Ann. Biol. anim. Bioch. Biophys., 1977, **17** (5 A), 673-678.

Stimulation of gonadotropin secretion in goldfish by elevation of rearing temperature

C. GILLET, R. BILLARD

Laboratoire de Physiologie des Poissons, I. N. R. A.
78350 *Jouy-en-Josas, France*

Summary. Adult goldfish put under controlled-raising photoperiod were subjected to a progressive rise in rearing temperature between February and May, or to a sudden rise in February when gametogenesis is initiated or in May when it is in progress. Plasma gonadotropin (c-GtH) was measured by radioimmunoassay. When temperature rose from 10 °C to 20 or 30 °C, a significant increase of immunoreactive c-GtH was observed. In a control group maintained at 10 °C from February to May, the cGtH level increased significantly, but remained significantly lower than the level of the group subjected to a progressive rise in rearing temperature and kept under the same photoperiod. This suggests an effect of both temperature and photoperiod on c-GtH release.

Introduction.

Temperature is one of the most important environmental factors controlling reproduction in teleost fish (De Vlaming, 1974). It may act at two levels :

On gonads : temperature controls the duration and yield of spermatogenesis (Egami and Hyodo-Taguchi, 1967 ; Billard, 1968) and the duration of oocyte maturation (Jalabert *et al.*, 1973). The temperature requirement for gametogenesis may depend on the stage of sexual development ; low temperatures are required for the initiation of spermatogenesis in *Fundulus* (Lofts *et al.*, 1968), meiosis in *Couesius plumbeus* (Ahsan, 1966) and ovarian maturation (Echelle *et al.*, 1973). Warm temperatures are required for the end of spermatogenesis and spermiation in *Couesius plumbeus* (Ahsan, 1966) and for ovulation and spawning in goldfish (Yamamoto *et al.*, 1966).

On the central nervous system and pituitary : gonadotropin secretion increases with temperature in tench (*Tinca tinca*, Breton *et al.*, 1975) and in goldfish (*Carassius auratus*, Gillet *et al.*, 1977). In carp, pituitary response to LH-RH injection has been shown to increase at the same time as the temperature (Weil *et al.*, 1975).

In the present experiment, the effect of slow rise in the rearing temperature on gonadotropin release was tested in goldfish between February when gametogenesis is initiated, and May when it is in progress ; the effects of a sudden rise were tested both in February and in May.

Material and methods.

Adult male and female goldfish weighing approximately 50 g were taken at the end of January from their natural environment in outside ponds in which the water temperature was 10 °C. Groups of 10 fish were put in 60 l thermoregulated plastic tanks under controlled-raising photoperiod ; fluorescent tubes provided 1 000 lux at water surface, similar to that recorded outside. Fish were fed pellets *ad libitum* twice a day.

Three groups of fish were kept under various rearing temperatures :

Experiment 1 : a progressive rise of 10 °C similar to the one occuring in a natural environment was planned over 4 months from February to May (10 °C in February, 12 °C in March, 16 °C in April and 20 °C in May). A control group was maintained at 10 °C during the 4-month period. Blood samples were taken by heart puncture every month between February and May (fig. 1, groups A and B).

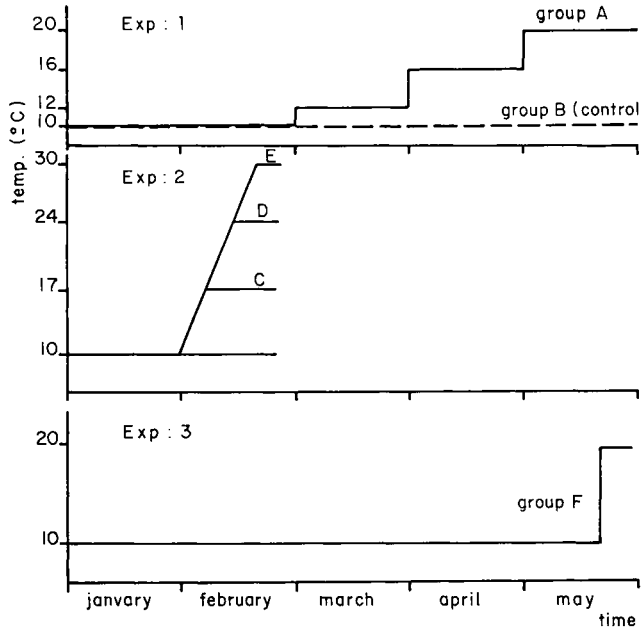

FIG. 1. — *Summary of the experimental procedures used to test the effects of a rise in rearing temperature on gonadotropin secretion in goldfish.*

Experiment 2 : at the beginning of February the temperature was raised in the 3 groups at the rate of 1 °C per day from 10 °C to 17, 24 or 30 °C. Blood samples were taken at the end of February when all the experimental temperatures were reached (fig. 1, groups C, D and E) ; they were also taken in the control group at 10 °C (group B).

Experiment 3 : one group was maintained at 10 °C until May and then transferred into tanks heated at 20 °C. Blood was sampled before the thermal shock and 15 mn, 6 h and 7 days afterwards (fig. 1, group F).

Blood was centrifuged at 3000 rpm for 20 mn and plasma samples stored at — 20 °C until analyzed by radioimmunoassay as previously described by Breton *et al.* (1971) for carp and extended to goldfish.

Results.

Results are reported in figure 2, 3 and 4. There is no difference between males and females in the circulating c-GtH recorded.

Experiment 1 : at the beginning of February at 10 °C, c-GtH blood level is low, i. e. less than 2 ng/ml plasma. When temperature is progressively increased by 10 °C during 3 months, c-GtH level reaches 11 ng/ml plasma which is a significant rise ($P < 0.005$) (fig. 2, group A). Also, the rise of c-GtH is proportional to temperature elevation. At

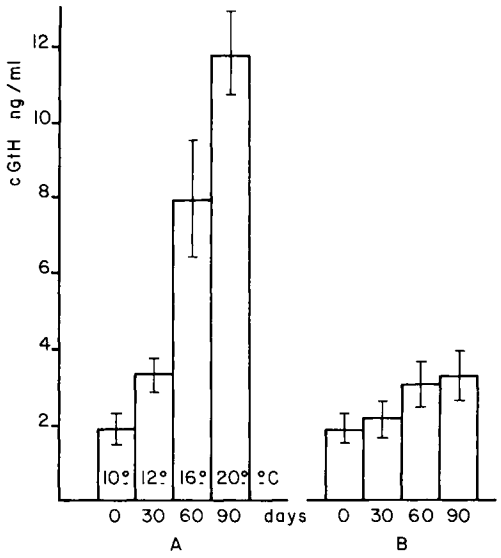

FIG. 2. — *Experiment* 1. *Variation of the amount of immunoreactive serum gonadotropin* (c-GtH).

A : group reared in temperature rising from 10 °C in february to 20 °C in may.
B : Control group maintained in a 10 °C constant temperature between february and may. Mean values are given with S. E.

constant rearing temperature of 10 °C, the c-GtH level increases significantly between February and May ($P < 0.05$) (fig. 2, group B). However, plasma gonadotropin level remains significantly lower in April and May ($P < 0.005$) in the control group (10 °C)

as compared to the group subjected to a progressive rise of rearing temperature. There was no significant difference between the two groups in March, but the rearing temperature was about the same (10 °C and 12 °C).

Experiment 2 : in fish subjected to a rise of temperature in February the c-GtH level significantly increased up to 6-8 ng/ml ($P < 0.001$) in all the temperatures tested (fig. 3). No statistical difference was observed among the 3 groups kept in warm temperatures (17, 24 and 30 °C).

Experiment 3 : c-GtH concentration was not modified 15 mn and 6 h after the fish received a thermal shock, but a significant increase (9 ng/ml) ($P < 0.05$) was observed seven days later. No mortality occurred following thermal shock.

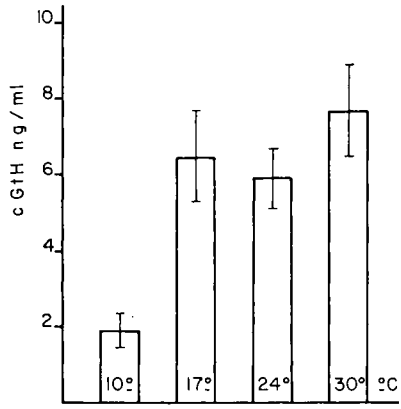

FIG. 3.

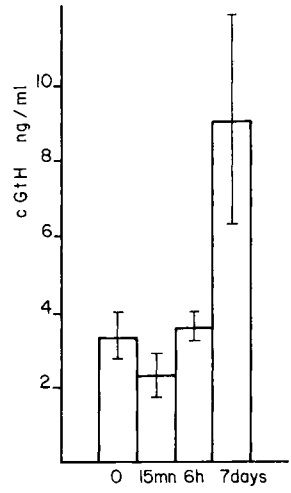

FIG. 4.

FIG. 3. — *Experiment* 2. *Variation in c-GtH titers in goldfish subjected to a progressive rise of* 1 °C *per day of rearing temperature in february.* Initial controls at 10 °C, experimental temperatures : 17, 24, 30 °C.

FIG. 4. — *Experiment* 3. *Circulating c-GtH level in fish kept at* 10 °C *between february and may, then subjected to a* 10 °C *thermal shock by raising the temperature from* 10 °C *to* 20 °C.

Discussion.

In the three experiments a sudden or progressive rise in temperature considerably increased blood c-GtH either in February at initiation of gametogenesis or in May when it is in progress. In every case, c-GtH level reached 7 to 11 ng/mg which is 3 to 4 times more and significantly higher than in the group kept at 10 °C. In the group kept at a constant temperature but under rising photoperiod, gonadotropin elevation was very limited, suggesting that increasing temperature is more important than increasing photoperiod. This is in agreement with many observations and

experiments showing that in Cyprinids temperature is the most important environmental factor in the control of gonadotropin secretion (De Vlaming, 1972).

However, in the control group maintained at a constant rearing temperature of 10 °C, plasma gonadotropin increased between February and May. This may be due to changes in photoperiodism or to the stage of sexual development which is more advanced in May than in February, even in fish kept at constant rearing temperature of 10 °C. The response to a similar thermal shock was slightly different in February and May, but is not statistically significant. After the thermal shock in May, this stress did not induce a noticeable secretion in gonadotropin within 6 h. However, 7 days later c-GtH level was as high as in the other experiments when the rise in temperature was more progressive. Thus, the stimulation of secretion does not seem to be due to stress, but to a true stimulation of the gonadotropic activity of the hypothalamo-pituitary complex. Other investigations have shown that temperature-stimulated secretion of gonadotropin is not continuous but lasts 3 months (Gillet *et al.*, 1977).

Within certain limits, raising rearing temperature accelerates gonad maturation in goldfish (Sasayama and Takahashi, 1972), but the gonads regress when fish are kept at a warm rearing temperature for more than 3 months (Gillet *et al.*, 1977).

In conclusion, a rise in the rearing temperature goldfish strongly stimulates gonadotropin secretion.

Reçu en janvier 1977.
Accepté en février 1977.

Acknowledgment. — This work was supported by the EEC (grant N° 045-74-1 ENV F) and the EDF (grant n° 8375 F). Editorial assistance of Mrs. Jacqueline Marcel is gratefully acknowledged.

Résumé. Des poissons rouges adultes placés en photopériode artificielle et croissante sont soumis à une élévation progressive de leur température d'élevage entre février et mai. D'autres subissent une élévation de température rapide, soit en février lors de l'initiation de la gamétogenèse ou en mai lorsque les animaux sont en pleine gamétogenèse. La gonadotropine plasmatique (c-GtH) est dosée par une méthode radioimmunologique. Lorsque la température s'élève de 10 à 20 ou 30 °C, il se produit un accroissement significatif de la c-GtH immunoréactive. Dans les groupes d'animaux maintenus sous photopériode croissante et soumis à des températures d'élevage constantes (10 °C) ou croissantes (passage de 10 à 20 °C entre février et mai) les niveaux circulant de c-GtH augmentent significativement, mais l'augmentation est plus marquée pour le lot soumis à l'élévation de température ($P < 0{,}005$). Ceci suggère la possibilité d'une action combinée de la température et de la photopériode sur la sécrétion de c-GtH.

References

AHSAN S. N., 1966. Effects of temperature and light on the cyclical changes in the spermatogenesis activity of the lake chub *Couesius plumbeus* Agassiz. *Can J. Zool.*, **44**, 161-171.

BILLARD R., 1968. Influence de la température sur la durée et l'efficacité de la spermatogenèse de Guppy *Poecilia reticulata*. *C. R. Acad. Sci. Paris, série* D, **266**, 2287-2290.

BRETON B., KANN G., BURZAWA-GÉRARD E., BILLARD R., 1971. Dosage radioimmunologique d'une hormone gonadotrope de Carpe *Cyprinus carpio*. *C. R. Acad. Sci. Paris, série D*, **272**, 1515-1517.

BRETON B., JALABERT B., FOSTIER A., BILLARD R., 1975. Etude sur le cycle reproducteur de la truite Arc-en-ciel et de la tanche. *J. Physiol.*, **70**, 561-564.

DE VLAMING V. L., 1972. Environmental control of teleost reproductive cycles : a brief review. *J. Fish. Biol.*, **4**, 131-140.

DE VLAMING V. L., 1974. Environmental and endocrine control of teleost reproduction. In C. B. SCHRECK, *Control of sex in fishes*, 13-83. Virginia Polytechnic Inst. and State University Blacksburg.

ECHELLE A. A., WILSON S., HILL L. G., 1973. The effects of few temperature daylight combinations on ovogenesis in the red river pup fish *Cyprinodon rubrofluviatilis* Cyprinodonticae. *Sthwest. Nat.*, **18**, 229-239.

EGAMI N., HYODO-TAGUCHI Y., 1967. An autoradiographic examination of rate of spermatogenesis at different temperatures in the fish *Oryzias latipes*. *Exp. Cell Res.*, **47**, 665-667.

GILLET C., BILLARD R., BRETON B., 1977. Effets de la température sur le taux de gonadotropine plasmatique et la spermatogenèse du poisson rouge *Carassius auratus*. *Can J. Zool.*, **55**, 242-245.

JALABERT B., BRY C., SZÖLLÖSI D., FOSTIER A., 1973. Etude comparée de l'action des hormones hypophysaires et stéroïdes sur la maturation *in vitro* des ovocytes de la truite et du carassin. *Ann. Biol. anim. Bioch. Biophys.*, **13**, hors série, 59-72.

LOFTS B., PICKFORD G. E., ATZ J. W., 1968. The effects of low temperature and cortisol on testicular regression in the hypophysectomized fish *Fundulus heteroclitus*. *Biol. Bull.*, **134**, 74-86.

SASAYAMA Y., TAKAHASHI H., 1972. Effects of starvation and unilateral castration in male goldfish *Carassius auratus* and a design of bioassay for fish gonadotropin using starved goldfish. *Bull. Fac. Fish. Hokkaido Univ.*, **22**, 267-279.

YAMAMOTO K., NAGAHAMA Y., YAMAZAKI F., 1966. A method to induce artificial spawning of goldfish all through the year. *Bull. Soc. Sci. Fish. Hokkaido Univ.*, **12**, 977-980.

WEIL C., BRETON B., REINAUD P., 1975. Etude de la réponse hypophysaire à l'administration de Gn-RH exogène au cours du cycle reproducteur annuel chez la carpe *Cyprinus carpio* L. *C. R. Acad. Sci. Paris, série D*, **280**, 2469-2472.