Maturational gonadotropin hormone (GtH) and gonadotropin releasing hormone (GnRH) changes during growth and sexual maturation of female carp (Cyprinus carpio L.)

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MATURATIONAL GONADOTROPIN HORMONE (GtH) AND GONADOTROPIN RELEASING HORMONE (GnRH) CHANGES DURING GROWTH AND SEXUAL MATURATION OF FEMALE CARP (*CYPRINUS CARPIO* L.)

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ABSTRACT

Fish originating from one couple of spawners were investigated with respect to GtH in the blood and pituitary, GnRH in the hypothalamus and pituitary (RIA), gonadosomatic index (GSI) and histology of gonads. GSI was showing great variability between specimens. The circulatory GtH levels did not fluctuate much troughout the investigated period except at the beginning of vacuolisation of the oocytes when peaks of GtH were observed. GtH in the pituitary started to accumulate from 13 months and GnRH from 33 months. GnRH contents in the hypothalami of the young females before 35 months were also very low, thereafter they increased and showed great variation.

Key words: *Cyprinus carpio*, hormones, maturation

1. INTRODUCTION

The profile of hormones during the life of carp has been reported so far only for steroids (Godovich et al. 1984, Galas, Bieniarz 1989, Galas et al., unpubl.).

Reports on the fluctuation in gonadotropin are limited to the sexual cycle (Billard, Breton 1978, Billard et al., 1978, Weil 1981, Yaron, Levavi-Zermonsky 1986) or during particular events such as ovulation (Santos et al. 1986a).

In carp, only one paper deals with GnRH changes but results are preliminary (The Peptide Hormone Group, 1977). More results are available for other cyprinids goldfish – Yu et al. (1987), roach – Breton et al. (1988) but they described only a very short time of the life of animals (pre-spawning periods).

It was the reason why it was decided to measure GtH levels in the blood and pituitary as well as GnRH in the pituitary and hypothalamus of common carp from 5 months after hatching to the first sexual maturity.

2. MATERIALS AND METHODS

In this experiment about 500 females carps were used, which originated from 1 pair of spawners. Spawning took place on 1 June 1981.

Experiment lasted from June 1981 till August 1985. Fish were kept in an earthen pond. Each day at 10:00 water temperature was recorded 20 cm over bottom.

Sampling was performed on the following days: 29 November 1981, 26 March 1982, 28 June 1982, 20 October 1982, 16 December 1982, 24 March 1983 and later monthly till August 1985, on 10–20 females.

Blood samples were taken from fish 5 h after sunrise and after killing, gonads (from 20 October 1982), hypothalami (from 19 June 1984) as well as pituitaries (from 29 October 1981) were collected.

Blood serum was preserved with merthiolate and kept at –20°C as well as the hypothalami and pituitaries until the measurement of GtH by RIA (according to B r e t o n et al. 1971) and GnRH (according to B r e t o n et al. 1986).

Pituitaries and hypothalami have been homogenized in acetic acid 0.01 N for GnRH measurement. An aliquot was incubated (volume 1/1) with phosphate buffer (pH 8) in order to have a pH of 7 and reassociate the GtH subunits. A fragment ($1cm^3$) of each gonad was taken for conventional (staining with eosin and hematoxylin) histological procedure.

Histological preparations were used for defining maturity stage, taking into consideration the number and size of all types of oocytes in the ovary and quantitative relationship between them.

The following maturity stages were distinguished:

stage 1 – ovary composed of about 100% of oocytes before vacuolisation;

stage 2 – ovary composed of oocytes before vacuolisation (70—95%) and oocytes at the onset of vacuolisation (5–30 %);

stage 3 – ovary which contains 5–90% of oocytes fully vacuolised;

stage 4 – ovary which contains besides oocytes before vacuolisation, at the beginnig of vacuolisation and at full vacuolisation, and also 10–85% oocytes at the onset of vitellogenesis;

stage 5 – ovary in which dominate oocytes with completed vitellogenesis.

The obtained data on GtH levels in the blood and pituitaries as well as on GnRH levels in the hypothalami and pituitaries during the different periods of female life were analysed statistically using correlation method and analysis of variance with the Duncan's test.

3. RESULTS

From hatching until October 82 gonads could not be visually identified. Then during the 3 first years of life, all the females had the gonads at the same stage of maturity (stage 1). Stage 2 was observed for the first time in females over 3 years old. Stage 3 appeared in 39.5-month-old females, stage 4 in 40.5-month-old fish, while gonads in stage 5 were observed only in nearly 4-year-old females. As shown in Table I, an ovarian development was not synchronous in all the females since different stages of gonadal maturity occurred in the fish of the same age.

In general, GSI increased during female growth and sexual maturation but always the period of an increase there was followed by a period of a slight decrease; however, differences were not statistically significant (Fig. 1).

In general, females more advanced in age displayed higher GSI but only females over 36 months had GSI higher ($P<0.01$) than females 27 months old and younger. GSI of females aged 28 to 35 months did not differ significantly from GSI of females below 27 and over 36 months.

Females at more advanced stages of maturity displayed higher GSI (Table I) but only females at stage maturity 5 had GSI significantly higher ($P<0.01$) than that of

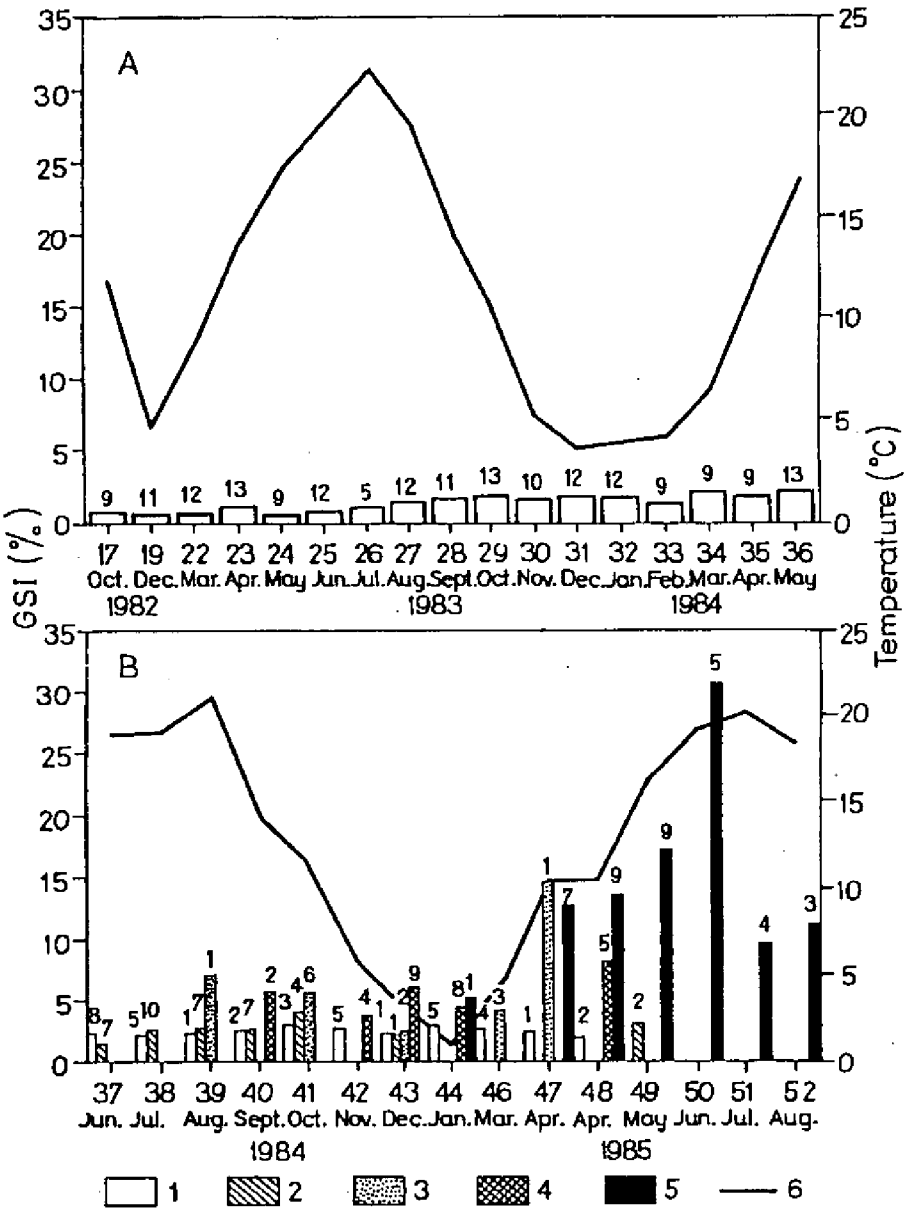

Fig. 1. GSI changes during growth and sexual maturation at 17 to 36-month (A) and 37 to 52-month-old (B) female carps. Values are mean. 1, 2, 3, 4, 5 – maturity stages, 6 – temperature; 17–52 – age in months; numbers over bars – no of specimens

females at stages of maturity 1, 2, 3 and 4. GSI values were not statistically significant in the females at maturity stages 1–4.

No correlations between the value of GSI and water temperature neither between the value of GSI and the stage of maturity were found. A significant correlation, ($P<0.01$) between GSI and age was found only for females at maturity stage 1.

GtH in the blood was at levels 9.79–33.25 ng · cm^{-3} in all females, irrespective of age and stage of maturity (Fig. 2). Only in 38 and 39-month-old females at stages of maturity 1 or 2 very high values of GtH in the blood were observed. These values were 168.76 and 138.83 ng · cm^{-3} for females at stage 1 and 214.51 and 161.41 ng · cm^{-3} for females at stage 2. Only these values differed significantly from all others.

The lowest values of the pituitary GtH content (2.64–50.47 ng · $pituitary^{-1}$) were observed in the youngest (5–13-months-old) females at maturity stage 1 (Fig. 3).

Table I. Stages of gonad maturity and GSI of females of the same age during the sampling period

Date	Age (months)	Stage of gonad maturity and GSI (mean, SE, *n*)
29 October 81	5	gonads no differenciated
28 June 82	13.5	gonads no differenciated
20 October 82	17	1(0.63, 0.26, 9)
17 May 84	36.5	1(1.98, 0.10, 13)
19 June 84	37	1(2.34, 0.23, 8); 2(1.39, 0.17, 7)
24 July 84	38	1(2.11,0.33, 5); 2(2.59, 0.37, 10)
15 August 84	39	1(2.22,–, 1); 2(2.65,0.23,7); 3(3.51,–, 1)
20 September 84	40	1(2.44,0.54,2); 2(2.57,0.16,7); 4(5.67, 1.13,2)
23 October 84	42	1(3.02, 1.72,3); 2(3.98, 0.44, 4); 3(5.65, 1.38, 6)
21 November 84	43	1(2.67, 0.59, 5); 4(3.86, 0.86, 4)
18 December 84	44	1(2.33, –, 1); 2(2.22, , 1); 3(2.36, 0.02, 2); 4(6.12, 1.09, 9)
24 January 85	45	1(2.92, 0.47, 5); 4(4.48, 1.02, 8); 5(5.02, 0.0, 1)
25 March 85	46	1(2.67, 0.66, 4); 3(4.10, 1.00, 3)
02 April 85	47	1(2.37, –, 1); 3(14.56, –, 1); 5(12.69, 2.51, 7)
23April 85	48	1(1.86, 1.70, 2); 4(8.16, 0.52, 5); 5(13.45, 1.14, 9)
21 May 85	49	2(3.13, 0.10, 2); 5(18.31, 4.69, 9)
18 June 85	50	5(29.68, 7.81, 5)
05 August 85	52	5(11.13, 0.45, 3)

The mean value for females at stage 1 was 212.79 ng · pituitary^{-1}, the highest values were observed in the oldest (45–52-month-old) females at stage 5 (mean value was 617.35 ng · pituitary^{-1}); the differences were highly significant ($P<0.01$). For females at remaining stages of maturity the mean values varied from 391.85 to 422.30 and differences between these means were not statistically significant.

Generally GtH in the pituitary increased with the age of the fish but a significant correlation ($P<0.01$) between age and GtH content in the pituitary was found only for fish at maturity stage 1.

No correlation was found between GtH content in the pituitry and water temperature.

The GnRH conten in the pituitary was low (below 1 ng · pituitary^{-1}) in females at maturity stage 1 till 34 months of age (Fig. 4), then it increased rapidly ($P<0.01$) reaching 3.55 ng · pituitary^{-1} at 35 months. From 35 months to 44 months (December 1984), GnRH levels showed a variation with a significant ($P<0.01$) decrease at 40 and 42 months. After December 1984, GnRH levels decreased rapidly ($P<0.01$) to the values below 1 ng · pituitary^{-1}.

Differences in GnRH contents per pituitary between females at various stages of maturity were not statistically significant. No correlations between GnRH contents in the pituitary and age neither between GnRH contents in the pituitary and temperature were found.

GnRH content in the hypothalamus in females at maturity stages 1, 2, 3, and 4 (Fig. 5) varied from 0.06 to 2.11 hypothalamus^{-1}. However, these variations were related neither to the age of the animals nor to the stage of maturity (Fig. 5).

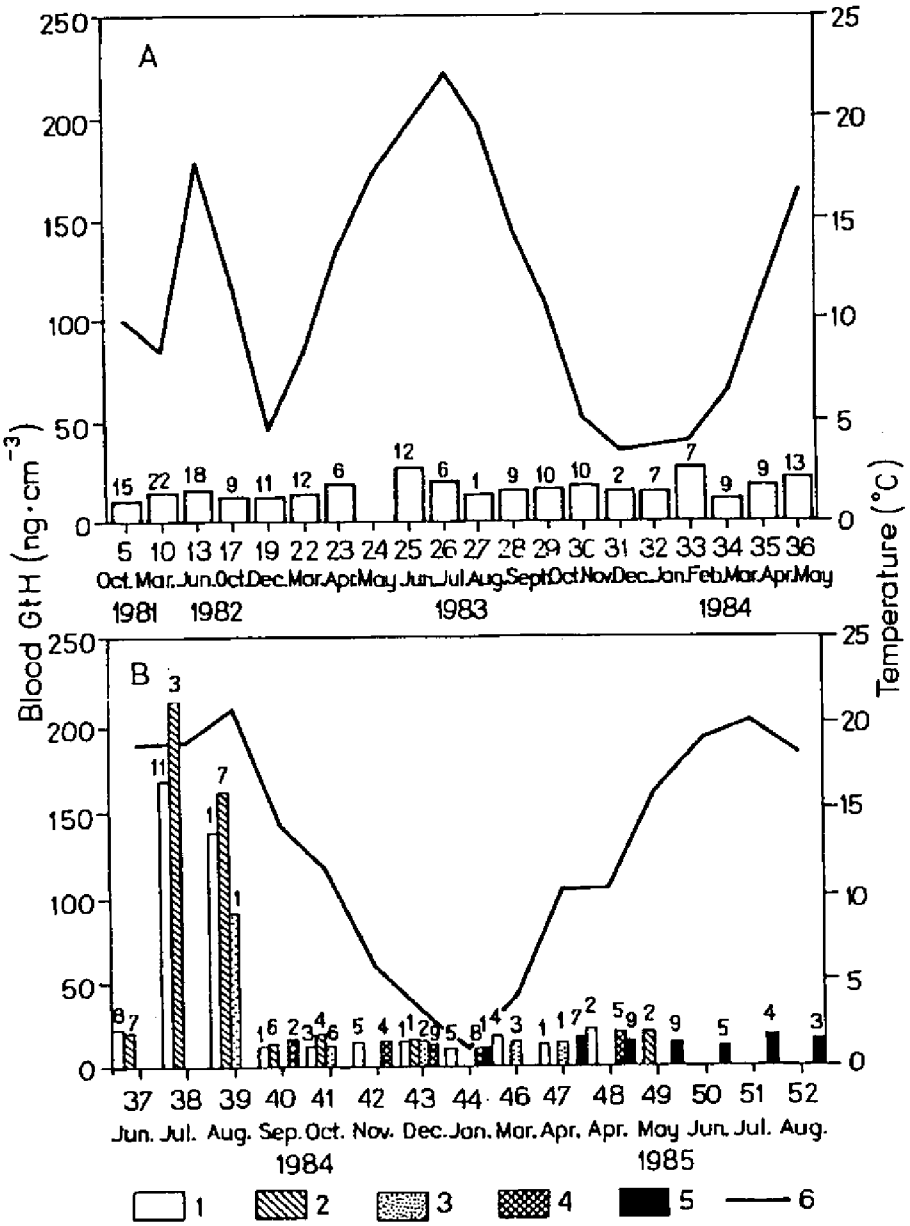

Fig. 2. Changes in blood GtH during growth and sexual maturation in 5 to 36-month (A) and 37 to 52-month-old (B) female carps. Values are mean. For explanation see Fig. 1

The 47 month-old-females at stage 5 had high GnRH content (4.12 ng · hypothalamus^{-1}); it was higher (P<0.01) than other females at the same stage of maturity. Furthermore females at stage 5 had significantly higher (P<0.01) mean GnRH content in the hypothalamus (1.5 ng · hypothalamus^{-1}) than females at other stages of maturity.

No correlation between GnRH content in the hypothalamus and age, neither between GnRH contents in the hypothalamus and temperature were found.

No statistically significant correlations between investigated traits (GSI, stage of gonads maturity, GtH in the blood and pituitary, GnRH in the pituitary and hypothalamus) were found.

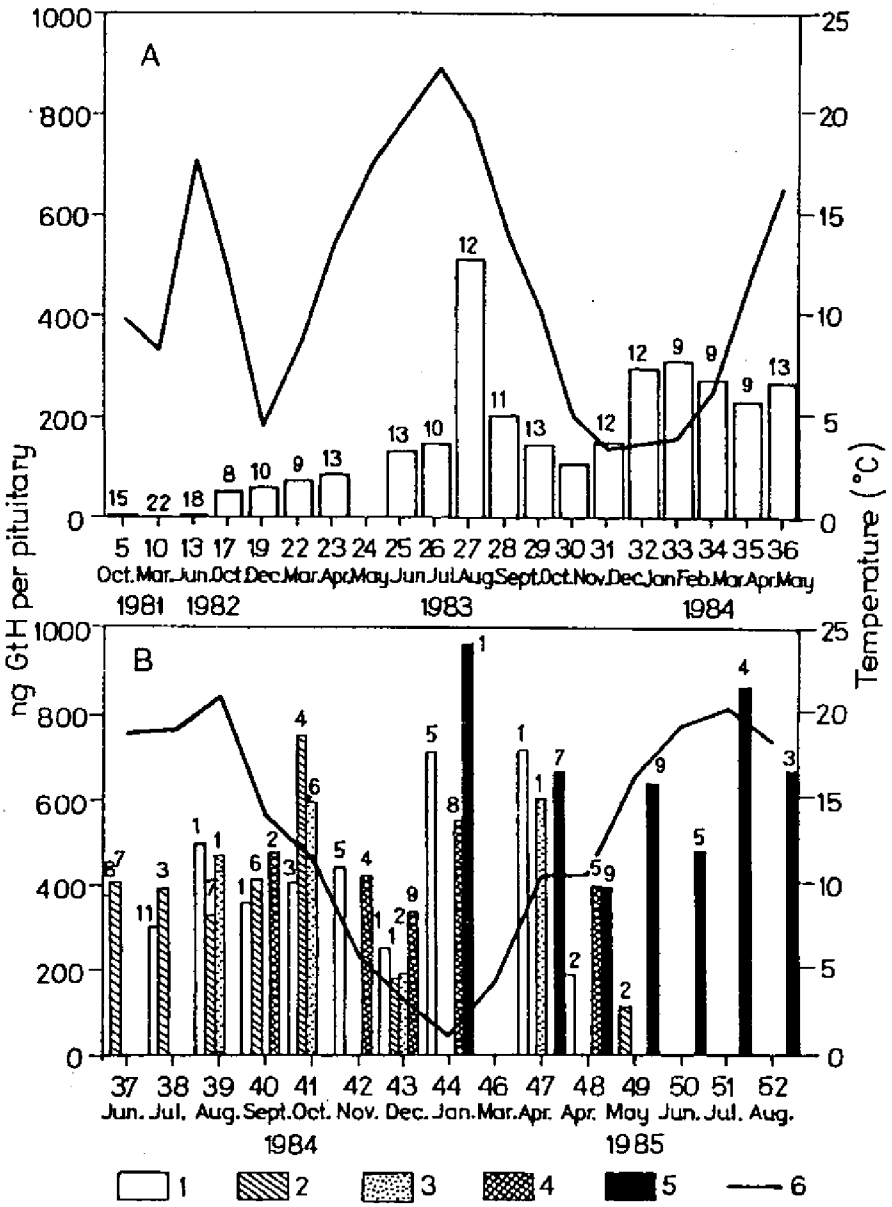

Fig. 3. Changes in the pituitary GtH during growth and sexual maturation in 5 to 36-month (A) and 37 to 52-month-old (B) female carps. Values are mean. For explanation see Fig. 1

4. DISCUSSION

An increase in GSI during carp life, more or less consistent with the increasing gonadal maturity, was expected. However, it was found that the growth of GSI was fluctuating, an increase being followed by a shorter period of decrease. This could be explained by an asynchronous growth of oocytes in ovaries connected with a great individual variation in their development (S a k u n, B u c k a y a 1968). In the regions with temperate climate, female carps in general reach sexual maturity at five years, but few percent of them reach maturity at four years of age. In consequence, sister carps raised in the same pond can have gonads at different stages of development. This might explain that in the present work, females with the same GSI were at various stages of gonad development and that at 3 years of age ovaries showed an asynchronous development of oocytes in the investigated females.

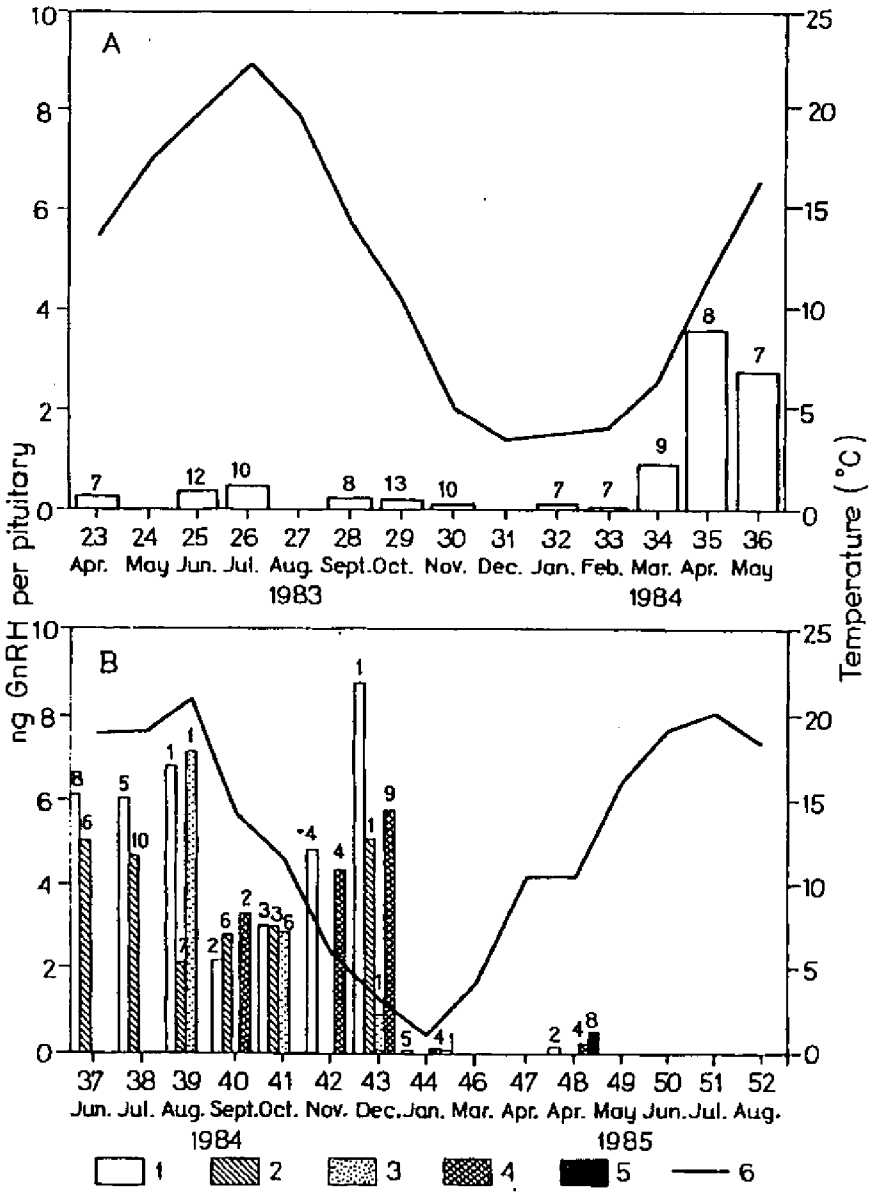

Fig. 4. Changes in the pituitary GnRH during growth and sexual maturation in 23 to 36-month (A) and 37 to 48-month-old (B) female carps. Values are mean. For explanation see Fig. 1

The GtH investigated in this study corresponds to GtH II of Itoh et al. (1988) and Suzuki et al. (1988), which in salmonids is accumulated in the pituitary up to the ovulation and then is released to the blood (Dickhoff, Swanson 1989).

In the present work, ovulation was not recorded since animals were kept in growing ponds. During five years of experimentation, GtH contents in blood were low and variable (9.79 to 33.25 ng · cm^{-3}, Fig. 2) except in July and August 1984 (third year of animal life) where GtH levels peaked to reach 186.8 and 138.8 ng · cm^{-3} in females at stage 1 and 214.5 and 161.0 ng · cm^{-3} in females, just starting vacuolisation (stage 2) stage at which Galas et al. (1987) did not observe any increase in blood oestradiol levels. On the other hand, these GtH high levels were not observed in fish at more advanced stages of vitellogenesis (3, 4, 5) although Galas et al. (1987) observed increases in plasma oestradiol levels in carps at stage 3 and 4.

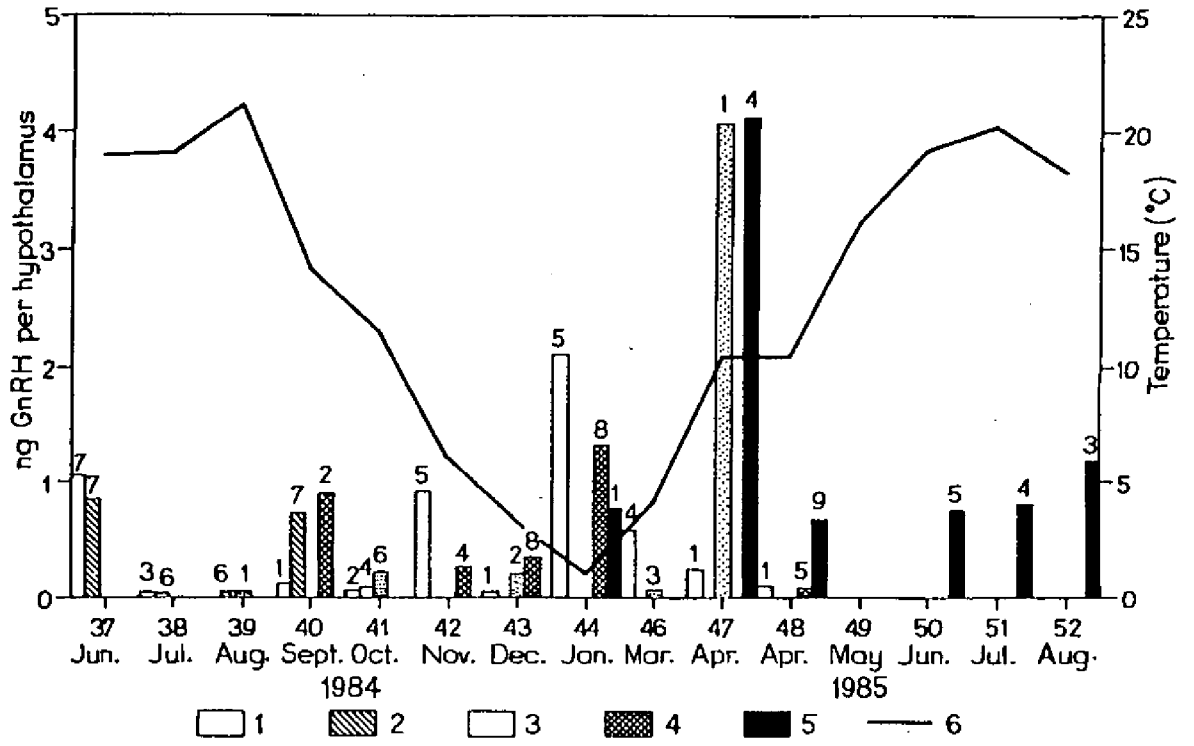

Fig. 5. Changes in the hypothalamus GnRH (females 37 to 52 months old). Values are mean. For other explanations see Fig. 1

Our observations were not consistent with the observations on *Salmo trutta* (Breton et al. 1983) in which a gradual increase in GtH levels was synchronised with the advancement of vitellogenesis. This increase in GtH observed in females at stages 1 and 2 could be related to pulsatile secretion of GtH, as it is known in trout (Zohar et al. 1986).

However, further studies should be done to confirm or invalidate this hypothesis. It is supposed that this increase in GtH levels in females 38 and 39 months-old corresponds to a wave of GtH necessary to induce the onset of oocytes vacuolisation (beginning of vitellogenesis) and the onset of asynchronic development of oocytes in the ovaries which to this moment have developed synchronicaly.

Turning to pituitary GtH content, it begins to increase after 13 months of age, reaching highest values in older specimens. This increase in pituitary GtH content in maturing females is in accordance with the work of Weil (1981) in carp for a maturational gonadotropin as well as with the work of Dickhoff, Swanson (1989) for GtH II in Salmonids. In the present work the variation in the pituitary GtH content is not related with the variation of water temperature since for example in females 42- to 45-month-old at all maturity stages, levels of GtH in the pituitary remained high in winter. Such an absence of relation pituitary GtH with the season has been already reported for female carp during their third year of life (Weil 1981).

An increase in pituitary GnRH level (Fig. 4) began at 35 months. Then, with the advancement of oogenesis some variation was observed but it was not precisely related to the maturity stages. However, when fish were ready to spawn (stage 5) at the end of sampling period, the lowest values were recorded, the same was observed in goldfish by Yu et al. (1987).

At the hypothalamus level, despite of a great variation observed in GnRH content it can be said that there was also an increase with the maturation of the ovary, since the highest values were recorded for females at stage 5 of maturity. This is not consistent with the results obtained by Y u et al. (1987), since in that work the hypothalamus GnRH contents were lower in sexually mature fish. In the present work could be stated no relationship between blood GtH levels, the pituitary and hypothalamus GnRH contents. However, such relationship have been described for another cyprinid – the roach maintained under natural conditions was able to ovulate spontaneously (B r e t o n et al. 1988). In the present work fish did not ovulate since they were kept in growing ponds and not in spawning ponds.

In conclusion, this work allows us to propose a model concerning changes in the ovary as related to gonadotropin and GnRH variations for carp in a temperate climate. During the first 3 years of female life there is a synchronous development of the ovary characterized by a gradual increase in pituitary GtH levels while pituitary GnRH contents increases lately. During this period, blood GtH levels does not change substantially. During the second phase ovaries undergo an asynchronous development. This phase is initiated on the ovaries beginning vacuolisation by a release to the blood of a great amount of GtH. It ends up when fish are five years old and at this time the ovary contains oocytes at the end of vitellogenesis. This asynchronous development of the ovaries is characterized by great variations in GtH (plasma and pituitary) and in GnRH (pituitary and hypothalamus) contents without clear parallel changes in all these parameters.

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5. SUMMARY

Fish 5- to 52-month-old originating from one couple of spawners were growing in a conventional carp pond.

GtH in the blood and pituitary, GnRH in the hypothalamus and pituitary (RIA), gonado-somatic index (GSI) and histology of gonads were investigated.

GSI showed high variability between specimens.

Circulatory GtH levels did not fluctuate much throughout the investigated period (9.79–33.25 ng. cm^{-3}) except at the beginning of oocyte vacuolisation when peaks of GtH (161.40–214.51 ng · cm^{-3}) were observed.

GtH in the pituitary started to accumulate from 13 months (from 2.64 to 617.35 ng · cm^{-3}) and GnRH from 33 months (from below 1.00 to 3.55 ng · $pituitary^{-1}$).

GnRH contents in the hypothalami of the females before 35 months were also low (0.06–2.11 ng · $hypothalamus^{-1}$) thereafter they increased up to 4.12 ng · $hypothalamus^{-1}$ showing great variations.

Significant correlations were only found between GSI and age as well as between GnRH content in the pituitary and age.

6. STRESZCZENIE

Ryby w wieku od 5 do 52 miesięcy pochodzące od jednej pary tarlaków chowano w zwyczajnym stawie karpiowym.

Badano (RIA) zawartość GtH we krwi i przysadce, GnRH w podwzgórzu i przysadce oraz współczynnik gonado-somatyczny (GSI) i histologicznie gonady.

GSI wykazywał bardzo dużą zmienność osobniczą.

Poziom GtH we krwi nie zmieniał się bardzo w czasie całego okresu badań (9,79–33,25 ng · cm^{-3}) z wyjątkiem początku wakuolizacji oocytów, gdy obserwowano najwyższe poziomy GtH (161,40–214,51 ng · cm^{-3}).

GtH w przysadce zaczęła się akumulować od 13 miesiąca (od 2,64 do 617,35 ng · cm^{-3}), a GnRH od 33 miesiąca (od koncentracji poniżej 1 do 3,55 ng · cm^{-3}).

Poziom GnRH w podwzgórzu samic przed 35 miesiącami był także bardzo niski (0,06–2,11 ng · podwzgórze^{-1}) a potem osiągnął wartość 4,12 ng · podwzgórze^{-1} wykazując równocześnie dużą zmienność.

Statystycznie istotną korelację stwierdzono tylko pomiedzy GSI i wiekiem oraz pomiędzy zawartością GnRH w przysadce i wiekiem.

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