

## **Plasma estradiol-17 $\beta$ and gonadotropin during ovulation in rainbow trout (*Salmo gairdneri* R.)**

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**Summary.** A radioimmunoassay (RIA) for estradiol-17 $\beta$  (E2-17 $\beta$ ) with a chromatographic step has been tested on rainbow trout plasma. Overloading and dilution tests gave satisfactory results. Intracardiac injection of E2-17 $\beta$  (10  $\mu$ g/kg) in July gave a peak in the first 30 min after injection ; return to the basic level occurred within the next 24 hrs. Using RIA, trout-gonadotropin (t-GTH) and E2-17 $\beta$  were followed in trout plasma during the very last stages of the reproductive cycle. Oocyte maturity was determined simultaneously. t-GTH increase during maturation and ovulation was confirmed while E2-17 $\beta$  dropped before oocyte maturation to reach a low level when t-GTH was high.

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### **Introduction.**

Since egg yolk precursor synthesis is known to be stimulated in fish, like in other lower vertebrates, by estrogens (Chester Jones *et al.*, 1972) those have been almost only related to vitellogenesis. While this latter process is associated with high levels of plasma estrogen, low values have been found during the spawning season in salmonid females (Cedard, Fontaine and Nomura, 1961 ; Breton *et al.*, 1975a ; Whitehead, Bromage and Forster, 1977) ; similar results have been reported in other species (Eleftheriou, Norman and Summerfelt, 1968 ; Wingfield and Grimm, 1977). The highest levels of plasma gonadotropin have been detected in the same period, even in ovulated fish (Crim, Meyer and Donaldson, 1973 ; Crim, Watts and Evans, 1975 ; Breton *et al.*, 1975a ; Jalabert *et al.*, 1976) but none of these studies have correlated estrogen and gonadotropin levels with accurate maturation stages at the end of the reproductive cycle. In the present work, we used frequent blood sampling for estradiol-17 $\beta$  (E2) and trout gonadotropin (t-GTH) determination combined with oocyte biopsy at different stages.

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## Material and methods.

**Blood and oocyte samples.** — Two-year old females weighing 500 to 800 g were kept in a recirculating water system (Petit, 1974) at 12 °C during December 1976 and submitted to natural photoperiod ; they were anesthetized with phenoxyethanol solution before sampling (Sehdev, McBride and Fagerlund, 1963). Blood was withdrawn from a caudal vessel using a syringe rinsed with sodium heparinate at 700 IU/ml. The sample was kept on crushed ice until centrifugation at 3 000 g for 15 min., the plasma was stored at — 20 °C.

Maturity stages were determined under binocular on fresh oocytes collected by abdominal stripping (Jalabert *et al.*, 1976).

**Estradiol-17 $\beta$  assay.** — Except for cyclohexane, analytical grade solvents were distilled before use ; glass and polypropylene tubes by LKB were silanized (water soluble, Silicad Clay-Adams). E2-17 $\beta$ -2, 4, 6, 7 (n)-<sup>3</sup>H was obtained from the Radiochemical Center (Amersham) ; purity was checked on thin-layer chromatography in chloroform-ethyl acetate system (80 : 20) with cold steroid as a reference (Steraloids). After adding 2 000 dpm E2-<sup>3</sup>H as recovery tracer to each sample, 100  $\mu$ l of plasma were extracted twice with 2 ml cyclohexane-ethyl acetate (50 : 50 v/v). After evaporation, the dry residue was dissolved in 200  $\mu$ l of benzene-methanol (85 : 15), transferred on a small Sephadex LH 20 column (80 mm  $\times$  5 mm), then rinsed with an additional 100  $\mu$ l. Elution was performed in the same solvent system ; the first 1.8 ml « estrone fraction » was discarded, and the next 2.4 ml « estradiol fraction » collected (adapted from Camerun and Jones, 1972). After evaporation, these fractions were

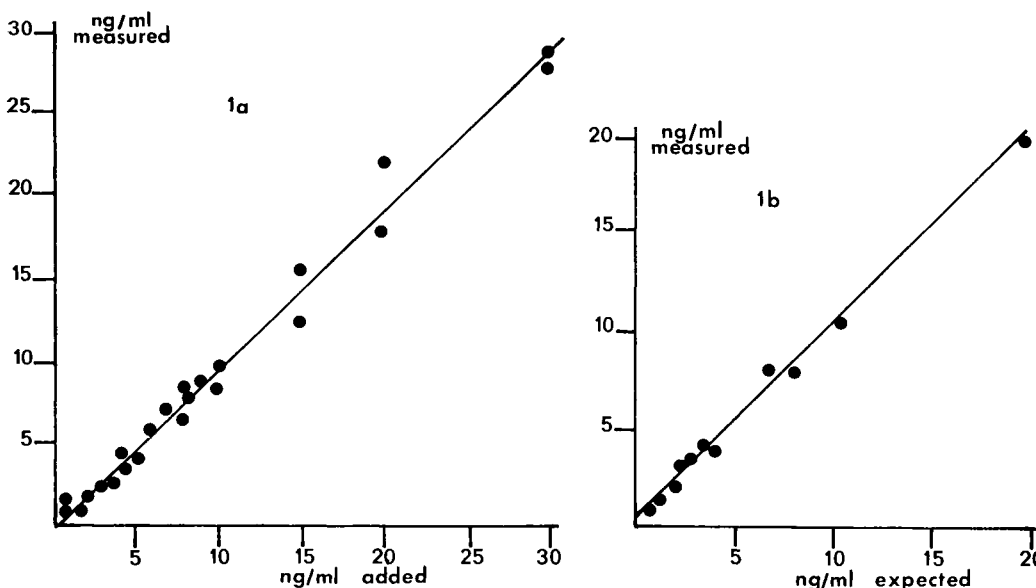


FIG. 1. — a) Overloading test :  $Y = 0.95 X - 0.14$   $\rho = 0.993$  ;  
 b) Dilution test :  $Y = 0.97 X - 0.49$   $\rho = 0.995$ .

dissolved in 500  $\mu$ l or 1 ml of phosphate buffer. Two aliquots of 100  $\mu$ l were used for the radioimmunoassay (RIA) ; 200  $\mu$ l or 600  $\mu$ l were counted for recovery. Double antibody RIA was then performed according to Terqui, Dray and Cotta (1973) using a rabbit anti-E2-17 $\beta$ -6-CMO-BSA (Dray *et al.*, 1971). After precipitation and centrifugation, the precipitate was counted according to Saumande and André (1975).

*Trout-gonadotropin assay.* — Gonadotropin was measured as described by Breton *et al.* (1971) using a pure trout gonadotropin as a standard against guinea-pig anti-SG-G-100.

## Results.

### Assessment of E2 RIA for trout plasma.

*Overloading tests.* — Increasing quantities of cold E2 (1 to 30 ng/ml) were added to different plasmas and measured for E2 (fig. 1a). The regression line slope between added and measured quantities does not differ from 1 and the zero intercept does not differ from 0 (variance analysis :  $p > 0.05$ ). Cold E2 was injected in the heart of 5 fish at 10  $\mu$ g/kg of body weight. In the first sample, a peak of E2 was detected in 3 fish after 10 min ; it was detected after 30 min. in the rest of the fish in the second sample. Following a regular decrease, the basic level was reached within 24 hrs (fig. 2).

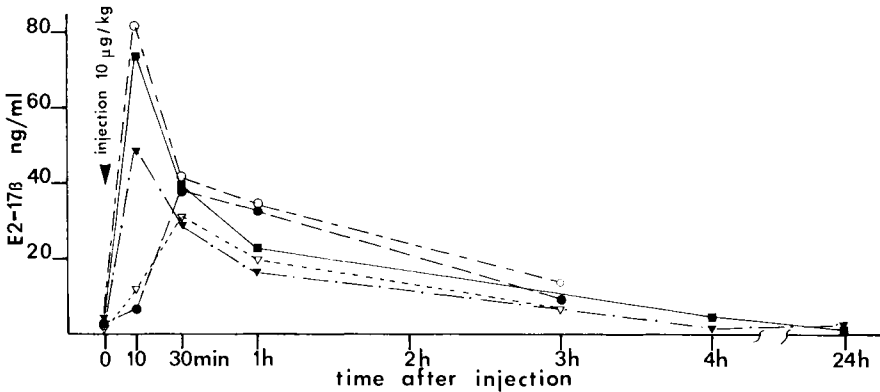


FIG. 2. — Profile of E2-17 $\beta$  in plasma after intracardiac injection of E2-17 $\beta$  (10  $\mu$ g/kg) in 5 females in July.

*Dilution test.* — Different plasmas were diluted (1/2, 1/3, 1/4, 1/6, 1/8) and each dilution measured for E2. The regression line slope between the expected and the measured quantities does not differ from 1 and the zero intercept does not differ from 0 (variance analysis :  $p > 0.05$ ). No difference is found when the slope of logit versus log dose curves, drawn with known quantities of cold E2 (standard curve) or serial plasma dilution, are compared (covariance analysis :  $p > 0.05$ ). There is therefore no immunological difference between pure E2 and measured E2.

*E2 ant t-GTH during ovulation.*

In a first experiment, 64 fish were divided into 7 groups according to maturity stage, and sampled once. In fish not ready to spawn, hormone levels were low ( $E2-17\beta = 2.8 \pm 0.2$  ng/ml ;  $t-GTH = 2.6 \pm 0.2$  ng/ml). In the other groups,  $E2-17\beta$  decreased before meiotic maturation while  $t-GTH$  rose during the last stages (fig. 3). Unequal variance analysis shows a very highly significant difference ( $p < 0.005$ ) for the two hormones letween the subpopulations « end of vitellogenesis + migrating germinal vesicle » ( $E2 = 8.82 \pm 1.8$  ng/ml ;  $t-GTH = 4.0 \pm 0.7$  ng/ml), and « maturation without germinal vesicle breakdown (GVBD) + maturation with GVBD » ( $E2 = 2.6 \pm 0,3$  ng/ml ;  $t-GTH = 11.1 \pm 1.6$  ng/ml).

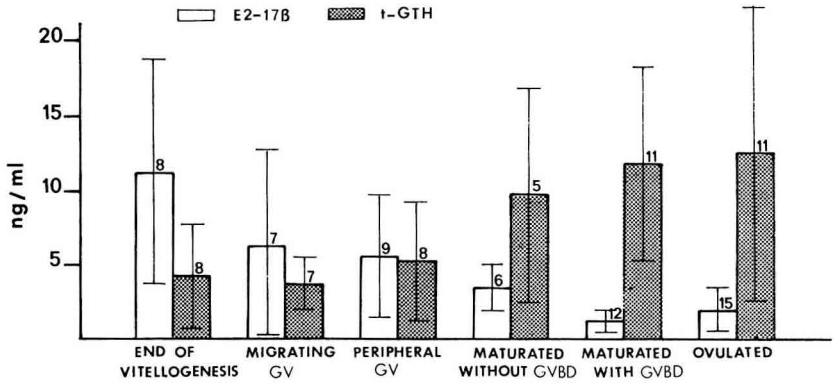


FIG. 3. — Plasma  $E2-17\beta$  (clear) and  $t-GTH$  (shaded) in female trout at different maturity stages (mean  $\pm$  SD).

In a second experiment, 10 fish with migrating GV oocytes were isolated and sampled every 2 or 3 days (fig. 4). When ovulation was detected, the same hormonal profiles as in the first experiment were observed, except for one fish (N° 8), showing a higher  $E2-17\beta$  level with a low  $t-GTH$  level.  $E2-17\beta$  remained high with low  $t-GTH$  levels in non-ovulating fish (N° 9 and 10).

**Discussion.**

RIA specificity is a difficult problem in new species where an unknown metabolite may cross-react with the antiserum. However, the LH-20 chromatographic step increases specificity, and measurement of diluted plasma shows that the substance being measured is immunologically  $E2$ .  $E2$  values, which are high as compared to mammals, confirm those described by other authors studying salmonides (Cedard, Fontaine and Nomura, 1961 ; Schreck, Lackey and Hopwood 1973 ; Whitehead, Bromage and Forster, 1977).

No ovulatory  $t-GTH$  peak has been detected in trout. This level increases slowly before ovulation and remains high afterwards. Such late high levels have been found in different salmonid species by Crim, Watts and Evans (1975) who suggest the role of

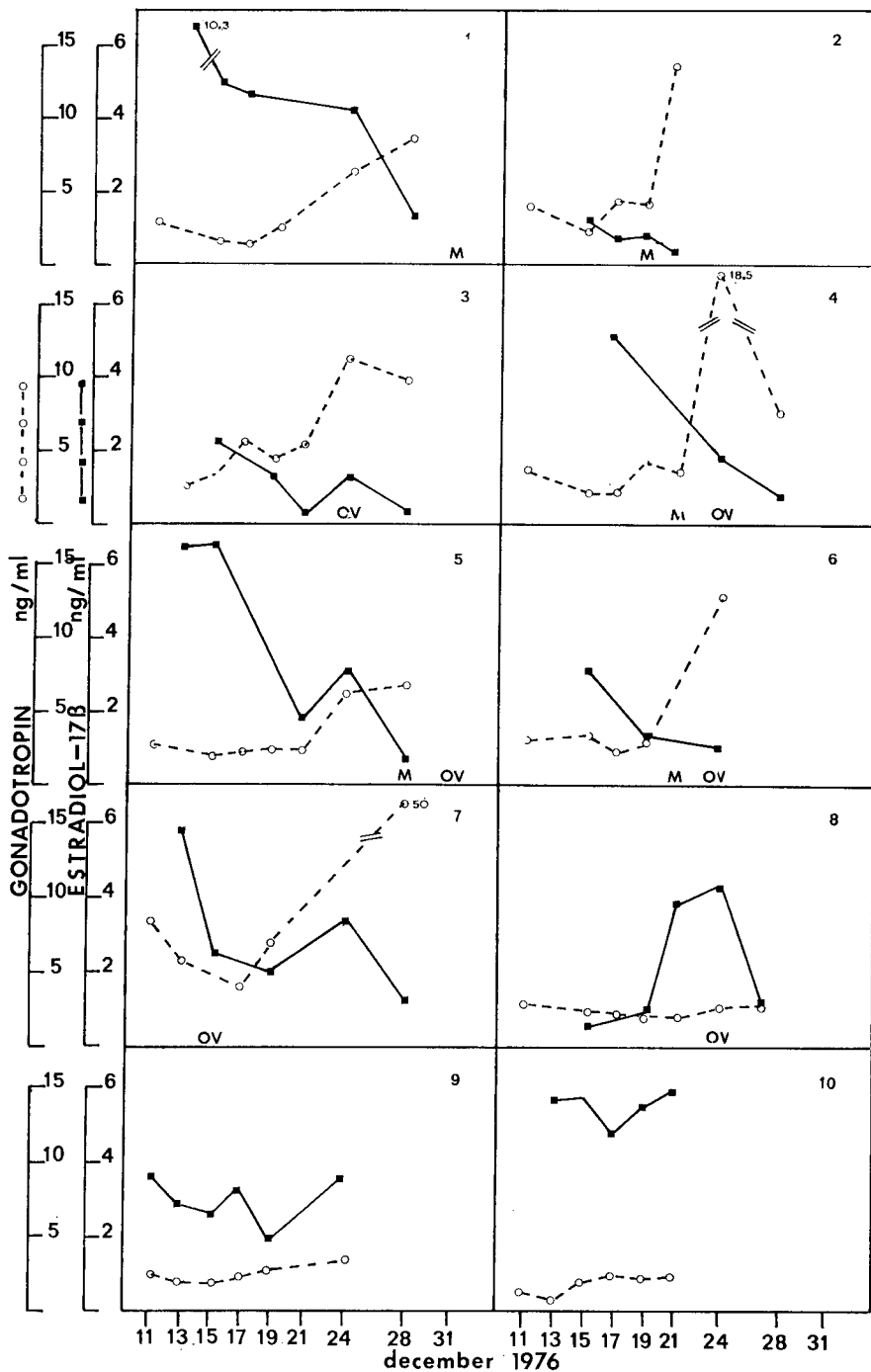


FIG. 4. — Individual plasma profiles of E2-17 $\beta$  (■—■—■) and t-GTH (○—○—○) in trout during ovulation. (M : maturation ; OV : ovulation).

gonadotropin in spawning behavior. We cannot eliminate the possibility of pulsative discharges for both hormones, undetectable with the sampling frequency, and we must find an answer to the need for numerous samples during a very short time in fish. In any case, E2 and t-GTH patterns at the end of the cycle are different from those in mammals (Pelletier and Thimonier, 1975).

A negative feedback has been seen on ovary development in fish (De Vlaming, 1974) and an anti-estrogen, clomiphene citrate, stimulates carp gonadotropin secretion (Breton *et al.*, 1975b) and ovulation in goldfish (Pandey and Hoar, 1972) and loach (Ueda and Takahashi, 1976).

There is direct action on the ovary besides these indirect effects. In trout, estrogen depresses *in vitro* the gonadotropic effect on oocyte maturation (Jalabert, 1975); similar results were obtained with amphibian oocytes (Wright, 1961; Schuetz, 1972). No studies in fish permit us to generalize on the role of estrogen in cytoplasmic maturation, as has been suggested in mammals (Thibault, 1977).

TABLE 1  
Assessment of E2-17 $\beta$  RIA in trout plasma

Antibody specificity (1) .....	Estradiol 17 $\beta$	100
	Estrone	11
	16 ketoestradiol 17 $\beta$	9
	16 epiestriol	8
	Estriol	< 0.5
	Estradiol-17 $\alpha$	< 0.5
	Testosterone	< 0.5
	11 ketotestosterone	< 0.5
	17 $\alpha$ -hydroxy-20 $\beta$ -dihydro Pg	< 0.5
Recovery for the chromatographic step (2) .....	E2 = 89 $\pm$ 9 p. 100 (n = 105)	
(LH 20 benzene-methanol 85/15) .....	E1 = 8.5 $\pm$ 0.4 p. 100 (n = 30)	
Total recovery (3) .....	E2 = 73 $\pm$ 7 p. 100 (n = 323)	
Sensitivity of the standard curve .....	5 pg/tube	
Usuable limits .....	20-800 pg/tube	
Blank (buffer) .....	< 5 pg/tube (n = 30)	
Variability (4)	Intra assay .....	X = 11.6 ng/ml SD = 0.5
	n = 5 .....	CV = 0.04
		X = 7.7 ng/ml SD = 0.3
		CV = 0.04
		X = 4.6 ng/ml SD = 0.2
		CV = 0.05
	Inter assay .....	X = 3.1 ng/ml SD = 0.5
	n = 6 .....	CV = 0.15

(1) Defined as 100 x/y, where X is the weight of the unlabelled E2-17 $\beta$  and y the weight of the heterologous steroid required to produce 50 p. 100 inhibition of the binding of  $^3\text{H}$ -E2-17 $\beta$  tracer.

(2) Estimated by chromatography of 8 000 dpm E2-17 $\beta$ - $^3\text{H}$ .

(3) Estimated from samples.

(4) Means, standard deviations and coefficient of variation for 4 blood samples.

The regulation of gonadotropin secretion during spawning season in trout appears original. If E2-17 $\beta$  in laying hens has no effect on LH surge (Laguë, VanTienhoven and Cunningham, 1975), other steroids, and especially progesterone, can stimulate it (Wilson and Sharp, 1976). However, in trout, 17-hydroxy 20 $\beta$ -dihydro progesterone induces *in vivo* maturation and ovulation without raising t-GTH (Jalabert *et al.*, 1976). The drop in E2 may be a signal.

*Symposium sur la Reproduction des Poissons  
Paimpont, France, 19-21 septembre 1977.*

**Acknowledgements.** — We are grateful to Miss Aline Solari and Dr. B. Chevassus for advices on statistical analysis. English manuscript has been read by Mrs. Alice Daifuku.

**Résumé.** Un dosage radioimmunologique de l'œstradiol-17 $\beta$  comprenant une étape de purification chromatographique a été éprouvé sur le plasma de truite Arc-en-ciel. Les tests de surcharge et de dilution plasmatique ont donné des résultats satisfaisants. Une injection intracardiaque d'E2-17 $\beta$  (10  $\mu$ g/kg) réalisée au mois de juillet provoque l'apparition d'un pic d'E2-17 $\beta$  dans les premières 30 mn après l'injection, puis le niveau de base est retrouvé au cours des 24 h suivantes. La gonadotropine de truite et l'EA-17 $\beta$ , dosées par radioimmunologie, ont été suivies dans le plasma de femelles pendant les tous derniers stades du cycle de reproduction. Simultanément, l'état de maturité des ovocytes a été déterminé. L'augmentation du niveau de t-GTH pendant la maturation et l'ovulation est confirmée, tandis que l'E2-17 $\beta$  chute avant la maturation méiotique pour atteindre un minimum quand la t-GTH est élevée.

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