Fish Physiology vol. 3 no. 3 pp 121-126 (1987)
Kugler Publications, Amsterdam/Berkeley

Androgen levels and erythrocytosis in maturing brown trout, *Salmo trutta* L.

T.G. Pottinger and A.D. Pickering
Freshwater Biological Association, The Ferry House, Far Sawrey, Ambleside, Cumbria, UK

Keywords: brown trout, androgens, erythrocytes, erythropoiesis, sexual maturation

Abstract

The number of circulating erythrocytes was monitored in two strains of brown trout during the spawning season. Erythrocyte numbers were significantly elevated in mature male fish of both strains compared with mature females or immature fish. This period of erythrocytosis coincided with elevated plasma 11-keto-testosterone levels in mature male fish, was out of phase with the testosterone peak in males and was not observed in females despite high levels of plasma testosterone. The results are discussed with reference to the control of erythropoiesis in higher vertebrates.

Introduction

The brown trout, *Salmo trutta*, in common with all salmonids shows a distinct seasonal breeding cycle. In addition to physiological changes in the reproductive system itself, changes also occur in other tissues. Among these are significant and consistent alterations in the blood cell composition of maturing brown trout (Pickering 1986). These include an elevation in the number of circulating erythrocytes in mature male fish, compared with mature females or immature fish, and a marked lymphocytopenia in mature fish of both sexes. Hormonal control of the lymphocytopenic response has been considered elsewhere (Pickering and Pottinger 1986) but no attention has yet been given to the factors which might influence red blood cell numbers during sexual maturation.

In higher vertebrates, it is clearly established that the induction of red cell differentiation (erythropoiesis) is mediated by a glycoprotein, erythropoietin, secreted primarily by the kidney (see Goldwasser 1985) and limited evidence suggests that a similar system may operate in fish (Zanjani *et al.*1969; Weinberg *et al.* 1976). The stimulatory effect of androgens on the erythropoietic system in mammals is well documented (Naets and Witteck 1966; Fried and Gurney 1968) and it has been shown that the experimental administration of androgens can, under certain circumstances, stimulate erythropoiesis in fish (Slicher 1958; Garavini and Martelli 1978). As far as we are aware, however, no study has attempted to correlate changes in the natural androgen profile with erythropoiesis in fish. The present investigation examines the possibility that changes in erythrocyte numbers during sexual maturation in brown trout are related to the levels of gonadal steroids occurring at this time.

Materials and methods

Fish

Eight hundred one year old (1+) Hungerford strain, (mean weight 214 g ± 31 g), and 800 two

year old (2+) Ferry House strain brown trout (mean weight 267 ± 16 g) were maintained in 16 outdoor fibreglass tanks (1500 l), 100 fish of either strain per tank. Each tank was supplied with a constant flow of lake water (35 l min^{-1}). The fish were fed daily with commercial trout pellets at a rate of 1–2% body weight day^{-1} and treated daily with malachite green (2.27 ppm) to prevent fungal infection. Water temperature during the experimental period declined from 16°C in August to 2°C in February.

Sampling procedure

Four fish were removed from each tank at fourteen day intervals during the period August 1984 – February 1985, using a hand-net. Fish were anaesthetized in MS-222 (Sandoz, 100 mg l^{-1}) and blood samples taken from the caudal vessels by means of heparinized syringes. A 1.0–2.0 ml aliquot of blood from each fish was spun at 1000 g at 4°C for 15 mins and the resultant plasma stored at −70°C until required for radioimmunoassay. Further aliquots of blood were used to determine total and differential blood cell counts as described previously (Pickering 1986). After the removal of blood samples, each fish was weighed and measured before being killed by a blow to the head. The state of maturation of the gonads was determined by internal examination.

Radioimmunoassay

Plasma samples were assayed for testosterone and 11-ketotestosterone using previously validated techniques (Pottinger and Pickering 1985a). Estradiol was measured in a limited number of samples from mature female fish using the same assay protocol as for the androgens with an antiserum supplied by Dr Z. Yaron (Tel Aviv). For details of cross-reactivities see Bogomolnaya and Yaron (1984).

Statistics

Data for the erythrocyte counts were analysed by analysis of variance (Genstat) with time, tank, sex and state of maturation and their interactions as factors. A multiple regression approach was adopted because of the non-orthogonality of the data with respect to sex and state of maturation. Probability levels given in the paper are derived from these analyses, data are presented as arithmetic means ± SEM.

Results

Erythrocyte counts

Comparison of cell counts of mature 1+ males with immature fish and those of 2+ mature males with 2+ mature females revealed a significant sex/maturity × time interaction in each case ($p < 0.05$ and $p < 0.01$ respectively, Figs 1a and 2a). The interactions were resolved as higher erythrocyte counts in sexually mature males for a proportion of the spawning period (mid September–December, see Figs 1 and 2).

Hormone levels

11-Ketotestosterone levels in both 1+ and 2+ mature males rose during September and October from approximately 2.5 ng ml^{-1} to reach a peak at the end of October of approximately 30.0 ng ml^{-1}. Levels subsequently declined to less than 1.0 ng ml^{-1} by February (Figs 1b and 2b). Testosterone levels in 1+ and 2+ mature males rose steadily from 1.0–2.0 ng ml^{-1} during August to peak at 6.0–8.0 ng ml^{-1} during October before declining to less than 1.0 ng ml^{-1} by February (Figs 1b and 2b). Testosterone levels in mature 2+ females rose from approximately 5.0 ng ml^{-1} in August to peak at approximately 52.0 ng ml^{-1} at the end of October, before declining to less than 1.0 ng ml^{-1} at the end of December (Fig. 2c). Estradiol levels were measured in mature females from mid-October onwards, and declined from approximately 4.0 ng

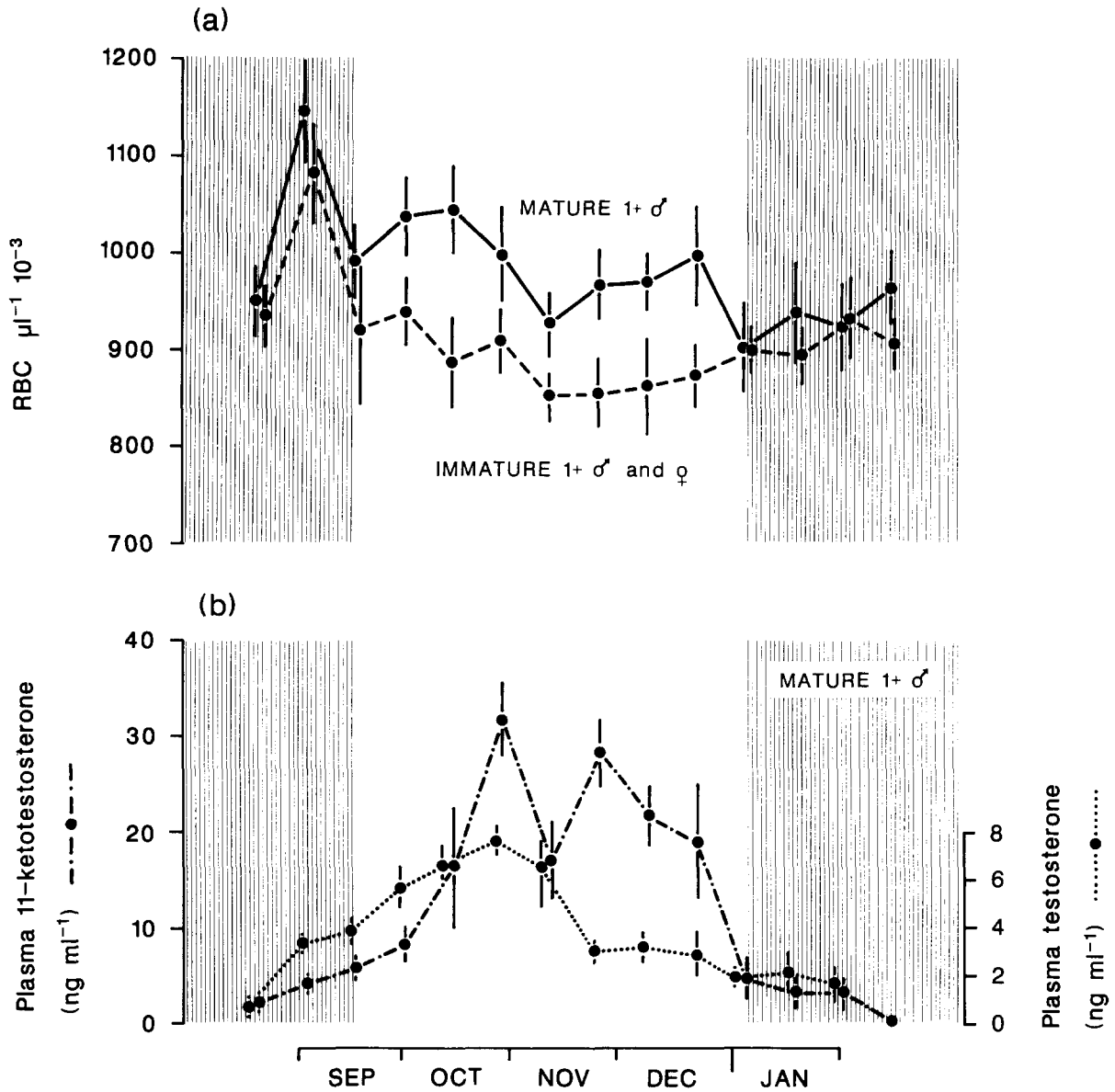


Fig. 1. (a) Changes in the number of circulating erythrocytes in 1+ Hungerford brown trout between August and February. Mature males are represented by -- • — immature fish by — — • — — Each point is the mean ± SEM (n = 5–9).

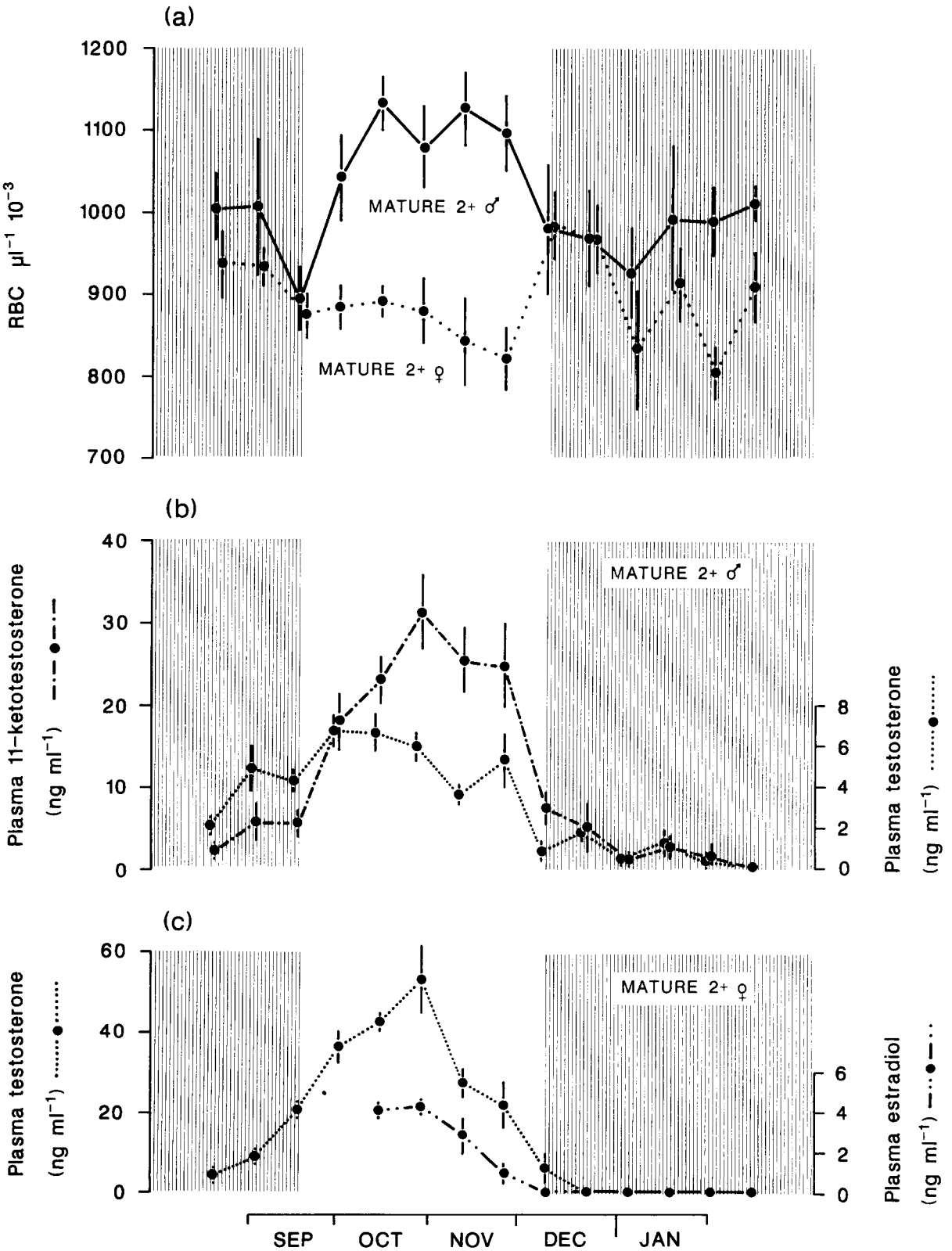
(b) Changes in plasma steroid levels in mature male 1+ Hungerford brown trout between August and February. Plasma 11-ketotestosterone is denoted by —·—•—·— plasma testosterone by · · · · • · · · · Each point is the mean ± SEM (n = 5–9)

The unshaded area indicates the period during which maximal differences in erythrocyte numbers occur.

ml^{-1} to less than 0.5 ng ml^{-1} by mid-December (Fig. 2c). Previous studies have shown that gonadal steroid levels in sexually immature brown trout remain low during the spawning period (Pottinger and Pickering 1985a).

Discussion

The present study demonstrates clearly that the increase in erythrocyte numbers observed in mature male brown trout during the spawning period oc-


(a)
RBC μl⁻¹ 10⁻³
1200
1100
1000
900
800
700
MATURE 2+ ♂
MATURE 2+ ♀
(b)
Plasma 11-ketotestosterone (ng ml⁻¹)
40
30
20
10
0
MATURE 2+ ♂
8
6
4
2
0
Plasma testosterone (ng ml⁻¹)
(c)
Plasma testosterone (ng ml⁻¹)
60
40
20
0
MATURE 2+ ♀
6
4
2
0
Plasma estradiol (ng ml⁻¹)
SEP
OCT
NOV
DEC
JAN

curs at a time when plasma androgen levels are elevated (mid September–December). Outside this period, no differences in erythrocyte number were discernible between mature males and mature females or immature fish of either sex. Throughout the period of study, no differences in neutrophil or thrombocyte count ascribable to sex or maturity occurred (Pickering 1986) but lymphocyte numbers in mature fish of both sexes declined (Pickering and Pottinger 1986). Therefore, the rise in erythrocyte numbers is not simply the result of a change in blood volume but a specific increase due to erythrocyte proliferation. That the haematological changes reported here are part of a normal seasonal cycle in brown trout is demonstrated by their consistency over the three successive spawning periods studied by Pickering (1986). In higher vertebrates, the stimulation of erythropoiesis by androgens is well documented (see Alexanian, 1969 and references therein) and, more pertinently, testosterone has been shown to increase the number of circulating erythrocytes in *Fundulus heteroclitus* (Slicher 1958) and to promote a rise in the proportion of erythroblasts in haematopoietic organs (kidney and heart) of *Ictalurus melas* (Garavini and Martelli 1978). In the present study however, the period of elevated erythrocyte numbers in mature male fish coincided more closely with peak levels of 11-ketotestosterone than testosterone (Figs 1 and 2). The testosterone peak was five-fold lower than that of 11-ketotestosterone and slightly preceded it. Furthermore testosterone levels in mature 2+ females were nearly twice as high as 11-ketotestosterone levels in mature males, and showed a very similar time-course (Figs 2b and 2c). Despite this, there were no detectable increases in erythrocyte numbers in mature female fish. Androgen levels reported in the present study are similar to those previously measured in brown trout (Kime and Manning 1982; Pottinger and Pickering 1985a). 11-Ketotestosterone, which, on a quantitative basis at least, is considered to be the major androgen in salmonids (Fostier *et al.* 1983), has been shown to stimulate secondary sexual characters in salmonids (Idler *et al.* 1961). Furthermore, 11-ketotestosterone is more effective than testosterone in stimulating at least one of these characters in the brown trout, the integumental changes associated with sexual maturation (Pottinger and Pickering 1985b). This, together with the evidence from the present study makes it reasonable to conclude that 11-ketotestosterone is also the most important androgen with regard to the stimulation of erythropoiesis. However, a further factor must also be taken into consideration. In some higher vertebrates the administration of estrogens is known to suppress erythropoiesis, and it has been suggested that estrogens are part of the regulatory mechanism controlling erythrocyte production (Dukes and Goldwasser 1961). An increase in estradiol levels occurs in maturing female salmonids, associated with vitellogenesis, and the levels measured in this study on the brown trout are similar to those reported for the same species by Breton *et al.* (1983). It is possible therefore, that a suppressive effect of estrogens contributes to the absence of a maturation-related erythrocytosis in the female brown trout.

In functional terms, the benefit to mature males of an elevation in erythrocyte numbers is presumably related to improved oxygen carrying capacity of the blood. An obvious inference is that the physical demands of spawning require an enhanced ability to deliver oxygen to the tissues, although this hypothesis presupposes that the observed erythro-

←

Fig. 2. (a) Changes in the number of circulating erythrocytes in 2+ FBA brown trout between August and February. Mature males are represented by — • — mature females by · · · • · · · . Each point is the mean ± SEM (n = 5 – 9).

(b) Changes in plasma steroid levels in mature male 2+ FBA brown trout between August and February. Plasma 11-ketotestosterone is denoted by — · — • — · — plasma testosterone by · · · · • · · · · Each point is the mean ± SEM (n = 5 – 9).

(c) Changes in plasma steroid levels in mature female 2+ FBA brown trout between August and February. Plasma testosterone is denoted by · · · · • · · · · plasma estradiol by · · — • · · — Each point is the mean ± SEM (n = 5 – 9).

The unshaded area indicates the period during which maximal differences in erythrocyte numbers occur.

cytosis is accompanied by a proportional increase in total haemoglobin levels. This explanation is supported by the work of Beamish (1964) who observed that in male brown trout standard oxygen uptake increased significantly during the spawning period. Furthermore, no increase in oxygen uptake occurred in mature female brown trout during the same period, and as we report here, no erythrocytosis occurs in the female.

In conclusion, the period of erythrocytosis observed in mature male brown trout closely parallels the maturational increase in plasma 11-ketotestosterone in males, is out of phase with the testosterone peak in males and is absent in females despite a high plasma testosterone titre. Thus, in contrast to higher vertebrates and the two fish species so far examined, it is suggested that maturity-related erythropoiesis in salmonids is stimulated by 11-ketotestosterone rather than testosterone. Further work is now needed on the effects of experimental administration of androgens and estrogens on erythropoiesis in salmonid fish if this hypothesis is to be validated.

Acknowledgements

The authors would like to thank Janet Pollard and Janice Fletcher for care and maintenance of the experimental fish, Margaret Hurley for statistical advice, Trevor Furnass for preparing the figures, and Dr Zvi Yaron (Tel Aviv) for kindly supplying estradiol antiserum.

References cited

Alexanian, R. 1969. Erythropoietin and erythropoiesis in anemic man following androgens. Blood 33: 564 – 572.

Beamish, F.W.H. 1964. Seasonal changes in the standard rate of oxygen consumption of fishes. Can. J. Zool. 42: 189–194.

Bogomolnaya, A. and Yaron, Z. 1984. Stimulation *in vitro* of estradiol secretion by the ovary of a cichlid fish, *Sarotherodon aureus*. Gen. Comp. Endocrinol. 53: 187–196.

Breton, B., Fostier, A., Zohar, Y., Le Bail, P.Y. and Billard, R. 1983. Gonadotropine glycoproteique et estradiol – 17β pendant le cycle reproducteur chez la truite fario (*Salmo trutta*) femelle. Gen. Comp. Endocrinol. 49: 220–231.

Dukes, P.P. and Goldwasser, E. 1961. Inhibition of erythropoiesis by estrogens. Endocrinology 69: 21–29.

Fostier, A., Jalabert, B., Billard, R., Breton, B. and Zohar, Y. 1983. The gonadal steroids. *In* Fish Physiology, vol. IXA, pp 277–372. Edited by W.S. Hoar, D.J. Randall and E.M. Donaldson. Academic Press, London.

Fried, W. and Gurney, C.W. 1968. The erythropoietic-stimulating effects of androgens. Ann. N.Y. Acad.Sci. 149: 356–365.

Garavini, C. and Martelli, P. 1978. Effect of testosterone on the erythropoiesis of the catfish (*Ictalurus melas*). Boll. Zool. 45: 383–387.

Goldwasser, E. 1984. Erythropoietin and its mode of action. Blood Cells 10: 147–162.

Idler, D.R., Bitners, I.I. and Schmidt, P.J. 1961. 11-Ketotestosterone: an androgen for sockeye salmon. Can. J. Biochem. Physiol. 39: 1737–1742.

Kime, D.E. and Manning, N.J. 1982. Seasonal patterns of free and conjugated androgens in the brown trout *Salmo trutta*. Gen. Comp. Endocrinol. 48: 222–231.

Naets, J.P. and Witteck, M. 1966. Mechanism of action of androgens on erythropoiesis. Am. J. Physiol. 210: 315–320.

Pickering, A.D. 1986. Changes in blood cell composition of the brown trout, *Salmo trutta*, during the spawning season. J. Fish Biol. In press.

Pickering, A.D. and Pottinger, T.G. 1986. Lymphocytopenia and interrenal activity during sexual maturation in the brown trout, *Salmo trutta* L. J. Fish Biol. In press.

Pottinger, T.G. and Pickering, A.D. 1985a. Changes in skin structure associated with elevated androgen levels in maturing male brown trout, *Salmo trutta* L. J. Fish Biol. 26: 745–753.

Pottinger, T.G. and Pickering, A.D. 1985b. The effects of 11-ketotestosterone and testosterone on the skin structure of brown trout, *Salmo trutta* L. Gen. Comp. Endocrinol. 59: 335–342.

Slicher, A.M. 1958. Seasonal changes in the erythrocytes of *Fundulus heteroclitus*, with observations on the effects of hypophysectomy and replacement therapy with methyl testosterone. Anat. Rec. 132: 508–509.

Weinberg, S.R., LoBue, J., Siegel, C.D.and Gordon, A.S. 1976. Hematopoiesis of the kissing gourami (*Helostoma temmincki*). Effects of starvation, bleeding, and plasma-stimulating factors on its erythropoiesis. Can. J. Zool. 54: 1115–1127.

Zanjani, E.D., Yu, M.-L., Pearlmutter, A. and Gordon, A.S. 1969. Humoral factors influencing erythropoiesis in the fish (Blue Gourami – *Trichogaster trichopterus*). Blood 33: 573–581.