely attached to the thecal connective tissue layer.(Fig. 4)

- Postovulatory Follicles B. The granulosa is folded and the lumen is narrow, the nucleus are linearly arranged and there is not pycnotics.(Fig. 5)

- Postovulatory Follicles C. They showed pronounced signs of degeneration, the follicle is much smaller. The granulosa cells dont have the nucleus linearly arranged, some of them are piycnotics. The granulosa is attached to the thecal conective tissue layer. The lumen is much reduced or absent. (Fig. 6)

RESULTS.

Table III shows the frecuence of microscopics structures at diferent stages of maturity. They correspond with the following reproductive stages:

Stage III. Ovaries with yolked oocytes but without starting maturation. There is not postovulatory follicles. Spawning do not begin.

Stage IV. It is distinquised by the presence of maturing oocytes. Postovulatory follicles are present in those where previous spawnings have occurred. They are ovaries maturing the next batch.

Stage V. Ovaries with completely hydrated oocytes and presenting eggs in the lumen. In the most advanced steps, all the hidrated oocytes have been ovulated and the next batch is ending the nuclear migration. They are ovaries in the ovulation or ready for the next spawning.

Stage VI. All of them showing postovulatory follicles in advanced stage of reabsorption. There are not yolked oocytes. Only in one ovary was observed an small group of oocytes in nuclear migration. There are not atretic oocytes. Those ovaries have finished the spawning.

Table I shows the results obtained. In the earlier samples the 50% of fish was in spawning (stage IV and V) and the 11.7% have not started it.

In fig. 7 is represented the evolution of the percentage of spawning fish through the sampling period.

In order to know whether it exists a trend in the proportion of individuals in stage V whithin those witch started the spawning, the results have been grouped according to the sampling hour (from 6 to 11h, from 14 to 15 and from 19 to 23). Comparing the percentage of individuals in stage V with the overall mean through a Chi cuadrado test, resulted no significant differences at 99% level of confidence. The overall mean obtained was that 44.5% of spawning fish were in the stage V of maturity.

The relative fecundity was 300±30 eggs by female gr.

With the presented data it is not found any significant relationship between batch fecundity and size, weight (ovary free), and number of yolked oocytes within the ovary.

DISCUSSION

The oocytes of yellowtail flounder have a development described as "group synchronous" (Wallace and Selman 1981), all of those to be spawned in the next spawning season are clearly distinguished of the others being also at the same level of vitellogenesis. Most of the oocytes reach the egg phase and they are espeled, the incidence of oocyte atresia (degeneration of oocytes) were very small.

The limanda is a serial spawner, the ovaries during the spawning, they present one group of oocytes full of yolk and another one in process of maturation, the later ones will be spawned in the next batch. Maturation is continuous so when ovulation of one batch finish the next one is ending the nuclear migration.

The number of oocytes maturing at the same time (batch fecundity) have been estimated in 200.000120.000, so a female of 42cm, with a total fecundity of 1.456.000 eggs (Pitt 1971) woul spawwn 7±1 times during the spawning season.

In the sample analysed the proportion of individuals in spawning with eggs in the lumen or in ovulation was 44.55%. The values obtained at diferent hours of the day did not show any significant difference. This result seems to support the lack of synchronism in the spawning hour.

Two methods are currently used to calculate the frecuency of spawning in fish populations. One is the"hydrated oocytes" method (De Martini and fountain 1981) and other is the "postovulatory follicles" (Hunter and Goldberg 1980). The former is based on the percentage of females with hydrated oocytes and requires the synchronism in the process to be used; the later based on the percentage of females showing postovulatory follicles requires to now their age. So it was not possible to use any of them in this case.

Fulton (1898) described the process of hidratation within the fish ovaries with pelagic eggs. The lasting of it has been studied in several species of fishes: Oryzias latipes (Yamamoto 1964), Seriphus politus (De Martini and Fountain 1981), Engraulis mordax (Hunter and Golberg, 1980), Engraulis ringens (Santander et al., 1984), Sardina pilchardus (Perez y Cal, 1988). Resulting to be about 12 hours in all of them.

If it is valid also in yellowtail flounder, and taking into account that the percentages of individuals in degree V kept constant in all the samples examined, it would be expected the degree V to last 12 hours, and the frecuence of spawning to be daily.

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TABLE I

Maturity stages found through the sampling period

	DATE	N	HOUR	%II	%III	%IV	%V	%VI
1	21,VI	34	19,30	2,9	11,7	35,3	14,7	35,3
2	22,VI	40	9,30	5	12,5	37,5	10	35
3	23,VI	52	23,30	5,7	3,8	38,5	25	26,9
4	24,VI	45	14,30	8,9	17,8	35,5	24,4	13,3
5	25,VI	54	10	3,7	25,9	37	12,9	20,4
6	27,VI	82	7	4,9	13,4	23,2	12,2	46,3
7	27,VI	68	10,30	1,5	17,6	22,1	25	47,1
8	27,VI	50	15,30	8	18	16	30	28
9	28,VI	90	10,30	3,3	18,9	17,8	20	40
10	20,VI	34	10	2,9	8,8	23,5	23,5	41,2
11	30,VI	58	10,30	5,2	3,5	29,3	25,8	36,2
12	30,VI	67	20,30	11,9	7,5	26,9	28,4	25,4
13	1,VII	78	10	5,1	17,9	15,4	14,1	7,4
14	2,VII	60	10,45	0	16,7	25	15	38,3
15	3,VII	54	10	3,7	9,3	25,9	14,8	46,3
16	3,VII	66	20,30	7,6	4,5	16,7	18,2	53
17	4,VII	41	10,30	4,9	14,6	17,1	17,1	46,3
18	7,VII	50	20,30	4	6	18	12	60
19	7,VII	45	10	11,1	4,4	22,2	15,6	46,7
20	8,VII	61	10	9,2	3,3	8,2	6,6	73,8
21	9,VII	69	8	0	2,9	2,9	10,1	84
22	9,VII	57	10,30	5,3	5,3	8,8	14	66,7
23	12,VII	32	15,30	6,3	3,1	25	12,5	53,1
24	13,VII	39	6,30	20,5	0	17,9	10,3	51,3
25	14,VII	69	10	6,8	1,2	7,3	2,9	82,6
26	15,VII	38	10	13,2	0	15,8	2,6	68,4
27	16,VII	62	10,30	11,3	0	1,6	11,3	75,8
28	19,VII	42	9,30	7,1	0	16,7	11,9	64,3
29	20,VII	38	10,30	2,6	2,6	7,9	7,9	78,9
30	22,VII	42	10,30	9,5	0	2,4	4,8	83,3
31	23,VII	47	21,30	2,1	0	12,8	6,4	78,7
32	23,VII	52	10,30	9,6	0	5,8	9,6	75
33	27,VII	72	20	30,3	0	0	4,5	65,2
34	28,VII	72	14	40,2	0	0	0	59,7
35	29,VII	32	7,30	46,8	0	0	0	53,1

TABLE II

Macroscopic characteristics of the different maturity stages in Limanda ferruginea. Stages modified from Howell (1983)

I. Ovaries small (2-6cm), cónical,pinkish,and generally translúcent, No oocytes visible to the naked eye

II. Ovaries relatively small (6-12cm), reddish, and translucent. Ovarian wall thick

III. Ovaries larger in size (> 12cm) and occupying most of ovarian cavity. Visibles oocytes large, yellowish in color, and opaque. Ovarian wall thin, translucent.

IV. Ovaries very large. Some oocytes yellowish and opaque, others transparent (hydrated)

V. Ovaries very large. Some oocytes yellowish and opaque, others transparent (hydrated). Ova run from vent upon slight pressure

VI. Ovaries flaccid, bloodshot.

TABLE III.

Microscopic characteristics of the diferent maturity stages in Limanda ferruginea.

MAT	N	YO%	NM%	HO%	F.A%	F.B%	F.C%
III	20	100	0	0	0	0	0
IV	42	88	26	78	31	74	86
V	16	94	31	100	81	87	100
VI	25	0	4	0	4	4	100

MAT = Maturity stages

N = Number of ovaries

YO = Yolked oocytes

NM = Oocytes ending the nuclear migration

HO = Hydrated oocytes

F.A = Postovulatory follicles A

F.B = Postovulatory follicles B

F.C = Postovulatory follicles C

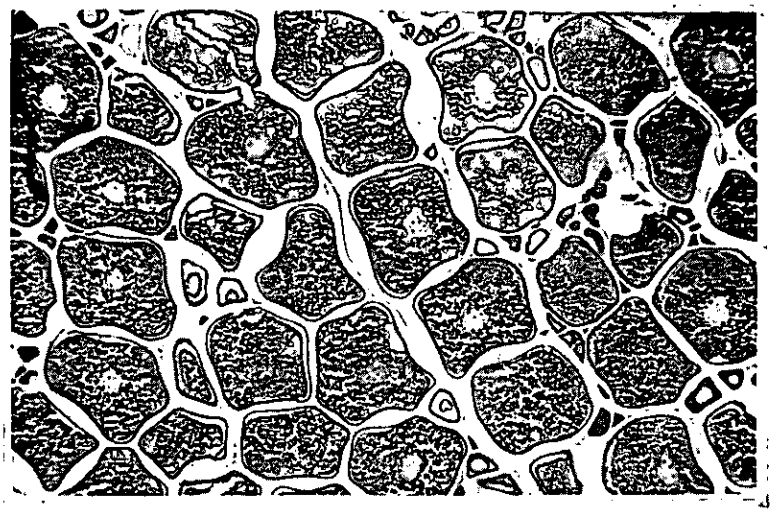

Fig. 1. Yolke. oocytes

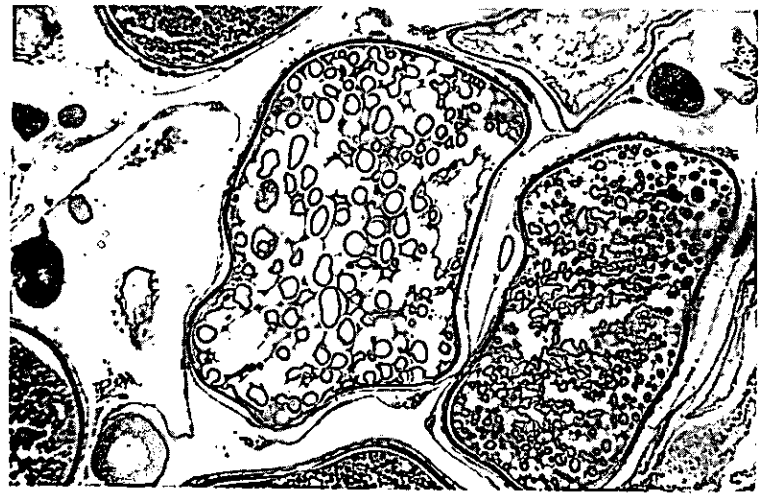

Fig. 2. Oocyte ending nuclear migration

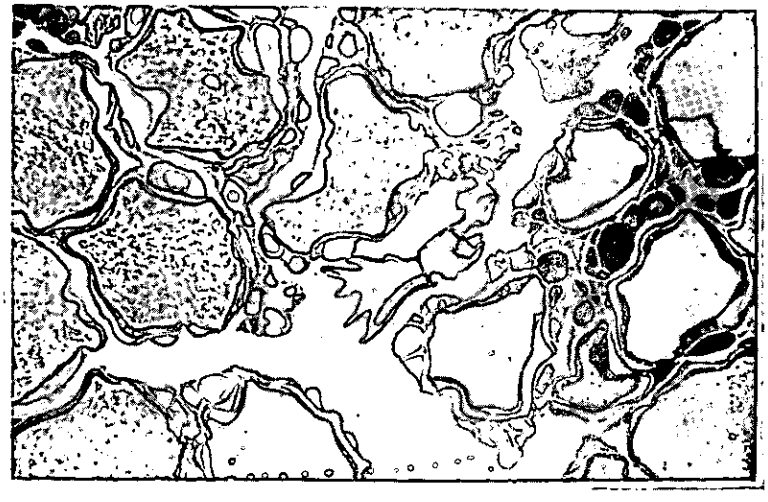

Fig. 3 Hydrated oocytes

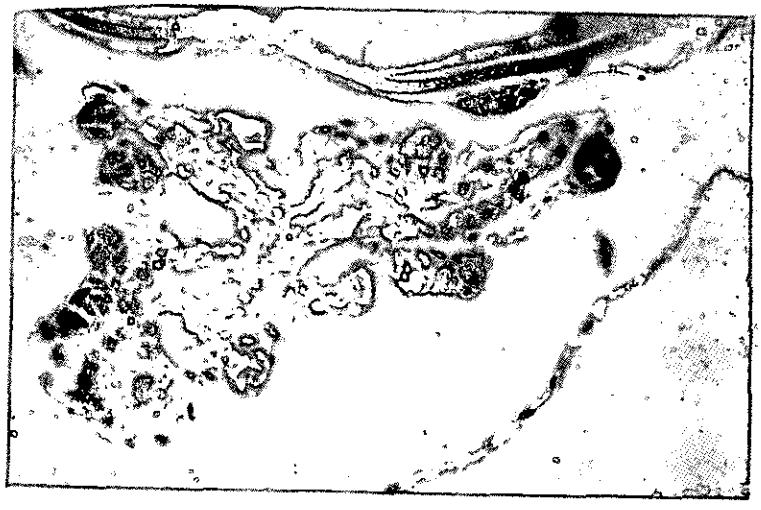

Fig. 4A Postovulatory follicle A

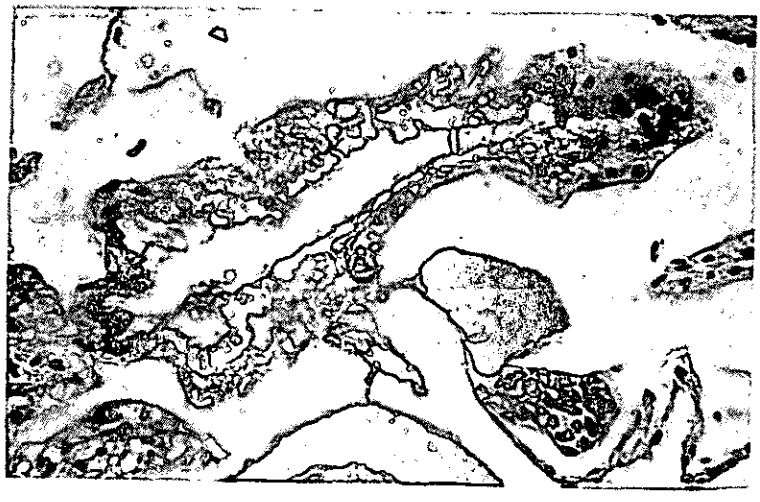

Fig. 4B Postovulatory follicle A

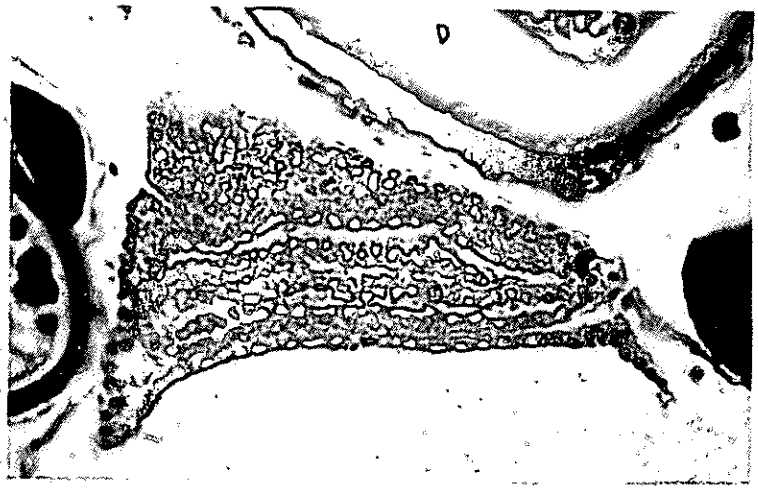

Fig. 5 Postovulatory follicle B

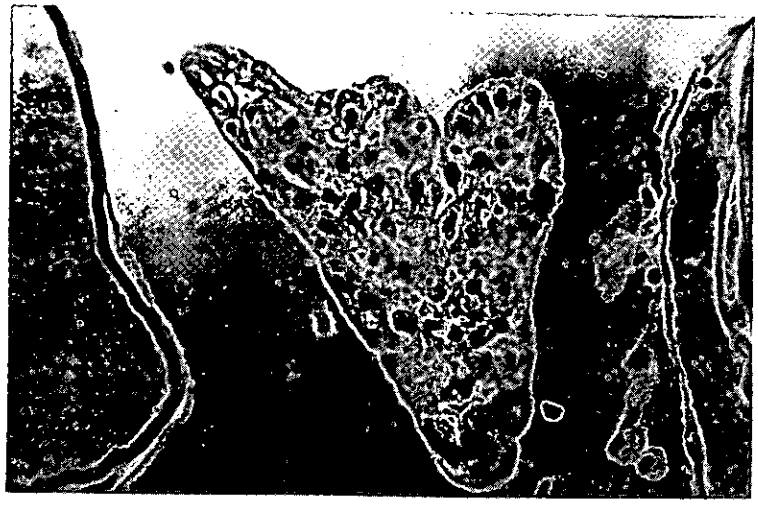

Fig. 6A Postovulatory follicle C

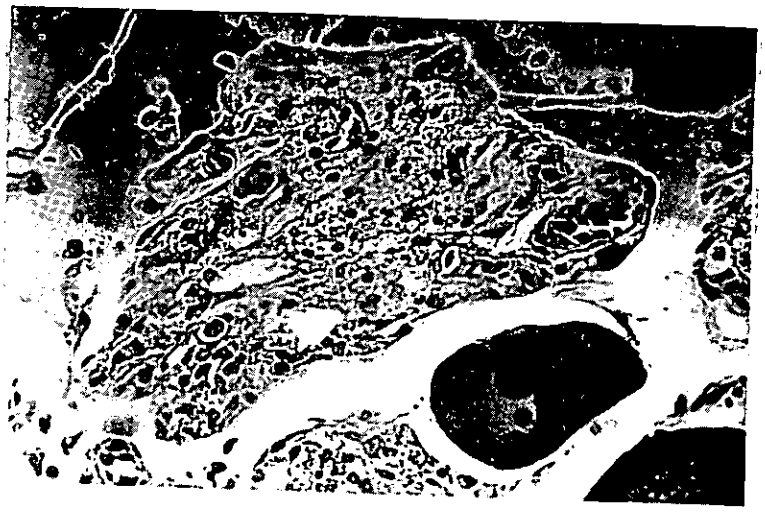

Fig. 6B Postovulatory follicle C

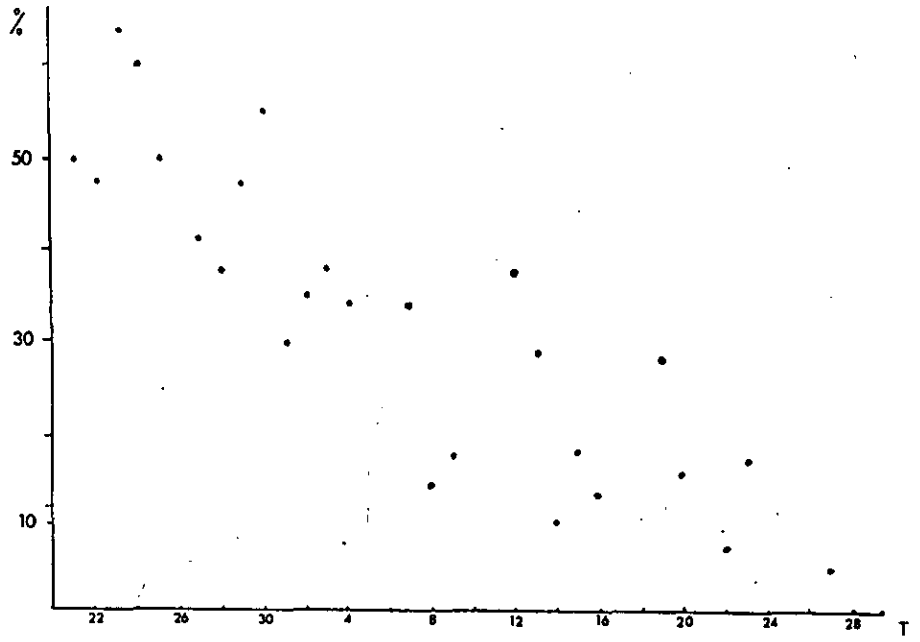

Fig. 7 Percentage of females in spawning (stages IV and V) during the samping period