

Temperature and reproduction in tench : Effect of a rise in the annual temperature regime on gonadotropin level, gametogenesis and spawning. II. The female

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Summary. Female tench were bred for two consecutive years under different thermo-periods ; the effect of the thermoperiods on vitellogenesis, spawning, plasma and pituitary gonadotropin (GTH) levels was studied. The temperature did not affect development during the preparatory period. We found no ovarian development below 10 °C. The temperature accelerated vitellogenesis only if the mean daily average was more than 10 °C, and the rate of increase of that parameter from 10 to 23 °C (spawning temperature) determined female fertility. The date of first spawning may be predicted by the summation of degree-days higher than 10 °C. GTH secretion increased during vitellogenesis to reach maximal values during spawning. The data indicate a gonadotropin surge at the beginning of each spawning. There was no difference between plasma GTH levels at the various temperatures, but pituitary GTH was higher in the groups bred under the highest temperatures.

Introduction.

In the northern hemisphere, cyprinid fish undergo ovarian development at high temperature, and an increase of temperature accelerates ovarian recrudescence (review of De Vlaming, 1972a, 1974 ; Peter and Hontela, 1978). Depending on the species, vitellogenesis may occur just after the spawning period, if the water temperature remains high (carp : Bieniarz *et al.*, 1978). In other species, there is a period of sexual quiescence between the summer and the following spring (lake chub, *Couescius plumbeus* : Ashan, 1966 ; tench, *Tinca tinca*). Recent studies suggest that temperature effect may be mediated by the pituitary which shows an increased response to hypothalamic hypophysiotropic hormones (LH, FSH/RH) as the temperature rises (Weil *et al.*, 1975). This hypothesis has been partly confirmed by studies of the effects of temperature on gonadotropin secretion (Gillet *et al.*, 1977, 1978).

In the present investigation, we have tried to determine the effects of temperature on ovarian development, pituitary gonadotropin content and plasma gonadotropin secretion in an intermittent spawner, the tench, bred over a 2-year period in natural ponds under three different thermoperiods. We wished to ascertain the temperatures necessary to trigger the different phases of the reproductive cycle : sexual quiescence, prespawning and spawning.

Material and methods.

These experiments were carried out simultaneously with those described for the male (Breton *et al.*, 1980) during a period extending from December 1973 to October 1975. Most of the experimental conditions and parameters have already been described during a study of temperature effects on male reproductive endocrinology (Breton *et al.*, 1980).

Three-year old tench females were bred in natural ponds (300 m² × 1.3 m deep). The water temperature either fluctuated naturally, or was obtained by mixing natural

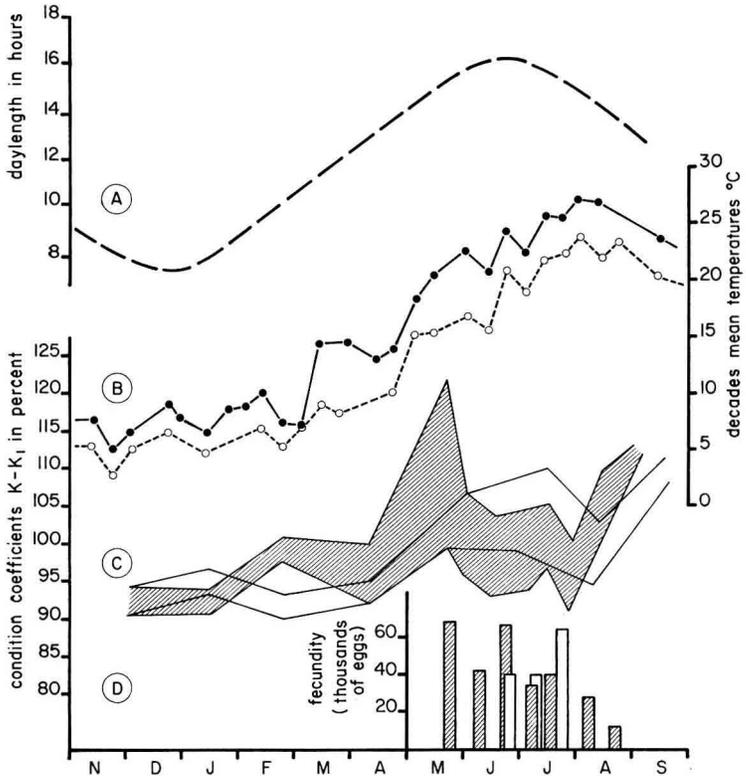


FIG. 1. — A : Photoperiod profile ; B : Temperature profile ; C : Pattern of condition coefficients $K-K_1$. Shaded area represents variations of K (upper line) and K_1 (lower line) in fish kept in heated water (group III-74) ; the open area represents variations in the same parameters in fish kept under natural temperature ; D : Fertility pattern.

and heated water, maintaining the temperatures at mean variations above the natural. The fish were supplied by a local fish farm.

Two series of experiments were carried out ; all manipulations were done in the morning.

Experiment A (December 1973 to October 1974). — The three thermoperiods included one natural and two experimental one (groups I, II and III-74) with mean temperature variations of 3 and 6 °C above the natural thermoperiod. Figure 1 shows the temperature profile under the three regimes. When the experiment began, each pond contained about 200 male and female tench. Seven females were killed monthly from December to April, and more frequently from May to October, depending on the spawning period. We determined the stage of ovarian development and measured the levels of pituitary and plasma gonadotropins in those 7 fish.

Experiment B (November 1974 to September 1975). — We used only two temperature regimes (fig. 2) corresponding, respectively, to the natural regime and to one having a

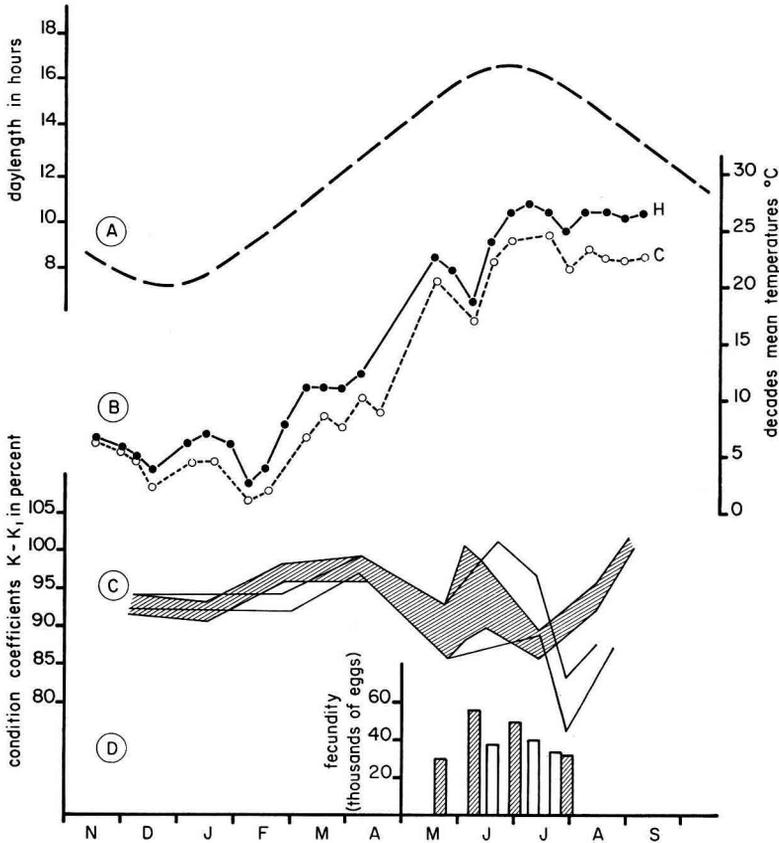


FIG. 2. — A : Photoperiod profile ; B : Temperature profile ; C : Pattern of condition coefficients $K - K_1$. Shaded area represents the variations of K (upper line) and K_1 (lower line) in fish kept in heated water (group III-75) ; open area represents variations in the same parameters in fish kept under natural temperature ; D : Fertility patterns in ponds I and III-75.

mean variation of 6 °C. Each group (groups I and III-75) had 220 to 260 male and female tench. Six females were killed monthly, or more frequently, to study the same parameters as in Experiment A. In addition, 10 females were tagged for repeated blood sampling and ovarian puncture, using the method of Bieniarz and Epler (1976).

Determination of stages of ovarian development. We used two methods :

1. The histological analysis of Sakun and Buckaja (1968). Eight stages of gametogenesis were defined (plate I) :

— Stage A. Complete vitellogenesis : oocyte nucleus central or shifted to the periphery ;

— Stage A'. Vitellogenesis in progress : enlarged oocyte with a central nucleus surrounded by a yolk mass ; oocyte periphery filled with vacuoles ;

— Stage a. Previtellogenesis : oocyte nucleus central ; all spaces between the nucleus and the zona radiata filled with large vacuoles ;

— Stages b and b'. Previtellogenesis : oocyte nucleus central and surrounded by cytoplasm ; space between the cytoplasm and the zona radiata filled with vacuoles of all sizes ;

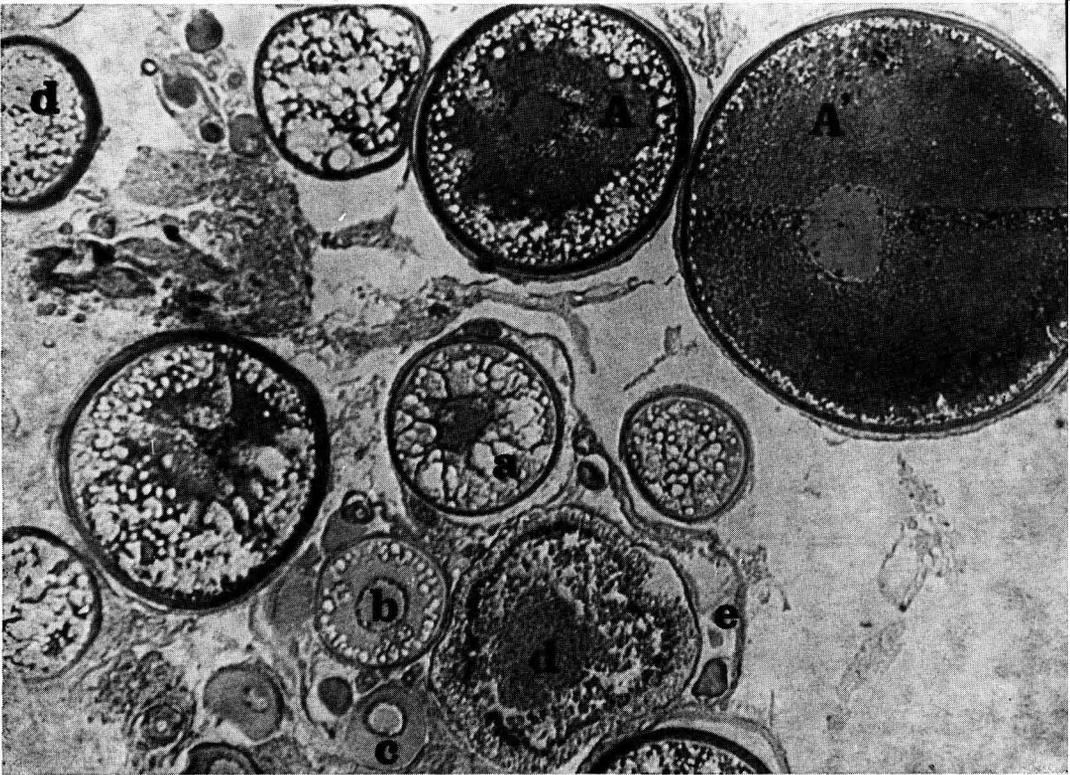


PLATE I. — Tench ovary showing the different types of oocytes. Staining with Azocarmin G, Mallory's solution. $\times 20$. A : complete vitellogenesis, A' : vitellogenesis in progress, a, b, b' : previtellogenesis, c : oogonia, d : resorbing oocyte, e : follicle after ovulation.

- Stage c. Small oocyte has a perinuclear space containing vacuole-free cytoplasm ;
- Stage d. Oocyte resorbing ;
- Stage e. Post-ovulatory follicle empty.

Every ovarian sample was histologically analyzed to determine the frequency of the different stages. This analysis provided a quantitative index for estimating gonadal development.

2. Ovarian development was also evaluated by measuring the different egg diameter classes in correlation to the gonadosomatic index (GSI).

Female fertility was determined after each spawning. Spawning was detected either by direct observation of egg-laying in the ponds, or more precisely by histological studies.

A criterion of gonadal mass development was also obtained after the coefficients of condition were determined (Le Crenn, 1951) either in reference to intact fish :

$$K = \frac{\text{weight of intact fish in grams} \times 100}{(\text{standard length})^3}$$

or in relation to the gonads :

$$K_1 = \frac{\text{weight of gonads in grams} \times 100}{(\text{standard length})^3}$$

K-K₁ gave an estimation of gonadal mass development as correlated to the animals' condition. That value was represented by the area between the profiles of those two parameters.

Determination of endocrinological parameters. Plasma and pituitary gonadotropin (GTH) levels were measured by radioimmunoassay (RIA) according to the technique described by Breton *et al.* (1971). The plasmas were frozen and stored at — 20 °C, and the pituitaries were acetone-dried until assay.

The t-test and the analysis of variance were used for statistical analysis.

Results.

All results on RIA specificity of tench GTH, temperature profiles and spawning times were the same as described in experiments on the male (Breton *et al.*, 1980).

First spawning depended strictly on water temperature and never occurred below 20-22 °C. Temperature rise thus accelerated the onset of the first spawning and increased the number of spawnings. Seven spawning periods were detected in pond III-74 under the highest thermoperiod as against 3 under the natural thermoperiod (P < 0.001) in the same year.

Ovarian cycle.

The ovarian cycle was divided into three periods :

- *preparatory* : there was no variation in mean oocyte diameter or gonadal mass, whatever the number of degree-days given the animals (table 1). This period corres-

ponded to the time when the water temperature was under 10 °C in all the temperature regimes ;

TABLE 1

Changes in egg diameter in correlation with sum of degrees-days received from the beginning of December to the end of February

Group	Degrees-days	Mean temperature	Oocyte diameter	K-K ₁
I-75	290.6	3.4	0.25 < 0.41 < 0.52	1.81
III-75	432.8	5.2	0.30 < 0.44 < 0.57	1.97
I-74	518	6.1	0.22 < 0.43 < 0.95	2.72
II-74	628.8	7.4	0.29 < 0.42 < 0.62	2.21
III-74	802.8	8.4	0.40 < 0.54 < 0.83	3.89

— *pre-spawning* : this period, corresponding to gonadal development, began when the water temperature rose above 10 °C. The temperature fluctuations determined female fertility, this is, the number of eggs laid during first spawning. Ovarian growth depended on the rate of temperature increase when the temperature was markedly above 10-11 °C. When that increase was gradual (III-74, fig. 1), gonadal development paralleled an increment in the coefficient of condition and maximal fertility was obtained at first spawning (68 000 eggs). Histological study of the ovaries after first spawning revealed the three classes of oocytes constituting the products of the following spawnings (fig. 3). When the temperature rise was rapid (III-75), the gonads developed rapidly also, but the coefficient of condition decreased and fertility was lower at first spawning (30 000 eggs) ;

TABLE 2

Σ degrees-days > 10 °C until first spawning

Pond No.	Σ temperature ($\theta > 10$ °C)	Number of efficient days ($\theta > 10$ °C)
I-74	1 103.0	73
II-74	1 161.5	84
III-74	1 047.5	70
I-75	1 053.5	63
III-75	1 022.5	70

— *spawning* : spawning only occurred if the mean daily temperature was 20 °C with maxima higher than 22 °C. In those conditions, eggs with a minimum diameter of 1 mm were ovulated and laid (fig. 3). The temperature of 10 °C seemed to be the main factor determining the date of the first spawning. The number of degree-days received

by each fish, when the temperature was over 10 °C, was constant ($1\ 077 \pm 24$) in the five groups, of all the years, from the onset of pre-spawning to first reproduction.

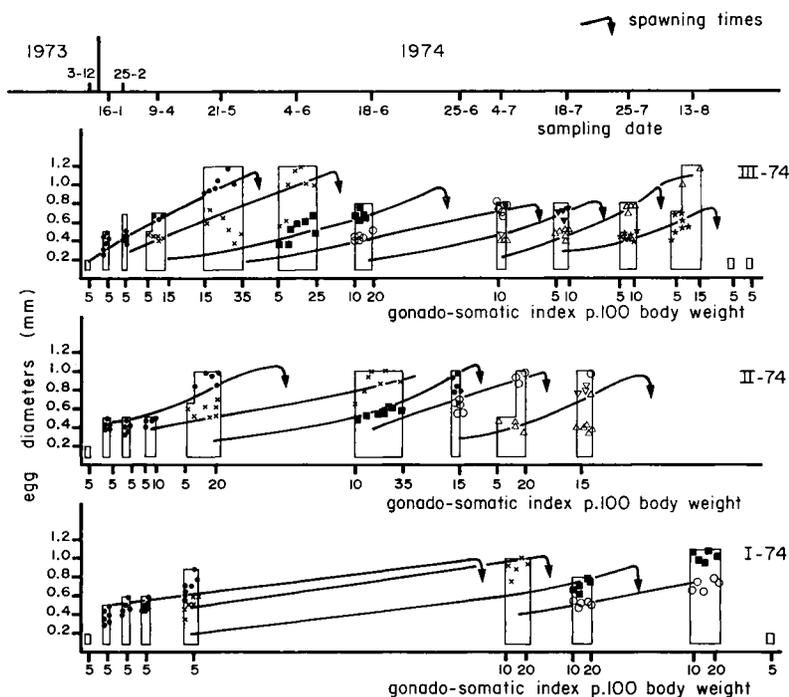


FIG. 3. — Pattern of egg diameter classes in relation to the gonado-somatic index in the 1974 ponds. The individual values for each sampling date were obtained by classifying egg diameter classes in relation to the corresponding gonado-somatic index.

Histological studies confirmed these observations. Table 3 shows that until February 25, 1974, all the fish were in previtellogenesis. At the onset of pre-spawning

TABLE 3

Changes in different types of oocytes in the ovaries of fishes from groups I and III 1974a.

The values are given as a quantitative index corresponding to the number and area of each category. bf_1 = before first spawning

Date	A'	A	a	b	c	d	e	GSI	\emptyset A'
14.01 ...	0	0	3.5	2.7	4.4	0.3	0	3	
25.02 ...	0	0	4.5	1.2	3.7	0.7	0	3.3	
8.04 ...	0	4	4	1	2	1	0	7.0	Group I
	0	0	4.7	1.5	3.5	0.5	0	4.1	
20.05 ...	3.1	2.3	2.7	1.3	2.4	1.7	0	8.3 bf_1	597.8
14.01 ...	0	0	3.4	2.6	4.3	0	0	3.3	
25.02 ...	0	0	4.6	1	2.8	1	0	2.8	
8.04 ...	3.2	3	3.5	1	2	2	0	12.0 bf_1	689.2
	0	4	4	1.5	3	3	0	5.9	Group III
20.05 ...	4.7	2.2	2.2	1.7	2.2	3.3	0.8	27.6	726.1

in April, differences appeared between groups I and III-74. All fish in the latter group were in vitellogenesis, while in group I, some were still in previtellogenesis. The gonadosomatic index of fish raised under the highest temperatures was significantly more elevated ($P < 0.001$) than in the other experimental lots. Comparative results were obtained in 1975, but the differences were less significant ($P < 0.05$) (table 4). There was no statistical difference between the GSI of groups I and III-75 before first spawning. The profile of gonadal mass development (K-K₁) confirms this (fig. 2).

TABLE 4

Changes in different types of oocytes in the ovaries of fishes from groups I and III-1975 (killed fishes)

Dates	A'	A	a	b	c	d	e	∅ A'	GSI	Plasma GTH ng/ml	Pituitary GTH µg/mg	
3.12	0	0	2.8	2.5	3.8	1	0		2.2	0.179	0.96	
13.01	0	0	2.8	3	4	1	0		2.2	1.894	2.267	
24.02	0	0	2.8	2.9	3.8	1	0		2.2	2.957	3.658	Group I
7.04	0	0	3	2.7	3.3	1	0		2.8	2.497	2.982	
19.05	3.3	3	3	0.3	2.7	1.7	0	0.630	10.5	4.609	3.504 bf ₁	
	0	3.7	3.3	2.3	2.3	0.7	0		6.4	5.056	6.265 af ₁	
2.12	0	0	2.8	2.5	3.7	1	0		2.4	0.283	2.142	
13.01	0	0	2.8	3.2	4	1	0		2.8	2.301	3.608	
24.02	0	0	3.3	3.5	4	1	0		2.4	3.633	2.131	Group III
7.04	0	0	3.5	3.5	4	1	0		3.1	1.615	1.991	
19.05	3.4	2.6	2.2	1.4	2	1.6	0	0.616	10.2	5.449	4.375 bf ₁	

Repeated sampling of the blood and ovaries of the same fish revealed the patterns of gonadal development and of GTH during natural spawning (table 5). During spawning, several classes of egg diameters were always found in the ovary (fig. 3). Those eggs began growth at regular intervals during pre-spawning and spawning, the temperature accelerating vitellogenesis.

TABLE 5

Changes in different types of oocytes in continuously sampled fishes in pond III-75 during spawning time

Spawning time	A'	A	a	b	c	d	e	Egg diameters			GSI	Plasma GTH ng/ml	Pituitary GTH µg/mg
								A'	A	a			
Before	2.8	2	2.7	1.6	3.6	3.4	0.7	0.572	0.417	0.297	2	3.918	6.786
During	4.7	1.1	1.3	1.4	2.8	2.5	1.9	0.642	0.425	0.312	15.9	4.646	
After	0.3	1.3	2.3	1.9	3.8	3.3	1.8	0.592	0.425	0.302	1.9	3.871	9.583
Before	4	3	4	0	2	0	2	0.654	0.378	0.308			
Just before	5	1.3	1.5	0.8	3.3	3.5	1.3	0.665	0.430			12.457	
Just after . .	1	3	4	0	3	3	4	0.525	0.402				

The ovaries of fish which were repeatedly sampled also showed a significantly higher number or resorbing oocytes, probably due to injury during biopsy.

Plasma gonadotropin levels.

Experiment A-1974 (fig. 4). There was a significant increment in the plasma GTH ($P < 0.05$) level in February, which decreased in March and then rose again but was not significantly different during vitellogenesis. The GTH level rose again at the onset of spawning ($P < 0.05$) and remained higher during that whole period ; it showed fluctuations, possibly due to successive spawnings. There was no significant temperature effect on those levels.

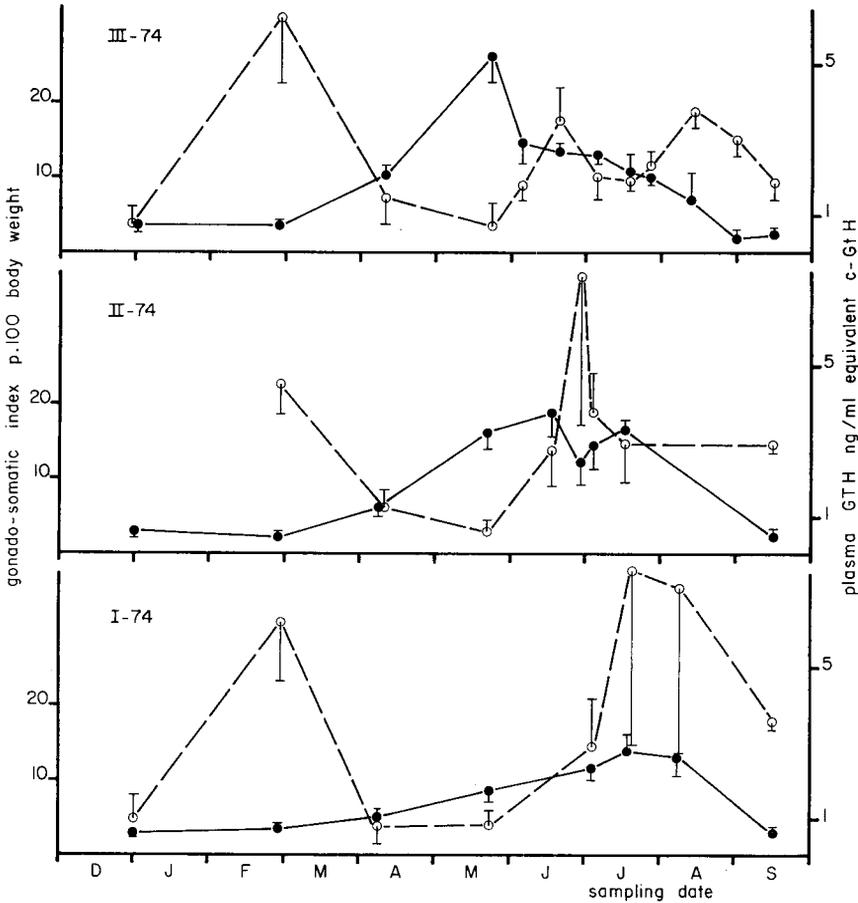


FIG. 4. — Pattern of plasma gonadotropin secretion (dashed line) and the gonado-somatic index (solid line) in 1974 fish

Experiment B-1975 (fig. 5). As compared to 1974, there was no increase of plasma GTH in February, but the levels were always higher during vitellogenesis in the killed and the repeatedly sampled fish. The fluctuations during spawning could not be attributed to ovulatory-type GTH surges in the killed fish.

On the contrary, the histological data showed that the rise in plasma GTH (up to 10 ng/ml) occurred just before ovulation (table 5).

Although the mean levels of plasma GTH did not vary with the mean temperature, rapid fluctuations in temperature could affect the GTH secretion, as on July 7, 1974 (group III-74 ; fig. 5).

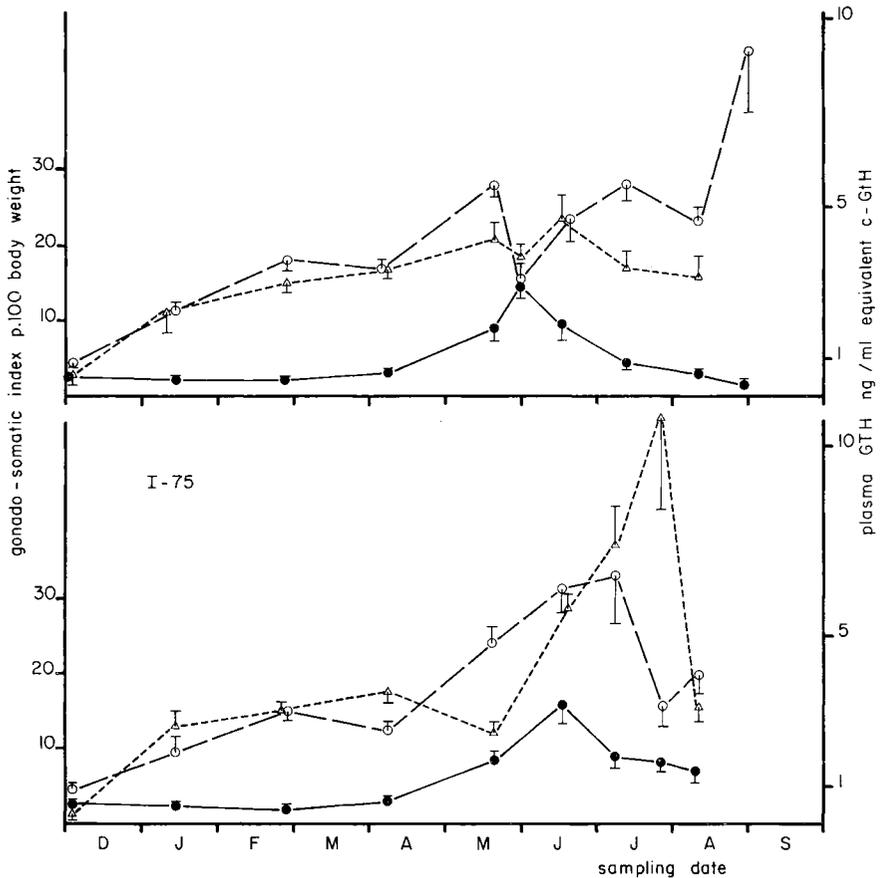


FIG. 5. — Pattern of plasma gonadotropin in killed fish (dashed line) and continuously sampled fish (dotted line) and the gonado-somatic index in 1975 (solid line)

Pituitary gonadotropin levels.

Experiment A-1974. The results were similar to those obtained in the males (Breton *et al.*, 1980). Pituitary GTH levels remained low up to April 8 ; there was no significant difference between the three groups (fig. 6). From May 6 on, they increased, reaching maximal values during reproduction. The pituitary contents were significantly higher in the group kept under the highest temperature. As the plasma GTH, the pituitary GTH levels dropped on July 7.

Experiment B-1975. The results were the same as in 1974, although mean GTH levels were lower.

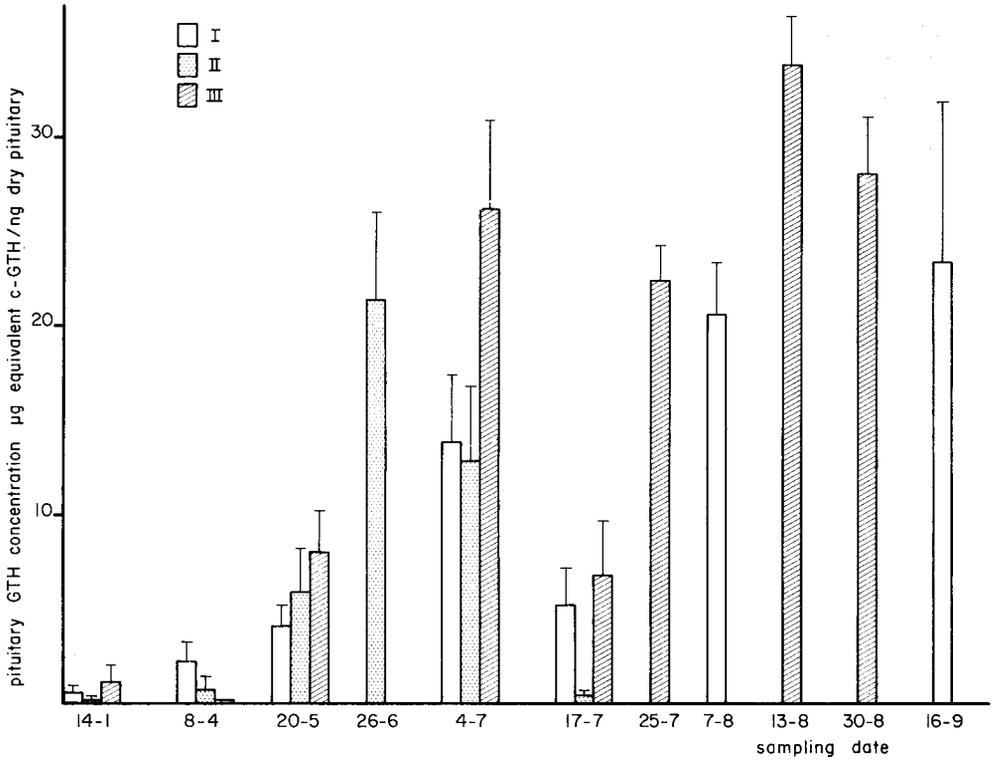


FIG. 6. — Variations in pituitary gonadotropin contents in 1974.

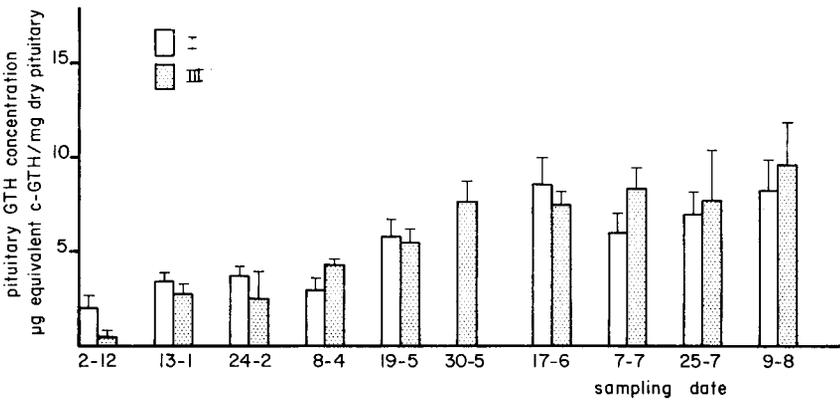


FIG. 7. — Variations in pituitary gonadotropin contents in 1975.

Discussion.

Marza (1938) classed the tench as a fish having asynchronous ovarian development. Our histological results and analysis of the distribution of various oocyte classes, according to their GSI, confirm that classification. This method may be compared to that of Yamamoto and Yamazaki (1961) studying the goldfish, *Carassius auratus*.

As in other species (*Heteropneustes fossilis*, Sundararaj and Vasal, 1976), the reproductive cycle of the female tench can be divided into preparatory, pre-spawning and spawning periods during which temperature effects act differently, but usually stimulate gametogenesis when the annual temperature pattern has a thermoperiod. Eight stages of gametogenesis, comparable to those described in other cyprinids, such as the goldfish (Yamazaki, 1965) or the carp (Gupta, 1975), have been determined. In contrast to the carp (Bieniarz *et al.*, 1978), which shows new vitellogenesis after spawning if the temperature remains sufficiently high (23 °C) (Gupta, 1975), the tench has a period of ovarian quiescence between the last reproduction and the following spring. Spawning lasts all summer and only stops when the water temperature drops below 20 °C. The minimum time needed for the post-ovulatory and preparatory phases has not yet been determined, and its knowledge would permit earlier overall increased fertility. During the preparatory period, the temperature does not affect previtellogenesis. Its effect appears at the onset of prespawning by stimulating vitellogenesis, only if the number of degree-days is higher than 10 °C. Similar observations were reported in *Gyllichthys mirabilis* (de Vlaming, 1972b,c) in which ovarian development occurs between 10 and 20 °C, regardless of the photoperiod. Thus, temperatures around 10 °C improve the coefficients of condition, and the ovaries develop slowly, while higher temperatures enhance the rate of ovarian growth. The final stages of vitellogenesis depend more on high temperatures, as in *Fundulus confluentus* (Harrington, 1959) and the gudgeon (Mackay, 1974). Moreover, the rate of temperature increase seems to influence fertility in tench, especially during first spawning, which is enhanced when the temperature rises slowly.

Aside from this factor, it seems possible to predict the date of first spawning and thus to program it. Egg laying could be determined using a criterion based on a degree-days constant ($1\,077 \pm 24$), integrating all the temperatures higher than a daily mean of 10 °C. The effects of higher temperatures, applied early in the year, need to be studied, if the date of first spawning is to be advanced. This would necessitate defining the length of the post-ovulatory and preparatory periods needed.

As in the tench in 1974, the trout (*Salmo trutta*) shows a GTH peak (Billard *et al.*, 1978). However, in that fish it seems to coincide with the initiation of previtellogenesis; no data indicate such a case in the tench. The 1975 experiment showed no stimulation of GTH secretion. The circadian rhythms of GTH secretion, demonstrated in cyprinids (Gillet *et al.*, 1978; Peter and Hontela, 1978), do not explain the diverse results of the two experiments in which blood samples were always taken during the same dark/light period. On the other hand, short-term pulsations of GTH secretion, as in the rainbow trout (Zohar, personal communication), cannot be eliminated as an explanation of this difference. In all the groups, mean levels of GTH increased during vitellogenesis. The highest levels occurred just before first spawning and fluctuated during

the whole period. A typical ovulatory surge of GTH, analogous to that in the goldfish (Breton *et al.*, 1972 ; Stacey *et al.*, 1979), has not been found in the tench, but rapid, wide variations of pituitary GTH indicate that it should also exist in that fish.

Some authors have shown the existence of interactions between the temperature and the photoperiod, stimulating gonadal development as in the goldfish (Kawamura and Otsuka, 1950) and the golden shiner, *Notemigonus chrysoleucas* (De Vlaming, 1975). The interactions might also stimulate GTH secretion in the goldfish (Gillet *et al.*, 1978 ; Peter and Hontela, 1978). Our experiments do not indicate that these mechanisms are involved in the reproductive cycle of the tench.

Acknowledgements. — This work was carried out under an exchange program between the I.N.R.A. (France) and the Inland Institute of Fisheries (Poland). It was partly supported by E.D.F. grants.

Reçu en avril 1979.

Accepté en février 1980.

Résumé. Des tanches femelles ont été élevées pendant deux années consécutives sous différentes thermopériodes. La dynamique de la vitellogenèse, la sécrétion gonadotrope plasmatique, et la concentration hypophysaire en gonadotropine ont été suivies. La température n'a aucun effet pendant la période préparatoire. Un seuil thermique évalué à 10 °C a été déterminé pour l'entrée en vitellogenèse qui est accélérée par la température. Seules les températures situées au-dessus de ce seuil induisent la vitellogenèse. La vitesse de croissance de la température de 10 à 23 °C (température du déclenchement de la fraie) influe sur la fertilité des femelles. Un indice de prévision de date de première fraie a été déterminé par les degrés-jours reçus par les animaux, uniquement lorsque la température dépasse 10 °C. L'étude de la sécrétion gonadotrope plasmatique montre que la sécrétion de GTH croît au cours de la vitellogenèse pour être maximum en période de fraie. Une décharge de GTH peut être suspectée pour le déclenchement de chaque ponte. Il n'existe pas de différence entre les niveaux de GTH plasmatique sous les différentes thermopériodes. Par contre la teneur hypophysaire en GTH est plus élevée à haute température.

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