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ENDOCRINE CONTROL OF THE TELEOST REPRODUCTIVE CYCLE AND ITS RELATION TO EXTERNAL FACTORS : SALMONID AND CYPRINID MODELS.

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INTRODUCTION

Most of the fish living in the temperate zone exhibit an annual reproductive cycle. Reproduction, or more precisely hatching, occurs when food is available for the fry in the wild. Therefore, reproduction is closely related to environment. Endocrine control of the reproductive cycle, one of the links between environment and reproduction, is examined in this paper. Two main groups presenting different reproductive strategies are considered :

1) Salmonids spawning in autumn and winter ; the large amount of yolk and the duration of embryogenesis and vesicle resorption delay release of the fry so that spawning is not strictly environment-dependent.

2) Cyprinids usually laying small eggs in spring and summer ; these fish are more environment-dependent, at least for spawning. Swimming up fry do not rely on the same food as salmonids.

Two strategies are discussed from recent data mainly gathered in our laboratory.

SALMONIDS

Data on the endocrinology of the reproductive cycle were first obtained from histophysiological studies on the pituitary gonad axis^{1, 2, 3}. Measurement of gonadotropin (GTH) in pituitary was first attempted by bioassay⁴. Later, plasma and pituitary GTH were measured by radioimmunoassay (RIA) following achievement of a suitable bioassay⁵, and purification of GTH⁶. Methodologies for measurements of sex steroids have been discussed⁷ and RIA is now available^{8, 9, 10, 11}.

Descriptive and experimental studies were carried out on the reproductive cycle in rainbow trout (*Salmo gairdneri*) and brown trout (*Salmo trutta fario*), including quantitative analysis of gametogenesis¹² and measurement of pituitary and plasma GTH¹³, estrogen 17 β (E₂)⁹ and androgen¹⁴. For the description of the cycle, 10 males and 10 females were sacrificed once or twice a month. Graph values are given with the standard deviation.

Male cycle. The reproductive cycle in males was divided into 8 stages¹⁵. Brown trout values for stage VIII (spermiation) are given for each sampling (twice a month) (Fig. 1). GTH increased at stage II in the plasma (9.9 \pm 7.8 ng/ml) as well as in the pituitary. At this time E₂ was detected in the plasma but no androgen was found. As in rainbow trout, GTH rose more rapidly in the plasma than in the pituitary during spermatogenesis. Most of the time E₂ was undetectable and androgens,

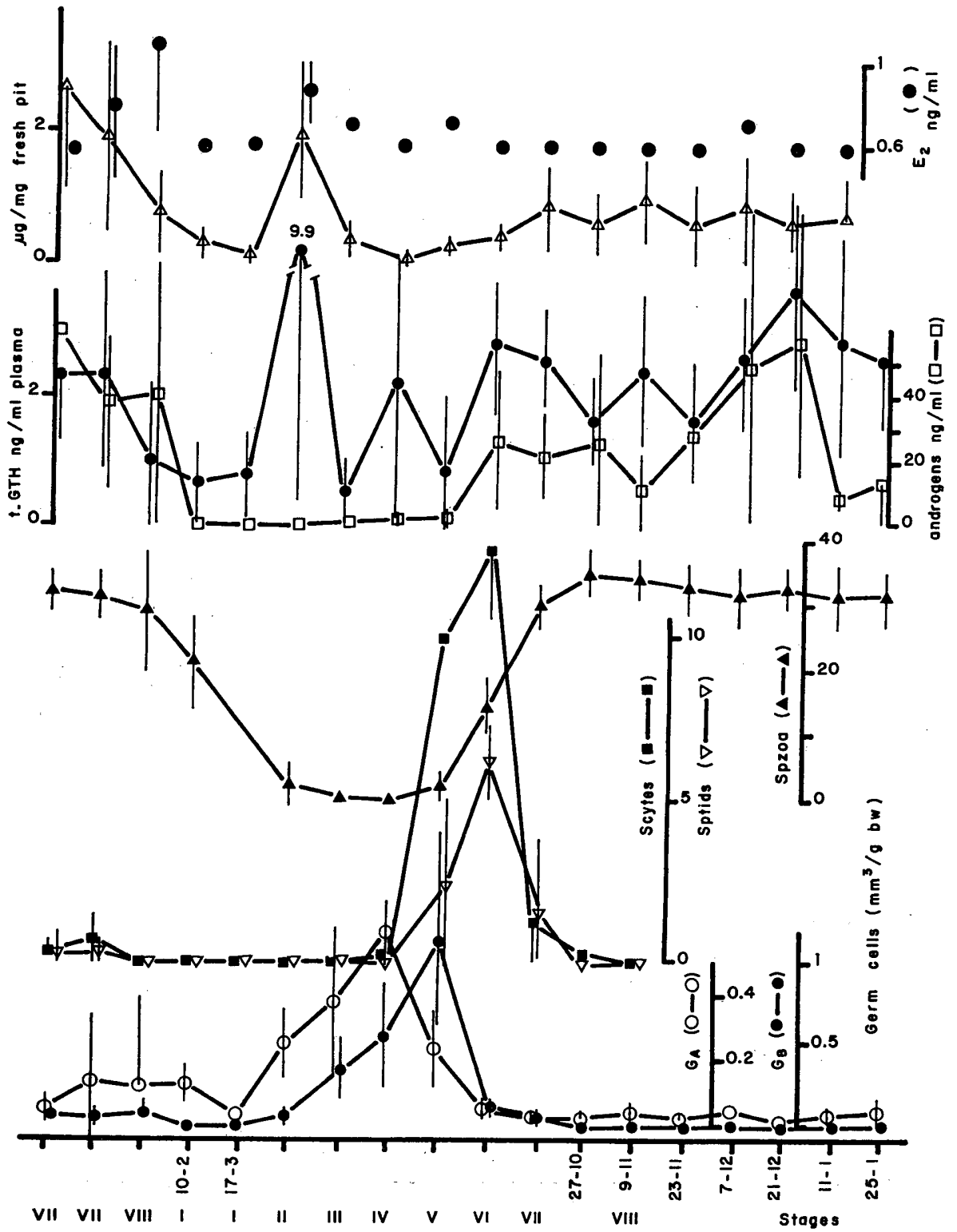


Fig. 1. Spermatogenic cycle and hormonal changes in brown trout.

which were low at the beginning, rose toward the end of spermatogenesis. During the period of spermiation, plasma GTH and androgen were high at the beginning, decreased slightly after one month, rose toward the end of December and dropped in January. In rainbow trout, a rise of plasma GTH (1.81 ± 0.77 ng/ml) was observed in spring when the number of type A spermatogonia (GA) and cysts of type B spermatogonia (GB) first appeared (Fig. 2). As spermatogenesis progressed, GTH increased rapidly in plasma (0.18 ± 0.14 to 3.79 ± 0.76 ng/ml) but more slowly in pituitary.

Female cycle. Brown trout oogenesis was divided in stages based on egg diameter and sequence of oocyte maturation and ovulation (Fig. 3). In spring there was a surge of GTH in pituitary and plasma; E_2 increased during vitellogenesis and reached its maximum value (10 ng/ml) 2 months before ovulation when egg diameter was 4-4.5 mm. GTH pituitary content increased slowly during vitellogenesis and more rapidly during oocyte maturation. Plasma GTH did not augment much during vitellogenesis, but rose at the time of oocyte maturation and ovulation, as already observed in this species at spawning time⁸. The coefficients of correlation between some parameters at various stages of the cycle are given on the following table :

	Previtellogenesis	Vitellogenesis	End of reproductive cycle
<u>Plasma GTH</u> <u>Plasma E_2</u>	- 0.07 (49) NS	+ 0.17 (54) NS	- 0.23 (78) *
<u>Pituitary GTH</u> <u>Plasma E_2</u>	+ 0.06 (49) NS	- 0.16 (51) NS	- 0.09 (52) NS
<u>Pituitary GTH</u> <u>Plasma GTH</u>	+ 0.48 (50) **	+ 0.02 (55) NS	- 0.11 (54) NS
<u>Egg diameter</u> <u>Plasma E_2</u>		+ 0.35 (45) **	
<u>Egg diameter</u> <u>Plasma GTH</u>		+ 0.35 (81) **	

() : Number of data ; * : $P < 0.05$; ** : $P < 0.01$

Rainbow trout stages of oogenesis were defined by the median egg diameter recorded in the ovary (Fig. 4). A rise of plasma GTH was observed when the number of oocytes in previtellogenesis increased. Females in active vitellogenesis exhibited an important rise of E_2 as previously observed^{10, 11}. A rise of GTH occurred later either in plasma or pituitary. When females reached the oocyte maturation stage (GV+), E_2 decreased and GTH was higher.

Some original aspects of the salmonid reproductive cycle. Environmental factor effects. Photoperiod and temperature changes are the main environmental factors involved in the determinism of the reproductive cycle. Many experiments have been performed in salmonids and recently reviewed¹⁶. Full gametogenesis is induced in decreasing photoperiod in both females¹⁰ and males¹³. Although a constant 24L regime

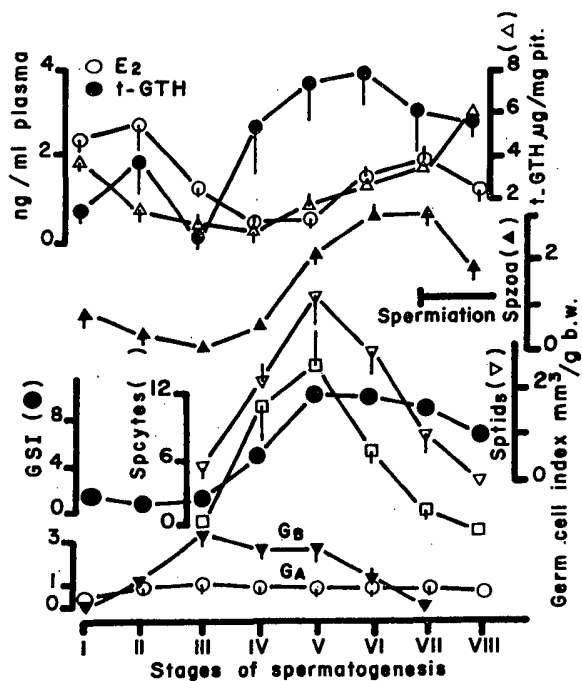


Fig. 2. Spermatogenetic cycle and hormonal changes in rainbow trout.

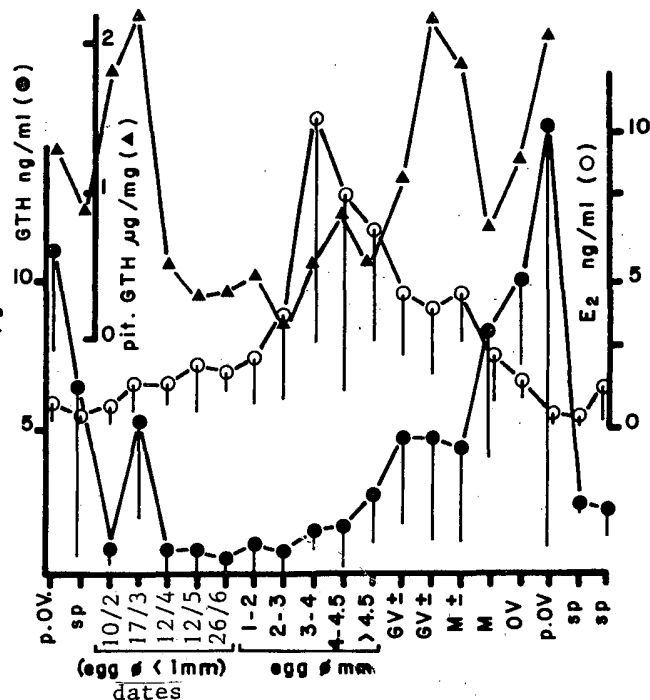


Fig. 3. Hormonal changes during the reproductive cycle of female brown trout

starting in June or a photoperiod rising from 16L in June to 24L in December were used, gametogenesis and spawning were delayed 2 months (Chevassus et al., unpublished). Effects of temperature have been investigated in males¹³: full spermatogenesis occurred at either 8 or 16°C, but the first step (GB multiplication and appearance of spermatocytes) was favoured by low temperature while spermiogenesis was stimulated at high temperature. There were wider plasma GTH variations at 16°C than at 8°C; for instance at 16°C plasma GTH reached its highest value at 12L-12D, 3 months after the photoperiod started to decrease. However, in females kept under various photoperiod regimes¹⁰, the rise of plasma E₂ occurred in July, even in the constant 12L-12D regime and whatever the time at which the photoperiod started to decrease. On the contrary, the other parameters studied (calcium and phosphoproteins) followed this photoperiodic change. During the period of spermiation sperm production was not modified by photoperiod changes but decreased at a high temperature regime¹⁷. Among the environmental factors, change in photoperiod probably plays a role in controlling the reproductive cycle, but endogenous rhythms are also highly responsible.

Vitellogenesis. Studies on the endocrine control of vitellogenesis are somewhat difficult for two main reasons:

- 1) The process is difficult to define: GSI, easily available and used in many studies, is too global. Histological data, such as endogenous or exogenous vitellogenesis employed in the above reproductive cycles, do not clearly establish the limits of the process.

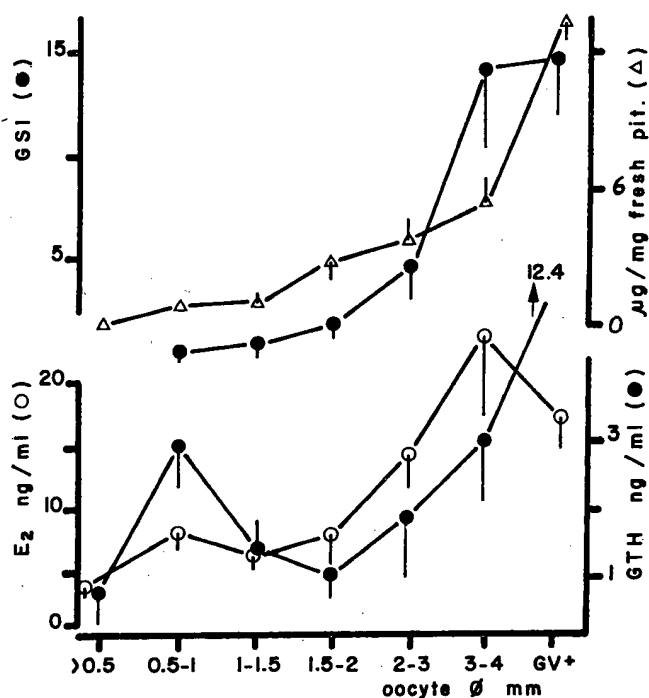


Fig. 4. Hormonal changes during the reproductive cycle of females rainbow trout



Fig. 5. Stimulation of E₂ secretion following intraperitoneal injection of s-GTH (50 $\mu\text{g}/\text{kg}$ b.w. n=4) in rainbow trout female during the first part of vitellogenesis (July 18 at 8h30) ; oocyte diameter (1.5-2 mm). Controls were given saline (NaCl 9‰, 1 ml/kg b.w. n=5)

2) Several organs are involved in the synthesis of vitellus precursors as related to nutrition and food availability before and during vitellogenesis.

The presence of pituitary is necessary for vitellogenesis¹⁸, and ovary regression following steroid injection is probably due to negative feedback¹⁹. Crude pituitary extracts stimulate vitellogenesis²¹. In goldfish replacement therapy with c-GTH (a unique carp GTH) initiates vitellogenesis²¹, while t-GTH or SG G100 do not stimulate vitellogenesis in immature trout²⁰ or Pacific salmon²². However, histochemical studies have shown that E₂ is synthesized in the interstitial tissue and granulosa cells of the ovary¹¹ and t-GTH elevates plasma E₂ in adult female at the beginning of vitellogenesis (Fig. 5). During a normal cycle E₂ is positively correlated with plasma vitellogenin⁸ and GSI (see Table, ¹¹). E₂ injections stimulate synthesis of phosphoproteins²³ and vitellogenin²⁴. However, E₂ or E₂ + t-GTH do not stimulate vitellogenin incorporation into trout oocytes²⁰ which seems to require a non-glycoprotein pituitary factor²⁴. A regulation of vitellogenesis mediated by pituitary through E₂ is too simple a view ; several factors including metabolic hormones^{25, 26} are obviously involved in its endocrine control.

Oocyte maturation and ovulation. Salmonid plasma t-GTH levels, during the sequence of events oocyte maturation-ovulation-oviposition, vary according to the species. The maximum level reaches 10 ng/ml in rainbow trout²⁷ or less in coho salmon²⁸ (species which are reproduced in hatcheries) and are 10 to 30 times more in some other species such as pink salmon²⁹ or chilko sockeye salmon³⁰, allowed to spawn naturally. This might indicate that the degree of domestication of these animals plays a part in these differences or that GTH acts on reproductive behaviour as already suggested³⁰.

In rainbow trout, peripheral migration of the germinal vesicle inside the oocytes is accompanied by a gradual rise of GTH from around 3.5 ng/ml to 6 ng/ml, preceding oocyte maturation ; then, during oocyte maturation GTH rises again to 8-10 ng/ml²⁷. During the same periods E₂ decreases so that E₂ is usually negatively correlated with GTH⁹. The same decrease can also be observed in coho salmon²⁸. However, injection of E₂ (1 mg/kg every 3 days) does not prevent ovulation.

Spermiation. After completion of salmonid spermatogenesis the seminiferous lobules are filled with spermatozoa. These spermatozoa remain several weeks in a non-motile state inside the testis until they begin to be released. This phenomenon of spermatozoon release from the sertoli cells has been called spermiation in amphibians³¹ and extended to higher vertebrates. As there is no implantation of the spermatid head in the cytoplasm of most teleost fish sertoli cells, the word "spermiation" corresponds to a "thinning of semen"³², allowing its migration toward the vas deferens from which it can be extruded by handstripping (sperm release). A peak of plasma GTH has been observed at about the time of initiation of spermiation in some experiments³³, but no peaks were found in cycle reproductive studies (Fig. 1., 2)³⁰ (sampling may not have been frequent enough). In another experiment, spermiation was seen to start with steady or even decreasing levels of GTH ; later on when sperm production increased a parallel rise of GTH was observed¹⁴. GTH effect on spermiation is possibly mediated by androgens (Fig. 1), such as 11-ketotestosterone³⁴, but exogenous progesterone is more potent than either testosterone or 11-ketotestosterone (Montalembert and Billard, unpublished).

CYPRINIDS

Only a few publications deal with the whole reproductive cycle in cyprinids. The available data show that the pattern of this cycle is species-dependent. Two main examples are given in this paper : carp (work carried out at Jouy en Josas) and tench (joint program with Poland). Results given in graphs include spermatogenic analysis¹² and GTH RIA³⁵ ($\bar{x} \pm SE$).

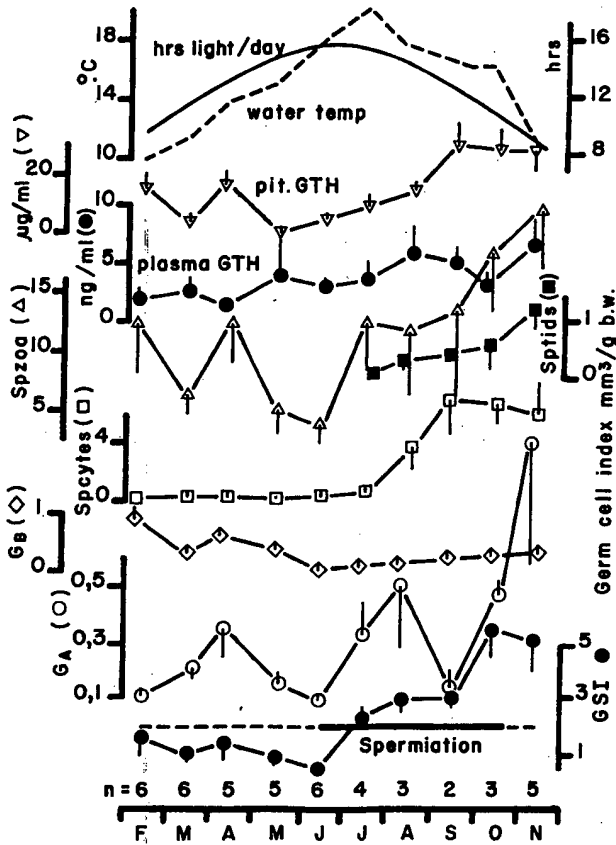


Fig. 6. Spermatogenic cycle and hormonal changes in common carp

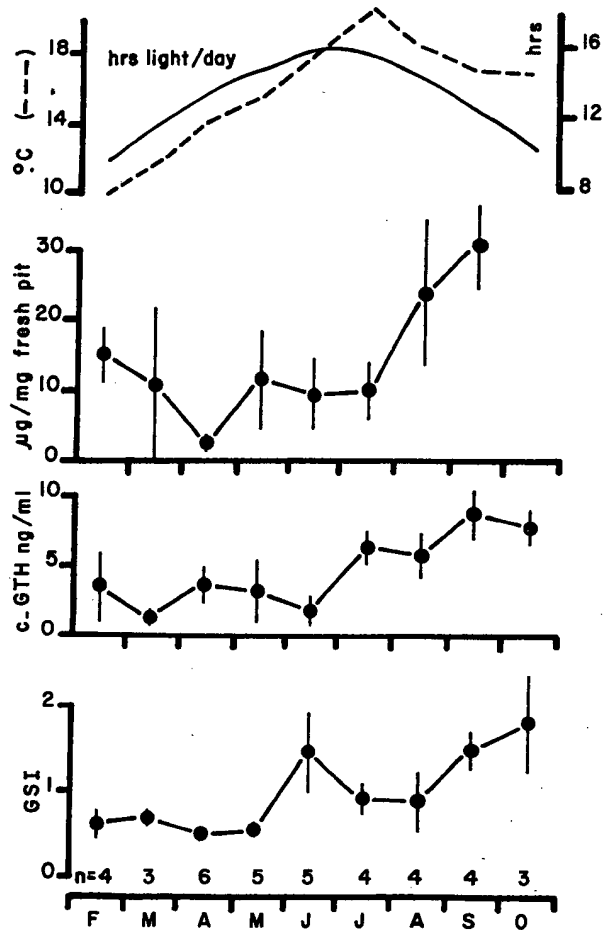


Fig. 7. Changes in GSI pituitary and plasma GTH on two years old common carp

Carp. Male cycle. (Fig. 6) The main spermatogenic activity, as shown by a rise of GSI and germ cells, occurred in summer and early autumn. In November the number of spermatids and spermatozoa was still rising as well as that of GA. Similarly, plasma and pituitary GTH increased during the same period. A discrete spermatogenic activity was also detected in April with a peak of GA and GB and spermatozoa but there was no noticeable rise in the number of spermatocytes and no spermatid was identified. A peak in pituitary GTH content was observed at the same period. Most of the males were found in spermiation from June to October, and only a few during the rest of the year. There seemed to be several spermatogenic waves occurring throughout the year, leading to a permanent stock of spermatozoa in the testis. This fluctuating stock was composed of spermatozoa of different ages.

Female cycle. Carp kept in a natural environment usually reach maturity during the third year of life. During the second year females are immature, but a significant rise of plasma GTH occurs in the summer (Fig. 7). The slight rise observed in pituitary GTH and GSI is not significant. It may therefore be that puberty is reached in the second year of life. During the reproductive cycle of the adult female (more than

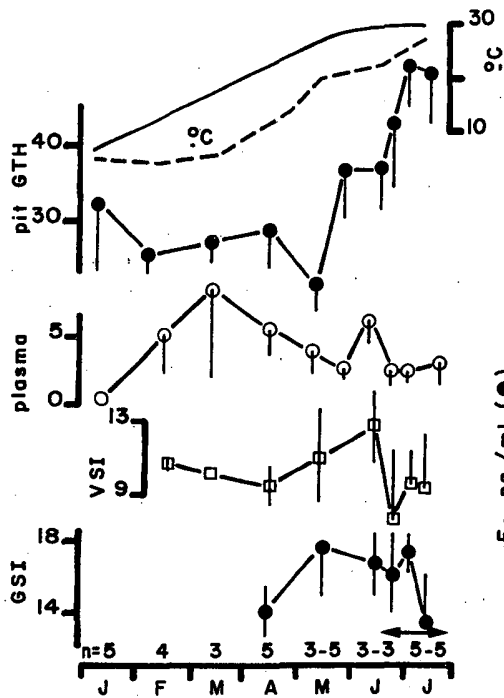


Fig. 8. Changes in GSI, VSI (vitello-somatic index), c-GTH (plasma in ng/ml and pituitary in $\mu\text{g}/\text{mg}$ fresh tissue) in adult common carp (\longleftrightarrow) oocyte atresia in the ovary. (\uparrow) changes in photoperiod from 8 to 16 hrs light/day

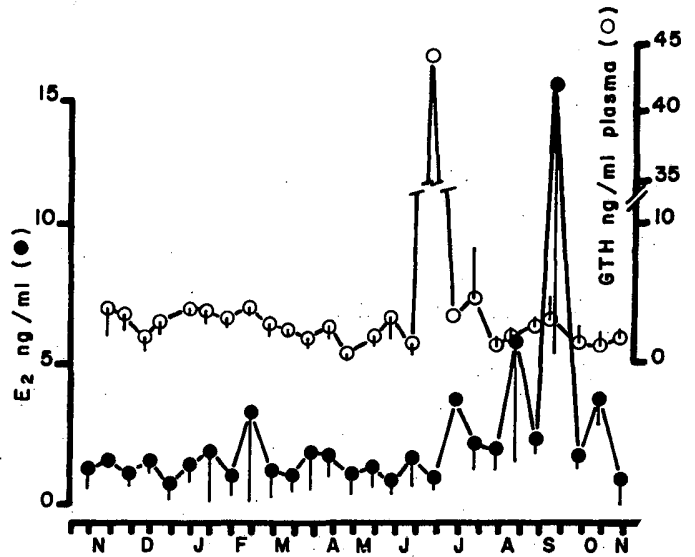


Fig. 9. Plasma GTH and E_2 changes throughout one year in adult female carp ($n = 5$) \pm SD

3 years old) pituitary GTH was low and constant in winter and rose dramatically from May to July. Plasma GTH increased in late winter and slightly decreased in spring when GSI reaches its maximum value (Fig. 8). In June some females kept in concrete raceways exhibited a surge of plasma GTH, but no spontaneous ovulation occurred and atretic oocytes were later found in the ovaries. In another experiment 5 females kept in a fiberglass aquarium were sampled for GTH and E_2 twice a month all the year round (Fig. 9). Plasma GTH did not vary much during the year, except that in July a huge surge of GTH was observed in 4 females. This surge was not followed by ovulation, but a small peak of E_2 was recorded afterwards on two females and other peaks also occurred in August-September and October, although GTH levels were low. There is no satisfactory explanation for the lack of ovulation after the GTH surge. This surge might not have been sufficient or perhaps other factors were involved having a facilitating effect due to the suitable environment, which is essential for natural spawning in carp. This GTH surge might have another role in inducing a new reproductive cycle since a new wave of oogenesis starts and vitellogenesis is completed in October³⁶. Pulsations in plasma E_2 recorded between July and October may therefore be involved in vitellogenesis.

Tench. The tench, as the carp, is an intermittent spawner but the pattern of the reproductive cycle is quite different. Vitellogenesis started in late winter and spawning in early summer. Plasma GTH levels were low (1-2 ng/ml) during vitellogenesis and higher (3-4 ng/ml) during the spawning season ; a depletion of GTH in the pituitary observed after one spawning suggests that a surge of GTH had occurred at this time.

Spermatogenic activity was initiated in winter and early spring ; GA decreased and GB and spermatocytes increased. In May, spermatids and spermatozoa were present and reached their maximum values in July when spawning started. Plasma GTH exhibited a peak in February when spermatogenesis was initiated and rose during active spermatogenesis. Pituitary GTH increased during the spawning season.

Goldfish. Plasma c-GTH increased with the gonadal recrudescence while immunofluorescent GTH decreased in the pituitary³³. Androgens rise prior to the spawning season in males ; androgens and estrogens were present in female plasma during the spawning season³⁷.

Some original aspects of the cyprinid reproductive cycle. Environmental factor effects. Available data in the literature indicate that temperature is the most important factor involved in the determinism of the sexual cycle although few experiments have been carried out to demonstrate the role of photoperiod. Some data have shown that rise of pituitary GTH observed under normal photoperiod in male carp does not occur under short photoperiod ; in addition GSI and the number of spermatozoa decrease but spermatocytes and GB remain stable. However, pituitary responsiveness to LH-RH, which is maximum from July to November, is not modified by short photoperiod (Weil, unpublished). In goldfish a circadian rhythm of plasma GTH level may be under photoperiod control³⁸. The effects of temperature are more obvious ; carp reproduction always occurs in warm conditions, and under a temperature of 20-22°C continuous gametogenesis has been reported³⁹. Temporary inhibition of gametogenesis occurred in goldfish taken from the wild and kept at 30°C⁴⁰ but after a recovery period of several months, normal gametogenesis was again observed⁴¹. Heated water (+ 6°C above ambient temperature) increased the number of tench spawning and the total duration of the spawning period³³ (7 spawnings over 3 months at + 6°C and 2 over 1 month in controls). After 3 months, reproduction stopped although temperature was still high, suggesting that gametogenesis is not a continuous process in this species as it is in carp.

Spermiation. Most of the cyprinid species present a quasi-permanent stock of spermatozoa throughout the year, and spermiation may occur almost all the year round. Therefore, it does not seem that a GTH surge is needed to induce spermiation ; a tonic release is probably sufficient. However, injections of GTH increase sperm yield; a species specificity for GTH has been shown in goldfish ; c-GTH is much more potent than t-GTH (Billard, unpublished). Hypophysation does not induce spermiation in goldfish when the rearing temperature is 10°C⁴⁰.

Ovulation. If gametogenesis may occur in a wide range of temperatures, thermal requirements are more strict for ovulation. In goldfish during the spawning period basal GTH level followed temperature fluctuation and at the time of ovulation, which occurred after several warm days, a GTH surge was recorded³⁸. Temperature probably acts differently at central nervous, pituitary and ovarian levels since exogenous 17α -hydroxy- 20β -dihydroprogesterone, supposedly produced by the ovary, can induce ovulation at 14°C ⁴²; however this treatment is not efficient at 10 - 12°C (Jalabert et al., unpublished). In goldfish hypophysation failed to induce ovulation at 10°C ⁴⁰

CONCLUSION

Reproductive strategy is quite different in the two groups studied. There are some similarities and some basic differences in the endocrine control of the reproductive cycle. The GTH levels detected in blood and pituitary are lower in both groups during gametogenesis strictly speaking and higher during the final stages of the cycle (oocyte maturation, ovulation and spawning). In addition, a rise of plasma and pituitary GTH is observed at the beginning of gametogenesis in carp. The meaning of changes in the concentration of circulating hormones is open to debate. While it is reasonable to think it reflects a release from the pituitary glands³⁰, gonadal plasma clearance rate and changes in gonad receptivity during the sexual cycle are not known in fish.

The differences between salmonids and cyprinids lie in the sequence of the reproductive cycle. In salmonids vitellogenesis and spermatogenesis usually occur in summer and are controlled by decreasing photoperiod. Spawning occurs in autumn or winter under lower temperature⁴³. In a cyprinid such as carp, gametogenesis starts immediately after the previous reproductive cycle and may be completed by October. It is then blocked until the following spring or summer when favorable thermal conditions favorize spawning. In tench gametogenesis only starts in spring; generally speaking gametogenesis in cyprinids may occur at any season, but spawning requires relatively high temperatures. Finally temperature is the main factor controlling the time of fry release in the wild; in cyprinids its action is mediated through final maturation and spawning but in salmonids temperature does not act directly on gametogenesis and spawning but mainly on the duration of embryonic development.

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