Plasma Oestrogen Levels in Rainbow Trout
Salmo gairdneri Richardson

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Plasma oestrogen levels in rainbow trout *Salmo gairdneri* Richardson

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Levels of oestrogens in plasma of mature fall-spawning rainbow trout *Salmo gairdneri* were determined using a radio-immunoassay. No significant difference was found in oestrogen concentration between the sexes (male $\bar{x}=2{\cdot}5$ ng/ml; female $\bar{x}=4{\cdot}4$ ng/ml); between individual variability was great. Four blood samplings over a 24-h period via cardiac puncture of males revealed no diel variation or change in estrogen levels due to the stress of bleedings. Although no gonado-somatic index-estrogen relationship could be demonstrated for either sex, there was correlation between oestrogen and androgen levels in the female.

I. INTRODUCTION

There are few studies concerning levels of circulating oestrogens in fishes. Eleftheriou *et al.* (1966) reported channel catfish *Ictalurus punctatus* have an increase in free plasma oestrogens with an increase in ovarian weight. They also observed an increase in oestrogens from prespawning to the end of the spawning period. Cedard *et al.* (1961) demonstrated fluctuations in plasma oestrogens in Atlantic salmon *Salmo salar* of both sexes during maturation with levels highest in ripe fish and lowest in the post-spawned group. The levels of female sex hormone in rainbow trout determined on relatively large numbers of individuals under treatment conditions are reported. The use of a recent solid-phase radioimmunoassay (Abraham, 1969) allows quantification of oestrogens in minute plasma volumes, thus eliminating possible error of pooled samples. This technique allowed us to investigate the possibility of diel variation. The effects of stress due to repeated cardiac puncture blood sampling were also evaluated for the male trout. The relationship of gonadal development to endogenous plasma oestrogens was determined for both sexes. The radioimmunoassay for oestrogens has been shown to measure oestradiol-17β with oestrone and oestriol

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implicated to a lesser extent (Abraham, 1969). Testes and ovaries of rainbow trout reportedly contain oestradiol-17β, oestriol, and 16-epioestriol (Galzigna, 1961). The steroids were quantified as oestrogens.

II. METHODS AND MATERIALS

EXPERIMENTAL DESIGN

Two-year-old fall-spawning hatchery rainbow trout (300–400 mm) were bled via cardiac puncture during spawning season (October 1970). Blood (1·0–1·5 ml) was collected from males at 1800, 2400, 0600 and 1200 h during a 24-h period. Females were sampled only at 1800 h. Males were divided into two groups, referred to as ‘ terminals ’ and ‘ repeats ’. Terminals consisted of 28 fish sampled at 1800 h and 10 different fish sampled once and then killed at each of the other three times, respectively. Only fish from which adequate blood was obtained were used in the analysis (Table I). Terminal fish were not sampled more than once. The 20 repeat fish were bled once at each of the sampling times; i.e. each fish was bled four times, once every 6 h, if they survived the study period. Plasma samples were stored at −15° C. Fish were internally sexed and gonado-somatic indices (GSI) determined. The experimental design is also discussed by Schreck *et al.* (1972*b*).

TABLE I. Oestrogen levels (ng/ml) in plasma of rainbow trout and statistical analysis

Treatment and time	*n*	Mean	Range	S.D.
Terminal males				
1800	24	2·5	1·0–5·6	1·2
2400	9	2·0	1·1–3·1	0·7
0600	10	3·3	1·5–5·9	1·2
1200	9	2·8	1·5–4·1	0·8
Repeat males				
1800	17	2·4	1·0–4·3	0·9
2400	17	2·0	1·1–4·4	0·8
0600	11	2·4	1·1–3·6	0·9
1200	9	1·8	0·7–3·8	1·0
Repeat males (survivors)*				
1800	9	2·6	1·3–4·3	1·0
2400	9	2·2	1·1–4·4	1·0
0600	9	2·4	1·1–3·6	0·9
1200	9	1·8	0·7–3·8	1·0
Females				
1800	9	4·4	1·8–5·0	2·0

*Includes only the nine individuals which survived to be bled four times.

ASSAY

The oestrogen assay was essentially as reported by Abraham (1969) except that an anti-oestradoil-17β antibody prepared by Ekre & Foote (1971) was used at a dilution of 1 : 1000 to coat the plastic tubes. The specificity of the assay for several steroids was tested at five different levels, including physiological ranges. The antibody was unreactive to androgens (androstenedione, testosterone), progestins (progesterone, 17α-hydroxyprogesterone), and corticoids (cortisol, corticosterone) but bound oestradiol-17β and oestrone equally and oestriol slightly. The sensitivity of the method is 12–15 pg for estradiol-17β. Authentic oestradiol-17β at five different concentrations ranging from 12·5–400 pg were assayed 32 times each. Essentially no variability was found at each of the respective concentrations, indicating reproducibility of the method. Plasma was extracted twice with 2 ml diethyl-ether, and recoveries were adjusted as suggested by Abraham (1969) using ^{3}H-oestradiol-17β to estimate losses.

III. RESULTS AND DISCUSSION

No significant difference in oestrogen levels (Table I) between the sexes at 1800 h (male $\bar{x}=2{\cdot}54$ ng/ml; female $\bar{x}=4{\cdot}38$ ng/ml) could be demonstrated using a modified *t*-test to account for unequal variances ($t'=2{\cdot}49$, $\alpha=0{\cdot}05$). The non-significance may be the case only in a statistical sense, for the between individual variability (male $s^2=1{\cdot}44$; female $s^2=4{\cdot}0$) is too large to permit definite conclusions. This suggests that caution should be exercised in interpreting results of small or pooled samples. A similar situation was found for the plasma androgens in these fish. Although males had an average of 45·9 ng/ml and females had an average of 78·3 ng/ml circulating androgen, no significant difference could be shown (Schreck *et al.*, 1972*b*). Schreck *et al.* (1972*b*) also found that androgens in rainbow trout were similar between the sexes when the sexes were of similar maturity. These findings supported those of Cedard *et al.* (1961) who demonstrated that Atlantic salmon males had as much, or in certain cases perhaps more, oestrogen than females.

Not enough is known about fish reproductive endocrinology to allow definite statements concerning why the male trout can have as much oestrogen as the female or why the female may have as much androgen as the male. Two mechanisms may be tentatively proposed to explain this: (1) the metabolic machinery influencing sex steroid hormone production, action and elimination is inefficient, creating a surplus, or (2) in addition to sexual roles, the fish are using the steroids in some other, yet unknown, capacity. The action of sex hormones as anabolic agents deserves attention.

Other non-significant comparisons ($\alpha=0{\cdot}05$) indicate: (1) there is no apparent diel variation. The oestrogen levels of the terminal fish do not change over the sampling periods (one-way analysis of variance); (2) the oestrogen concentration of the repeat group is not changed by repeated cardiac puncture (one-way analysis of variance and complete randomized block design of the nine individuals surviving to the fourth bleeding); (3) no significant linear regression could be found between oestrogen levels and GSI's of males or females.

A significant positive correlation between oestrogen levels reported here and androgen levels for the same fish given by Schreck *et al.* (1972*b*) was found for the female ($r=0{\cdot}78$) but not the male. One possible explanation may be that there is a larger non-gonadal source of oestrogens in the male than in the female. While a positive androgen-GSI relationship exists for both sexes, we could not statistically demonstrate a similar correlation for the oestrogens. A marked stress-induced change in male sex hormone levels has been reported (Schreck *et al.*, 1972*b*), but none was apparent in the oestrogen levels of the trout. Perhaps the physiological range in levels of circulating oestrogens is small, thus any endogenous fluctuation may not be evident.

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