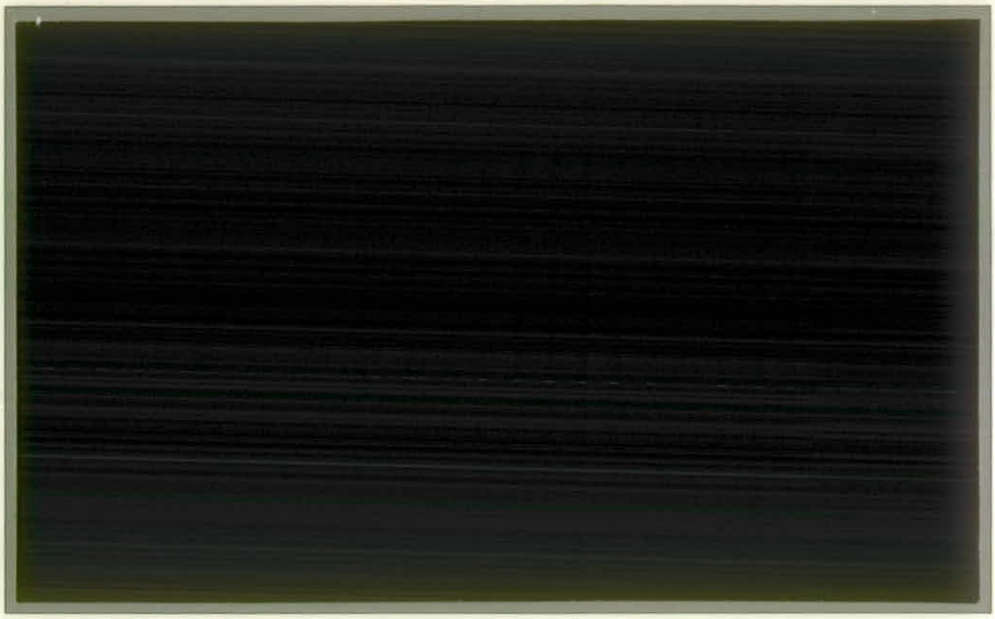
T11050h5/1

APRIL 1990

Institute of Freshwater Ecology



Natural Environment Research Council

INSTITUTE OF FRESHWATER ECOLOGY

Windermere Laboratory, Far Sawrey, Ambleside, Cumbria, LA22 OLP

The physiology of stress responses in freshwater fish

Final report for the period

1 April 1985 to 31 March 1990

A. D. Pickering and T.G. Pottinger

Project Leader: A.D. Pickering

Report date : April 1990

Report to : Ministry of Agriculture, Fisheries and Food

IFE Report Ref: W1/T11050h5/1

TFS Project No: T11050h5

CONTENTS

SUMMARY

1. Plasma cortisol levels are elevated in salmonid fish subjected to all forms of environmental stress.
2. In the short-term, this is an adaptive response but under chronic conditions, elevated cortisol levels may be maladaptive.
3. Chronic cortisol elevation is directly responsible for many of the damaging effects on survival (disease resistance), growth and reproduction.
4. Rainbow trout appear to be less sensitive than either brown trout or Atlantic salmon to environmental stress. However, this may reflect strain differences rather than genuine species differences.
5. A series of guidelines for fish farmers has been developed to minimize stress-induced damage under aquaculture conditions.
6. Current work is directed towards selecting strains of rainbow trout with a reduced cortisol response to common aquacultural stresses.

I INTRODUCTION

The term 'environmental stress' is frequently used to describe those conditions detrimental to the state of well-being of an animal, although it is a difficult concept to define in precise terms (Pickering, 1981). In the past, considerable attention has been given to conditions which are directly lethal to fish and, consequently, the factors leading to sudden fish kills are usually well-understood. However, most environmental disturbances are not directly lethal, yet can still damage fish populations by increasing their susceptibility to disease, by reducing fish growth and by interfering with reproductive processes. Such disturbances, or stresses, may take the form of sudden temperature or pH changes, water quality deterioration, sublethal pollution or stresses associated with intensive fish husbandry. Indeed, it has been suggested that most outbreaks of infectious diseases in the expanding aquaculture industry are related to poor husbandry techniques. An understanding of the basic physiological and endocrinological processes occurring in fish subjected to such types of stress is, therefore essential if we are to avoid or minimize the worst consequences of stress on the fish's performance capacity (survival, growth and reproductive success).

This report summarizes the results of a research programme, part-funded by the Ministry of Agriculture, Fisheries and Food (Project NBB 13) from 1 April 1985 to 31 March 1990, designed to provide such an understanding of the response of salmonid fish to various forms of environmental fish.

II INITIAL OBJECTIVES OF THE RESEARCH

1. The development of techniques capable of measuring physiological stress responses without the inherent problems of changes related to the processes of experimentation themselves.

2. Elucidation of the links between the immediate physiological/endocrinological changes occurring in stressed fish and their ultimate expression in terms of survival, growth and reproduction.

3. To investigate the possibility of using changes in the physiology or endocrinology of the fish as a predictive indicator of longer-term survival probabilities (ie. as an 'early warning system').

4. To develop methods for controlling the stress response or for minimizing the subsequent damage to the fish.

III BACKGROUND

Studies on the brown trout, Salmo trutta, prior to 1985 had established several important facts. It is possible, by means of careful experimental design and with sufficient tank to tank replication, to measure some of the more sensitive and rapid changes that occur in stressed fish without the results being obscured by changes caused by disturbance of the fish during the sampling procedures - Objective 1 (Pickering et al., 1982). However, work of this type can only be successfully undertaken at an organisation such as the FBA (now the IFE) with extensive fish-rearing and experimental facilities. An invariable response of stressed fish to all forms of environmental stress is an activation of the hypothalamic-pituitary-interrenal axis

with a resultant elevation in the blood plasma of the steroid hormone, cortisol (Pickering & Pottinger, 1984, 1985b; Pickering and Stewart, 1984). This aspect of the stress response, in association with other hormonal changes, is believed to be of adaptive significance in the short-term by mobilizing energy reserves not normally available to the fish as it attempts to avoid and overcome the immediate threat. However, during chronic stress or under aquaculture conditions, where there is no escape, elevated cortisol levels may be maladaptive. Thus, administration of cortisol to otherwise unstressed fish causes a decrease in white blood cell counts (Pickering, 1984) and an increase in susceptibility to common forms of disease (Pickering & Duston, 1983; Pickering & Pottinger, 1985b). This link between elevated cortisol levels and disease (Objective 2) may explain why, in chronically-stressed fish, plasma cortisol levels may ultimately be reduced to basal values (acclimation) despite the continued presence of the stress (Pickering & Pottinger, 1985a; Pickering & Stewart, 1984). The use of elevated plasma cortisol levels as an indicator of stress (Objective 3) is further complicated by seasonal and diel rhythms of cortisol levels, by post-prandial feeding peaks (Pickering & Pottinger, 1983) and by changes associated with sexual maturity (Pickering & Christie, 1981). Interestingly, periods of chronic cortisol elevation during the later stages of sexual maturation are associated with lymphocytopenia (Pickering, 1986; Pickering & Pottinger, 1987) and an increase in the susceptibility of post-spawning fish to disease (Richards & Pickering, 1978; Pickering and Christie, 1980; Pickering & Willoughby, 1982 a,b). This increased susceptibility to disease is exacerbated by androgen-induced changes in the skin (Pickering, 1977; Pottinger & Pickering, 1985 a,b). Environmental stress can also activate other aspects of the pituitary gland, including the pars intermedia (Sumpter et al., 1985; Pickering et al., 1986).

The research programme was part-funded by MAFF from April 1985 and, against the above background of information on the stress response of brown trout, greater emphasis was then given to studies of the rainbow trout, Oncorhynchus mykiss (Walbaum), (formerly Salmo gairdneri Richardson) because this species forms the basis of the expanding aquaculture in England. However, for comparative purposes, some work on the brown trout was maintained. The rest of this report summarizes the work from 1985-1990 and is organized on the basis of subject matter, rather than as a chronological record. During this period, a total of 21 papers from the project have been published, or have been accepted for publication, and these are included as Appendix 1 at the end of the report.

IV ENDOCRINOLOGY OF THE RESPONSE OF SALMONID FISH TO STRESS

In qualitative terms, rainbow trout respond to various forms of environmental stress in a similar manner to brown trout. Thus, the hypothalamic-pituitary-interrenal axis is activated but the magnitude of the stress-induced cortisol elevation is, in general, lower than that of the brown trout (Pickering & Pottinger, 1989). However, this apparent species difference might well be the result of strain to strain differences in sensitivity to stress because, from a survey of 5 different strains of rainbow trout, we found one strain (Caribou) which consistently responded to a standard form of confinement stress with a higher cortisol response than the other 4 strains and, in this respect at least, was similar to the brown trout (Pickering & Pottinger, 1989). Clearly, further work on different strains of both domesticated and wild rainbow trout is needed if we are to resolve this problem. Regarding wild strains of fish, during the course of this work we had the opportunity to sample 2 natural populations of Salmo trutta and found that, contrary to speculative suggestions by other workers in this field,

wild brown trout had extremely low plasma cortisol levels (< 2 ng ml^{-1}) for much of the year. This agrees with our studies on hatchery-reared fish. Preliminary evidence, however, suggests that sea trout in Dale Park Beck (SD 353 927) may have significantly higher and more variable plasma cortisol levels than resident trout in Stainton Beck (SD 528 865) (Pickering & Pottinger, unpublished). Sexual maturation in wild fish, as in hatchery-reared fish, appears to be associated with elevated plasma cortisol levels.

For a steroid hormone, such as cortisol, to have a physiological effect it must first combine with specific hormone receptors in the cells of the target tissues. In rainbow trout, continuous (chronic) confinement causes the expected elevation of plasma cortisol levels but the cortisol binding capacity of one of the principal target organs, the liver, is significantly reduced within 48 h of confinement (Pottinger, 1990). Moreover, implantation of physiological doses of cortisol into otherwise unstressed fish also caused a reduction in hepatic cortisol binding capacity. In view of the potentially damaging effects of cortisol on the defence systems, growth processes and reproductive physiology (see below), this 'down-regulation' of target tissue sensitivity might represent an acclimatory response prior to the ultimate reduction of plasma cortisol levels in chronically-stressed fish (Pickering & Pottinger, 1989). Clearly, this is an important area for further study because change in target tissue sensitivity is yet another form of control which must influence the physiological consequences of hormonal changes during the stress response of fish.

V STRESS AND DISEASE RESISTANCE

We have shown that chronic elevation of plasma cortisol in response to most forms of environmental stress is sufficient to cause a significant increase in the susceptibility of salmonid fish to disease. Thus there is a direct, and highly significant, correlation between mean plasma cortisol levels in hormonally-implanted brown trout and the mortality rate, due to disease, within the experimental population (Pickering, 1989a). Indeed, brown trout are so sensitive that chronic elevation of plasma cortisol from a basal value of < 2 ng ml^{-1} to only 10 ng ml^{-1} is sufficient to cause a significant increase in susceptibility to Saprolegnia infection, furunculosis and fin-rot (Pickering, 1989b). Rainbow trout also respond to plasma cortisol elevation with an increase in susceptibility to disease but evidence by Pickering et al., (1989) indicates that this species is less sensitive than the brown trout to physiological doses of cortisol. Nevertheless, a chronic stress such as social domination of one fish by another, under conditions of prolonged confinement, is capable of elevating plasma cortisol, reducing the number of circulating lymphocytes and increasing the susceptibility of rainbow trout to Flexibacter infection (Pickering & Pottinger, in preparation). Thus, the species difference again seems to be quantitative rather than qualitative. This comparative approach was extended to Atlantic salmon, Salmo salar, and we were able to show that during the fish's first winter in freshwater, the increased mortality rate was also associated with elevated plasma cortisol levels and reduced white blood cell counts (Pickering & Pottinger, 1988). It seems likely, therefore, that the results of our studies can be applied, in principle, to all salmonid species.

The most important conclusion from this aspect of our work is that chronic plasma cortisol elevation, as a result of environmental stress, is a major factor in predisposing fish to common bacterial and fungal infections and to parasitic infestations. Moreover, this has been demonstrated at cortisol levels well within the physiological range for the species and at levels which have frequently been reported in the literature as being typical of 'unstressed' fish. It is clear, therefore, that the defence systems of salmonid fish are much more sensitive to chronically-elevated plasma cortisol levels than was hitherto realised. The quantitative relationship between plasma cortisol and mortality due to disease also suggests that from a knowledge of the state of activity of the hypothalamic-pituitary-interrenal axis and target tissue sensitivity to cortisol, one might be able to predict, and thus take steps to prevent, potential disease outbreaks (Objective 3).

VI STRESS AND GROWTH

We have demonstrated 3 possible hormonal pathways involved in the growth suppression of stressed, salmonid fish (Pickering, 1990 a,b). The catabolic action of elevated plasma cortisol levels is one mechanism which can suppress growth in fish. We have shown that cortisol elevation in both brown trout and rainbow trout causes a significant reduction in the coefficient of condition of the fish (Pickering et al., 1989), a result of the mobilisation of body reserves. Ultimately, growth rate is suppressed in cortisol-treated fish. However, growth suppression continues in chronically-stressed brown trout long after plasma cortisol levels have acclimated to basal values (Pickering & Stewart, 1984). Clearly, the control of growth processes in salmonid is a complex subject, probably involving many other aspects of the fish's endocrine system. One such aspect concerns the effect of environmental stress on the

levels of pituitary growth hormone circulating in the blood of rainbow trout. However, this study was the subject of a separate MAFF Commission and full details can be found in the Final Customer's Report (FBA Report Reference Number W1/272/1F, May 1988). In summary, it was already known that pituitary growth hormone is an important and highly effective growth promoter in salmonid fish and we have shown that an acute stress, such as short-term handling and confinement, is capable of suppressing circulating growth hormone levels in the rainbow trout. Paradoxically, chronic stresses such as overcrowding cause a significant elevation of growth hormone levels, an effect which we interpret as a physiological response to starvation in the stressed fish. In the short-term, however, stress might contribute to growth suppression by reducing circulating growth hormone levels.

A third hormonal pathway which could cause growth suppression, at least during certain parts of the animal's life cycle, is via the suppression of circulating anabolic steroids, such as some of the reproductive hormones. The effect of stress on androgens and oestrogens will be dealt with in the next section on reproduction. It is apparent that the control of growth processes is a complex subject involving many factors and ought to be the focus of continuing investigation, particularly in relation to recent advances in the techniques for measuring growth hormones, insulins and somatomedins (insulin-like growth factors) in fish.

VII STRESS AND REPRODUCTION

The investigation of endocrine processes governing the relationship between environmental stress and reproductive physiology of salmonid fish is a relatively new area of research, much of which originates directly from this MAFF Commission. Progress has been facilitated by

a fruitful collaboration with Dr. J. P. Sumpter and colleagues (Brunel University), a collaboration for which we are most grateful. Both acute and chronic stresses have been shown to suppress, significantly, the levels of circulating androgens, testosterone and 11-ketotestosterone, in sexually maturing male brown trout (Pickering et al., 1987; Sumpter et al., 1987). This effect could be mimicked by cortisol administration to otherwise unstressed fish (Carragher et al., 1989). Furthermore, the reproductive endocrinology of sexually maturing female trout (both brown and rainbow) was suppressed by cortisol treatment, resulting in a significant suppression of pituitary gonadotropin levels, of plasma oestradiol and testosterone and of circulating yolk precursors in transit from the liver to the ovary. The net effect after 3 weeks of cortisol treatment was a significant reduction in the size of the gonad in both male and female fish. Thus, cortisol elevation as a result of chronic stress is capable of seriously interfering with the maturational processes of salmonid fish. Many of the mechanisms linking the hypothalamic-pituitary-interrenal axis to the pituitary-gonadal axis require elucidation but in vitro studies at Brunel University have already shown that physiological doses of cortisol can suppress oestradiol release from the oocytes (Sumpter et al., 1987) and gonadotropin secretion from the pituitary gland (Carragher & Sumpter, 1990). Moreover, Pottinger & Pickering (1990) have demonstrated that in vitro cortisol administration reduces the number of oestradiol receptors in the liver of female rainbow trout (vitellogenin production by the liver is stimulated by oestradiol from the ovary of maturing fish). Thus, cortisol has the potential to suppress the pituitary-gonadal axis at several different sites. Future studies should attempt to elucidate the effects of environmental stress and cortisol elevation on the reproductive system in terms of the quantity and quality of the gametes.

VIII PRACTICAL MEASURES TO AVOID OR MINIMIZE THE DAMAGING EFFECTS OF STRESS

One of the requirements of MAFF was that the results of this work should be fed back to the aquaculture industry in the form of advice for avoiding or minimizing stress-related damage in the fish farm. As a consequence, papers have been given at several meetings, including the prestigious Aqua Nor meeting (Pickering, 1988) which is attended by large numbers of U.K. fish farmers, and a series of guidelines has been published in Trout News (Pickering, 1989c). A fuller version of these guidelines, with additional background information, has also been accepted for publication in Recent Advances in Aquaculture (Pickering, 1990b). The guidelines can be briefly summarized as follows:-

1. Minimize the duration of unavoidable stress.
2. Avoid stressing at high water temperatures.
3. Avoid multiple stresses at all times.
4. Use dilute salt solutions for transportation of freshwater fish.
5. Withdraw food prior to stressing (e.g. grading).
6. Use mild anaesthesia under extreme conditions.

In addition to producing these guidelines (many of which constitute good husbandry practice), we have also investigated the value of floating, overhead cover in attempting to minimize stress responses (Pickering et al., 1987a). Although ineffective with both brown trout and rainbow trout, the provision of overhead cover more than doubled the growth rate of underyearling Atlantic salmon, resulting in a doubling of the proportion of potential S1 smolts

(upper size mode at the end of the first summer's growth). Moreover, overhead cover halved the mortality rate due to disease of potential S2 fish (lower size mode parr) during their first winter in freshwater (Pickering & Pottinger, unpublished). It is not yet clear whether these beneficial effects of cover on Atlantic salmon, but not on brown trout or rainbow trout, represent a species or a strain difference. It is, perhaps, relevant that the brown trout and rainbow trout were from domesticated strains whereas the salmon were first generation from wild fish. Nevertheless, overhead cover is a valuable tool that the fish farmer should consider, particularly if breeding from wild stocks for restocking purposes.

IV FUTURE DEVELOPMENTS

This study has clearly shown that many of the damaging effects of environmental stress are mediated by chronically-elevated plasma cortisol levels. Consequently, it is appropriate to examine methods for reducing this aspect of the stress response or for blocking the action of cortisol on the target tissues. As already pointed out, the hormonal effects of cortisol (including its potentially damaging effects) are mediated via specific cortisol receptors. It is possible, by means of dexamethasone treatment, to block these receptors thereby inactivating the hypothalamic-pituitary-interrenal axis (pituitary ACTH secretion is ultimately controlled by a feedback mechanism involving cortisol receptors). Thus, dexamethasone treatment completely abolishes the stress-induced elevation of plasma ACTH and cortisol (Pickering et al., 1987b). However, dexamethasone is itself a potent synthetic corticosteroid and produces cortisol-like effects such as lymphocytopenia (Pickering et al., 1987b). It is, therefore, an inappropriate tool to minimize stress-induced damage on, for example, the fish's defence systems. Other synthetic steroids have since been developed for mammalian use and

one of these, RU 486, appears to bind effectively to the rainbow trout cortisol receptor (Pottinger, 1990). If this steroid behaves in fish as it does in mammals, it ought not to act as a corticosteroid in its own right and might, therefore, be useful in minimizing stress-induced damage. However, steroid treatment is not acceptable to the industry and this approach could only be used for research purposes. Nevertheless, it could provide a powerful tool for investigating other roles of cortisol in the stress response.

An alternative approach to minimizing the stress response is to attempt to develop strains which only produce limited amounts of corticosteroids in response to husbandry stresses, an approach which has been successfully adopted in the poultry industry. By means of careful, repeated screening of the cortisol response of individually marked rainbow trout, we have been able to identify fish which give a consistently low or a consistently high cortisol response to the stress of confinement (Pickering, Pottinger & Hurley, in preparation). Eggs from these fish have been fertilized and have recently hatched. The resultant offspring will be examined for differences in the magnitude of their cortisol response (evidence from Norwegian studies on the Atlantic salmon suggests that stress-induced cortisol elevation has a heritable component). If it is possible to develop new strains of fish with different physiological/endocrinological stress responses, further work will be required to monitor the performance of these fish (survival, growth, reproduction) under conditions of aquacultural stress. In addition, attention must also be given to the possibility of differences in target tissue sensitivity to cortisol. Clearly, this research is long-term in nature with a high basic science component.

ACKNOWLEDGEMENTS

Specific acknowledgements have been made in the original research papers on which this report is based. In addition, the authors are grateful to Mrs. Sybil Smethurst for manuscript preparation and to NERC and MAFF for financial support.

REFERENCES

Carragher, J.F. and Sumpter, J.P. (1990). The effect of cortisol on the in vitro secretion of gonadotropin by rainbow trout (Oncorhynchus mykiss) pituitary glands Gen. Comp. Endocrinol. (in press).

Carragher, J.F., Sumpter, J.P., Pottinger, T.G. and Pickering, A.D. (1989). The deleterious effect of cortisol implantation on reproductive function in two species of trout, Salmo trutta L. and Salmo gairdneri Richardson. Gen. Comp. Endocrinol. 76, 310-321.

Pickering, A.D. (1977). Seasonal changes in the epidermis of the brown trout Salmo trutta (L.). J. Fish Biol. 10, 561-566.

Pickering, A.D. (1981). Introduction : the concept of biological stress. In : Stress and Fish (A.D. Pickering, ed.) pp. 1-9. Academic Press. London and New York.

Pickering, A.D. (1984). Cortisol-induced lymphocytopenia in brown trout, Salmo trutta L. Gen. Comp. Endocrinol. 53, 252-259.

Pickering, A.D. (1986). Changes in blood cell composition of the brown trout, Salmo trutta L. during the spawning season. J. Fish Biol. 29, 335-347.

Pickering, A.D. (1988). Stress responses and disease resistance in farmed fish. Aqua Nor 1987, Trodheim, Proc. Conf. 3, 35-49.

Pickering, A.D. (1989a). Environmental Stress and the Survival of brown trout, Salmo trutta. Freshwat. Biol. 21, 47-55.

Pickering, A.D. (1989b). Factors affecting the susceptibility of salmonid fish to disease. Ann. Rep. Freshwat. Biol. Assoc. 57, 61-80.

Pickering, A.D. (1989c). Husbandry and Stress in fish. Trout News 8, 12-14.

Pickering, A.D. (1990a). Stress and the suppression of somatic growth in teleost fish. In : Progress in Comparative Endocrinology (in press).

Pickering, A.D. (1990b). Husbandry and Stress in Fish. In : Recent Advances in Aquaculture (in press).

Pickering, A.D. and Christie, P. (1980). Sexual differences in the incidence and severity of ectoparasitic infestation of the brown trout, Salmo trutta L. J. Fish Biol. 16, 669-683.

Pickering, A.D. and Christie, P. (1981). Changes in the concentrations of plasma cortisol and thyroxine during sexual maturation of the hatchery-reared brown trout, Salmo trutta L. Gen. Comp. Endocrinol. 44, 487-496.

Pickering, A.D. and Duston, J. (1983). Administration of cortisol to brown trout, Salmo trutta L., and its effects on the susceptibility to Saprolegnia infection and furunculosis. J. Fish Biol. 23, 163-175.

Pickering, A.D. and Pottinger, T.G. (1983). Seasonal and diel changes in plasma cortisol levels of the brown trout, Salmo trutta L. Gen. Comp. Endocrinol. 49, 232-239.

Pickering, A.D. and Pottinger, T.G. (1985a). Acclimation of the brown trout. Salmo trutta L., to the stress of daily malachite green treatment. Aquaculture 44, 145-152.

Pickering, A.D. and Pottinger, T.G. (1985b). Factors influencing blood cortisol levels of brown trout under intensive culture conditions. In : Current Trends in Comparative Endocrinology. (B. Lofts and W.N. Holmes, eds). pp. 1239-1242. Hong Kong University Press : Hong Kong.

Pickering, A.D. and Pottinger, T.G. (1985c). Cortisol can increase the susceptibility of brown trout, Salmo trutta L., to disease without affecting the white blood cell count. J. Fish Biol. 27, 611-619.

Pickering, A.D. and Pottinger, T.G. (1987). Lymphocytopenia and interrenal activity during sexual maturation in the brown trout, Salmo trutta L. J. Fish Biol. 30, 41-50.

Pickering, A.D. and Pottinger, T.G. (1988). Lymphocytopenia and the overwinter survival of Atlantic salmon parr, Salmo salar L. J. Fish Biol. 32, 689-697.

Pickering, A.D. and Pottinger, T.G. (1989). Stress responses and disease resistance in salmonid fish : Effects of chronic elevation of plasma cortisol. Fish Physiol. Biochem. 7, 253-258.

Pickering, A.D. and Stewart, A. (1984). Acclimation of the interrenal tissue of the brown trout, Salmo trutta L., to chronic crowding stress. J. Fish Biol. 24, 731-740.

Pickering, A.D. and Willoughby, L.G. (1982a). Saprolegnia infections of salmonid fish. In : Microbial Diseases of Fish (R.J. Roberts, ed.) pp. 271-297. Academic Press : London and New York.

Pickering, A.D. and Willoughby, L.G. (1982b). Saprolegnia infections of salmonid fish. Ann. Rep. Freshwat. Biol. Assoc. 50, 38-48.

Pickering, A.D., Griffiths, R and Pottinger, T.G. (1987a). A comparison of the effects of overhead cover on the growth, survival and haematology of juvenile Atlantic salmon, Salmo salar L., brown trout, Salmo trutta L., and rainbow trout, Salmo gairdneri Richardson. Aquaculture 66, 109-124.

Pickering, A.D., Pottinger, T.G. and Carragher, J.F. (1989). Differences in the sensitivity of brown trout, Salmo trutta L., and rainbow trout, Salmo gairdneri Richardson, to physiological doses of cortisol. J. Fish Biol. 34, 757-768.

Pickering, A.D., Pottinger, T.G. and Christie, P (1982). Recovery of the brown trout, Salmo trutta L., from acute handling stress : a time-course study. J. Fish Biol. 20, 229-244.

Pickering, A.D., Pottinger, T.G. and Sumpter, J.P. (1986). Independence of the pituitary-interrenal axis and melanotroph activity in the brown trout, Salmo trutta L., under conditions of environmental stress. Gen. Comp. Endocrinol. 64, 206-211.

Pickering, A.D., Pottinger, T.G., Carragher, J.F. and Sumpter, J.P. (1987b). The effects of acute and chronic stress on the levels of reproduction hormones in the plasma of mature male brown trout, Salmo trutta L. Gen. Comp. Endocrinol. 68, 245-259.

Pickering, A.D., Pottinger, T.G., Sumpter, J.P., Carragher, J.F. and Le Bail, P.-Y. (1990). Effects of acute and chronic stress on the levels of circulating growth hormone in the rainbow trout, Oncorhynchus mykiss. Gen. Comp. Endocrinol. (submitted).

Pottinger, T.G. (1990). The effect of stress and exogenous cortisol on receptor-like binding of cortisol in the liver of rainbow trout, Oncorhynchus mykiss. Gen. Comp. Endocrinol. (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.

Pottinger, T.G. and Pickering, A.D. (1990). The effect of cortisol administration on hepatic and plasma estradiol-binding capacity in immature female rainbow trout (Oncorhynchus mykiss). Gen. Comp. Endocrinol. (in press).

Richards, R.H. and Pickering, A.D. (1978). Frequency and distribution patterns of Saprolegnia infection in wild and hatchery-reared brown trout Salmo trutta L. and char salvelinus alpinus L. J. Fish Dis. 1, 69-82.

Sumpter, J.P., Pickering, A.D. and Pottinger, T.G. (1985). Stress-induced elevation of plasma α-MSH and endorphin in the brown trout, Salmo trutta L. Gen. Comp. Endocrinol. 59, 257-265.

Sumpter, J.P., Carragher, J.F., Pottinger, T.G. and Pickering, A.D. (1987). The interaction of stress and reproduction in trout. In : Reproductive Physiology of Fish (D.R. Idler, L.W. Crim and J.M. Walsh, eds.) pp. 299-302. Memorial University of Newfoundland : St. Johns.

APPENDIX I

Copies of the scientific papers published (or in the process of publication) as a result of this commission. Papers are bound in chronological order.

J. Fish Biol. (1987) 30, 363–374

Poor water quality suppresses the cortisol response of salmonid fish to handling and confinement

A. D. PICKERING AND T. G. POTTINGER

Freshwater Biological Association, Windermere Laboratory, The Ferry House, Far Sawrey, Ambleside, Cumbria LA22 0LP, England

(Received 23 July 1986, Accepted 19 September 1986)

Confinement of brown trout in small troughs of static water for 1 h at a density of six fish 25 l^{-1} stimulated the hypothalamic-pituitary-interrenal axis and resulted in an elevation of plasma cortisol from basal levels (less than 2 ng ml^{-1}) to about 100 ng ml^{-1}, the degree of stimulation being dependent upon water temperature. Confinement at a density of 30 fish 25 l^{-1} resulted in a 50% suppression of this response. It is demonstrated that this effect is mediated by changes in water chemistry and not by crowding *per se*. Experimental manipulation of the water chemistry showed that reduced pH (7·1→6·3), elevated free CO_2 (63→520 μmol l^{-1}) or elevated ammonia (8→1300 μg l^{-1} as total ammonia nitrogen) had no individual effects on the interrenal response to acute confinement. Elevated ammonia in combination with reduced pH significantly increased the plasma cortisol levels in response to acute confinement, whereas a combination of reduced oxygen (100→20% saturation), elevated free CO_2 and elevated ammonia markedly suppressed (≃50%) the cortisol response of both brown trout and rainbow trout to acute confinement in a manner similar to that observed with trout at high densities. A compensatory increase in plasma cortisol levels was observed during the subsequent recovery of fish which had been confined for 1 h in water of poor quality. These findings are discussed in relation to the exposure of fish to multiple stresses and to the role of corticosteroids in the stress response.

I. INTRODUCTION

Secretion of the steroid hormone cortisol by the interrenal tissue is a characteristic reaction of teleost fish to almost all forms of environmental stress (Donaldson, 1981). It is assumed that this represents part of a more general adaptive response (see Pickering, 1981) in which cortisol mobilizes, by virtue of its catabolic properties, stored food reserves, thereby enabling the fish to cope with increased energy demands (Dave *et al.*, 1979; Lidman *et al.*, 1979; Leach & Taylor, 1982; Paxton *et al.*, 1984). However, excessive or prolonged elevation of plasma cortisol levels may have deleterious effects on the fish's defence systems, with a resultant increase in the susceptibility of the fish to disease (Robertson *et al.*, 1963; Pickering & Duston, 1983: Pickering & Pottinger, 1985). This apparent conflict between advantageous and disadvantageous effects of interrenal stimulation has promoted an intense interest in the identification of factors which modify the activity of the hypothalamic-pituitary-interrenal (HPI) axis in fish.

To date, most studies have examined the effects of single environmental stresses under carefully controlled experimental conditions. Within this group of studies it has been shown that handling, confinement and water quality deterioration can all stimulate the HPI axis in teleost fish (e.g. Barton *et al.*, 1980; Swift, 1981; Tomasso *et al.*, 1981*a*; Pickering *et al.*, 1982). However, as pointed out by Leatherland & Sonstegard (1984), environmental stresses rarely occur in isolation, and much more attention needs to be given to the effects of combined stresses. Unfortunately, few studies have specifically addressed this problem. Tomasso *et al.*

0022–1112/87/030363+12 $03.00/0

(1981*b*) failed to find any additive or synergistic effects of ammonia and nitrite on the interrenal response of channel catfish, *Ictalurus punctatus*, exposed to pollutants, whereas Barton *et al.* (1985) reported an exaggerated interrenal response of the rainbow trout, *Salmo gairdneri*, to acute handling stress in fish previously stressed by chronic exposure to low pH.

The present investigation originates from a pilot study in which we observed that the mean elevation of blood cortisol levels in fish handled and confined at high densities was significantly lower than that in fish handled and confined at low densities (A. D. Pickering & T. G. Pottinger, unpubl.) thus raising the intriguing possibility that one form of stress (crowding) may suppress the effects of another form of stress (handling and confinement) on the HPI axis of salmonid fish. The present paper examines the effects of crowding and water quality deterioration on the interrenal response of brown trout, *Salmo trutta*, and rainbow trout to handling and confinement, a combination of stresses frequently encountered in aquacultural operations such as grading, transport and stocking.

II. MATERIALS AND METHODS

EXPERIMENTAL FISH

Seven hundred and forty-four 2-year-old brown trout (Dunsop Bridge strain) and 48 1-year-old rainbow trout (Annandale strain) were used during the present investigation. All the fish were sexually immature and the sex ratios (♂:♀) within the populations were 0·45 (brown trout) and 0·58 (rainbow trout). The fish were reared at the FBA's experimental fish hatchery in large (1500-l) outdoor fibreglass tanks each supplied with a constant flow of Windermere lake water (35 l min^{-1}). The fish were fed once daily with commercial trout pellets at 1–2% body weight day^{-1} (exact rate dependent upon temperature). Mean body weights (±S.D.) for the two species were 333·5±86·3 g (brown trout) and 203·9±38·1 g (rainbow trout). The water temperature during the experimental period (January–May, 1986) ranged from 1·9 to 9·1° C.

EXPERIMENT 1. EFFECTS OF CROWDING

A. The effects of crowding on water chemistry and on cortisol levels in brown trout during confinement stress

Thirty-six brown trout were taken from each of two rearing tanks and distributed between four small (60 × 40 × 25 cm) fibreglass troughs each containing 25 l Windermere lake water, so that two troughs each contained six fish and two troughs each contained thirty fish. The fish were confined under these conditions for a period of 1 h. Water samples were taken from each trough at 0, 15, 30, 45 and 60 min for subsequent chemical analysis (see below) and six fish were sampled from each trough at 60 min. The remaining fish were then returned to their rearing tanks. The sampled fish were rapidly anaesthetized in phenoxyethanol (Sigma, 500 mg l^{-1}) and blood samples were taken from the caudal vessels by means of heparinized syringes, stored in ice (up to 30 min) and centrifuged at 2000 rev min^{-1} in a refrigerated centrifuge. Plasma was stored at −70° C until assayed for cortisol (see below). Each fish was then killed by a blow to the head and weighed, measured and sexed. The whole experiment was then repeated using fish from two other rearing tanks.

B. The influence of water flow on the effect of crowding during confinement

An identical procedure (see above) was adopted except that the troughs used for confinement were adapted to receive a constant flow-through of Windermere lake water at a rate of 10 l min^{-1}.

C. The effect of post-confinement effluent on the response to confinement

Twelve brown trout were taken from each of two rearing tanks and distributed equally into four small fibreglass troughs (i.e., six fish trough^{-1}). Two of the troughs contained 25 l

TABLE 1. Comparison of certain aspects of the chemistry of untreated Windermere lake water, lake water containing brown trout for 1 h at a density of six fish $25l^{-1}$, lake water containing brown trout for 1 h at a density of 30 fish $25l^{-1}$ and artificially modified Windermere lake water (WLW). Values are arithmetic mean ± S.E.M. (*n*)

Medium	Stocking density fish $25l^{-1}$	O_2 saturation	pH	Free CO_2 ($\mu mol\ l^{-1}$)	Total NH_3.N ($\mu g\ l^{-1}$)
Windermere lake water	0	97·1 ± 0·47 (44)	7·07 ± 0·03 (44)	63 ± 4·6 (44)	8 ± 1·1 (44)
Windermere lake water	6	30·4 ± 1·51 (44)	6·35 ± 0·01 (44)	368 ± 14 (44)	429 ± 24 (44)
Windermere lake water	30	22·1 ± 1·6 (8)	6·31 ± 0·02 (8)	480 ± 43·2 (8)	1198 ± 169 (8)
Artificially modified WLW	0	21·6 ± 0·6 (16)	6·29 ± 0·04 (24)	526 ± 21·7 (16)	1325 ± 27 (20)

fresh Windermere lake water (no flow-through); the other two contained 25 l lake water which had held 30 brown trout during the preceding 1 h (i.e., effluent water). Water samples were taken from all four troughs at 0 and 60 min post-confinement and blood samples were taken from each fish (as described for Expt. 1A) at the end of the 60-min confinement period. Again, the whole experiment was repeated using fish from different rearing tanks.

EXPERIMENT 2. THE EFFECT OF ALTERATIONS IN WATER CHEMISTRY ON THE INTERRENAL RESPONSE TO HANDLING AND CONFINEMENT

The chemistry of Windermere lake water was artificially modified to reproduce those changes in O_2 concentration, pH, free CO_2 levels and total ammonia nitrogen (NH_3.N) that occurred when 30 brown trout were confined for 1 h in 25 l lake water (Table I). Oxygen concentration was reduced to ≈20% by passing nitrogen gas through lake water. Free CO_2 levels were increased to about 500 $\mu mol\ l^{-1}$ by adding 1 g $NaHCO_3$ to 25 l lake water and then reducing the pH to 6·3 with 0·1 N HCl. Total ammonia nitrogen was increased to ≈1300 $\mu g\ l^{-1}$ by the addition of 107 mg NH_4Cl to 25 l lake water. The experimental procedure was identical to Experiment 1C except that artificially modified lake water was used in two of the troughs instead of effluent water. Water chemistry parameters (low O_2, reduced pH, high free CO_2 and high NH_3.N) were examined separately and in various combinations for their effects on the interrenal response of brown trout to 1-h confinement. In addition, the effects of a combination of low O_2, high free CO_2 and high NH_3.N on the interrenal response of rainbow trout to acute confinement stress was also investigated.

EXPERIMENT 3. RECOVERY OF BROWN TROUT AFTER ACUTE CONFINEMENT AND POOR WATER QUALITY

Twelve groups of six fish were confined in either 25 l Windermere lake water or 25 l artificially modified lake water (low O_2, high free CO_2, high NH_3.N) for 1 h and then returned to large, outdoor rearing tanks for subsequent recovery. Samples of 12 fish from each treatment were taken (in two groups of six) at 20 min (during confinement), 1 h (immediately at the end of the period of confinement) and at 2, 4, 7 and 24 h post-confinement, for plasma cortisol determination. In view of the known effects of repeated sampling of individual rearing tanks on plasma cortisol levels in the brown trout (Pickering *et al.*, 1982) and the limited tank space available for this study, the experiment was carried out over a period of 4 days in such a way that no tank was sampled more than once during the recovery period. Care was taken to ensure that experimental fish (confined in artificially

modified lake water) were sampled at the same time as their appropriate controls (confined in natural lake water).

Water chemistry

Oxygen determinations were made with a Yellow Springs Instrument oxygen probe (Model 5739) calibrated immediately prior to use in air-saturated water; pH was measured with a Radiometer combined electrode (Model GK 2401C); and alkalinity was determined by potentiometric titration. Free CO_2 levels were then calculated from pH, alkalinity and temperature data (Mackereth *et al.*, 1978). Total ammonia nitrogen was determined using the indophenol-blue technique (Chaney & Marbach, 1962).

CORTISOL RADIOIMMUNOASSAY

Plasma samples were assayed for cortisol using a modification of the technique described by Pickering & Pottinger (1983). The full characteristics of the assay, which uses Steranti anti-cortisol-3 (CMO) HSA as antiserum, are given by Pickering *et al.* (in press).

STATISTICAL ANALYSES

Cortisol data were subjected to analysis of variance (Genstat) with stocking density (six or 30 fish $25l^{-1}$), water quality (Windermere lake water, modified lake water or effluent water), experiment number (duplicate experiments) and time (experiment 3) as factors. Troughs and fish were used as blocking effects to give a nested error structure with which to assess the significance of the factors and their interactions. From a plot of the residuals against fitted values appropriate transformations ($\sqrt{}$ or log) were selected, where necessary, to improve homogeneity of variance. The levels of significance given in the paper are derived from these analyses but, for ease of presentation, data are given as arithmetic means ± S.E.M. The degree of correlation between mean plasma cortisol levels in brown trout after 1 h confinement and environmental water temperature was measured by simple linear regression on the untransformed data.

III. RESULTS

Confinement of brown trout at a density of six fish $25l^{-1}$ for 1 h elevated plasma cortisol levels from basal levels (less than 2 ng ml^{-1}) to 87 ng ml^{-1}, whereas confinement at a density of 30 fish $25l^{-1}$ only elevated cortisol levels to 40 ng ml^{-1} [Fig. 1(a)]. This highly significant difference in response ($P<0·001$) was totally abolished when a continuous flow-through of fresh water was maintained to each of the troughs used for confinement [Fig. 1(b)], indicating that the original difference in response was related to changes in water chemistry and not to crowding *per se*. This was confirmed by experiment 1C in which the interrenal response of brown trout confined at a density of six fish $25l^{-1}$ in water which had previously contained 30 fish $25l^{-1}$ was significantly less ($P<0·001$) than that of fish confined at a similar density in fresh lake water [Fig. 1(c)].

Changes in water chemistry during the 1-h confinement period are illustrated in Fig. 2. As expected, O_2 depletion, pH drop and CO_2 and NH_3.N elevation were all more rapid and more severe in troughs containing 30 fish $25l^{-1}$ than in those containing six fish $25l^{-1}$. Based on these changes in water chemistry, natural lake water was modified by simple chemical manipulation (see Materials and Methods) to mimic those changes (with regard to O_2, pH, CO_2 and NH_3.N) that occur when 30 fish are confined for 1 h in 25 l water (Table I). Oxygen depletion alone (down to 20% saturation) produced a slight, but statistically significant ($P<0·01$), depression of the interrenal response of brown trout confined for 1 h at a density of six fish $25l^{-1}$ [Fig. 3(a)]. However, oxygen depletion combined with either high

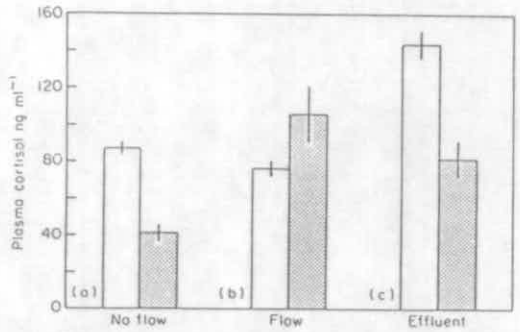


FIG. 1. Effect of crowding on the elevation of plasma cortisol levels in brown trout in response to handling and acute confinement for 1 h. Values are arithmetic means ± S.E.M. ($n = 24$); ambient water temperatures are indicated for each experiment. (a) Open column represents control fish confined in groups of six in small tanks each containing 25 l static Windermere lake water. Stippled column represents fish confined in groups of 30 in similar small tanks each containing 25 l static Windermere lake water. 4·1° C, $P < 0.001$. (b) As for (a) except that a constant flow of Windermere lake water (10 l min^{-1}) was maintained to both groups during the period of confinement. 4·0° C, N.S. (c) Open column represents control fish confined in groups of six in small tanks each containing 25 l static Windermere lake water. Stippled column represents experimental fish confined in groups of six in small tanks each containing 25 l static Windermere lake water which had contained 30 fish during the hour immediately preceding this experiment. 7·6° C, $P < 0.001$.

free CO_2 levels ($\approx 500\ \mu mol\ l^{-1}$) or high NH_3.N concentrations (up to $1300\ \mu g\ l^{-1}$) had no significant effect on the cortisol response to confinement (Fig. 3b, c). Reduced pH (down to 6·3), elevated CO_2, elevated NH_3.N, or a combination of elevated CO_2 and NH_3.N, also had no effect on the cortisol response to confinement [Fig. 3 (d)–(g)]. Reduced pH combined with elevated NH_3.N caused a highly significant ($P < 0.001$) increase in plasma cortisol levels in response to confinement [120 ng ml^{-1} cf. 75 ng ml^{-1}, Fig. 3(h)]. When brown trout were exposed to a combination of O_2 depletion, elevated free CO_2 levels (with associated pH drop) and elevated NH_3.N concentrations, the interrenal response to 1-h confinement was markedly suppressed to approximately 50% of that in control fish confined in natural lake water [Fig. 3(i), $P < 0.001$]. The magnitude of this suppression was very similar to that which occurred in brown trout confined at high stocking density (Fig. 1). In both cases, several of the fish showed an inability to maintain physical equilibrium, and all were generally less responsive to any form of handling. However, no stress-related mortalities occurred during subsequent recovery periods.

During the course of the study, it became apparent that the interrenal response of control brown trout to 1-h confinement varied significantly between experiments. Mean plasma cortisol levels after 1-h confinement showed a highly significant correlation ($P < 0.001$) with water temperature (Fig. 4), the sensitivity of the system being such that an increase in water temperature of 5° C (from 2 to 7° C) resulted in a doubling of the cortisol response to 1-h confinement from 60 ng ml^{-1} to 135 ng ml^{-1}.

The suppression of the interrenal response to acute confinement stress by exposure of the fish to a combination of low O_2, high free CO_2 and high NH_3.N

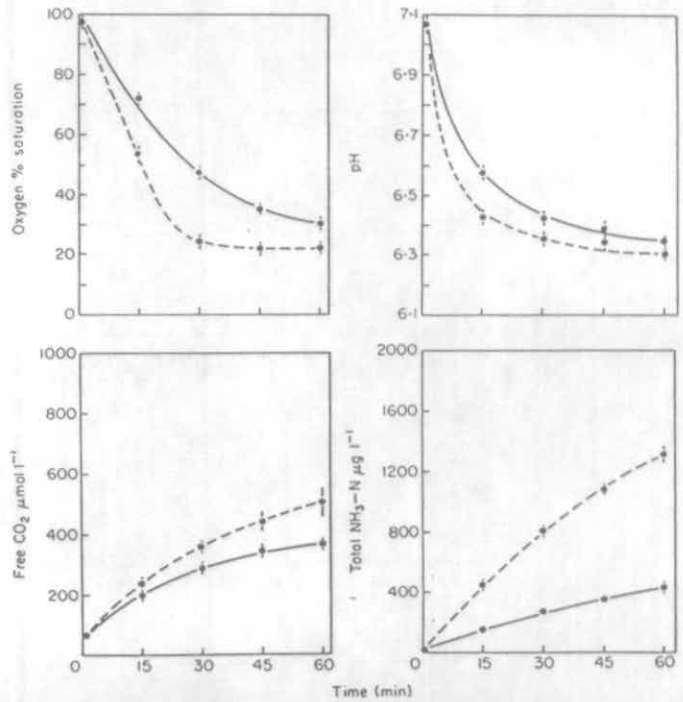


FIG. 2. Changes in water chemistry during 1-h confinement of brown trout in small tanks containing 25 l static Windermere lake water. Continuous lines represent trout confined in groups of six fish $25\ l^{-1}$, broken lines represent trout confined in groups of 30 fish $25\ l^{-1}$. Values are arithmetic mean ± S.E.M. ($n = 4$).

also occurred in rainbow trout. The mean plasma cortisol level in rainbow trout after 1-h confinement at a density of six fish $25\ l^{-1}$ in natural lake water was 120·6 ± 14·7 ng ml^{-1} compared with 45·4 ± 10·6 ng ml^{-1} ($n = 24$ in each case) for rainbow trout confined at a similar stocking density in artificially modified lake water ($P < 0.001$).

Monitoring the plasma cortisol levels of brown trout during confinement in artificially modified lake water and subsequent recovery showed that the interrenal response was suppressed during the whole of the confinement period (Fig. 5) but cortisol levels then rapidly increased during the first hour of recovery period to values similar to those of control fish (i.e., brown trout which were confined at six fish $25\ l^{-1}$ in fresh lake water). The subsequent decline in plasma cortisol (towards basal levels) during the next 24 h was similar in both groups (Fig. 5).

IV. DISCUSSION

It is well established that maintenance of fish at high stocking densities for prolonged periods of time results in a chronic stimulation of the HPI axis, with

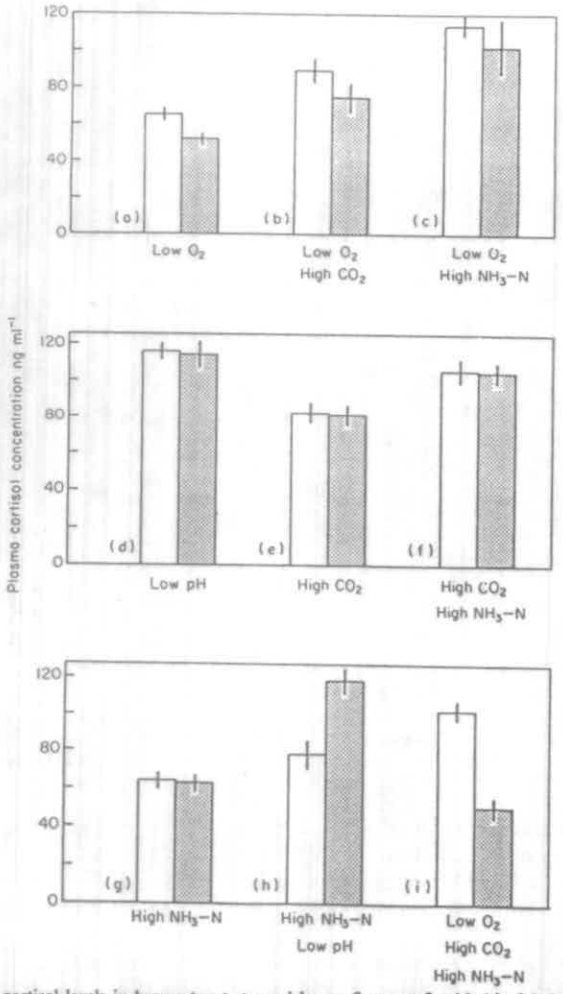


FIG. 3. Plasma cortisol levels in brown trout stressed by confinement for 1 h (six fish $25l^{-1}$ water) in Windermere lake water (open columns) and artificially modified lake water (shaded columns). Values are arithmetic means ± S.E.M. ($n=24$) and the water temperature for each experiment is indicated. (a) 2·0° C, $P<0.01$; (b) 3·6° C, N.S.; (c) 4·7° C, N.S.; (d) 5·3° C, N.S.; (e) 3·4° C, N.S.; (f) 4·8° C, N.S.; (g) 1·9° C, N.S.; (h) 3·6° C, $P<0.001$; (i) 4·2° C, $P<0.001$.

acclimation of the system to this form of stress requiring a time period ranging from days to weeks (Schreck, 1981; Pickering & Stewart, 1984). This effect of high stocking density has been shown to be independent of water quality (Pickering & Stewart, 1984). The present investigation has shown that high densities can also *suppress* the short-term activation of the HPI axis in acutely confined fish. In this

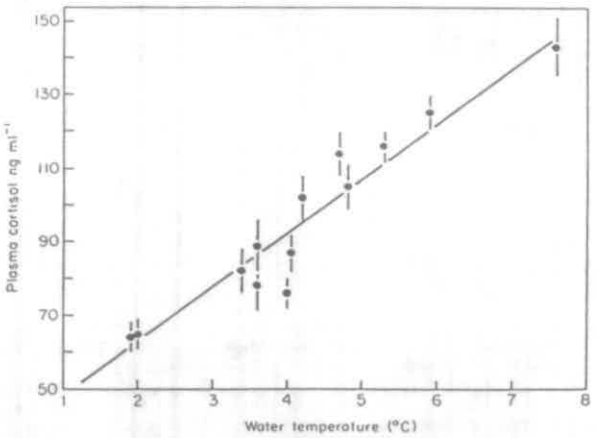


FIG. 4. Relationship between the mean plasma cortisol levels of brown trout stressed by confinement for 1 h (six fish $25l^{-1}$ water) in Windermere lake water and water temperature. Values are arithmetic mean ± S.E.M. ($n=24$). Linear regression analysis gave the formula $y=14{\cdot}7x+32{\cdot}9$, $r=0{\cdot}948$, $P<0{\cdot}001$.

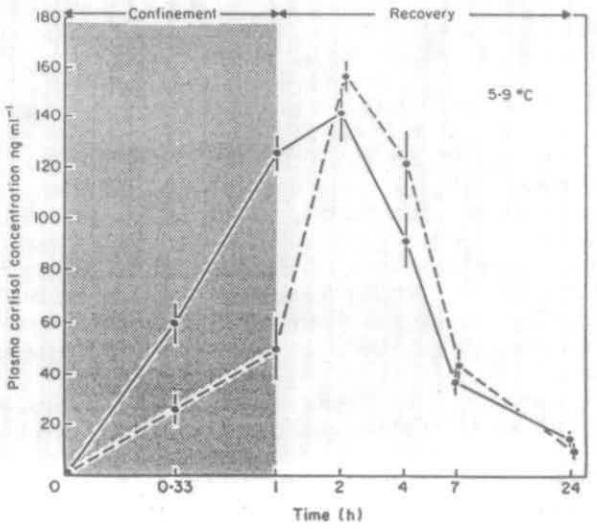


FIG. 5. Recovery of plasma cortisol levels in the brown trout following confinement for 1 h at a density of six fish $25l^{-1}$. The continuous line represents fish confined in Windermere lake water, the broken line represents fish confined in artificially modified lake water (low O_2, high free CO_2, high NH_3-N). Values are arithmetic mean ± S.E.M. ($n=12$).

case, however, the effect is mediated by changes in water chemistry and not by crowding as such. Matty (1985) reported that rainbow trout respond better to stress in 'conditioned' water: 'If the water in which trout are subject to thermal stress until heat death is used to thermal stress a second batch of trout then this second batch take longer for heat death to occur'. He implies that the mechanism behind this phenomenon involves some form of pheromone or chemical communication between the fish. On the other hand, Schreck (1981) failed to find any evidence of chemical communication between stressed and unstressed coho salmon, *Oncorhynchus kisutch*.

In the present study we have shown that the suppressive effects of high density on the cortisol response to acute confinement can be accurately reproduced by simple manipulation of the water chemistry and, consequently, there is no need to infer any form of pheromonal communication between fish. Reduced oxygen concentration alone can cause a minor suppression of the stress response to confinement, but suppression is markedly greater when the fish are also subjected to elevated free CO_2 and ammonia levels. In this respect, therefore, the chemical components of the environmental stress appear to act synergistically, but their overall effect on the response of the fish to acute confinement is suppressive. However, the present study has also shown that another combination of chemical stresses, reduced pH and elevated ammonia levels significantly increases the interrenal response of brown trout to acute confinement. In consideration of the studies by Tomasso *et al.* (1981*b*), this result is somewhat surprising because they demonstrated that reducing the pH of the water also reduces plasma corticosteroid levels in channel catfish exposed to elevated ammonia concentrations, an effect almost certainly mediated by the shift from NH_3 to NH_4^+, NH_3 being much more toxic to fish than NH_4^+ (see Smart, 1981).

These complex interactions between multiple environmental stresses with both synergistic and suppressive components emphasize the need for greater consideration to be given to experimental design in studies attempting to elucidate the role of the HPI axis in the stress response to teleost fish.

At the present time we can only speculate as to the possible mechanisms behind the suppressive effects of poor water quality on subsequent HPI sensitivity. With the exception of oxygen levels (down to 20% saturation), the changes in water chemistry during the study were well within the limits normally recommended for salmonid cultivation. For example, the maximum total ammonia nitrogen (NH_3.N) level of 1300 $\mu g\,l^{-1}$ at a pH of 6·3 and a temperature of 5° C corresponds to an unionized ammonia concentration of 0·325 $\mu g\,l^{-1}$, approximately 20% of the 'safe' level of 1·6 $\mu g\,l^{-1}$ recommended by the U.S. Environment Protection Agency (1976). Similarly, the maximum free CO_2 concentration of 526 $\mu mol\,l^{-1}$ (or 23·1 $mg\,l^{-1}$) during the present study is within the limits of free CO_2 concentrations known to occur in many U.K. fish hatcheries (Smart, 1981). However, there can be little doubt from our results that a combination of reduced O_2, elevated CO_2 and elevated ammonia produces a more profound suppression of the cortisol response to acute confinement than reduced O_2 alone. It is known that low oxygen may considerably increase the toxicity of ammonia to fish, probably by its effect on respiratory flow (Lloyd, 1961) and that ammonia reduces the oxygen-carrying capacity of the blood (Brockway, 1950; Sousa & Meade, 1977) but increases the overall oxygen consumption (Smart, 1978). Moreover, elevated CO_2

levels may also reduce the oxygen affinity and oxygen capacity of the blood (Alabaster *et al.*, 1957; Basu, 1959) resulting in a reduced efficiency of oxygen uptake from the environment (Saunders, 1962). Thus, considerable scope exists for interactions between reduced O_2, elevated CO_2 and elevated NH_3.N levels on the respiratory physiology of freshwater fish. However, it seems to us somewhat paradoxical that the result of combining what are presumably stressful factors is a suppression of HPI activity rather than an increase in activity.

Observations on the behaviour of brown trout and rainbow trout subjected to the triple combination of low O_2, elevated CO_2 and elevated NH_3.N indicated a lack of responsiveness to physical disturbance in a manner similar to that of fish under mild anaesthesia (McFarland, 1959). The importance of a sense of 'awareness' in the stimulation of the HPI axis has been emphasized by Schreck (1981), and it has been demonstrated that mild anaesthesia may ameliorate some of the physiological and endocrinological changes that occur during the stress response of fish (Strange & Schreck, 1978; Limsuwan *et al.*, 1983) although other studies have shown that anaesthetics may actually induce a stress response in otherwise unstressed fish (Wedemeyer, 1970; Davis *et al.*, 1982). It is possible that the water quality deterioration during the present investigation reduced the level of consciousness of the fish during acute confinement.

A further interesting observation during the present investigation was that blood cortisol levels in brown trout confined for 1 h in water of poor quality increased rapidly during the first hour of subsequent recovery in their rearing tanks to the levels found in control fish which had been confined in natural lake water. Thus, although the environmental stresses had been removed, it appears that there was still a requirement for cortisol during the subsequent recovery of homeostasis, possibly reflecting major physiological disturbances caused by the poor water quality. If this interpretation is correct, it follows that elevation of plasma cortisol levels is not a totally reliable indicator of the degree of physiological disturbance of salmonid fish during an acute stress.

The authors are particularly grateful to Mrs J. Pollard and Miss J. M. Fletcher for maintaining the experimental fish, Mr R. Griffiths for practical assistance, Mr E. Rigg and Mr N. Hetherington for the pH, alkalinity and ammonia determinations, and Mr T. I. Furnass for the artwork, and to NERC and MAFF for financial support.

References

Alabaster, J. S., Herbert, D. W. M. & Hemens, J. (1957). The survival of rainbow trout (*Salmo gairdneri* Richardson) and perch (*Perca fluviatilis* L.) at various concentrations of dissolved oxygen and carbon dioxide. *Ann. appl. Biol.* **45**, 177–188.

Barton, B. A., Peter, R. E. & Paulencu, C. R. (1980). Plasma cortisol levels of fingerling rainbow trout (*Salmo gairdneri*) at rest, and subjected to handling, confinement, transport and stocking. *Can. J. Fish. Aquat. Sci.* **37**, 805–811.

Barton, B. A., Weiner, G. S. & Schreck, C. B. (1985). Effect of prior acid exposure on physiological responses of juvenile rainbow trout (*Salmo gairdneri*) to acute handling stress. *Can J. Fish. Aquat. Sci.* **42**, 710–717.

Basu, S. P. (1959). Active respiration of fish in relation to ambient concentrations of oxygen and carbon dioxide. *J. Fish. Res. Bd Can.* **16**, 175–212.

Brockway, D. R. (1950). Metabolic products and their effects. *Progve Fish-Cult.* **12**, 127–129.

Chaney, A. L. & Marbach, E. P. (1962). Modified reagents for the determination of urea and ammonia. *Clin. Chem.* **8**, 130–132.

Dave, G., Johansson-Sjobeck, M.-L., Larsson, A., Lewander, K. & Lidman, U. (1979). Effects of cortisol on the fatty acid composition of the total blood plasma lipids in the European eel, *Anguilla anguilla* L. *Comp. Biochem. Physiol.* **64A**, 37–40.

Davis, K. B., Parker, N. C. & Suttle, M. A. (1982). Plasma corticosteroids and chlorides in striped bass exposed to tricaine methanesulfonate, quinaldine, etomidate, and salt. *Progve Fish-Cult.* **44**, 205–207.

Donaldson, E. M. (1981). The pituitary-interrenal axis as an indicator of stress in fish. In *Stress and Fish* (A. D. Pickering, ed.), pp. 11–47. London, New York: Academic Press.

Leach, G. J. & Taylor, M. H. (1982). The effect of cortisol treatment on carbohydrate and protein metabolism in *Fundulus heteroclitus*. *Gen. comp. Endocrinol.* **48**, 76–83.

Leatherland, J. F. & Sonstegard, R. A. (1984). Pathobiological responses of feral teleosts to environmental stressors: interlake studies of the physiology of Great Lakes salmon. In *Contaminant Effects on Fisheries* (V. W. Cairns, P. V. Hodson and J. O. Nriagu, eds), pp. 115–149. New York: John Wiley & Sons.

Lidman, V., Dave, G., Johansson-Sjobeck, M.-L., Larsson, A. & Lewander, K. (1979). Metabolic effects of cortisol in the European eel, *Anguilla anguilla* (L.). *Comp. Biochem. Physiol.* **63A**, 339–344.

Limsuwan, C., Limsuwan, T., Grizzle, J. M. & Plumb, J. A. (1983). Stress response and blood characteristics of channel catfish (*Ictalurus punctatus*) after anaesthesia with etomidate. *Can. J. Fish. Aquat. Sci.* **40**, 2105–2112.

Lloyd, R. (1961). Effect of dissolved oxygen concentrations on the toxicity of several poisons to rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **38**, 447–455.

Mackereth, F. J. H., Heron, J. & Talling, J. F. (1978). Water analysis: some revised methods for limnologists. *Freshwat. Biol. Ass. Sci. Publ. No.* **36**.

Matty, A. J. (1985). *Fish Endocrinology*. Beckenham, Kent: Croom Helm. 267 pp.

McFarland, W. N. (1959). A study of the effects of anaesthetics on the behaviour and physiology of fishes. *Publs Inst. Mar. Sci., Texas* **6**, 23–55.

Paxton, R., Gist, D. H. & Umminger, B. L. (1984). Serum cortisol levels in thermally acclimated goldfish (*Carassius auratus*) and killifish (*Fundulus heteroclitus*): implications in control of hepatic glycogen metabolism. *Comp. Biochem. Physiol.* **78B**, 813–816.

Pickering, A. D. (1981). Introduction: the concept of biological stress. In *Stress and Fish* (A. D. Pickering, ed.), pp. 1–9. London, New York: Academic Press.

Pickering, A. D. & Duston, J. (1983). Administration of cortisol to brown trout, *Salmo trutta* L., and its effects on the susceptibility to *Saprolegnia* infection and furunculosis. *J. Fish Biol.* **23**, 163–175.

Pickering, A. D. & Pottinger, T. G. (1983). Seasonal and diel changes in plasma cortisol levels of the brown trout, *Salmo trutta* L. *Gen. comp. Endocrinol.* **49**, 232–239.

Pickering, A. D. & Pottinger, T. G. (1985). Cortisol can increase the susceptibility of brown trout, *Salmo trutta* L., to disease without affecting the white blood cell count. *J. Fish Biol.* **27**, 611–619.

Pickering, A. D. & Stewart, A. (1984). Acclimation of the interrenal tissue of the brown trout, *Salmo trutta* L., to chronic crowding stress. *J. Fish Biol.* **24**, 731–740.

Pickering, A. D., Pottinger, T. G. & Christie, P. (1982). Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: a time course study. *J. Fish Biol.* **20**, 229–244.

Pickering, A. D., Pottinger, T. G. & Sumpter, J. P. (in press). On the use of dexamethasone to block the pituitary-interrenal axis in the brown trout, *Salmo trutta* L. *Gen. comp. Endocrinol.*

Robertson, O. H., Hane, S., Wexler, D. C. & Rinfret, A. P. (1963). The effect of hydrocortisone on immature rainbow trout (*Salmo gairdneri*). *Gen. comp. Endocrinol.* **3**, 422–436.

Saunders, R. L. (1962). The irrigation of the gills in fishes. II. Efficiency of oxygen uptake in relation to respiratory flow activity and concentrations of oxygen and carbon dioxide. *Can. J. Zool.* **40**, 817–862.

Schreck, C. B. (1981). Stress and compensation in teleostean fishes: response to social and physical factors. In *Stress and Fish* (A. D. Pickering, ed.), pp. 295–321. London, New York: Academic Press.

Smart, G. R. (1978). Investigations of the toxic mechanisms of ammonia to fish—gas exchange in rainbow trout (*Salmo gairdneri*) exposed to acutely lethal concentrations. *J. Fish Biol.* **12**, 93–104.

Smart, G. R. (1981). Aspects of water quality producing stress in intensive fish culture. In *Stress and Fish* (A. D. Pickering, ed.), pp. 277–293. London, New York: Academic Press.

Sousa, R. J. & Meade, T. L. (1977). The influence of ammonia on the oxygen delivery system of coho salmon hemoglobin. *Comp. Biochem. Physiol.* **58A**, 23–28.

Strange, R. J. & Schreck, C. B. (1978). Anaesthetic and handling stress on survival and cortisol concentration in yearling chinook salmon (*Oncorhynchus tshawytscha*). *J. Fish. Res. Bd Can.* **35**, 345–349.

Swift, D. J. (1981). Changes in selected blood component concentrations of rainbow trout, *Salmo gairdneri* Richardson, exposed to hypoxia or sublethal concentrations of phenol or ammonia. *J. Fish Biol.* **19**, 45–61.

Tomasso, J. R., Davis, K. B. & Parker, N. C. (1981*a*). Plasma corticosteroid dynamics in channel catfish, *Ictalurus punctatus* (Rafinesque), during and after oxygen depletion. *J. Fish Biol.* **18**, 519–526.

Tomasso, J. R., Davis, K. B. & Simco, B. A. (1981*b*). Plasma corticosteroid dynamics in channel catfish (*Ictalurus punctatus*) exposed to ammonia and nitrite. *Can. J. Fish. Aquat. Sci.* **38**, 1106–1112.

U.S. Environmental Protection Agency (1976). *Quality Criteria for Water*. Washington, D.C.: U.S. Government Printing Office, 256 pp.

Wedemeyer, G. (1970). Stress of anaesthesia with MS 222 and benzocaine in rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Bd Can.* **27**, 909–914.

J. Fish Biol. (1987) 30, 701–712

Crowding causes prolonged leucopenia in salmonid fish, despite interrenal acclimation

A. D. PICKERING AND T. G. POTTINGER

Freshwater Biological Association, The Ferry House, Far Sawrey, Near Ambleside, Cumbria LA22 0LP, England

(Received 25 September 1986, Accepted 15 November 1986)

Crowding for 3 weeks significantly reduced the coefficient of condition of both brown trout and rainbow trout. However, acclimation of the hypothalamic–pituitary–interrenal (HPI) axis, as assessed by changes in plasma cortisol levels, occurred within 6 days for brown trout and within 10 days for rainbow trout. Blood lactate levels were significantly reduced in the crowded fish of both species throughout the experiment. Sexual maturation of the male fish significantly elevated the number of circulating red blood cells in both species, reduced the lactate levels in brown trout and elevated cortisol levels in the rainbow trout. Despite the relatively rapid interrenal acclimation, the numbers of thrombocytes and lymphocytes in the blood of both species were significantly reduced during the period of crowding and it is concluded that changes in the composition of circulating blood cells are more reliable indicators of chronic crowding stress than are plasma cortisol levels. These findings are discussed in relation to the role of the HPI axis in suppressing the defence systems of salmonid fish during periods of chronic stress.

I. INTRODUCTION

Cortisol, the major corticosteroid in salmonid fish (Idler & Truscott, 1972), is secreted by the interrenal tissue in response to almost any form of environmental stress (Donaldson, 1981). It is assumed that the adaptive nature of this response is related to the catabolic action of the steroid as the fish increases its energy expenditure in an attempt to maintain homeostasis (Schreck, 1981). However, it is now well established that corticosteroids can also have a suppressive effect on the defence systems of teleost fish, resulting in a predisposition to common bacterial, fungal and protozoan infestations (Robertson *et al.*, 1963; Roth, 1972; Pickering & Duston, 1983; Pickering & Pottinger, 1985). This appears to be mediated by a reduction in the number of circulating white blood cells, particularly lymphocytes (McLeay, 1973*a*,*b*; Pickering, 1984), and/or a suppression of their activity (Stave & Roberson, 1985; Ellsaesser & Clem, 1986; Pickering & Pottinger, 1985).

Under conditions of chronic stress, such as crowding, the fish ultimately may acclimate to the changed circumstances and plasma cortisol concentrations will, after several days (or even weeks), return to basal levels (Strange *et al.*, 1978; Barton *et al.*, 1980; Pickering & Stewart, 1984). Despite this 'ideal' compensation (Schreck, 1981) of the hypothalamic–pituitary–interrenal (HPI) axis, chronically crowded fish may still be limited in their performance capacities. For example, crowding results in both reduced growth rates and increased mortality rates (Refstie & Kittelsen, 1976; Refstie, 1977; Fagerlund *et al.*, 1981; Soderberg & Krise, 1986). This raises the possibility that the defence systems of chronically stressed fish may still be impaired for some time after the return of plasma corticosteroid concentrations to basal levels. In the present investigation we examine the

0022–1112/87/060701 + 12 $03.00/0

changes in interrenal activity and circulating blood cell concentrations following the onset of crowding in brown trout, *Salmo trutta* L., and rainbow trout, *Salmo gairdneri* Richardson. In addition, blood lactate has been monitored because we have shown in previous studies (Pickering *et al.*, 1982) that changes in lactate levels can, under certain circumstances, be as sensitive as cortisol as indicators of a physiological stress response.

II. MATERIALS AND METHODS

EXPERIMENTAL FISH

Two thousand two hundred each of 1 + brown trout (Hungerford stock) and rainbow trout (Howietoun stock) were used during the present investigation. The brown trout had a mean body weight of 290 g and a sex ratio (♂:♀) of 0·67. Forty-five per cent of the males and 1% of the females were sexually mature for the first time. The rainbow trout had a mean body weight of 389 g and a sex ratio (♂:♀) of 1·22. Forty-nine per cent of the males and 3% of the females were sexually mature for the first time. The fish had been reared from eggs at the FBA's hatchery and fed once daily on commercial trout food at the manufacturer's recommended feeding rates.

EXPERIMENTAL DESIGN

Eight large (1 m dia.) outdoor fibreglass tanks were each stocked with 75 brown trout from a randomly mixed population. A further eight tanks were each stocked with 200 brown trout from the same population and left for a period of 2 weeks to recover from the effects of handling stress (Pickering *et al.*, 1982). Each tank was supplied with a constant flow (35 l min^{-1}) of Windermere lake water, the temperature of which declined from 14·7 to 12·6° C during the course of the experiment (September–October, 1985). After the recovery period, the water level of the tanks containing 200 fish was lowered from 45 cm (water volume 1220 l) to 15 cm (water volume 450 l). This produced a final stock density of 123 g l^{-1} for the crowded fish compared with 18 g l^{-1} for the uncrowded control fish (75 fish $tank^{-1}$, water volume 1220 l). Two fish were then sampled from each tank at 2, 6, 10, 14 and 21 days post-crowding. It was considered inadvisable to sample the tanks at '0-h' (*i.e.* immediately prior to the lowering of water levels) because we have shown that recovery from some of the haematological effects of handling (and, possibly, disturbance due to sampling) requires at least 3 days (Pickering *et al.*, 1982). However, in an attempt to assess the physiological state of the fish prior to the experimental procedures described above, a sample of 24 fish was taken from the undisturbed parent population (kept at similar stocking densities to the uncrowded control fish). Data from these '0-h' fish are included in the graphical presentations but excluded, on the grounds of non-orthogonality, from the statistical analyses.

Thus, the experimental design attempted to dissociate the more obvious effects of acute handling stress from those caused by an increase in stocking density. The limitations of our hatchery facilities, however, meant that the crowded fish had to be maintained at an intermediate stocking density (200 fish 1220 l^{-1} = 48 g l^{-1}) for 2 weeks prior to the final crowding. The experimental design for the rainbow trout was identical to that described above for the brown trout except that the stocking densities differed because of differences in the mean body weight of the fish (uncrowded 24 g l^{-1}, intermediate 64 g l^{-1}, crowded 172 g l^{-1}).

SAMPLING PROCEDURE

At each sampling time fish were rapidly netted with as little disturbance as possible to the fish remaining in the tank and anaesthetized in phenoxyethanol (Sigma, 500 mg l^{-1}). A blood sample was taken from the caudal vessels by means of a heparinized syringe within 1 min of netting and blood smears made. A 50-µl aliquot of fresh blood was diluted with trout Ringer solution (final dilution 1:40 000), fixed with Lugol's iodine and sedimented in a polypropylene tube mounted on a glass microscope slide. The rest of the blood sample was kept on ice for 30 min, centrifuged at 4° C and a subsample of the plasma was deproteinized

with 0·6 N perchloric acid and stored at −20° C for subsequent lactate determination. The remaining plasma was stored at −70° C until assayed for cortisol. The fish was killed by a blow to the head, weighed, measured and the sex and state of maturation assessed by internal examination. Total blood cell counts were made on the sedimented blood samples using an inverted microscope, and differential counts were made from air-dried, methanol-fixed, stained (haematoxylin and eosin) blood smears. Absolute concentrations of each cell type were calculated from the total and differential blood cells counts. Plasma cortisol levels were determined by radioimmunoassay using previously validated techniques (Pickering *et al.*, 1987). Plasma lactate was measured using a Boehringer Mannheim test kit which utilizes the enzymic oxidation of lactate to pyruvate by LDH and the shift in UV absorbance when NAD is reduced to NADH. Water samples were taken at regular intervals for total ammonium nitrogen determination (indophenol blue method) and dissolved oxygen was measured with a Yellow Springs Instrument oxygen probe (Model 5739).

STATISTICAL ANALYSES

The data for body weight, length, coefficient of condition (K factor = 100 W/L^3), plasma cortisol, plasma lactate and blood cell counts were separately subjected to analyses of covariance (Genstat) using treatment (crowded or uncrowded), time (2, 6, 10, 14 and 21 days) and fish sequence (one or two) as factors. Tank and fish were used as blocking effects to give a nested error structure with which to assess the significance of the factors and their interactions. Sex and state of maturity were used as covariates because it has been shown previously that blood cell counts and cortisol levels in the brown trout can be altered in sexually mature fish during the spawning period (Pickering, 1986; Pickering & Pottinger, 1987). From a plot of the residuals against fitted values appropriate transformations ($\sqrt{}$ or log) were selected, where necessary, to improve homogeneity of variance. The levels of significance given in the paper are derived from these analyses but, for ease of presentation, data are given as arithmetic means ± S.E.M.

III. RESULTS

Oxygen concentrations in the uncrowded control tanks never fell below 82% saturation and total ammonia nitrogen levels were in the range 87–129 μg l^{-1}. In the most severely crowded tanks of rainbow trout, dissolved oxygen was still maintained above 60% saturation and ammonia levels did not exceed 300 μg l^{-1} (total ammonia nitrogen). The duration of the experiment (3 weeks) was too short to allow the detection of significant effects of crowding on the body weight or length for either species. However, in both the brown trout and rainbow trout crowding resulted in significant reductions in the coefficient of condition ($P<0·001$ and $<0·01$, respectively, Fig. 1). It was also found that sexually mature male fish had a significantly higher coefficient of condition than immature fish of either sex ($P<0·001$ for each species).

Plasma cortisol concentrations in the uncrowded control fish remained very low in both species throughout the investigation (generally less than 2 ng ml^{-1}). In the crowded fish, however, cortisol levels were significantly elevated during the early stages of the experiment: the plasma cortisol levels of crowded brown trout were greater than 4 ng ml^{-1} at 2 days post-crowding ($P<0·001$) but had returned to basal levels by day 6 (Fig. 2(a)); in the rainbow trout they were still elevated at 6 days post-crowding (≈7 ng ml^{-1}, $P<0·001$) but had returned to basal levels by day 10 (Fig. 2(b)). In the rainbow trout, but not in the brown trout, a significant ($P<0·01$) covariate effect in the cortisol data was observed. This was caused by higher cortisol concentrations (by approximately 3 ng ml^{-1}) in the blood of sexually mature males when compared with immature fish. Crowding also had a

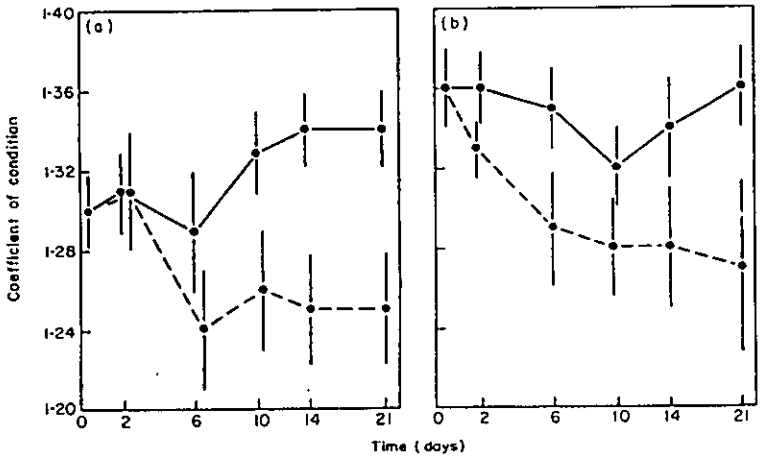


FIG. 1. Changes in the coefficient of condition (K factor = 100 W/L^3) of (a) brown trout and (b) rainbow trout during crowding. Continuous lines represent uncrowded control fish, broken lines represent chronically crowded fish. Values are arithmetic means ± S.E.M. $n=16$ (except for time zero values where $n=24$).

significant effect on blood lactate levels in both species ($P<0·05$ in each case). Somewhat surprisingly, this took the form of a *reduction* in the blood lactate of crowded fish compared with the uncrowded controls (Fig. 2(c), (d)). In addition, sexually mature male brown trout had significantly ($P<0·001$) lower lactate levels than immature fish (on average approximately 20 μg ml^{-1} lower). This covariate effect was not statistically significant, however, for the rainbow trout data ($P=0·085$).

No consistent effect of crowding on the concentration of circulating erythrocytes was observed. In the brown trout, crowding was associated with a significant elevation of erythrocyte counts at 2 days post-crowding ($P<0·05$) and a significant reduction at 21 days ($P<0·05$) when compared with the uncrowded controls (Fig. 3(a)). In the rainbow trout a significant difference between treatments was observed only at 14 days post-crowding, with the crowded fish having fewer circulating red blood cells than the controls ($P<0·001$), but by day 21 this difference had disappeared (Fig. 3(b)). Crowding did not influence the number of circulating neutrophils in either species, with counts remaining relatively constant throughout the experiment (Fig. 3(c), (d)). The mean neutrophil count for the blood of the brown trout was 2302 ± 245 cells μl^{-1} (mean ± S.E.M., $n=160$) compared with 4357 ± 299 for the rainbow trout ($P<0·001$).

In both species, crowding caused a prolonged reduction in the numbers of circulating thrombocytes and lymphocytes, as compared with uncrowded control fish (Fig. 4). Control brown trout had considerably more thrombocytes than control rainbow trout, a difference which was maintained throughout the experiment [17 500 ± 618 cf. 11 400 ± 549 cells μl^{-1}, respectively (mean ± S.E.M., $n=80$), $P<0·001$]. However, crowding suppressed the thrombocyte counts in both species ($P<0·005$ in each case) with no real signs of recovery during the 21 days post-

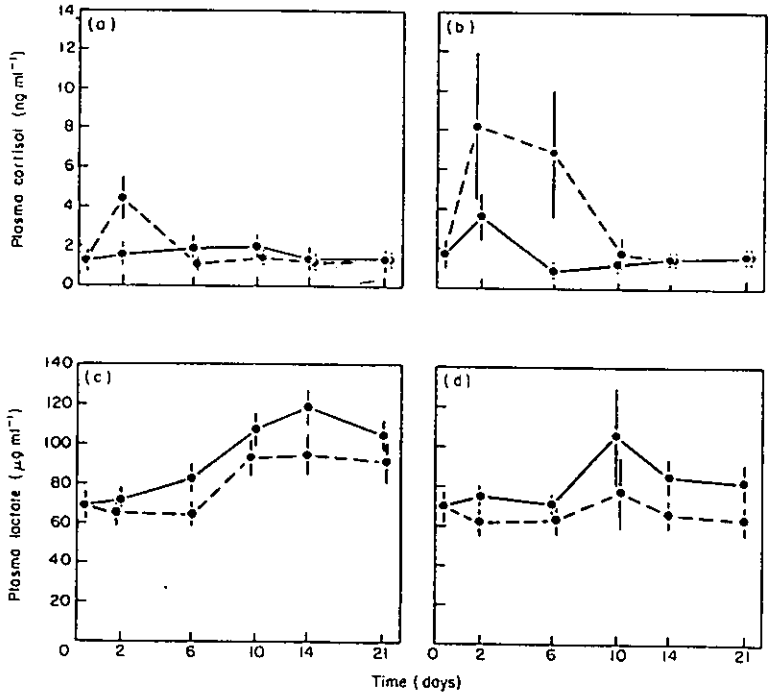


FIG. 2. Changes in plasma cortisol and lactate concentrations of (a), (c) brown trout and (b), (d) rainbow trout during chronic crowding. See legend to Fig. 1 for details.

crowding (i.e. no significant treatment × time interaction, see Fig. 4(a), (b)). An even greater difference between the two species was observed in the number of circulating lymphocytes in the blood of uncrowded control fish. The mean lymphocyte count of control brown trout was $52\,668 \pm 2095$ cells μl^{-1} (mean $\pm$ S.E.M., $n = 80$) compared with $15\,921 \pm 833$ for the rainbow trout ($P < 0{\cdot}001$). Crowding caused a marked reduction in the lymphocyte count of brown trout at 2 days post-crowding (down to 28 000 cells μl^{-1}) and the suppression was maintained throughout the experiment ($P < 0{\cdot}001$, no significant treatment × time interaction) although the data gave the impression of a certain degree of recovery during the later stages of the experiment (Fig. 4(c)). This was partly related to the fact that lymphocyte counts in the control fish dropped slightly at day 10. In the rainbow trout, crowding caused a less dramatic but highly significant ($P < 0{\cdot}01$) reduction in the number of circulating lymphocytes, a suppression which was maintained throughout the experiment (Fig. 4(d)).

IV. DISCUSSION

Crowding caused a reduction in the coefficient of condition (K) of both species of trout during the present investigation but the duration of the experiment (3 weeks)

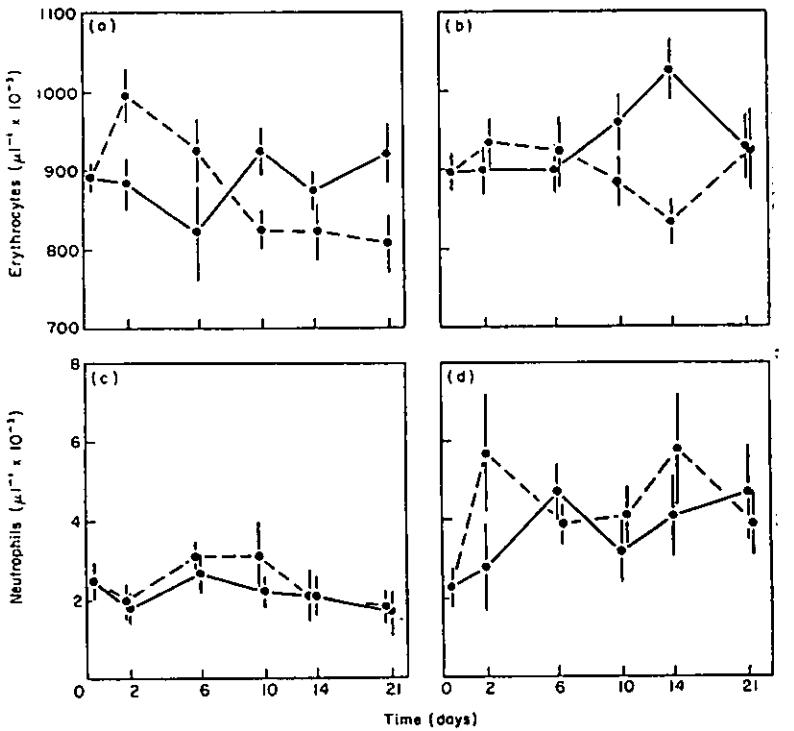


FIG. 3. Changes in the concentrations of circulating erythrocytes and neutrophils in (a), (c), brown trout and (b), (d), rainbow trout during chronic crowding. See legend to Fig. 1 for details.

was not sufficient to allow the demonstration of suppression of growth rate *per se*. It seems unlikely that the effect on K factor was mediated by deleterious changes in water quality as a result of the increased stocking density, because Smart (1981) reported that the growth of rainbow trout, was similar when reared at 60, 85 and 120% oxygen saturation, with no growth suppression even at the lowest oxygen concentration. In the present study the oxygen concentration in the most crowded tanks never dropped below 60% saturation ($= 6{\cdot}32\ mg\ l^{-1}$ at 13°C). Total ammonia concentrations were low in all tanks and unionized ammonia remained well below the levels recommended as 'safe' by the U.S. Environmental Protection Agency (1976). Thus, it seems likely that the important component of environmental stress was the degree of crowding and not a change in water quality as a result of crowding.

Plasma cortisol levels were elevated in both species of trout following the onset of crowding. However, cortisol returned to basal levels by day 6 in the brown trout and by day 10 in the rainbow trout despite the continued crowding. This relatively rapid acclimation of the plasma cortisol levels of salmonid fish to chronic stress is

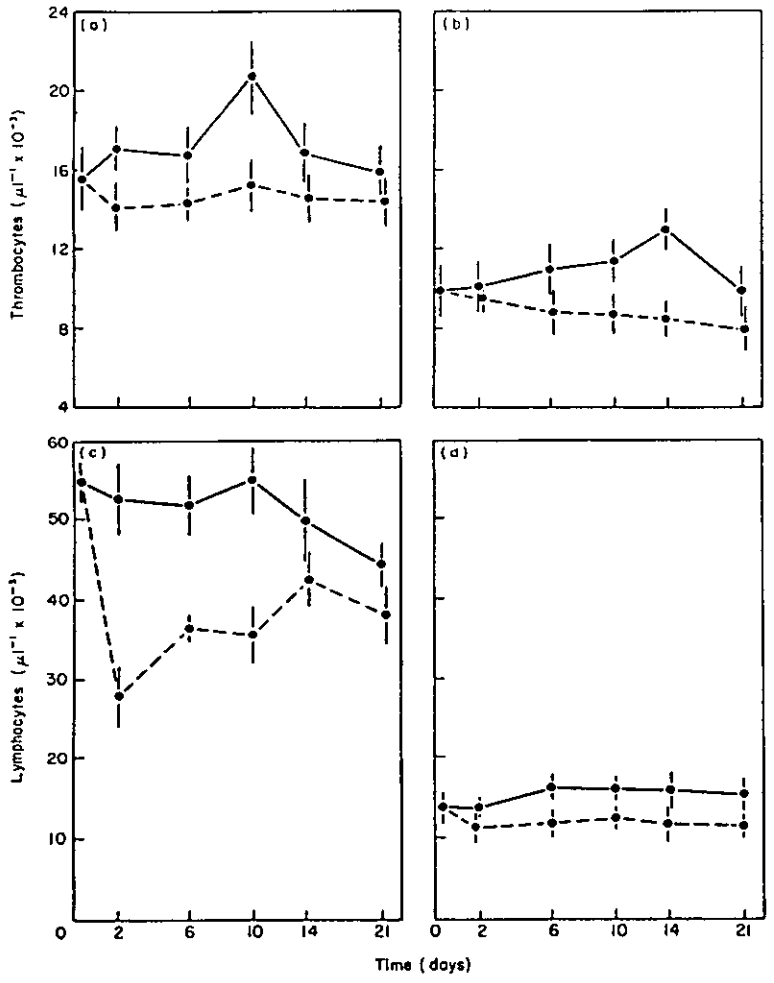


FIG. 4. Changes in the concentrations of circulating thrombocytes and lymphocytes in (a), (c), brown trout and (b), (d), rainbow trout during chronic crowding. See legend to Fig. 1 for details.

consistent with the findings of several other workers (see Schreck, 1981 for references) but contrasts somewhat with the study of Pickering & Stewart (1984) on the acclimation of the brown trout to chronic crowding; in their study, plasma cortisol levels were elevated for 28 days post-crowding but returned to basal values by day 40. It is not clear whether this difference in acclimation time is related to the different strains of fish used in the two studies or to differences in experimental conditions. Little information is available on the mechanism of acclimation to

chronic stress but Redding *et al.* (1984) present evidence suggesting that an increased metabolic clearance rate of corticosteroids from the blood might be an important component. Thus, the HPI axis of chronically crowded fish may still be more active than that of unstressed fish, despite the return of plasma cortisol to basal levels. Further work using the recently validated ACTH radioimmunoassay for salmonid blood plasma (Sumpter & Donaldson, 1986) would help to resolve this problem. The sex and state of maturation had no measurable effect on the plasma cortisol levels of the brown trout used during the present study (September–mid-October). This conforms with a recent investigation using the same stock of brown trout (Hungerford stock) in which we have shown that cortisol elevation as a result of sexual maturation does not occur until the end of October (Pickering & Pottinger, 1987). During the present study, however, sexually mature male rainbow trout did have significantly higher plasma cortisol levels than immature fish of either sex.

In both species crowding caused a persistent reduction in the levels of plasma lactate, an observation which seemed somewhat surprising in view of the lower oxygen concentrations in the crowded tanks. One possibility is that plasma lactate levels are more indicative of the degree of muscular activity of the fish (Black, 1957*a*,*b*) rather than the degree of oxygen availability as such. However, this is not supported by the work of Wendt & Saunders (1973) in which no differences were apparent between basal blood lactate levels of Atlantic salmon, *Salmo salar*, reared at high and low water velocities. An alternative explanation might be found in the studies of Wardle (1978) on carbohydrate metabolism in the plaice, *Pleuronectes platessa*, in which he demonstrated that the release of lactate from the muscle cells to the blood stream was reduced following stress, a mechanism activated by circulating catecholamines. In the present study, sexually mature male brown trout had significantly lower plasma lactate levels than immature fish. It seems probable that this was associated with the higher erythrocyte count (and, presumably, higher O_2 carrying capacity) in the blood of mature males (Pickering, 1986; Pottinger & Pickering, in press). This interpretation is complicated, however, by our failure to observe a similar effect of sexual maturation on the blood lactate levels of the rainbow trout, despite the fact that the mature males of this species also had elevated erythrocyte counts. This species difference could be related to the differences in plasma cortisol levels of the mature fish (see above) because Lidman *et al.* (1979) have shown that chronic plasma cortisol elevation in the eel, *Anguilla anguilla*, causes a significant elevation of blood lactate levels. Thus, in the mature rainbow trout, the effect on blood lactate levels of increased erythrocyte counts may be opposed by the effect of elevated cortisol concentrations.

Crowding failed to produce any consistent pattern of change in the numbers of circulating erythrocytes and had no significant effect on the concentration of neutrophils in the blood of either species of trout. By comparison, crowding caused a prolonged reduction in the number of circulating thrombocytes and lymphocytes in both species. In previous studies we have been unable to demonstrate any effect of acute stress or corticosteroid administration on the circulating thrombocyte population of brown trout (Pickering *et al.*, 1982, 1987; Pickering, 1984). Moreover, McLeay and colleagues could not find any effect of chronic stress (exposure to pollutants) on the thrombocytes of juvenile coho salmon, *Oncorhynchus kisutch*, (McLeay, 1973*c*; McLeay & Brown, 1974) and, despite

showing that ACTH and dexamethasone (but not cortisol) could cause thrombocytopenia (McLeay, 1973*a*,*b*), were unable to correlate seasonal variation in thrombocyte numbers with adrenocortical activity (McLeay, 1975). In the present study the thrombocytopenia outlasted the period of elevated cortisol levels and, taking all available information into consideration, it seems likely that mechanisms other than suppression by corticosteroids were responsible for the reduction in thrombocyte numbers in the crowded fish. Srivastava (1969) provided evidence that the clotting rate in teleosts is directly proportional to the number of thrombocytes present in the blood; therefore, it follows that the clotting rate of the crowded fish in the present study was probably reduced. This impairment of an important defence system to physical injury provides further evidence that the performance capacity of chronically crowded fish is reduced regardless of the state of activity of the HPI axis. In this respect, the response of salmonid fish to chronic stress is different from the response to acute stress. Casillas & Smith (1977) reported a *decrease* in clotting time in rainbow trout following an acute stress, a response which appears to be of adaptive significance.

Salmonid lymphocytes, unlike the thrombocytes, are known to be extremely sensitive to corticosteroids (McLeay, 1973*b*; Pickering, 1984). Thus, it might be argued that the dramatic suppression of the lymphocyte count of brown trout at 2 days post-crowding was related to a transient surge in plasma cortisol levels during the first few hours of crowding. However, in previous studies we have shown that lymphocyte counts return to normal within a day or two following the return of blood cortisol to basal levels (Pickering *et al.*, 1982; Pickering, 1984). The prolonged lymphocytopenia in the crowded fish of both species suggests that either the enhanced interrenal activity during the first week post-crowding had a prolonged effect on the lymphoid tissues, or other mechanisms are involved in the continued suppression of lymphocyte counts during chronic stress. Klinger *et al.* (1983) reported a similar leucopenia in channel catfish, *Ictalurus punctatus*, under crowded conditions, even though plasma cortisol levels remained low. Further work is now needed to establish the mechanisms behind this prolonged leucopenia in chronically crowded fish, including the possibility of some water-borne immunosuppressive factor(s) (see, e.g., Perlmutter *et al.*, 1973). It has been shown that a reduction in blood lymphocyte count may coincide with a period of increased susceptibility to disease (Pickering & Pottinger, 1987) and it seems likely, therefore, that lymphocytopenia is at least partly responsible for observed increases in mortality rate in crowded salmonid fish (Refstie, 1977; Soderberg & Krise, 1986).

In view of the marked difference between uncrowded, control brown trout and rainbow trout, caution is needed when interpreting absolute lymphocyte counts. In the present investigation the mean lymphocyte count of the brown trout was approximately three times greater than that of the rainbow trout, yet both species appeared to be in perfect health. It is not clear at this stage whether this marked difference in lymphocyte count represents a real species difference or a difference between strains, nor is it known what the implications are regarding the relative importance of the various components of the fish's defence system. Further study in this area is likely to be rewarding.

In summary, it has been shown that crowding produces a transient increase in plasma cortisol levels but a much more prolonged change in the composition of

circulating blood cells in both brown trout and rainbow trout. These haematological changes, which reflect some impairment of the fish's defence systems, may be a more useful index of chronic crowding stress than the degree of activation of the HPI axis. However, under different experimental conditions we have shown that plasma cortisol levels, and not the composition of circulating blood cells, can be more sensitive predictive indicators of reduced disease resistance (Pickering & Pottinger, 1985). This highlights some of the complexities of the teleost stress response and emphasizes the need to use many parameters when attempting to assess the nature and extent of the effects of environmental stress on salmonid fish.

The authors are particularly grateful to Mrs J. Pollard for care and maintenance of the experimental fish, Miss J. M. Fletcher for the lactate determinations, Dr J. Hilton and his colleagues and for the water analysis and Mrs M. A. Hurley for statistical advice, and NERC and MAFF for financial support.

References

Barton, B. A., Peter, R. E. & Paulencu, C. R. (1980). Plasma cortisol levels of fingerling rainbow trout (*Salmo gairdneri*) at rest, and subjected to handling, confinement, transport, and stocking. *Can. J. Fish. aquat. Sci.* 37, 805–811.

Black, E. C. (1957*a*). Alterations in the blood level of lactic acid in certain salmonoid fishes following muscular activity. I. Kamloops trout, *Salmo gairdneri*. *J. Fish. Res. Bd Can.* 14, 117–134.

Black, E. C. (1957*b*). Alterations in the blood level of lactic acid in certain salmonoid fishes following muscular activity. II. Lake trout, *Salvelinus namaycush*. *J. Fish. Res. Bd Can.* 14, 645–649.

Casillas, E. & Smith, L. S. (1977). Effects of stress on blood coagulation and haematology in rainbow trout (*Salmo gairdneri*). *J. Fish Biol.* 10, 481–491.

Donaldson, E. M. (1981). The pituitary–interrenal axis as an indicator of stress in fish. In *Stress and Fish* (A. D. Pickering, ed.), pp. 11–47. London and New York: Academic Press.

Ellsaesser, C. F. & Clem, L. W. (1986). Haematological and immunological changes in channel catfish stressed by handling and transport. *J. Fish Biol.* 28, 511–521.

Fagerlund, U. H. M., McBride, J. R. & Stone, E. T. (1981). Stress-related effects of hatchery rearing density on coho salmon. *Trans. Am. Fish. Soc.* 110, 644–649.

Idler, D. R. & Truscott, B. (1972). Corticosteroids in fish. In *Steroids in Nonmammalian Vertebrates* (D. R. Idler, ed.), pp. 126–252. London and New York: Academic Press.

Klinger, H., Delventhal, H. & Hilge, V. (1983). Water quality and stocking density as stressors of channel catfish (*Ictalurus punctatus* Raf.). *Aquaculture* 30, 263–272.

Lidman, U., Dave, G., Johansson-Sjöbeck, M.-L., Larsson, A. and Lewander, K. (1979). Metabolic effects of cortisol in the European eel, *Anguilla anguilla* (L.). *Comp. Biochem. Physiol.* 63A, 339–344.

McLeay, D. J. (1973*a*). Effects of ACTH on the pituitary-interrenal axis and abundance of white blood cell types in juvenile coho salmon, *Oncorhynchus kisutch*. *Gen. comp. Endocrinol.* 21, 431–440.

McLeay, D. J. (1973*b*). Effects of cortisol and dexamethasone on the pituitary-interrenal axis and abundance of white blood cell types in juvenile coho salmon, *Oncorhynchus kisutch*. *Gen. comp. Endocrinol.* 21, 441–450.

McLeay, D. J. (1973*c*). Effects of a 12-hr and 25-day exposure to kraft pulp mill effluent on the blood and tissues of juvenile coho salmon (*Oncorhynchus kisutch*). *J. Fish. Res. Bd Can.* 30, 395–400.

McLeay, D. J. (1975). Variations in the pituitary-interrenal axis and the abundance of circulating blood-cell types in juvenile coho salmon, *Oncorhynchus kisutch*, during stream residence. *Can. J. Zool.* 53, 1882–1891.

McLeay, D. J. & Brown, D. A. (1974). Growth stimulation and biochemical changes in juvenile coho salmon (*Oncorhynchus kisutch*) exposed to a bleached kraft pulpmill effluent for 200 days. *J. Fish. Res. Bd Can.* **31**, 1043–1049.

Perlmutter, A., Sarot, D. A., Yu, M.-L., Filazzola, R. J. & Seeley, R. J. (1973). The effect of crowding on the immune response of the blue gourami, *Trichogaster trichopterus*, to infectious pancreatic necrosis (IPN) virus. *Life Sci.* **13**, 363–375.

Pickering, A. D. (1984). Cortisol-induced lymphocytopenia in brown trout, *Salmo trutta* L. *Gen. comp. Endocrinol.* **53**, 252–259.

Pickering, A. D. (1986). Changes in blood cell composition of the brown trout, *Salmo trutta* L., during the spawning season. *J. Fish Biol.* **29**, 335–347.

Pickering, A. D. & Duston, J. (1983). Administration of cortisol to brown trout, *Salmo trutta* L., and its effects on the susceptibility to *Saprolegnia* infection and furunculosis. *J. Fish Biol.* **23**, 163–175.

Pickering, A. D. & Pottinger, T. G. (1985). Cortisol can increase the susceptibility of brown trout, *Salmo trutta* L., to disease without reducing the white blood cell count. *J. Fish Biol.* **27**, 611–619.

Pickering, A. D. & Pottinger, T. G. (1987). Lymphocytopenia and interrenal activity during sexual maturation in the brown trout, *Salmo trutta* L. *J. Fish Biol.* **30**, 41–50.

Pickering, A. D., Pottinger, T. G. & Christie, P. (1982). Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: a time-course study. *J. Fish Biol.* **20**, 229–244.

Pickering, A. D., Pottinger, T. G. & Sumpter, J.P. (1987). On the use of dexamethasone to block the pituitary-interrenal axis in the brown trout, *Salmo trutta* L. *Gen. comp. Endocrinol.* **65**, 346–353.

Pickering, A. D. & Stewart, A. (1984). Acclimation of the interrenal tissue of the brown trout, *Salmo trutta* L., to chronic crowding stress. *J. Fish Biol.* **24**, 731–740.

Pottinger, T. G. & Pickering, A. D. (in press). Androgen levels and erythrocytosis in maturing brown trout, *Salmo trutta* L. *Fish Physiol. Biochem.*

Redding, J. H., Patino, R. & Schreck, C. B. (1984). Clearance of corticosteroids in yearling coho salmon, *Oncorhynchus kisutch*, in fresh water and seawater and after stress. *Gen. comp. Endocrinol.* **54**, 433–443.

Refstie, T. (1977). Effect of density on growth and survival of rainbow trout. *Aquaculture* **11**, 329–334.

Refstie, T. & Kittelsen, A. (1976). Effect of density on growth and survival of artificially reared Atlantic salmon. *Aquaculture* **8**, 319–326.

Robertson, O. H., Hane, S., Wexler, B. C. & Rinfret, A. P. (1963). The effect of hydrocortisone on immature rainbow trout (*Salmo gairdnerii*). *Gen. comp. Endocrinol.* **3**, 422–436.

Roth, R. R. (1972). Some factors contributing to the development of fungus infection in freshwater fish. *J. Wildl. Dis.* **8**, 24–28.

Schreck, C. B. (1981). Stress and compensation in teleostean fishes: response to social and physical factors. In *Stress and Fish* (A. D. Pickering, ed.), pp. 295–321. London and New York: Academic Press.

Smart, G. R. (1981). Aspects of water quality producing stress in intensive fish culture. In *Stress and Fish* (A. D. Pickering, ed.), pp. 277–293. London and New York: Academic Press.

Soderberg, R. W. & Krise, W. F. (1986). Effects of rearing density on growth and survival of lake trout. *Progve Fish-Cult.* **48**, 30–32.

Srivastava, A. K. (1969). Studies on the haematology of certain freshwater teleosts—V. Thrombocytes and the clotting of blood. *Anat. Anz.* **124**, 368–374.

Stave, J. W. & Robertson, B. S. (1985). Hydrocortisone suppresses the chemiluminescent response of striped bass phagocytes. *Dev. comp. Immunol.* **9**, 77–84.

Strange, R. J., Schreck, C. B. & Ewing, R. D. (1978). Cortisol concentrations in confined juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Trans. Am. Fish. Soc.* **107**, 812–819.

Sumpter, J. P. & Donaldson, E. M. (1986). The development and validation of a radioimmunoassay to measure plasma ACTH levels in salmonid fishes. *Gen. comp. Endocrinol.* **62**, 367–376.

U.S. Environmental Protection Agency (1976). *Quality Criteria for Water*. Washington, D.C.: U.S. Government Printing Office. 256 pp.

Wardle, C. S. (1978). Non-release of lactic acid from anaerobic swimming muscle of plaice *Pleuronectes platessa* L.: a stress reaction. *J. exp. Biol.* **77**, 141–155.

Wendt, C. A. G. & Saunders, R. L. (1973). Changes in carbohydrate metabolism in young Atlantic salmon in response to various forms of stress. *Spec. Publ. Int. Atlant. Salm. Found.* **4**, 55–82.

GENERAL AND COMPARATIVE ENDOCRINOLOGY 65, 346–353 (1987)

On the Use of Dexamethasone to Block the Pituitary–Interrenal Axis in the Brown Trout, *Salmo trutta* L.

A. D. PICKERING, T. G. POTTINGER, AND J. P. SUMPTER*

*Freshwater Biological Association, The Ferry House, Far Sawrey, Nr Ambleside, Cumbria LA22 OLP, and *Department of Applied Biology, Faculty of Mathematics and Science, Brunel University, Uxbridge, Middlesex, UB8 3PH, United Kingdom*

Accepted September 21, 1986

Oral administration of dexamethasone in a single meal at a dose of 3 μg g^{-1} body wt was sufficient to block the secretion of ACTH and cortisol in brown trout subjected to a handling stress followed by 30 min confinement. This dose, however, also had a marked effect on several of the circulating blood cell types. Lymphocyte and neutrophil counts were reduced in dexamethasone-treated fish whereas erythrocyte counts increased. The value of dexamethasone as a tool for investigating the role of interrenal tissue during stress responses in teleost fish is limited by its cortisol-like effects on other steroid-sensitive tissues.

The hypothalamic–pituitary–interrenal (HPI) axis in teleost fish is activated in response to almost all forms of environmental stress (Donaldson, 1981). However, the role of cortisol (the major interrenal hormone in salmonid fish) in such stress responses is poorly understood although it is becoming apparent that components of the fishes' defence systems against pathogenic challenge are cortisol-sensitive (Pickering, 1984; Stave and Roberson, 1985; Ellsaesser and Clem, 1986) and that stress-induced predisposition to disease may be mediated, at least in part, by interrenal stimulation (Pickering and Duston, 1982; Pickering and Pottinger, 1985). Progress in this area of research is limited by the difficulty in experimentally suppressing the activity of the HPI axis under conditions of stress. The diffuse nature of the interrenal tissue makes surgical removal almost impossible, although at least two groups have attempted this operation (Butler *et al.*, 1969; Chan *et al.*, 1969). An alternative approach to suppressing interrenal activity is by hypophysectomy, but this interferes with so many other endocrine systems that results are often difficult to interpret.

Valuable information has been obtained by the administration of hormones and drugs to modify the activity at various points along the HPI axis. In this context, several workers have attempted to suppress the axis by means of the synthetic steroids, dexamethasone and betamethasone (Fagerlund, 1967; Donaldson and McBride, 1967; Bradshaw and Fontaine-Bertrand, 1968; Fagerlund and McBride, 1969; Hawkins and Ball, 1973; McLeay, 1973b; Fryer, 1975; Swift, 1982; Sumpter and Donaldson, 1986). In fish, as in mammals (Purves and Sirett, 1965; Plotsky and Vale, 1984), these steroids suppress ACTH secretion (Sumpter and Donaldson, 1986), an effect likely to be mediated via the hypothalamus (see Fryer and Peter, 1977a,b,c) as well as by direct action on the pituitary gland (Sage, 1968; Sage and Purrott, 1969). In all previous studies on fish, dexamethasone (and betamethasone) has been administered by intraperitoneal injection, either as a single dose or as a series of injections. However, in our experience we have found it impossible to inject salmonid fish without initially stimulating the HPI axis, a problem also encountered by Swift

0016-6480/87 $1.50

(1982). Oral administration, by incorporating the steroid in the normal diet, overcomes this problem and it has been shown previously that the natural steroid hormone, cortisol, can be successfully administered in physiological doses via this route (Pickering and Duston, 1982). However, in mammals dexamethasone is a potent glucocorticoid in its own right and at least one study indicates that it may have corticosteroid-like activities in fish (McLeay, 1973b).

The present study examines the use of orally administered dexamethasone as a means of blocking stress-induced ACTH and cortisol release in the brown trout, *Salmo trutta* L. Particular attention is given to the possibility that, at levels required to block the HPI axis, dexamethasone may exert agonistic influences on peripheral corticosteroid-sensitive tissues, such as the circulating lymphocyte population (Pickering, 1984). Consequently, we examine the haematology of dexamethasone-treated fish.

MATERIALS AND METHODS

Experimental fish. All experiments were carried out during May–June 1985 on hatchery-reared, 2+ brown trout (Hungerford strain) with a mean body weight of 253.4 ± 7.48 g (SD). The ratio (♂:♀) within the population was 0.96 and ≈70% of the males and ≈7% of the females had spawned the previous November. The fish were reared at the Freshwater Biological Association's experimental hatchery in large (1500-liter), outdoor, fibreglass tanks each supplied with a constant flow of Windermere lake water (35 liters min^{-1}) and fed once daily with commercial trout pellets at a rate of 1–2% body wt day^{-1} (exact rate dependent on temperature). Water temperature during the experimental period was in the range 8.3–12.2°.

Experiment 1: Dose of dexamethasone needed to block the HPI axis. Four hundred brown trout were distributed equally into four large outdoor tanks (identical to those in which the fish were reared) and left for 2 weeks to recover from the effects of handling and relocation (Pickering *et al.*, 1982). At the end of this period the normal daily ration of trout pellets (1% body wt day^{-1}) was replaced by an experimental meal consisting of dexamethasone-treated pellets. The pellets were prepared by spraying with dexamethasone dissolved in ethanol and allowing the ethanol to evaporate overnight at room temperature. The fish in one tank were fed a control meal (ethanol-treated pellets), and the fish in the other three tanks received pellets with a dexamethasone content of 100, 200, and 300 mg kg^{-1}, respectively. At intervals of 6, 24, 48, 72, 96, and 168 hr after dexamethasone treatment, groups of six fish were netted from each tank (taking care to keep the disturbance to the remaining fish to a minimum) and transferred to small tanks (60 × 40 × 25 cm) each containing 25 liters Windermere lake water. After 1 hr of this confinement stress, the fish were anaesthetized in phenoxyethanol (1:2000) and a blood sample was taken from the caudal vessels by means of a heparinized syringe, kept on ice for <30 min, and then centrifuged at 4°. The resultant blood plasma was stored at −70° until assayed for cortisol (see below). Immediately after blood sampling, each fish was killed by a blow to the head and its weight and sex were determined.

Experiment 2: The effect of dexamethasone on plasma ACTH levels. Two hundred eighty brown trout were distributed equally into four large outdoor tanks and left for 2 weeks to recover from the stress of handling and relocation. Duplicate tanks were then given a control meal of ethanol-treated pellets, the other two tanks receiving pellets containing dexamethasone at a dose of 300 mg kg^{-1} food. The feeding rate for all groups was 1% body wt meal^{-1}. After 48 hr, groups of six fish were rapidly netted from each tank and blood samples taken from the caudal vessels (unstressed fish). Further groups of six fish were then subjected to an acute confinement stress (see above for details) for a period of 30 min. (This shorter confinement (cf. experiment 1) was chosen because Sumpter *et al.* (1986) have shown that plasma ACTH levels are elevated rapidly and then plateau in rainbow trout, *Salmo gairdneri* subjected to a similar confinement stress.) Blood samples were then taken from the caudal vessels of the stressed fish. In this experiment, samples were taken with EDTA (750 μg ml^{-1}) as anticoagulant (heparin interferes with the ACTH assay) (Sumpter and Donaldson, 1986) and stored on ice in precooled tubes containing a protease inhibitor (Aprotinin, 5000 Kallikrein units tube^{-1}, Sigma). Blood samples were centrifuged within 30 min of collection and the plasma was stored at −70° until assayed for cortisol and ACTH (see below). The sex and body weight of each fish were determined as previously.

Experiment 3: Effect of dexamethasone on the haematology of brown trout. Five hundred sixty brown trout were distributed equally into eight large outdoor tanks and, as previously, were left for 2 weeks to recover from the handling stress. Four tanks of fish were then given a single control meal of ethanol-treated pellets, the other four tanks being given dexamethasone-treated pellets (300 mg kg^{-1} food) at a rate of 1% body wt meal^{-1}. Groups of six fish tank^{-1} were removed (taking care not to disturb the remaining fish)

24, 48, 72, and 96 hr posttreatment and blood samples rapidly taken by means of heparinized syringes from the caudal vessels. Plasma samples were prepared for cortisol determination as in experiment 1. No fish were sampled at 0 hr (i.e., immediately prior to dexamethasone administration) because of the possibility that the disturbance might suppress the feeding response (see Pickering *et al.*, 1982). Aliquots (50 µl) of fresh blood were diluted with trout Ringer solution (final dilution 1:40,000), fixed with Lugol's iodine, and sedimented in polypropylene tubes mounted on glass microscope slides. Total blood cell counts were made on the sedimented blood samples using an inverted microscope and differential counts were made from air-dried, methanol-fixed, stained (haematoxylin and eosin) blood smears. Absolute concentrations of erythrocytes, neutrophils, thrombocytes, and lymphocytes were calculated from the total and differential blood cell counts.

Radioimmunoassays. Plasma samples were assayed for ACTH using a previously validated radioimmunoassay (Sumpter and Donaldson, 1986). Pituitary extracts and plasma samples from both *Oncorhynchus* (on which the assay was originally developed) and *Salmo* species have parallel dose–response curves (Sumpter and Donaldson, 1986; Sumpter, unpublished observations) and we are confident, therefore, of the suitability of the assay for the present study. Cortisol was determined using a modification of the assay described by Pickering and Pottinger (1983). Aliquots (200 µl) of plasma were extracted with 1 ml Aristar ethyl acetate. After thorough mixing and centrifugation 20- to 200-µl aliquots of the organic supernatant were pipetted into assay tubes together with 20,000 dpm [1,2,6,7-^{3}H]cortisol (86 Ci mmol^{-1}, Amersham International). A range of standard tubes (0–800 pg cortisol tube^{-1}) containing 20,000 dpm [^{3}H]cortisol tube^{-1} was prepared in duplicate from a stock solution of inert cortisol in ethyl acetate. All tubes were evaporated to dryness under vacuum at 35°. BSA–saline (100 µl, 0.1% bovine serum albumin in 0.9% NaCl) was added to each tube followed by 100 µl of antiserum (Steranti anti-cortisol-3-(CMO)HSA) at a concentration sufficient to bind ≈50% of the [^{3}H]cortisol in the absence of inert steroid. The antiserum shows negligible cross reactivity with dexamethasone. After vortex mixing and incubation for 4–18 hr at 4°, 100 µl of chilled dextran–charcoal suspension (0.5% charcoal, 0.1% dextran, 0.9% NaCl) was added to each tube. After further mixing, tubes were incubated on ice for 5 min and centrifuged at 1200*g* for 5 min at 4°. Aliquots (200 µl) of supernatant were added to scintillation vials containing 5 ml Unisolve 1 liquid scintillation fluid (Koch–Light) and counted under standard ^{3}H conditions. A standard curve was constructed and unknowns were read from this curve.

Cortisol assay characteristics. Aliquots of ethyl acetate-extracted plasma diluted parallel to the standard curve over the complete range of 0–800 pg tube^{-1}. The smallest amount of cortisol statistically distinguishable from 0 was 10 pg tube^{-1} (≡0.2 ng ml^{-1} plasma). The following values for between-assay variation were obtained from 10 separate assays of three plasma pools: low pool 1.4 ± 0.1 ng ml^{-1} ($\bar{x}$ ± SEM), coefficient of variation (CV) = 20.8%; medium pool 7.6 ± 0.2 ng ml^{-1}, CV = 7.1%; high pool 37.2 ± 1.8 ng ml^{-1}, CV = 15.5%. The following values for within-assay variation were obtained from eight determinations of the three plasma pools: low pool 1.3 ± 0.1 ng ml^{-1}, CV = 21.2%; medium pool 6.7 ± 0.14 ng ml^{-1}, CV = 6.2%; high pool 39.1 ± 1.2 ng ml^{-1}, CV = 8.9%. Regression analysis of measured cortisol against cortisol added to stripped plasma gave a correlation coefficient (r) of 0.98 ($P < 0.01$) and a gradient of 0.9.

Statistical analysis. The data were subjected to analysis of variance (Genstat) with treatment (dexamethasone, control), confinement stress (stressed, unstressed), and time as factors. For experiments 2 and 3, tank and fish were used as blocking effects to give a nested error structure with which to assess the significance of the factors and their interactions. From a plot of the residuals against fitted values appropriate transformations (√ or log) were selected, where necessary, to improve homogeneity of variance. Square-root transformations were used for the ACTH data (experiment 2) and for the neutrophil and lymphocyte data (experiment 3); log transformations were used for the cortisol data (experiments 1 and 2); erythrocyte and thrombocyte data (experiment 3) were analysed without prior transformation. The levels of significance given in the paper are derived from these analyses but for ease of presentation, data are given as arithmetic means ± SEM.

RESULTS

Experiment 1: Dose of dexamethasone needed to block the HPI axis. Plasma cortisol values for brown trout stressed by confinement for 1 hr at various times post-dexamethasone treatment are presented in Fig. 1. In control fish, mean cortisol levels in stressed fish were always in the range of 70–160 ng ml^{-1} although there was some evidence that cortisol levels in stressed fish increased during the course of the experiment ($P < 0.05$). Dexamethasone at a dose of 100 mg kg^{-1} food had a slight suppressive effect on the stress-induced elevation of plasma cortisol levels at 24, 48, and 72 hr after steroid treatment ($P < 0.01$) although

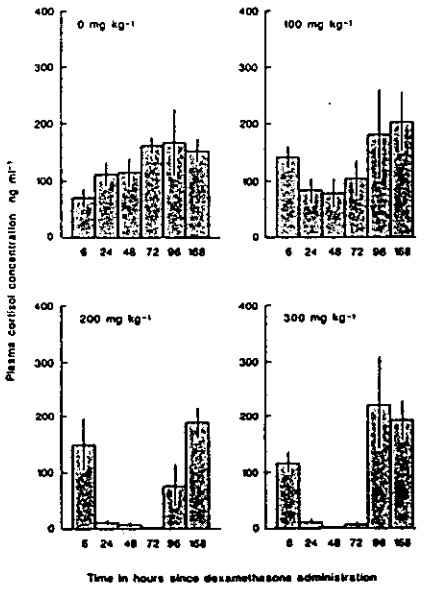


FIG. 1. Effect of oral administration of dexamethasone on the plasma cortisol levels in brown trout stressed by acute confinement for 1 hr at various times post-dexamethasone treatment. Values are arithmetic means; vertical lines indicate ± SEM (n = 6 in each case). Concentrations of dexamethasone are given as mg kg^{-1} food and fish were given a single meal of trout pellets (1% body wt) at 0 hr.

levels were not reduced to anywhere near those of unstressed fish (<1 ng ml^{-1}, Table 1). Dexamethasone at both 200 and 300 mg kg^{-1} food had a marked suppressive effect on the cortisol response to confinement stress ($P < 0.001$ in each case) with cortisol levels in stressed fish remaining very low (<5 ng ml^{-1}) at both 48 and 72 hr after administration of the steroid. However, at 96 hr posttreatment, the suppressive effect of dexamethasone had disappeared. For subsequent studies, a dose of 300 mg dexamethasone kg^{-1} food, given as a single meal (1% body wt), was selected. This dose, which amounts to ≈3 µg g^{-1} fish, gave maximum suppression 48 hr after administration.

TABLE 1
LEVELS OF PLASMA ACTH AND CORTISOL IN UNSTRESSED AND STRESSED BROWN TROUT BOTH WITH AND WITHOUT DEXAMETHASONE TREATMENT

	ACTH (pg ml^{-1})	Cortisol (ng ml^{-1})
Unstressed		
Control	66.3 ± 9.7 (12)	0.5 ± 0.3 (12)
Dexamethasone	10.6 ± 2.2 (12)	<0.2 (12)
Stressed (30 min confinement)		
Control	646.0 ± 56.8 (10)	76.3 ± 20.8 (12)
Dexamethasone	10.9 ± 1.8 (10)	1.7 ± 0.8 (11)

Note. Values are expressed as arithmetic means ± SEM (n).

Experiment 2: The effect of dexamethasone on plasma ACTH levels. Plasma ACTH and cortisol levels for stressed and unstressed brown trout both with and without dexamethasone treatment are presented in Table 1. Unstressed, untreated (control) brown trout had a mean plasma ACTH concentration of 66 pg ml^{-1} and mean cortisol level of 0.5 ng ml^{-1}. Dexamethasone treatment (300 mg kg food^{-1}, fed as a single meal 48 hr previously) significantly suppressed ACTH levels to 10 µg ml^{-1} ($P < 0.001$). Cortisol levels in unstressed, dexamethasone-treated fish all were below the limit of detection of our assay (<0.2 ng ml^{-1}). Thirty minutes confinement stress elevated plasma ACTH levels of untreated control fish from 66 to 646 pg ml^{-1} ($P < 0.001$) with a concomitant increase in plasma cortisol from 0.5 to 76 ng ml^{-1} ($P < 0.001$). In dexamethasone-treated fish, however, acute confinement stress did not significantly elevate plasma ACTH and cortisol levels which stayed at 10 pg ml^{-1} and <2 ng ml^{-1}, respectively.

Experiment 3: Effect of dexamethasone on the haematology of the brown trout. A single dose of dexamethasone (300 mg kg food^{-1}) had significant effects on three of the four major circulating blood cell types (Fig. 2). Erythrocyte counts were elevated in dexamethasone-treated fish at 24 and 48 hr posttreatment ($P < 0.001$) but had re-

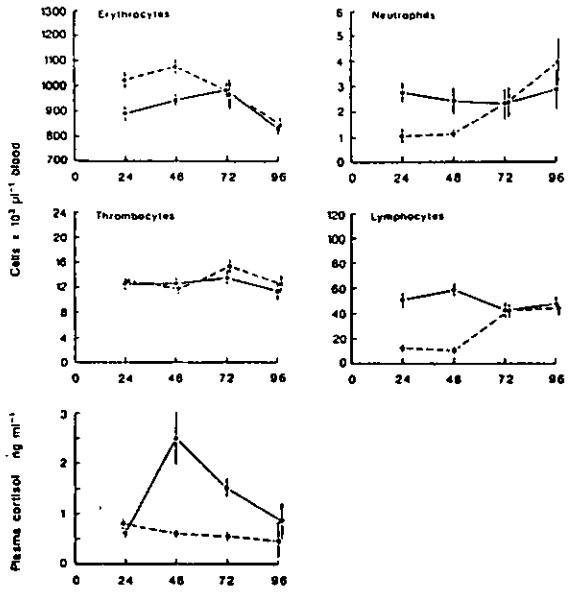


FIG. 2. Haematological changes in brown trout following oral administration of dexamethasone (300 mg kg^{-1} food) in a single meal (1% body wt) at 0 hr. Values are arithmetic means ± SEM (n = 24 in each case). Continuous line indicates control fish (fed ethanol-treated diet); broken line represents dexamethasone-treated fish. Plasma cortisol values for the same fish are given in the lower graph.

turned to control values by 72 hr. Circulating neutrophil numbers were significantly depressed by dexamethasone at 24 and 48 hr posttreatment ($P < 0.001$) as were lymphocyte counts ($P < 0.001$). Thrombocyte concentration was not significantly affected by dexamethasone treatment and remained stable in both groups throughout the experimental period. Cortisol values in unstressed control fish showed a slight, but highly significant ($P < 0.001$), elevation from 0.6 to 2.5 ng ml^{-1} at 48 hr posttreatment (i.e., 24 hr after the first sample) with a gradual return to <1 ng ml^{-1} at 96 hr. It is likely that this represents a minor stress response to unavoidable disturbance during the daily sampling of fish from each tank with a subsequent acclimation to the repeated stimulus (see Pickering and Pottinger, 1984). Dexamethasone treatment completely abolished this response, with levels remaining at ≈0.5 ng ml^{-1} throughout the experiment.

DISCUSSION

The present study has shown that oral administration of the synthetic steroid, dexamethasone, can be used to successfully block the HPI axis in brown trout. A single meal (1% bod · wt) containing dexamethasone at a concentration of 300 mg kg^{-1} food (3 µg g^{-1} fish) was sufficient to block the cortisol response to handling and confinement stress for a period of up to 3 days. Using intraperitoneal injection as the delivery system, previous workers have shown that the effective doses of dexamethasone (and betamethasone) for blocking the HPI axis in fish lie in the range 0.1–10 µg g^{-1} (Donaldson and McBride, 1967; McLeay, 1973b; Fryer, 1975; Swift, 1982).

Dexamethasone treatment significantly suppressed basal ACTH levels in the brown trout (down to 10 pg ml^{-1}), a result in accordance with those of Sumpter and Donaldson (1986) on salmonid fish. Acute handling stress combined with 30 min confinement caused a marked elevation of plasma ACTH (up to 650 pg ml^{-1}), a rise which was totally eliminated by dexamethasone treatment. This confirms the work of Sumpter and Donaldson (1986) and shows that dexamethasone acts in salmonid fish by suppressing the release of ACTH from the corticotrophs. However, its precise mode of action needs further study. Sage (1968) and Sage and Purrott (1969) present evidence from *in vitro* studies which suggests that corticosteroids, at high concentrations, can exert a direct negative feedback on the corticotrophs themselves whereas Fryer and Peter (1977a, b, c), in an elegant series of studies on the goldfish, argue convincingly that the sites of negative feedback are located in the hypothalamus and telencephalon.

As might be expected, changes in plasma ACTH levels were closely paralleled by changes in plasma cortisol with unstressed control brown trout having cortisol levels of <1 ng ml^{-1} (similar to those reported by Pickering and Pottinger (1984) for the same species). Cortisol levels in dexamethasone-treated fish were normally below the limit of detection of our assay and the elevation of cortisol in untreated fish (up to 76 ng ml^{-1}) following handling and confinement for 30 min was totally suppressed in treated fish. There can be little doubt, therefore, that oral administration of dexamethasone effectively blocks the HPI axis in stressed fish by suppressing ACTH release from the pituitary gland and, consequently, the secretion of cortisol from the interrenal tissue.

In mammals, dexamethasone can act as a glucocorticoid with a potency often several times greater than that of the natural corticosteroids, cortisol and corticosterone (Burton *et al.*, 1967). The fact that it effectively blocks ACTH release in the brown trout (presumably acting via specific corticosteroid receptors) also suggests that it may act peripherally on other corticosteroid-sensitive tissues. Previous studies on the brown trout using orally administered cortisol have shown that the lymphocytes are steroid-sensitive and that physiological doses of cortisol can result in a marked lymphocytopenia (Pickering, 1984). This steroid sensitivity has also been demonstrated in coho salmon, *Oncorhynchus kisutch*, by McLeay (1973a, b). Moreover, McLeay showed that dexamethasone, at either 1 or 10 µg g^{-1} fish, was effective in decreasing the number of circulating small lymphocytes. Other possible changes in cell numbers in McLeay's study are somewhat more difficult to interpret because all the cell counts are presented in relative terms. In the present study, absolute concentrations of each cell type were determined and dexamethasone had a clear suppressive effect on the number of circulating lymphocytes and neutrophils. In addition, dexamethasone also increased the number of circulating erythrocytes (assuming no changes in total blood volume). Swift (1982) found a similar effect of betamethasone on the haematocrit values in the rainbow trout and was unable to detect any changes in the concentration of major ions or total protein. It is unlikely, therefore, that the changes in blood cell concentrations resulted from any change in total blood volume.

The effects of dexamethasone differ in several ways from those of the natural corticosteroid, cortisol. For example, in previous studies on the brown trout, physiological doses of cortisol affected only the lymphocyte count, not the neutrophil or erythrocyte counts (Pickering, 1984). It is not clear whether this results from differences in dosage or potency between the

two steroids but it is interesting that McLeay (1973b) reported that 1 μg g^{-1} dexamethasone reduced the number of circulating thrombocytes in coho salmon whereas 200 μg g^{-1} cortisol had no significant effect on this cell type. This suggests that in addition to its cortisol-like effect on circulating lymphocytes, dexamethasone (and betamethasone) may also have other, more specific, effects on the blood cell population in salmonid fish. In the present study, however, dexamethasone had no significant effect on the number of circulating thrombocytes.

Thus, dexamethasone is an effective steroid for blocking the HPI axis of the brown trout. It acts by inhibiting the secretion of ACTH from the corticotrophs, probably via an effect on the secretion of corticotropin-releasing factor from the hypothalamus. However, its value as a tool for investigating the role of the interrenal during stress responses is limited by its cortisol-like activity on other steroid-sensitive tissues.

ACKNOWLEDGMENTS

The authors are particularly grateful to Mrs. J. Pollard and Miss J. M. Fletcher for maintaining the experimental fish, Mrs. M. A. Hurley for statistical advice, and Mr. T. I. Furnass for the artwork. This work was partly financed by a contract with MAFF.

REFERENCES

Bradshaw, S. D., and Fontaine-Bertrand, E. (1968). Le cortisol dans l'Anguille, dosé par fluorimétrie et par inhibition compétitive de la liaison spécifique cortisol-transcortine: Influence de diverses conditions expérimentales. *C.R. Acad. Sci. Paris Ser. D.* 267, 894–897.

Burton, A. F., Storr, J. M., and Dunn, W. L. (1967). Cytolytic action of corticosteroids on thymus and lymphoma cells in vitro. *Canad. J. Biochem.* 45, 289–297.

Butler, D. G., Clarke, W. C., Donaldson, E. M., and Langford, R. W. (1969). Surgical adrenalectomy of a teleost fish (*Anguilla rostrata* Le Sueur): Effect on plasma cortisol and tissue electrolyte and carbohydrate concentrations. *Gen. Comp. Endocrinol.* 12, 503–514.

Chan, D. K. O., Rankin, J. C., and Chester Jones, I. (1969). Influences of the adrenal cortex and the corpuscles of Stannius on osmoregulation in the European eel (*Anguilla anguilla* L.) adapted to freshwater. *Gen. Comp. Endocrinol. Suppl.* 2, 342–353.

Donaldson, E. M. (1981). The pituitary-interrenal axis as an indicator of stress in fish. *In* "Stress and Fish" (A. D. Pickering, ed.), pp. 11–47. Academic Press, New York/London.

Donaldson, E. M., and McBride, J. R. (1967). The effects of hypophysectomy in the rainbow trout *Salmo gairdnerii* (Rich.) with special reference to the pituitary-interrenal axis. *Gen. Comp. Endocrinol.* 9, 93–101.

Ellsaesser, C. F., and Clem, L. W. (1986). Haematological and immunological changes in channel catfish stressed by handling and transport. *J. Fish Biol.* 28, 511–521.

Fagerlund, U. H. M. (1967). Plasma cortisol concentration in relation to stress in adult sockeye salmon during the freshwater stage of their life cycle. *Gen. Comp. Endocrinol.* 8, 197–207.

Fagerlund, U. H. M., and McBride, J. R. (1969). Suppression by dexamethasone of interrenal activity in adult sockeye salmon (*Oncorhynchus nerka*). *Gen. Comp. Endocrinol.* 12, 651–657.

Fryer, J. N. (1975). Stress and adrenocorticosteroid dynamics in the goldfish, *Carassius auratus*. *Canad. J. Zool.* 53, 1012–1020.

Fryer, J. N., and Peter, R. E. (1977a). Hypothalamic control of ACTH in goldfish. I. Corticotrophin-releasing factor activity in teleost brain tissue extracts. *Gen. Comp. Endocrinol.* 33, 196–201.

Fryer, J. N., and Peter, R. E. (1977b). Hypothalamic control of ACTH secretion in goldfish. II. Hypothalamic lesioning studies. *Gen. Comp. Endocrinol.* 33, 202–214.

Fryer, J. N., and Peter, R. E. (1977c). Hypothalamic control of ACTH secretion in goldfish. III. Hypothalamic cortisol implant studies. *Gen. Comp. Endocrinol.* 33, 215–225.

Hawkins, E. F., and Ball, J. N. (1973). Current knowledge of the mechanisms involved in the control of ACTH secretion in teleost fishes. *In* "Brain-Pituitary-Adrenal Relationships" (A. Brodish and E. S. Redgate, eds.), pp. 293–315. Karger, Basel.

McLeay, D. J. (1973a). Effects of ACTH on the pituitary-interrenal axis and abundance of white blood cell types in juvenile coho salmon, *Oncorhynchus kisutch*. *Gen. Comp. Endocrinol.* 21, 431–440.

McLeay, D. J. (1973b). Effects of cortisol and dexamethasone on the pituitary-interrenal axis and abundance of white blood cell types in juvenile coho salmon, *Oncorhynchus kisutch*. *Gen. Comp. Endocrinol.* 21, 441–450.

Pickering, A. D. (1984). Cortisol-induced lymphocytopenia in brown trout, *Salmo trutta* L. *Gen. Comp. Endocrinol.* 53, 252–259.

Pickering, A. D., and Duston, J. (1982). Administration of cortisol to brown trout, *Salmo trutta* L., and its effects on the susceptibility to *Saprolegnia* infection and furunculosis. *J. Fish Biol.* 23, 163–175.

Pickering, A. D., and Pottinger, T. G. (1983). Seasonal and diel changes in plasma cortisol levels of the brown trout, *Salmo trutta* L. *Gen. Comp. Endocrinol.* 49, 232–239.

Pickering, A. D., and Pottinger, T. G. (1984). Acclimation of the brown trout, *Salmo trutta* L., to the stress of daily malachite green treatment. *Aquaculture* 44, 145–152.

Pickering, A. D., and Pottinger, T. G. (1985). Cortisol can increase the susceptibility of brown trout, *Salmo trutta* L., to disease without affecting the white blood cell count. *J. Fish Biol.* 27, 611–619.

Pickering, A. D., Pottinger, T. G., and Christie, P. (1982). Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: A time-course study. *J. Fish Biol.* 20, 229–244.

Plotsky, P. H., and Vale, W. (1984). Hemorrhage-induced secretion of corticotropin-releasing factor-like immunoreactivity into the rat hypophysial portal circulation and its inhibition by glucocorticoids. *Endocrinology* 114, 164–169.

Purves, H. D., and Sirett, N. E. (1965). Assay of corticotrophin in dexamethasone-treated rats. *Endocrinology* 77, 366–374.

Sage, M. (1968). Responses to steroids of *Xiphophorus* ACTH cells in organ culture. *Z. Zellforsch. Mikrosk. Anat.* 92, 34–42.

Sage, M., and Purrott, R. J. (1969). The control of teleost ACTH cells. *Z. Vgl. Physiol.* 63, 85–90.

Stave, J. W., and Roberson, B. S. (1985). Hydrocortisone suppresses the chemiluminescent response of striped bass phagocytes. *Dev. Comp. Immunol.* 9, 77–84.

Sumpter, J. P., and Donaldson, E. M. (1986). The development and validation of a radioimmunoassay to measure plasma ACTH levels in salmonid fishes. *Gen. Comp. Endocrinol.* 62, 367–376.

Sumpter, J. P., Dye, H. M., and Benfey, T. J. (1986). The effects of stress on plasma ACTH, α-MSH, and cortisol levels in salmonid fishes. *Gen. Comp. Endocrinol.* 62, 377–385.

Swift, D. J. (1982). Changes in selected blood component values of rainbow trout, *Salmo gairdneri* Richardson, following the blocking of the cortisol stress response with betamethasone and subsequent exposure to phenol or hypoxia. *J. Fish Biol.* 21, 269–277.

GENERAL AND COMPARATIVE ENDOCRINOLOGY 68, 249–259 (1987)

The Effects of Acute and Chronic Stress on the Levels of Reproductive Hormones in the Plasma of Mature Male Brown Trout, *Salmo trutta* L.

A. D. PICKERING,* T. G. POTTINGER,* J. CARRAGHER, AND J. P. SUMPTER

*Freshwater Biological Association, The Ferry House, Far Sawrey, Nr. Ambleside, Cumbria, LA22 OLP, and Department of Applied Biology, Brunel University, Uxbridge, Middlesex, UB8 3PH, United Kingdom

Accepted June 13, 1987

Chronic confinement for 1 month caused a significant elevation of plasma cortisol but suppressed the levels of plasma testosterone and 11-ketotestosterone in sexually mature male brown trout. An acute handling stress for 1 hr elevated blood cortisol and ACTH levels and also suppressed circulating androgens. This androgen suppression in response to acute stress was accompanied by an *elevation* of plasma gonadotropin levels. These findings are discussed in relation to stress-induced suppression of reproductive function in mammals and the possible biological consequences of such a suppression in fish are outlined.

It is well known that most forms of environmental stress can inhibit reproductive processes in mammals, an effect evident in domesticated animals (Grandin, 1984; Moberg, 1985) as well as in natural populations (Christian, 1961). This may be attributed to reduced hypothalamic LH-RH secretion (Kawakami and Higuchi, 1981; Sirinathsinghji *et al.*, 1983; Rivier *et al.*, 1986), suppressed LH (and, to a lesser extent, FSH) release from the pituitary gland (Krulich *et al.*, 1974; Gray *et al.*, 1978; Taché *et al.*, 1978), and a resultant reduction in the levels of circulating sex steroids (Kreuz *et al.*, 1972; Repčeková and Mikulaj, 1977; Francis, 1981). In stressed males, the suppression of testosterone levels may also be caused by elevated plasma corticosteroid levels, which can inhibit androgen synthesis and/or release (Saez *et al.*, 1977; Bambino and Hseuh, 1981; Welsh *et al.*, 1982; Sapolsky, 1985). It is also probable that corticosteroids suppress the levels of reproductive hormones by means of feedback mechanisms located in the hypothalamus and pituitary (Thibier and Rolland, 1976; Welsh and Johnson, 1981). A similar type of stress-induced suppression of the reproductive system has been observed in reptiles and amphibians (Licht *et al.*, 1983; Moore and Miller, 1984; Lance and Elsey, 1986) and Moore and Zoeller (1985) provide convincing evidence that corticosterone can suppress the hypothalamic–pituitary–gonadal axis in the newt, *Taricha granulosa*.

As in higher vertebrates, fish reproduction is influenced by environmental stress but it has generally been assumed that the primary impact is on the fish's growth rate and that this, in turn, has particular consequences for fecundity and age at maturity (Stearns and Crandall, 1984). Food supply and fecundity are positively linked (Scott, 1962; Bagenal, 1969) and population density and fecundity may be inversely related (Bagenal, 1966; Kipling and Frost, 1969; Kuznetsov and Khalitov, 1978), thus supporting the conclusion that variation in growth rate has a major influence on reproductive physiology. However, there is also evidence from the literature that stress-induced suppression of reproduction in teleost fish can act via mechanisms independent of food supply and growth rate. Thus, Dahlgren (1979) demonstrated a suppression of ovarian growth in guppies, *Poecilia reticulata*, at high population densities even when the food supply was constant and proportional to the population size. Similarly, Hedtke and Puglisi (1980) found that the stress of oil exposure impaired egg production in *Jordanella floridae*, even though growth was not affected. Mann and Mills (1985) reported that the egg number and mean egg size of the dace, *Leuciscus leuciscus*, were low during the spawning season of 1977 despite very high somatic growth during the previous, exceptionally warm summer. This was interpreted by Stearns and Crandall (1984) as a response to extreme temperature stress.

Hitherto, little attention has been given to the effects of stress on the endocrinology of reproduction in fish (see review by Billard *et al.*, 1981) despite the implications this may have for fishery management and for aquaculture. Gillet *et al.* (1981) found that hypoxia or starvation resulted in a decrease in both plasma and pituitary GTH levels in the goldfish, *Carassius auratus*. On the other hand, acute emersion stress failed to influence plasma GTH levels in rainbow trout, *Salmo gairdneri* (C. Bry, unpublished data, in Billard *et al.*, 1981), while Leatherland *et al.* (1982) found that electroshock elevated, rather than depressed, plasma GTH levels in mature female coho salmon, *Oncorhynchus kisutch*, although it had no effect in sexually mature males. Low pH can inhibit reproduction in teleost fish (Menendez, 1976; Craig and Baski, 1977; Ruby *et al.*, 1977; Lee and Gerking, 1980) although Weiner *et al.* (1986) failed to observe any suppression of sex steroid levels in rainbow trout exposed to low pH, despite a demonstrable effect of the acid conditions on gamete quality. By comparison, Freeman *et al.* (1983) found that the plasma levels of testosterone and 11-ketotestosterone in migrating Atlantic salmon, *Salmo salar*, in Nova Scotia were significantly lower in the Westfield River (pH 4.7) than in the Medway River (pH 5.6). Other pollutants have variously been reported to elevate plasma androgen levels (Sangalang and Freeman, 1974) or to suppress them (Truscott *et al.*, 1983). In a study designed primarily to investigate the reduced reproductive success of coho salmon in Lake Erie, Morrison *et al.* (1985) briefly stated that handling suppressed androgen levels in these fish but, surprisingly, it had no effect on blood cortisol levels. As yet, no convincing evidence of interrenal involvement in the suppression of reproductive endocrinology in teleost fish has been presented, although working with pharmacological doses of hormone, Rasquin and Atz (1952) reported that ACTH and cortisone depressed spermatogenesis and caused the resorption of mature eggs in *Astyanax mexicanus*, and Joshi (1982) found that cortisol treatment arrested spermiation and inhibited maturation in *Labeo gonius*.

Salmonid fish occupy a unique position with regard to the effects of environmental stress; first, because of their requirements for water of the highest quality the fish are extremely sensitive to deleterious changes in the environment and, second, because this group of fish forms the basis of an expanding aquaculture industry. Under these conditions, fish are subjected to such stresses as handling, grading, transport, confinement, overcrowding, and water quality deterioration. However, little is known about the effects of such treatments on salmonid reproductive physiology.

The present study was designed to investigate the effects of chronic confinement stress and acute handling stress on the reproductive endocrinology of male brown trout, *Salmo trutta* L. This species was chosen because we have evidence from previous studies that it is sensitive to the stress of handling and confinement (Pickering *et al.*, 1982, 1986; Pickering and Pottinger, 1985, 1987a; Sumpter *et al.*, 1985) and is a species for which we have background information on the changes in

0016-6480/87 $1.50

plasma hormone levels during the course of sexual maturation (Pickering and Christie, 1981; Pottinger and Pickering, 1985, 1987).

MATERIALS AND METHODS

Experimental fish. A stock of brown trout (FBA strain, hatched January 1983) was reared in large (1500 liter) outdoor,fibreglass tanks each supplied with a constant flow of Windermere lake water (35 liters min^{-1}, overall temperature range 2–18°). The fish were fed with commercial trout pellets at rates recommended by the manufacturers (exact rate dependent upon fish size and water temperature). In October 1985, 40 sexually mature males from this population (mean body wt 326 ± 25.2 g (± SEM)) were used in Experiment 1 (a study of the effects of chronic confinement) and in November 1986 a further 120 sexually mature males (mean body wt 470 ± 17.3 g) were used in Experiment 2 (a study of the effects of acute handling stress). During the spawning season (October–February) malachite green (2.77 ppm) was used on a twice-weekly basis to prevent colonization of the mature fish by the pathogenic fungus *Saprolegnia diclina* Type I (see Pickering and Willoughby, 1982).

Experiment 1: chronic confinement stress. Thirteen sexually mature male brown trout were taken from their rearing tanks and individually confined, under natural photoperiod, in small glass aquaria (60 × 30 × 30 cm) each supplied with a constant flow of Windermere lake water at ambient temperature (4 liters min^{-1}, temperature range 11.4–14.7°). After 1 month of confinement, each fish was rapidly anaesthetized in MS222 (Sandoz, 100 mg $liter^{-1}$) and a blood sample was taken from the caudal vessels by means of a heparinized syringe. On the same day, blood samples were also taken from 27 sexually mature brown trout from the stock population in the outdoor rearing tanks. With care it is possible to obtain blood samples from salmonid fish in large outdoor tanks without a stress-induced elevation of the apparent "basal" plasma cortisol level (Pickering and Pottinger, 1985). Blood plasma was stored at −70° until assayed for cortisol, testosterone, and 11-ketotestosterone.

Experiment 2: acute handling and confinement stress. In early November 1986, five sexually mature male brown trout from the stock population were placed in each of 24 large rearing tanks. After a recovery period of 2 weeks, which allowed the fish to overcome the effects of acute handling stress (Pickering *et al.*, 1982), the fish in each tank (with the exception of the 0-hr control fish) were subjected to an acute stress. This consisted of netting the fish from the rearing tank, transferring them to a small confinement tank (45 × 30 × 15 cm) supplied with a constant flow of Windermere lake water at ambient temperature (10 liters min^{-1}, 8.5°) for a period of 1 hr, and then returning the fish to their own rearing tank. Duplicate tanks of fish were then sampled at 0 hr (prestress) and at 1, 4, 8, 24, and 48 hr poststress. This experimental design avoided repeated disturbance of the fish during sampling (each tank was only sampled once), an important consideration in any investigation of acute stress responses in salmonid fish (see Pickering *et al.*, 1982; Pickering and Pottinger, 1985). Blood samples were taken from the caudal vessels of anaesthetized fish but EDTA-dusted syringes were used in this experiment because heparin interferes with the ACTH assay (Sumpter and Donaldson, 1986). Aprotinin (Sigma, 3750 kallikrein units ml^{-1} blood) was also used to protect the ACTH molecule from proteolytic degradation. Plasma samples were stored at −70° until assayed for cortisol, testosterone, 11-ketotestosterone, ACTH, and GTH.

Radioimmunoassays. In most cases plasma hormone levels were assayed using established and previously validated radioimmunoassays. Cortisol was determined according to Pickering *et al.* (1987); testosterone and 11-ketotestosterone were measured by the methods of Pottinger and Pickering (1985) but using antisera of higher specificity, thereby allowing the omission of the TLC separation stage. Details of the cross-reactivities for the testosterone and 11-ketotestosterone antisera can be found in Scott *et al.* (1984) and Dye *et al.* (1986), respectively. Gonadotropin was assayed by means of a recently developed radioimmunoassay, which was considerably more sensitive than the one used by us in earlier studies (see, for example, Scott and Sumpter, 1983). Gonadotropin was purified from chum salmon (*Oncorhynchus keta*) pituitary glands by ethanolic precipitation, gel-filtration, anion- and cation-exchange chromatography, affinity chromatography on Con A–Sepharose, and a final gel-filtration step. If biologically distinct gonadotropins exist in fish, the purified hormone represents the so-called maturational or ovulatory GTH (Idler, 1982). Antibodies were raised against this preparation and the antiserum producing the most sensitive standard curve was used. Tracer for the assay was prepared by iodination of the purified GTH. The standard employed was another highly purified GTH preparation (DEAE III, Fraction 13), from a chinook salmon (*Oncorhynchus tschawytscha*), kindly provided by Dr B. Breton. The practical sensitivity of the radioimmunoassay was 0.1 ng ml^{-1}. Plasma from brown trout diluted parallel to the standard GTH in this radioimmunoassay, suggesting that it was appropriate to use it for the measurement of brown trout GTH. It is possible that TSH cross-reacts in all of the GTH radioimmunoassays in use presently, although the physiological studies conducted with salmonid GTH radioimmunoassays suggest that this interference, if it occurs, is not significant. All of these problems concerned with the radioimmunoassay of GTH in fish are discussed extensively in Dodd and Sumpter (1984).

Statistics. The levels of plasma cortisol, testosterone, and 11-ketotestosterone from Experiment 1 were separately analysed by one-way analysis of variance (ANOVA, Genstat). From a plot of residuals against fitted values appropriate transformations were selected, when necessary, to improve homogeneity of variance. A log transformation was used for the cortisol and testosterone data; the 11-ketotestosterone levels were analysed without prior transformation. The hormone levels from Experiment 2 were separately analyzed by multifactorial analysis of variance (ANOVA, Genstat) with treatment (stressed, unstressed), time, and number (sequence within each sample) as factors. Tanks and fish were used as blocking effects to produce a nested error structure with which to assess the significance of the factors and their interactions. A square root transformation was used for the cortisol and ACTH data; androgen levels and GTH were analysed without prior transformation. Levels of significance given in this paper are derived from these analyses but for ease of presentation, data are given as arithmetic means ± SEM.

RESULTS

Sexually mature male brown trout subjected to chronic confinement (Experiment 1) did not fully acclimate to the conditions, as indicated by their reduced feeding during the course of the experiment. Figure 1 shows a comparison of the plasma steroid hormone levels of these fish (after 1 month's confinement) with the hormone levels of control fish from the rearing tanks. Confinement caused a highly significant ($P < 0.001$) elevation of blood cortisol levels from 2 to 14 ng ml^{-1}, but it suppressed plasma testosterone levels by more than 50% ($P < 0.05$) and also had a significant ($P < 0.05$) suppressive effect on 11-ketotestosterone levels.

Handling and confinement for 1 hr (Experiment 2) caused a marked elevation of blood ACTH levels from a mean of ~50 pg ml^{-1} in the unstressed control fish to over 120 pg ml^{-1} at the end of the 1-hr confinement period (Fig. 2a, $P < 0.01$). However, plasma ACTH returned to control levels within 4 hr and remained stable during the next 48 hr. The mean plasma cortisol concentration of sexually mature unstressed male brown trout in Experiment 2 was approximately 13 ng ml^{-1}. Handling and confinement caused a marked elevation of blood cortisol levels (to ~70 ng ml^{-1}), which lasted at least 8 hr before returning to the levels in unstressed control fish (Fig. 2b). Blood gonadotropin levels showed a marked and significant elevation in response to acute stress ($P < 0.01$), with levels being significantly elevated within 1 hr and remaining high (up to 4 ng ml^{-1}) for at least 4 hr (Fig. 3a). In the unstressed control fish, GTH levels remained stable at ~2 ng ml^{-1}. Acute stress caused a rapid suppression of plasma testosterone levels

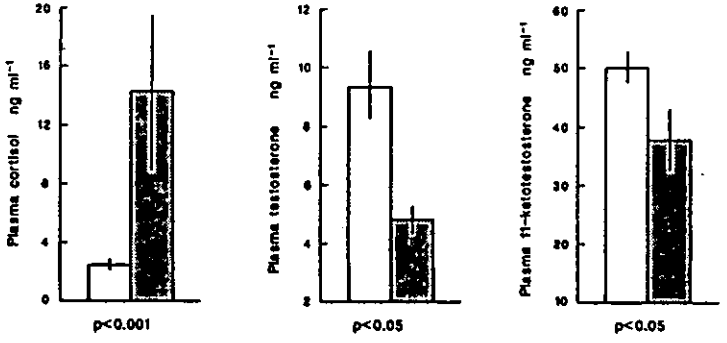


FIG. 1. The effect of confinement for 1 month on the levels of circulating cortisol, testosterone, and 11-ketotestosterone in sexually mature, male brown trout. Open columns represent the control fish; shaded columns represent the stressed fish. Values are means ± SEM (n = 27 for the control fish, n = 13 for the stressed fish).

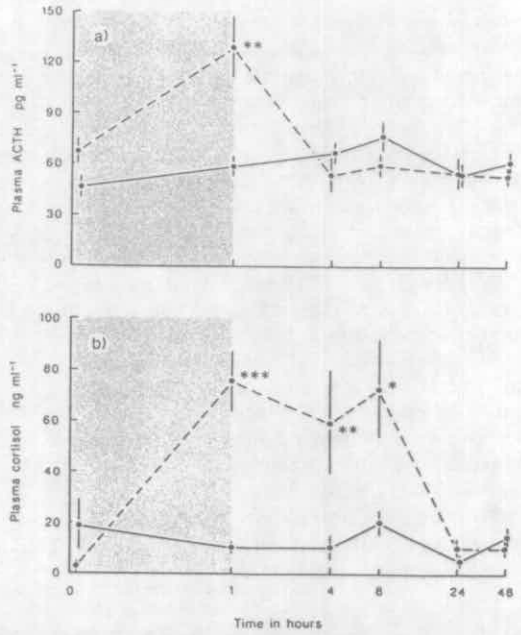


FIG. 2. The effect of acute handling and confinement stress on the levels of circulating ACTH (a) and cortisol (b) in sexually mature, male brown trout. The continuous lines represent unstressed control fish, the broken lines represent stressed fish. The shaded area denotes the duration of the period of stress. Values are means ± SEM (n = 10 in each case). The asterisks show the significance of the differences between control and stressed fish at each sampling time (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

(from ~12 down to 4 ng ml^{-1}, $P < 0.01$), and even at 8 hr poststress, plasma testosterone levels were still significantly lower than those of the unstressed control fish. By 24 hr, however, testosterone levels had returned to normal but were significantly elevated at 48 hr poststress (Fig. 3b). The effects of acute stress on 11-ketotestosterone levels were similar, but less marked, with a significant suppression ($P < 0.05$) at 4 hr poststress only (Fig. 3c).

DISCUSSION

The present study examines the effects of chronic (continuous) and acute (short-term) stress on the pituitary–interrenal and pituitary–gonadal axes of sexually mature male brown trout. Plasma cortisol levels of chronically confined trout (Experiment 1) were still elevated after 1 month's confinement, indicating that the fish had not fully acclimated to this form of stress. In a previous study, we have shown that brown trout will acclimate to another form of chronic stress, overcrowding, with blood cortisol levels eventually returning to normal (Pickering and Stewart, 1984), although complete acclimation may take several weeks. The chronically confined fish fed little during the present study and it was impossible, therefore, to dissociate the effects of confinement (and isolation?) per se from the effects of starvation. However, in other studies (T. G. Pottinger, unpublished)

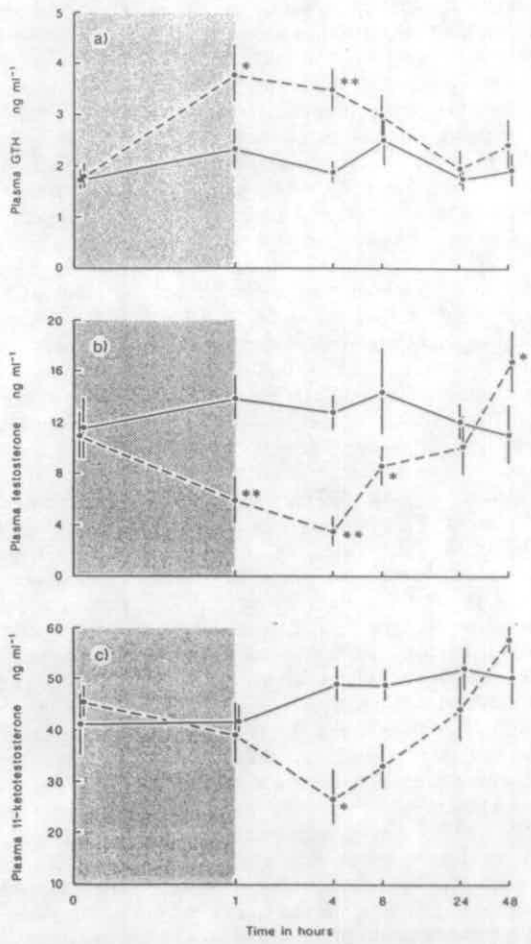


FIG. 3. The effect of acute handling and confinement stress on the levels of circulating GTH (a), testosterone (b), and 11-ketotestosterone (c) in sexually mature, male brown trout. The continuous lines represent unstressed control fish, the broken lines represent stressed fish. The shaded area denotes the duration of the period of stress. Values are means ± SEM (n = 10 in each case). The asterisks show the significance of the differences between control and stressed fish at each sampling time (*$P < 0.05$, **$P < 0.01$).

we have found that blood cortisol levels remain extremely low in starved, but otherwise unstressed, brown trout and so it would appear that confinement was responsible for the chronic interrenal activation of the fish in Experiment 1.

Acute handling stress (Experiment 2) caused a marked stimulation of the pitu-

itary–interrenal axis in sexually mature male brown trout, characterized by an elevation of both plasma ACTH and cortisol levels. The magnitude and duration of this response was similar to that previously reported for salmonid fish subjected to an acute handling stress (Pickering *et al.*, 1982; Sumpter *et al.*, 1986). In the present case, starvation was not a factor because both the control and stressed groups were feeding well prior to experimentation. Blood cortisol levels in the unstressed control fish from Experiment 2 (~13 ng ml^{-1}) were significantly higher than those from Experiment 1 (~2 ng ml^{-1}, $P < 0.001$). This almost certainly reflects differences in the timing of the two experiments. Although spermiation had taken place in both studies (gentle abdominal pressure resulted in the expression of milt), the study of chronic confinement stress was undertaken in October, at the beginning of the spawning season, whereas the study of acute handling stress took place in November. It is known that the blood cortisol levels of sexually mature male brown trout rise significantly during the spawning season (Pickering and Pottinger, 1987b) and the difference in timing between the two studies would account for the observed differences in cortisol levels between the unstressed control fish.

Despite these differences there can be little doubt from the present investigation that both chronic confinement stress and acute handling stress caused a marked suppression of the concentration of the circulating androgens, testosterone and 11-ketotestosterone. With both forms of stress plasma testosterone levels were reduced by more than 50% from ~10 to 4 ng ml^{-1}. Although the percentage of suppression of 11-ketotestosterone levels was not as marked, the reduction in each case was still significant. Schreck *et al.* (1972) found that repeated blood sampling caused a suppression of circulating androgen levels in the rainbow trout, but they were unable to determine whether this represented a stress response or simply a dilution effect.

Some discussion of the possible mechanisms behind this stress-induced androgen suppression is required at this stage. In the present study, whereas androgen levels were suppressed, plasma GTH levels were significantly *elevated* for a period of at least 4 hr following acute handling stress. This paradox is not immediately explicable, although low androgen levels, operating via a negative feedback mechanism located at the level of the hypothalamus and/or pituitary gland (see Peter, 1983), might be at least partly responsible. The simultaneous elevation of plasma gonadotropin (LH) levels and suppression of testosterone levels has also been observed in mammals subjected to acute surgery (and anaesthesia) stress (Nakashima *et al.*, 1975; Aono *et al.*, 1976). When the stress is prolonged or chronic, however, plasma LH levels in mammals ultimately decline (Gray *et al.*, 1978; Taché *et al.*, 1978). A similar difference in the response to acute or chronic stress might occur in fish, although supporting evidence is extremely fragmentary (unfortunately, plasma GTH levels of the chronically stressed fish were not measured during the present study). However, Gillet *et al.* (1981) reported that chronic starvation suppressed plasma GTH levels in the goldfish, and Bry and Zohar (1980) and Zohar (1980) found that plasma GTH levels were reduced in rainbow trout which had not acclimated to the stress of chronic cannulation. In contrast, acutely stressed (electroshock) female coho salmon had elevated plasma GTH levels (Leatherland *et al.*, 1982). This potential difference in the response of the gonadotropes to acute and chronic stress could account for the observed androgen suppression in chronically stressed fish (Experiment 1) but could not account for the observed androgen suppression in acutely stressed brown trout from Experiment 2. Taking our sampling regime into account, it is possible that we

missed a suppression of GTH levels that might have occurred during the early phase of acute stress but is difficult to see how such a short-term event could maintain the androgen suppression for more than 8 hr, particularly when GTH levels were subsequently elevated for at least 4 hr poststress.

In some mammals, corticosteroids appear to act directly on the testes by suppressing GTH-induced androgen secretion (Saez *et al.*, 1977; Bambino and Hseuh, 1981; Welsh *et al.*, 1982; Sapolsky, 1985). The close temporal correlation between elevated plasma cortisol levels and suppressed testosterone levels (compare Fig. 2b with Fig. 3b) is consistent with such a mechanism in salmonid fish although direct evidence, from studies involving cortisol administration, is lacking. Clearly, further work is needed on the possible relationships between interrenal activity, sexual development, and androgen secretion in salmonid fish.

The biological consequences of environmental stress on reproductive endocrinology can be expressed both in terms of reproductive behaviour and in terms of the quantity and quality of the gametes. However, the diverse nature of most environmental stresses makes generalisations extremely difficult, although almost any environmental disturbance can result in follicular atresia in female fish (Ball, 1960). This effect is clearly evident in natural fish populations (June, 1977) and must cause a reduction in fecundity. In addition, the stress of exposure to low pH levels (an increasing problem in many areas) can cause a reduction in yolk deposition (Ruby *et al.*, 1977), a delay in ovulation (Tam and Payson, 1986), and a loss of egg viability (Menendez, 1976; Craig and Baski, 1977; Lee and Gerking, 1980). Weiner *et al.* (1986), working with acid-stressed male rainbow trout, demonstrated that milt quality (as measured by resultant fry survival) could be significantly reduced. Similarly, Macek (1968) found that sublethal levels of DDT could suppress gamete quality in both male and female brook trout, *Salvelinus fontinalis*.

To summarize, the present investigation has demonstrated the principle that both acute and chronic stress can suppress androgen levels in sexually mature male brown trout. Further studies of both sexes, at different stages of the reproductive cycle, are needed if we are to interpret the consequences of such hormonal events on the reproductive success of salmonid fish, both in the wild and under aquaculture conditions.

ACKNOWLEDGMENTS

The authors thank Dr. P. Copeland (Port Aransas Marine Laboratory, Texas) who purified the GTH and helped establish the GTH radioimmunoassay, Dr. B. Breton (INRA, Rennes) for the GTH standard, Dr. H. M. Dye (Department of Fisheries and Oceans, West Vancouver) for providing the 11-ketotestosterone antiserum, Mrs. J. Pollard and Miss J. M. Fletcher (FBA) for maintaining the experimental fish, Mrs. M. A. Hurley (FBA) for statistical advice, and Mr. T. I. Furnass (FBA) for the artwork. We are grateful to NERC and MAFF for financial support.

REFERENCES

Aono, T., Kurachi, K., Miyata, M., Nakasima, A., Koshiyama, K., Uozumi, T., and Matsumoto, K. (1976). Influence of surgical stress under general anesthésia on serum gonadotropin levels in male and female patients. *J. Clin. Endocrinol. Metab.* 42, 144–148.

Bagenal, T. B. (1966). The ecological and geographical aspects of the fecundity of the plaice. *J. Mar. Biol. Assoc. UK* 46, 161–186.

Bagenal, T. B. (1969). The relationship between food supply and fecundity in brown trout *Salmo trutta* L. *J. Fish Biol.* 1, 167–182.

Ball, J. N. (1960). Reproduction in female bony fish. *Symp. Zool. Soc. London* 1, 105–135.

Bambino, T. H., and Hsueh, A. J. W. (1981). Direct inhibitory effect of glucocorticoids upon testicular luteinizing hormone receptor and steroidogenesis *in vivo* and *in vitro*. *Endocrinology* 108, 2142–2147.

Billard, R., Bry, C., and Gillet, C. (1981). Stress, environment and reproduction in teleost fish. *In* "Stress and Fish" (A. D. Pickering, Ed.), pp. 85–208. Academic Press, London/New York.

Bry, C., and Zohar, Y. (1980). Dorsal aorta catheterization in rainbow trout (*Salmo gairdneri*). Glu-

cocorticoid levels, haematological data and resumption of feeding during five days after surgery. *Reprod. Nutr. Dev.* 20, 1825–1834.

Christian, J. J. (1961). Phenomena associated with population density. *Proc. Natl. Acad. Sci. USA* 47, 428–449.

Craig, G. R., and Baski, W. F. (1977). The effects of depressed pH on flagfish reproduction, growth and survival. *Water Res.* 11, 621–626.

Dahlgren, B. T. (1979). The effects of population density on fecundity and fertility in the guppy, *Poecilia reticulata* (Peters). *J. Fish Biol.* 15, 71–91.

Dodd, J. M., and Sumpter, J. P. (1984). Reproductive cycles of cyclostomes, elasmobranchs, and bony fishes. *In* "Marshall's Physiology of Reproduction" (G. E. Lamming, Ed.), Vol. A, 4th ed., pp. 1–126. Livingstone, London.

Dye, H. M., Sumpter, J. P., Fagerlund, U. H. M., and Donaldson, E. M. (1986). Changes in reproductive parameters during the spawning migration of pink salmon, *Oncorhynchus gorbuscha* (Walbaum). *J. Fish Biol.* 29, 167–176.

Francis, K. T. (1981). The relationship between high and low trait psychological stress, serum testosterone, and serum cortisol. *Experientia* 37, 1296–1297.

Freeman, H. C., Sangalang, G. B., Burns, G., and McMenemy, M. (1983). The blood sex hormone levels in sexually mature male Atlantic salmon (*Salmo salar*) in the Westfield River (pH 4.7) and the Medway River (pH 5.6), Nova Scotia. *Sci. Total Environ.* 32, 87–91.

Gillet, C., Billard, R., and Breton, B. (1981). La reproduction du poisson rouge *Carassius auratus* élevé à 30°C. Effet de la photopériode, de l'alimentation et de l'oxygénation. *Cah. Lab. Montereau* 11, 49–56.

Grandin, T. (1984). Reduce stress of handling to improve productivity of livestock. *Vet. Med.* June, 827–831.

Gray, G. D., Smith, E. R., Damassa, D. A., Ehrenkranz, J. R. L., and Davidson, J. M. (1978). Neuroendocrine mechanisms mediating the suppression of circulating testosterone levels associated with chronic stress in male rats. *Neuroendocrinology* 25, 247–256.

Hane, S., and Robertson, O. H. (1959). Changes in plasma 17-hydroxycorticosteroids accompanying sexual maturation and spawning of the Pacific salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Salmo gairdneri*). *Proc. Natl. Acad. Sci. USA* 45, 886–893.

Hedtke, S. F., and Puglisi, F. A. (1980). Effects of waste oil on the survival and reproduction of the American flagfish, *Jordanella floridae*. *Canad. J. Fish. Aquat. Sci.* 37, 757–764.

Idler, D. R. (1982). Some perspectives on fish gonadotropins. *In* "Reproductive Physiology of Fish" (C. J. J. Richter and H. J. Th. Goos, Eds.), pp. 4–13. Pudoc, Wageningen.

Joshi, S. N. (1982). Effect of hydrocortisone acetate on the maturity of male *Labeo gonius* (Ham.). *Endokrinologie* 80, 299–303.

June, F. C. (1977). Reproductive patterns in seventeen species of warmwater fishes in a Missouri River reservoir. *Environ. Biol. Fish.* 2, 285–296.

Kawakami, M., and Higuchi, T. (1981). Effect of partial deafferentation of the hypothalamus on stress-induced LH suppression and prolactin release. *Neuroendocrinology* 32, 278–284.

Kipling, C., and Frost, W. E. (1969). Variations in the fecundity of pike *Esox lucius* L. in Windermere. *J. Fish Biol.* 1, 221–237.

Kreuz, L. E., Rose, R. M., and Jennings, J. R. (1972). Suppression of plasma testosterone levels and psychological stress. *Arch. Gen. Psychiatry* 26, 479–482.

Krulich, L., Hefco, E., Illner, P., and Read, C. B. (1974). The effects of acute stress on the secretion of LH, FSH and GH in the normal male rat, with comments on their statistical evaluation. *Neuroendocrinology* 16, 293–311.

Kuznetsov, V. A., and Khalitov, N. Kh. (1978). Alterations in the fecundity and egg quality of the roach, *Rutilus rutilus*, in connection with different feeding conditions. *J. Ichthyol.* 18, 63–70.

Lance, V. A., and Elsey, R. M. (1986). Stress-induced suppression of testosterone secretion in male alligators. *J. Exp. Zool.* 239, 241–246.

Leatherland, J. F., Copeland, P., Sumpter, J. P., and Sonstegard, R. A. (1982). Hormonal control of gonadal maturation and development of secondary sexual characteristics in coho salmon, *Oncorhynchus kisutch*, from Lakes Ontario, Erie, and Michigan. *Gen. Comp. Endocrinol.* 48, 196–204.

Lee, R. M., and Gerking, S. D. (1980). Survival and reproductive performance of the desert pupfish, *Cyprinodon n. nevadensis* (Eigenmann and Eigenmann), in acid waters. *J. Fish Biol.* 17, 507–515.

Licht, P., McCreery, B. R., Barnes, R., and Pang, R. (1983). Seasonal and stress related changes in plasma gonadotropins, sex steroids, and corticosterone in the bullfrog, *Rana catesbeiana*. *Gen. Comp. Endocrinol.* 50, 124–145.

Macek, K. J. (1968). Reproduction in brook trout (*Salvelinus fontinalis*) fed sublethal concentrations of DDT. *J. Fish Res. Board Canad.* 25, 1787–1796.

Mann, R. H. K., and Mills, C. A. (1985). Variations in the size of gonads, eggs and larvae of the dace, *Leuciscus leuciscus*. *Environ. Biol. Fish.* 13, 277–287.

Menendez, R. (1976). Chronic effects of reduced pH on brook trout (*Salvelinus fontinalis*). *J. Fish. Res. Board Canad.* 33, 118–123.

Moberg, G. P. (1985). Influence of stress on reproduction: Measure of well-being. *In* "Animal Stress" (G. P. Moberg, Ed.), pp. 245–267. Amer. Physiol. Soc., Bethesda.

Moore, F. L., and Miller, L. J. (1984). Stress-induced inhibition of sexual behaviour: Corticosterone inhibits courtship behaviors of a male amphibian (*Taricha granulosa*). Horm. Behav. 18, 400–410.

Moore, F. L., and Zoeller, R. T. (1985). Stress-induced inhibition of sexual reproduction: Evidence of suppressed secretion of LH–RH in an amphibian. *Gen. Comp. Endocrinol.* 60, 252–258.

Morrison, P. F., Leatherland, J. F., and Sonstegard, R. A. (1985). Plasma cortisol and sex steroid levels in Great Lakes coho salmon (*Oncorhynchus kisutch* Walbaum) in relation to fecundity and egg survival. *Comp. Biochem. Physiol. A* 80, 61–68.

Nakashima, A., Koshiyama, K., Uozumi, T., Monden, Y., Hamanaka, Y., Kurachi, K., Aono, T., Mizutani, S., and Matsumoto, K. (1975). Effects of general anaesthesia and severity of surgical stress on serum LH and testosterone in males. *Acta Endocrinol.* 78, 258–269.

Peter, R. E. (1983). The brain and neurohormones in teleost reproduction. *In* "Fish Physiology" (W. S. Hoar, D. J. Randall, and E. M. Donaldson, Eds.), Vol. 9, pp. 97–135. Academic Press, New York.

Pickering, A. D., and Christie, P. (1981). Changes in the concentrations of plasma cortisol and thyroxine during sexual maturation of the hatchery-reared brown trout, *Salmo trutta* L. *Gen. Comp. Endocrinol.* 44, 487–496.

Pickering, A. D., and Pottinger, T. G. (1985). Factors influencing blood cortisol levels of brown trout under intensive culture conditions. *In* "Current Trends in Comparative Endocrinology" (B. Lofts and W. N. Holmes, Eds.), Vol. 2, pp. 1239–1242. Hong Kong Univ. Press, Hong Kong.

Pickering, A. D., and Pottinger, T. G. (1987a). Poor water quality suppresses the cortisol response of salmonid fish to handling and confinement. *J. Fish Biol.* 30, 363–374.

Pickering, A. D., and Pottinger, T. G. (1987b). Lymphocytopenia and interrenal activity during sexual maturation in the brown trout, *Salmo trutta* L. *J. Fish Biol.* 30, 41–50.

Pickering, A. D., Pottinger, T. G., and Christie, P. (1982). Recovery of the brown trout, *Salmo trutta* L., from acute handling stress; a time-course study. *J. Fish Biol.* 20, 229–244.

Pickering, A. D., Pottinger, T. G., and Sumpter, J. P. (1986). Independence of the pituitary–interrenal axis and melanotroph activity in the brown trout, *Salmo trutta* L., under conditions of environmental stress. *Gen. Comp. Endocrinol.* 64, 206–211.

Pickering, A. D., Pottinger, T. G., and Sumpter, J. P. (1987). On the use of dexamethasone to block the pituitary–interrenal axis in the brown trout, *Salmo trutta* L. *Gen. Comp. Endocrinol.* 65, 346–353.

Pickering, A. D., and Stewart, A. (1984). Acclimation of the interrenal tissue of the brown trout, *Salmo trutta* L., to chronic crowding stress. *J. Fish Biol.* 25, 123–128.

Pickering, A. D., and Willoughby, L. G. (1982). *Saprolegnia* infections of salmonid fish. *In* "Microbial Diseases of Fish" (R. J. Roberts, Ed.), pp. 271–297. Academic Press, New York/London.

Pottinger, T. G., and Pickering, A. D. (1985). 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. (1987). Plasma androgen levels and erythrocytosis in maturing brown trout, *Salmo trutta* L. *Fish Physiol. Biochem.*, in press.

Rasquin, P., and Atz, E. H. (1952). Effects of ACTH and cortisone on the pituitary, thyroid and gonads of the teleost *Astyanax mexicanus*. *Zoologica N.Y.* 37, 77–87.

Repčeková, D., and Mikulaj, L. (1977). Plasma testosterone of rats subjected to immobilization stress and/or HCG administration. *Horm. Res.* 8, 51–57.

Rivier, C., Rivier, J., and Vale, W. (1986). Stress-induced inhibition of reproductive functions: Role of endogenous corticotropin-releasing factor. *Science* 231, 607–609.

Ruby, S. M., Aczel, J., and Craig, G. R. (1977). The effects of depressed pH on oogenesis in flagfish, *Jordanella floridae*. *Water Res.* 11, 757–762.

Saez, J. M., Morera, A. M., Haour, F., and Evain, D. (1977). Effects of *in vivo* administration of dexamethasone, corticotropin and human chorionic gonadotropin on steroidogenesis and protein and DNA synthesis of testicular interstitial cells in prepubertal rats. *Endocrinology* 101, 1256–1263.

Sangalang, G. B., and Freeman, H. C. (1974). Effects of sublethal cadmium on maturation and testosterone and 11-ketotestosterone production *in vivo* in brook trout. *Biol. Reprod.* 11, 429–435.

Sapolsky, R. M. (1985). Stress-induced suppression of testicular function in the wild baboon: Role of glucocorticoids. *Endocrinology* 116, 2273–2278.

Schreck, C. B., Lackey, R. T., and Hopwood, M. L. (1972). Evaluation of diel variation in androgen

levels of rainbow trout, *Salmo gairdneri*. *Copeia* 4, 865–868.

Scott, A. P., MacKenzie, D. S., and Stacey, N. E. (1984). Endocrine changes during natural spawning in the white sucker, *Catostomus commersoni*. II. Steroid hormones. *Gen. Comp. Endocrinol.* 56, 349–359.

Scott, A. P., and Sumpter, J. P. (1983). A comparison of the female reproductive cycle of autumn-spawning and winter-spawning strains of rainbow trout (*Salmo gairdneri* Richardson). *Gen. Comp. Endocrinol.* 52, 79–85.

Scott, D. P. (1962). Effect of food quantity on fecundity of rainbow trout, *Salmo gairdneri*. *J. Fish. Res. Board Canad.* 19, 715–731.

Sirinathsinghji, D. J. S., Rees, L. H., Rivier, J., and Vale, W. (1983). Corticotropin-releasing factor is a potent inhibitor of sexual receptivity in the female rat. *Nature (London)* 305, 232–235.

Stearns, S. C., and Crandall, R. E. (1984). Plasticity for age and size at sexual maturity: A life-history response to unavoidable stress. *In* "Fish Reproduction: Strategies and Tactics" (G. W. Potts and R. J. Wootton, Eds.), pp. 13–33. Academic Press, New York/London.

Sumpter, J. P., and Donaldson, E. M. (1986). The development and validation of a radioimmunoassay to measure plasma ACTH levels in salmonid fishes. *Gen. Comp. Endocrinol.* 62, 367–376.

Sumpter, J. P., Dye, H. M., and Benfey, T. J. (1986). The effects of stress on plasma ACTH, α-MSH, and cortisol levels in salmonid fishes. *Gen. Comp. Endocrinol.* 62, 377–385.

Sumpter, J. P., Pickering, A. D., and Pottinger, T. G. (1985). Stress-induced elevation of plasma α-MSH and endorphin in the brown trout, *Salmo trutta* L. *Gen. Comp. Endocrinol.* 59, 257–265.

Taché, Y., Du Ruisseau, P., Ducharme, J. R., and Collu, R. (1978). Pattern of adenohypophysial hormone changes in male rats following chronic stress. *Neuroendocrinology* 26, 208–219.

Tam, W. H., and Payson, P. D. (1986). Effects of chronic exposure to sublethal pH on growth, egg production, and ovulation in brook trout, *Salvelinus fontinalis*. *Canad. J. Fish. Aquat. Sci.* 43, 275–280.

Thibier, M., and Rolland, O. (1976). The effect of dexamethasone (DXM) on circulating testosterone (T) and luteinizing hormone (LH) in young postpubertal bulls. *Theriogenology* 5, 53–59.

Truscott, B., Walsh, J. M., Burton, M. P., Payne, J. F., and Idler, D. R. (1983). Effect of acute exposure to crude petroleum on some reproductive hormones in salmon and flounder. *Comp. Biochem. Physiol. C* 75, 121–130.

Weiner, G. S., Schreck, C. B., and Li, H. W. (1986). Effects of low pH on reproduction of rainbow trout. *Trans. Amer. Fish. Soc.* 15, 75–82.

Welsh, T. H., Bambino, T. H., and Hsueh, A. J. W. (1982). Mechanism of glucocorticoid-induced suppression of testicular androgen biosynthesis *in vitro*. *Biol. Reprod.* 27, 1138–1146.

Welsh, T. H., and Johnson, B. H. (1981). Stress-induced alterations in secretion of corticosteroids, progesterone, luteinizing hormone, and testosterone in bulls. *Endocrinology* 109, 185–190.

Zohar, Y. (1980). Dorsal aorta catheterization in rainbow trout (*Salmo gairdneri*): Its validity in the study of blood gonadotropin patterns. *Reprod. Nutr. Dev.* 20, 1811–1823.

Aquaculture, 66 (1987) 109–124
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

A Comparison of the Effects of Overhead Cover on the Growth, Survival and Haematology of Juvenile Atlantic Salmon, *Salmo salar* L., Brown Trout, *Salmo trutta* L., and Rainbow Trout, *Salmo gairdneri* Richardson

A.D. PICKERING, R. GRIFFITHS and T.G. POTTINGER

Freshwater Biological Association, The Ferry House, Far Sawrey, Nr. Ambleside, Cumbria (Great Britain)

(Accepted 9 June 1987)

ABSTRACT

Pickering, A.D., Griffiths, R. and Pottinger, T.G., 1987. A comparison of the effects of overhead cover on the growth, survival and haematology of juvenile Atlantic salmon, *Salmo salar* L., brown trout, *Salmo trutta* L., and rainbow trout, *Salmo gairdneri* Richardson. *Aquaculture*, 66: 109–124.

The provision of floating annular covers to conventional tangential-flow rearing tanks significantly increased the growth rate of underyearling Atlantic salmon during the period July–November. This increased the proportion of potential 1-year-old smolts from 22% to 38% of the population ($P<0.001$). Salmon without access to overhead cover grew slowly and showed haematological signs of a chronic stress response (thrombocytopenia and lymphocytopenia). Mortality rates and the incidence of disease in the young salmon were not influenced by the provision of cover. Overhead cover had no significant effects on any of these parameters in underyearling brown trout and rainbow trout. These findings are discussed in relation to feeding opportunity and behavioural differences between species.

INTRODUCTION

Overhead cover is an important feature of the natural environment of salmonid fish. Behavioural studies have shown that brown trout, *Salmo trutta* L., brook trout, *Salvelinus fontinalis* Mitchill, and, to a lesser extent, rainbow trout, *Salmo gairdneri* Richardson, are attracted to those parts of a stream with overhead cover (Butler and Hawthorne, 1968) and juvenile Atlantic salmon, *Salmo salar* L., move rapidly to areas of cover when alarmed (Kalleberg, 1958). Moreover, the carrying capacity of trout streams is strongly influenced by the degree

0044-8486/87/$03.50

of cover (Saunders and Smith, 1955) and this can be artificially increased to improve the standing crop of brook trout (Saunders and Smith, 1962).

Attempts to exploit this phenomenon for aquacultural purposes have been infrequent and the results equivocal. Roadhouse et al. (1986) demonstrated that the provision of overhead cover could significantly increase the growth rate of hatchery-reared lake trout, *Salvelinus namaycush* (Walbaum). Growth in juvenile Atlantic salmon is characterized by a size bimodality (Knutsson and Grav, 1976; Thorpe, 1977) which results from differences in the growth rates of potential smolts and parr (see Thorpe, 1987). Simpson and Thorpe (1976), using a radial-flow tank designed to optimize feeding opportunities of juvenile salmon (Thorpe, 1981), showed that this size bimodality was significantly influenced by the provision of annular tank covers, under which the fish tended to congregate. The use of covers resulted in a lower proportion of the population in the upper size mode, i.e., it restricted the growth of some fish. In other studies on the same species, the provision of cover had no significant influence on growth and bimodality (Thorpe and Morgan, 1978). However, when different degrees of overhead cover were compared, Thorpe and Wankowski (1979) found that increasing the size of the annular tank cover increased the proportion of the population in the upper modal group.

The present study examines the effects of overhead cover on the growth and survival of three species of salmonid fish reared under aquaculture conditions in conventional, tangential-flow tanks. Routine health checks were made by screening the fish for signs of disease, and chronic stress responses were identified by means of haematology (Pickering and Pottinger, 1987a).

MATERIALS AND METHODS

Fish

Brown trout eyed-ova were purchased from Dunsop Bridge Trout Farm, Nr. Clitheroe, Lancashire, in February 1986, rainbow trout eyed-ova were obtained from Annandale Trout Farm, Johnstone Bridge, Dumfriesshire, in May 1986, and freshly fertilized eggs from wild Atlantic salmon, taken on their spawning migration in the River Lune, Lancashire, were kindly provided by the North West Water Authority in December 1985. Eggs were incubated in a Heath incubator at the FBA's hatchery and alevins were transferred to fish-rearing troughs until the yolk-sac was fully resorbed and the fish were actively feeding on BP Nutrition "Mainstream" pellets (size 00). The resultant fry were then used for experimental studies (see below) commencing in late-May for the brown trout, mid-June for the rainbow trout and mid-July for the salmon.

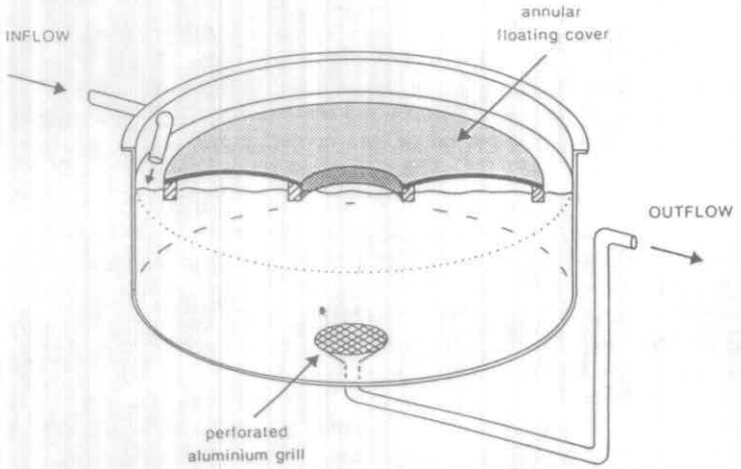


Fig. 1. Schematic diagram of the rearing tank and floating annular cover used during the present investigation. The cross-hatched region of the cover consists of fibreglass-reinforced expanded polystyrene.

Rearing tanks and covers

The experiments were performed in 2-m diameter, outdoor, grey fibreglass tanks each supplied with a tangential flow of aerated Windermere lake water (35 l min^{-1}). The water depth was adjusted by changing the height of the outflow pipe and the current speed (15–30 cm s^{-1}) was adjusted by altering the angle from the vertical of the inflow pipe. Annular floating covers were constructed of fibreglass and polystyrene to a design patented (U.K. 2052 931, U.S.A. 4271 788) by Knowles, Rines Associates (KRA Westbrook, Moniak Bridge, Inverness-shire, Scotland, Fig. 1). The cover, which occupied 67% of the water surface area, was prevented from rotating by means of four nylon guy-lines attached to the sides of the tank. Fine plastic netting (0.5-in. mesh) was used to prevent the accumulation of falling leaves and each tank was cleaned on a fortnightly basis without removing the fish.

Experimental design

Brown trout, rainbow trout and salmon fry were separately distributed into quadruplicate rearing tanks at a density of 1000 fish/tank. For each species, two of the four tanks were provided with floating annular covers and the allocation of species and covers between the 12 tanks available for this study was randomized by drawing lots. The fish were hand-fed with commercial trout

and salmon food three times daily during weekdays and once daily at the weekends. The exact feeding rate was dependent upon fish size and water temperature but within each species, the amount of food given at any one time was adjusted to the fastest growth rate and was identical for all four tanks. The water temperature during the period of this investigation (May–November 1986) was in the range 6.7–17.1°C. Daily mortalities were noted and weekly mortality rates were calculated after correction for losses due to sampling.

Samples of eight fish per tank were taken on a fortnightly basis and anaesthetized in phenoxyethanol (0.5 ml l^{-1}). Skin scrapes were taken from the flanks of the fish and examined for ectoparasites by dark-field and phase-contrast microscopy. Parasites were identified to the genus level and their abundance on each fish was estimated on a scale of 0 (absent) to 3 (abundant). Each fish was then weighed and measured (fork length), a blood sample was taken by severing the tail and collecting blood from the caudal vessels in heparinized capillary tubes, and the fish was killed by spinal section. Total blood cell counts were made on sedimented, diluted blood samples and differential cell counts were obtained from air-dried, methanol-fixed smears [see Pickering and Pottinger (1985) for technical details]. Growth studies and blood sampling were carried out on all species until September 1986 and the monitoring of mortalities and ectoparasite abundance was continued until November 1986, at which point the experiment was terminated. At the end of the experiment, the fork length of all the salmon from each treatment (covers or no covers) was measured and the length/frequency distributions were examined for bimodality.

Statistical analyses

Weight, length and blood cell concentrations were separately analysed for each species by analysis of variance (Genstat). Treatment (covers or no covers) and time were used as factors and tank and fish number were used as blocking effects to give a nested error structure with which to assess the significance of the factors and their interaction. From a plot of the residuals against fitted values, appropriate transformations (square root or logarithm) were selected, where necessary, to improve homogeneity of variance. Levels of significance were derived from these analyses but, for ease of presentation, data are given as arithmetic means ±SEM. The probability paper technique was used to identify bimodality in the salmon length frequency distributions (Cassie, 1954) and comparisons of total mortality within each treatment and of the number of salmon within each mode at the end of the experiment were made by χ^2 analysis.

RESULTS

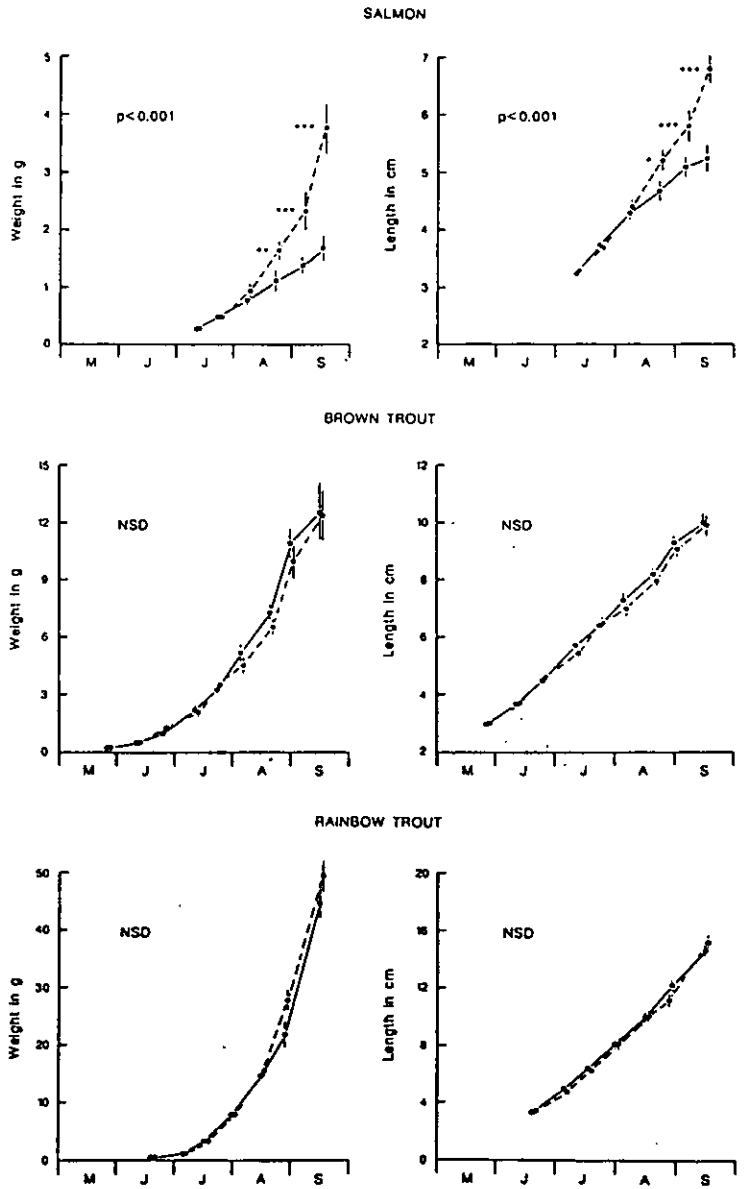
Growth

The provision of overhead cover had no significant effect on the growth rate of brown trout or rainbow trout (Fig. 2). During the period May–September the brown trout increased in weight to ≈12 g (mean length 10 cm), during the period June–September the rainbow trout increased in weight to ≈50 g (mean length 15 cm). By comparison, overhead cover had a marked and highly significant ($P<0.001$) effect on the growth of juvenile Atlantic salmon. During the period July–September salmon without access to overhead cover only increased in weight to ≈1.5 g (mean length 5 cm) whereas the salmon in tanks provided with floating covers increased in weight to almost 4 g (mean length 7 cm). This growth-promoting effect of overhead cover became apparent within 6 weeks of the onset of the experiment (Fig. 2).

An analysis of the length frequency distributions of salmon in November revealed a marked bimodality in the fish from both treatments (Fig. 3). However, both modal lengths were greater in the fish from tanks provided with overhead cover (6.5 and 9.5 cm cf. 5.5 and 8.25 cm). Moreover, in the covered tanks 38% of the population belonged to the upper mode whereas only 22% of the population belonged to the upper mode in the tanks without overhead cover. This difference in proportion was highly significant ($P<0.001$).

Disease and mortality

Skin smears do not provide rigorously quantitative data and it was necessary, therefore, to estimate parasitic abundance in arbitrary units. For each fish the abundance of each parasite was estimated on a scale of 0 (absent) to 3 (abundant). Simple addition of these estimates for each fish within a sample allowed a comparison of changes in the abundance of the five different genera of ectoparasitic protozoa that occurred on the experimental fish during the course of this study (Fig. 4). All species of fish were initially infested with the parasitic flagellate, *Ichthyobodo* (≡*Costia*). In the salmon, the infestation was relatively short-lived (July and August) and never reached the same degree of abundance that developed in both species of trout. The parasitic ciliate, *Trichodina*, succeeded *Ichthyobodo* on the salmon but did not appear on either species of trout. A minor and short-lived Myxosporidian infestation occurred on the rainbow trout during July. *Ichthyobodo* infestation was followed on the brown trout, but not on the rainbow trout, by colonization with the sessile peritrich, *Scyphidia*, and a related ciliate, *Apiosoma* (≡*Glossatella*), occurred on all three species of fish during the later stages of the study (August onwards). Interestingly, *Apiosoma* was found on the salmon in tanks provided with overhead cover but not on those without cover. With this one exception,



overhead cover appeared to have little effect on the severity or duration of ectoparasitic infestation.

When the mortality data were subjected to χ^2 analysis it was not possible to demonstrate any significant effects of overhead cover on the total mortality of each species. Nevertheless, mortality rates were not constant (Fig. 5) and in the salmon, the highest weekly mortality rates ($\approx 1\%$) coincided with the pe-

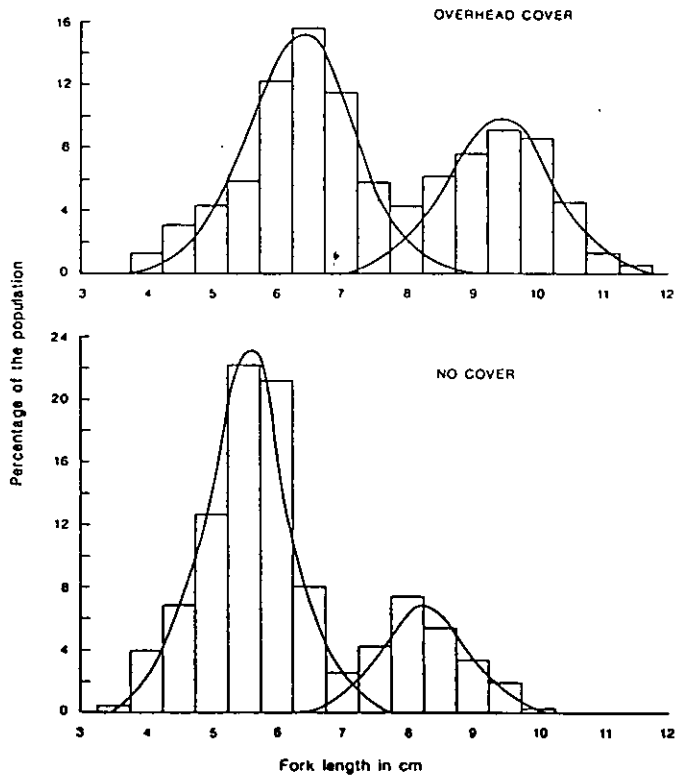


Fig. 3. Length frequency distributions of juvenile salmon reared from July to November in the presence of overhead cover (upper figure) or without cover (lower figure). The modal distribution was determined using Cassie's (1954) probability paper technique.

Fig. 2. The increase in body weight and fork length of the three species of salmonid fish during the course of the study. Continuous lines represent fish reared in tanks without overhead cover, broken lines represent fish from tanks provided with annular floating covers. Each point represents the arithmetic mean ±SEM ($n=16$) and the significance of the difference between means at each sampling time is indicated ($^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$). The probability value given for each graph is the interaction of treatment with time (derived from analysis of variance).

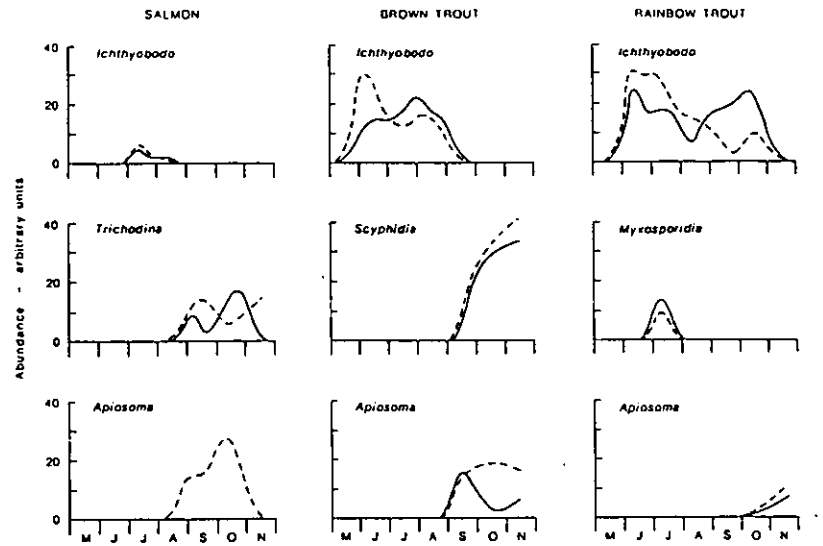


Fig. 4. Ectoparasitic infestations on juvenile salmon, brown trout and rainbow trout during the study period. The abundance of each parasite was estimated, at fortnightly intervals, from skin scrapes and is expressed in arbitrary units (see Results for details). Continuous lines represent fish in tanks without cover, broken lines represent fish in tanks fitted with floating annular covers.

riod of *Ichthyobodo* infestation. It was observed, however, that this elevated mortality rate was also associated with the failure of a few fry to feed properly and it is impossible, therefore, to dissociate the effects of malnutrition from those of disease per se. In the brown trout and rainbow trout, *Ichthyobodo* infestation was not associated with any marked increase in mortality rate. Survival of the brown trout was high throughout the whole of the study period with weekly mortality rates remaining below 1%. A marked increase in rainbow trout mortality rate was observed (particularly in the covered tanks) during September. Visual inspection of the dead fish revealed a few open lesions on the flanks of some fish together with evidence of internal haemorrhaging and it was concluded that some form of bacterial infection was probably responsible. This only developed when the carrying capacity of the tanks [as calculated according to Westers and Pratt (1977)] was exceeded. Under normal aquacultural operations, these fish would have been thinned-out and graded before such problems occurred.

Blood cell counts

In all species, the concentration of circulating erythrocytes showed a progressive increase during the course of this study (Fig. 6). In both the salmon

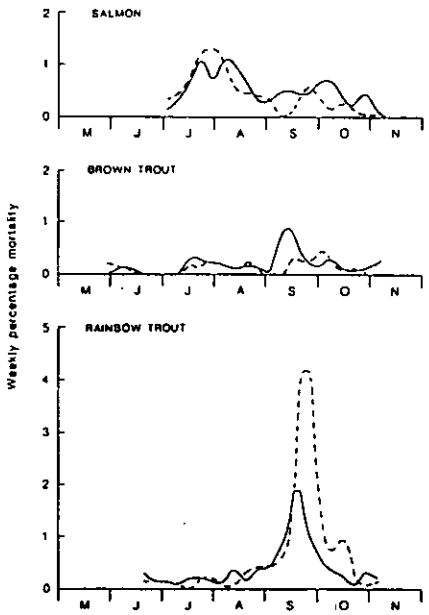
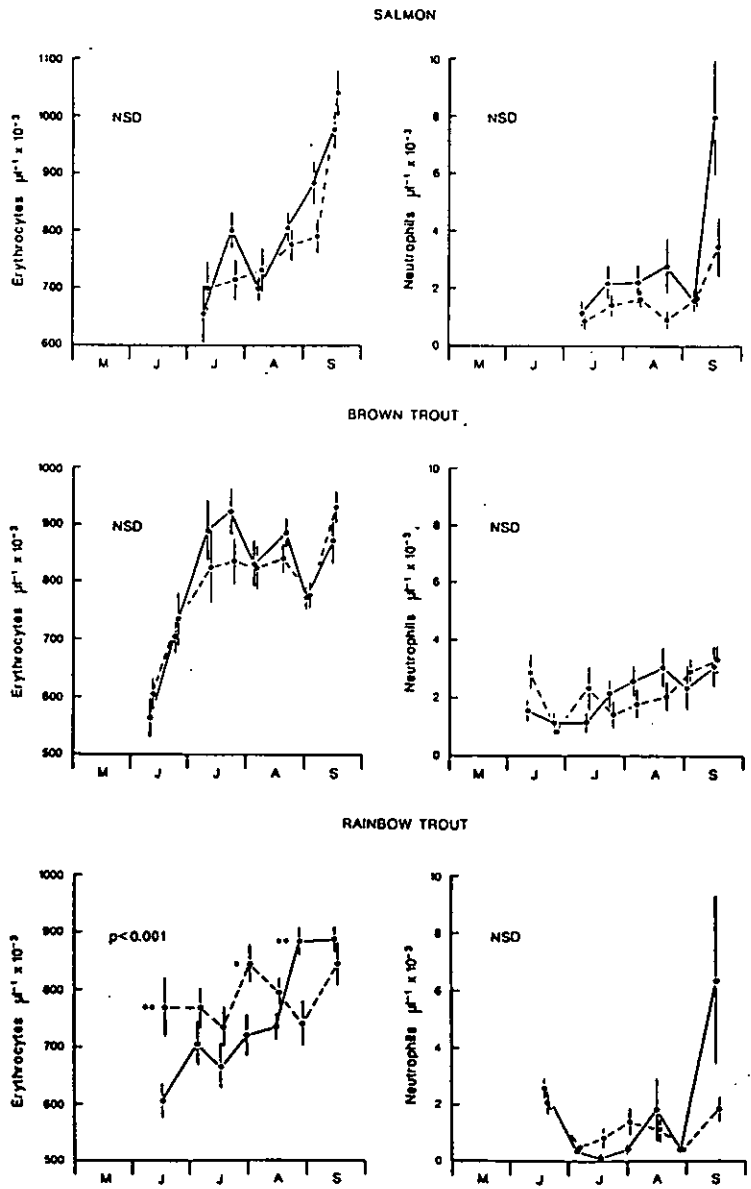


Fig. 5. Weekly mortality rates of juvenile salmon, brown trout and rainbow trout. The broken line represents fish reared in tanks with overhead annular covers, the continuous line represents fish without covers.

and the brown trout, no significant effects of overhead cover on the erythrocyte count were detected. A highly significant interaction of treatment with time ($P<0.001$) was noted for the rainbow trout but interpretation of this result is difficult because a significant treatment effect was found at the start of the experiment. This suggests that, for reasons which are not apparent, the distribution of rainbow trout fry between the original quadruplicate tanks was not random with regard to the concentration of circulating erythrocytes. However, by the end of the sampling period the red blood cell counts in fish from both treatments (covers, no covers) were similar.

Neutrophil counts in all species were not significantly affected by the provision of overhead cover although, in both the salmon and the rainbow trout, the neutrophil count in the fish from uncovered tanks tended to be high at the end of the study period (Fig. 6).

Thrombocyte counts in all species showed a marked increase during the course of the study (Fig. 7) and overhead cover significantly influenced the number of circulating thrombocytes in the salmon ($P<0.05$) but not in the



trout. This effect on the salmon appeared as an elevated thrombocyte count at the end of the experiment in the fish provided with overhead cover.

In a similar manner, lymphocyte counts in all species showed a marked increase during the investigation but again, overhead cover had a significant effect on this parameter only in the salmon ($P < 0.005$). This treatment × time effect took the form of a lower lymphocyte count during August and September in the fish without overhead cover when compared with those fish provided with cover (Fig. 7).

Although the haematology data were not analysed for species differences, it is apparent from a comparison of the graphs in Figs. 6 and 7 that marked species differences in most of the blood cell types did occur at sometime during this study. Whether these represent true species differences or reflect strain differences or differences in developmental age remains to be seen, and further work in this area is indicated.

DISCUSSION

Of the three species of salmonid fish used in this study, only the Atlantic salmon showed any marked response to the presence of overhead cover. This was immediately apparent in the behaviour of the fish, which distributed themselves underneath the covers within 2 days of the start of the experiment. By comparison, juvenile salmon in tanks without covers tended to congregate over the central perforated aluminium grill. Overhead cover made little difference to the distribution of either species of trout which, even in tanks without cover, distributed themselves more or less evenly to occupy all the available space. It seems reasonable to suppose that this change in the distribution of salmon fry in the presence of overhead cover increased their feeding opportunities, a supposition which is supported by the marked stimulatory effect of cover on growth rate. Thorpe (1987) concluded that the growth rate of juvenile Atlantic salmon, in specifically designed radial-flow tanks with food delivered to the incoming water supply, is strongly influenced by feeding opportunity. The present study has shown that the provision of simple floating covers can markedly increase the growth rate of juvenile salmon in conventional tangential-flow tanks in which the food is delivered at the water surface.

Growth stimulation was apparent by November of the salmon's first growing season in both the upper and lower size modes. The upper mode represents fish

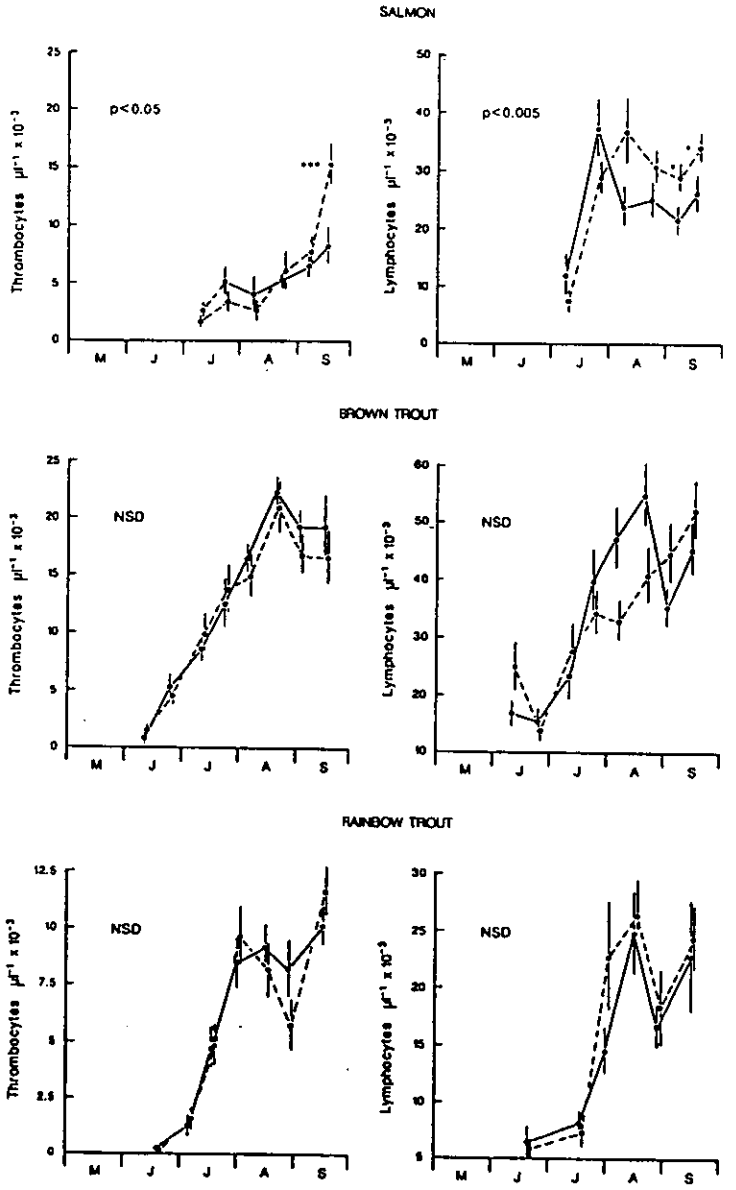


Fig. 6. Changes in the erythrocyte and neutrophil counts of the three species of salmonid fish during the course of the study. Continuous lines represent fish reared in tanks without overhead cover, broken lines represent fish from tanks provided with annular floating covers. Each point represents the arithmetic mean ±SEM ($n = 16$) and the significance of the difference between means at each sampling point is indicated ($^*P < 0.05$, $^{**}P < 0.01$). The probability value for each graph is the treatment × time interaction (derived from analysis of variance).

that will smoltify the following spring whereas fish in the lower mode will remain in fresh water, as parr, for at least one more year (Simpson and Thorpe, 1976; Thorpe, 1977). It is well known that genetic factors, stocking density and photoperiod can have major influences on the proportion of fish within a population in the upper size mode (Refstie and Kittelsen, 1976; Refstie et al., 1977; Thorpe, 1977, 1987; Thorpe and Morgan, 1978; Bailey et al., 1980) but in the present study, these factors were similar for both treatments. The provision of overhead cover during the first few months of feeding almost doubled the proportion of 1-year-old smolts, presumably as a result of the growth-promoting effect during the critical period of mid–late summer, at which time the physiological decision to smoltify is taken (Thorpe, 1987). From a practical point of view, inconvenience of removal of the covers for cleaning purposes was largely offset by reduced algal growth on the tanks as a result of light limitation.

The haematological data suggest that, towards the end of the study period, the juvenile salmon without access to overhead cover were showing signs of a chronic stress response. This took the form of reduced lymphocyte and thrombocyte counts, similar to those observed by Pickering and Pottinger (1987b) for brown trout and rainbow trout subjected to chronic crowding stress, and leucopenia has been shown to be a characteristic response of salmonid fish to other forms of stress (Weinreb, 1958; McLeay, 1973a, 1975; McLeay and Gordon, 1977; Pickering et al., 1982). This response is believed to be mediated, at least in part, by an activation of the hypothalamic–pituitary–interrenal axis (McLeay, 1973b; Pickering, 1984; Pickering and Pottinger, 1987a).

Environmental stress can reduce the performance capacity of the fish in various ways (Schreck, 1981). Cover had no significant effect on the mortality rate of any species and seemed to have little influence on the degree of ectoparasitic loading of the fish. Thus, in this study it was not possible to correlate the reduced white blood cell count with an increased susceptibility to disease. However, as discussed above, another aspect of the fish's performance capacity, the growth rate, was clearly reduced in those fish showing signs of a chronic stress response.

The difference in response to overhead cover of the three species of salmonid fish calls for some comment. Firstly, Stuart (1953), working with natural fish populations, concluded that "salmon fry show all the signs of a gregarious nature and on emergence from the gravel remain side by side and head to tail in 'shoal' formation. This characteristic persists throughout the initial fresh-water

Fig. 7. Changes in the thrombocyte and lymphocyte counts of the three species of salmonid fish during the course of the study. Continuous lines represent fish reared in tanks without overhead cover, broken lines represent fish from tanks provided with annular floating covers. Each point represents the arithmetic mean ±SEM ($n=16$) and the significance of the difference between means at each sampling point is indicated ($^{*}P<0.05$, $^{***}P<0.001$). The probability value given for each graph is the interaction of treatment with time (derived from analysis of variance).

phase of their life history and may persist throughout their lives." The tendency for salmon without cover to congregate on the central grill of the tanks used in the present study may represent a similar response. By comparison, brown trout fry rapidly disperse so that "there is usually a minimum distance within which one fry will not tolerate another" (Stuart, 1953). However, Stradmeyer and Thorpe (1987), also working on a natural population of salmon parr, observed marked territoriality and agonistic behaviour. It is possible, therefore, that the "congregating" behaviour of salmon in tanks is atypical, and a consequence of their confinement (J.E. Thorpe, personal communication, 1986). Secondly, salmonid fish can show marked species differences in their response to light (Hoar et al., 1957) and to the substratum. Thorpe (1981) found that young Atlantic salmon show a preference for resting on a non-reflecting dark surface and although the tanks used in our study were uniformly grey, the salmon showed a preference for the dark areas underneath the covers. On the other hand, young rainbow trout do not show preference for a dark background until they are approximately 1 year old (Kwain and MacCrimmon, 1969). The underyearling rainbow trout used in the present study distributed themselves throughout the grey fibreglass tank, even in the absence of overhead cover and a similar distribution of fish occurred in the tanks containing underyearling brown trout. Thirdly, juvenile salmon are known to establish and maintain a bottom-feeding posture with only minimal excursions into mid-water (Kalleberg, 1958) whereas brown trout fry swim more actively, maintaining station in mid-water (Stuart, 1953). Young rainbow trout also occupy mid-water feeding stations (Newman, 1956) and, therefore, the usable feeding space within the experimental tanks is potentially greater for both trout species than for the salmon. The net result of these three factors is that brown trout and rainbow trout will occupy all the potential feeding sites in the type of rearing tank used in this study, whereas juvenile salmon appear to require overhead cover if they are to disperse and use the available tank space to maximal effect.

In the present study, the floating annular cover occupied 67% of the water surface. If this technique is to be exploited for smolt-rearing purposes it would seem, from the studies of J.E. Thorpe and his colleagues, that large overhead covers offer the most potential. Indeed, Simpson and Thorpe (1976) found that the provision of a relatively limited area of overhead cover caused local crowding with a resultant growth suppression and a reduction in the proportion of potential 1-year-old smolts.

In summary, the provision of floating annular covers to conventional, tangential-flow rearing tanks significantly increased the growth rate of underyearling Atlantic salmon. This resulted in a greater proportion of potential 1-year-old smolts in the population. Salmon without access to overhead cover grew slowly and showed haematological signs of a chronic stress response.

Overhead cover had no significant effects on the growth, mortality or haematology of brown trout or rainbow trout reared under similar conditions.

ACKNOWLEDGEMENTS

The authors are grateful to Mrs. J. Pollard and Miss J.M. Fletcher (FBA) for maintaining the experimental fish, Mr. C. Durie, Mr. A.P. Atkinson and colleagues (NWWA) for providing the salmon eggs, Mrs. M.A. Hurley and Dr. J.M. Elliott (FBA) for statistical advice, Mr. T.I. Furnass (FBA) for the artwork, Dr. J.E. Thorpe (Pitlochry) for his critical comments on the manuscript, and NERC and MAFF for financial support. The idea for this study originated from a discussion with Mr. A.H. Knowles (Knowles, Rines Associates).

REFERENCES

Bailey, J.K., Saunders, R.L. and Buzeta, M.I., 1980. Influence of parental smolt age and sea age on growth and smolting of hatchery-reared Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci., 37: 1379-1386.

Butler, R.L. and Hawthorne, V.M., 1968. The reactions of dominant trout to changes in overhead artificial cover. Trans. Am. fish. Soc., 97: 37-41.

Cassie, R.M., 1954. Some uses of probability paper in the analysis of size frequency distributions. Aust. J. Mar. Freshwater Res., 5: 513-522.

Hoar, W.S., Keenleyside, M.H.A. and Goodall, R.G., 1957. Reactions of juvenile Pacific salmon to light. J. Fish. Res. Board Can., 14: 815-830.

Kalleberg, H., 1958. Observations in a stream tank of territoriality and competition in juvenile salmon and trout (*Salmo salar* L. and *S. trutta* L.). Rep. Inst. Freshwater Res. Drottningholm, 39: 55-98.

Knutsson, S. and Grav, T., 1976. Seawater adaptation in Atlantic salmon (*Salmo salar* L.) at different experimental temperatures and photoperiods. Aquaculture, 8: 169-187.

Kwain, W.-H. and MacCrimmon, H.R., 1969. Age and vision as factors in bottom colour selection by rainbow trout, *Salmo gairdneri*. J. Fish. Res. Board Can., 26: 687-693.

McLeay, D.J., 1973a. Effects of a 12-hr and 25-day exposure to kraft pulp mill effluent on the blood and tissues of juvenile coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can., 30: 395-400.

McLeay, D.J., 1973b. Effects of cortisol and dexamethasone on the pituitary-interrenal axis and abundance of white blood cell types in juvenile coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol., 21: 441-450.

McLeay, D.J., 1975. Sensitivity of blood cell counts in juvenile coho salmon (*Oncorhynchus kisutch*) to stressors including sublethal concentrations of pulp mill effluent and zinc. J. Fish. Res. Board Can., 32: 2357-2364.

McLeay, D.J. and Gordon, M.R., 1977. Leucocrit: a simple haematological technique for measuring acute stress in salmonid fish, including stressful concentrations of pulp mill effluent. J. Fish. Res. Board Can., 34: 2164-2175.

Newman, M.A., 1956. Social behaviour and interspecific competition in two trout species. Physiol. Zool., 29: 64-81.

Pickering, A.D., 1984. Cortisol-induced lymphocytopenia in brown trout, *Salmo trutta* L. Gen. Comp. Endocrinol., 53: 252-259.

Pickering, A.D. and Pottinger, T.G., 1985. Acclimation of the brown trout, *Salmo trutta* L., to the stress of daily exposure to malachite green. Aquaculture, 44: 145-152.

Pickering, A.D. and Pottinger, T.G., 1987a. Lymphocytopenia and interrenal activity during sexual maturation in the brown trout, *Salmo trutta* L. J. Fish Biol., 30: 41-50.

Pickering, A.D. and Pottinger, T.G., 1987b. Crowding causes prolonged leucopenia in salmonid fish, despite interrenal acclimation. J. Fish Biol., 30: 701-712.

Pickering, A.D., Pottinger, T.G. and Christie, P., 1982. Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: a time-course study. J. Fish Biol., 20: 229-244.

Refstie, T. and Kittelsen, A., 1976. Effect of density on growth and survival of artificially reared Atlantic salmon. Aquaculture, 8: 319-326.

Refstie, T., Steine, T.A. and Gjedrem, T., 1977. Selection experiments with salmon. II. Proportion of Atlantic salmon smoltifying at 1 year of age. Aquaculture, 10: 231-242.

Roadhouse, S., Saari, M.J. and Roadhouse, D., 1986. Behavioural and biochemical correlates of hatchery rearing methods on lake trout. Prog. Fish Cult., 48: 38-42.

Saunders, J.W. and Smith, M.W., 1955. Standing crops of trout in a small Prince Edward Island stream. Can. Fish-Cult., 17: 1-8.

Saunders, J.W. and Smith, M.W., 1962. Physical alteration of stream habitat to improve brook trout production. Trans. Am. Fish. Soc., 91: 185-188.

Schreck, C.B., 1981. Stress and compensation in teleostean fishes: response to social and physical factors. In: A.D. Pickering (Editor), Stress and Fish. Academic Press, London, New York, NY, pp. 295-321.

Simpson, T.H. and Thorpe, J.E., 1976. Growth bimodality in the Atlantic salmon. Int. Counc. Explor. Sea, C.M. 1976/M: 22, 7 pp.

Stradmeyer, L. and Thorpe, J.E., 1987. Feeding behaviour of wild Atlantic salmon, *Salmo salar* L., parr in mid- to late summer in a Scottish river. Aquacult. Fish Manage., 18: 33-49.

Stuart, T.A., 1953. Spawning migration, reproduction and young stages of loch trout (*Salmo trutta* L.). Scott. Home Dept. Freshwater Salmon Fish. Res., 5: 1-39.

Thorpe, J.E., 1977. Bimodal distribution of length of juvenile Atlantic salmon (*Salmo salar* L.) under artificial rearing conditions. J. Fish Biol., 11: 175-184.

Thorpe, J.E., 1981. Rearing salmonids in freshwater. In: A.D. Hawkins (Editor), Aquarium Systems. Academic Press, London, New York, NY, pp. 325-344.

Thorpe, J.E., 1987. Environmental regulation of some growth patterns in Atlantic salmon. In: R.C. Summerfelt and G.E. Hall (Editors), Age and Growth of Fish. Iowa State University Press, Ames, IA, pp. 463-474.

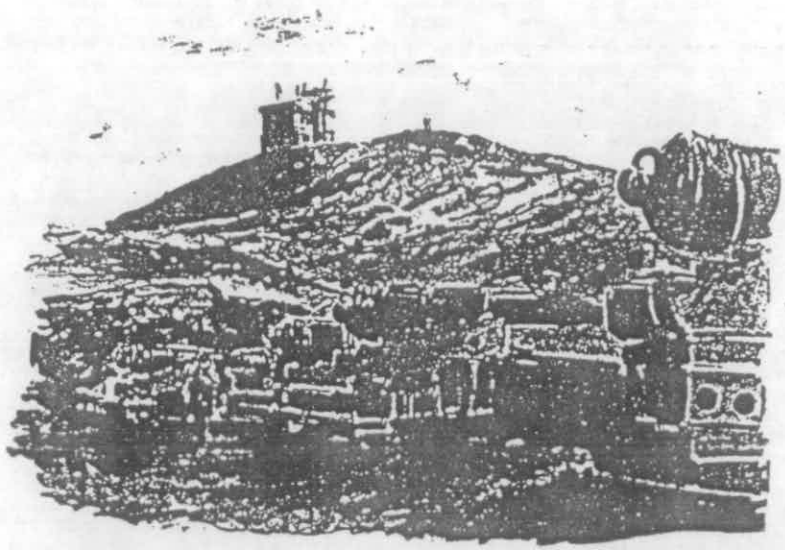
Thorpe, J.E. and Morgan, R.I.G., 1978. Parental influence on growth rate, smolting rate and survival in hatchery reared juvenile Atlantic salmon, *Salmo salar*. J. Fish. Biol., 13: 549-556.

Thorpe, J.E. and Wankowski, J.W.J., 1979. Feed presentation and food particle size for juvenile Atlantic salmon, *Salmo salar* L. In: J.E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 1. Heeneman, Berlin, pp. 501-513.

Weinreb, E.L., 1958. Studies on the histology and histopathology of the rainbow trout, *Salmo gairdneri*. I. Haematology: under normal and experimental conditions of inflammation. Zoologica (NY), 43: 145-154.

Westers, H. and Pratt, K.M., 1977. Rational design of hatcheries for intensive salmonid culture, based on metabolic characteristics. Prog. Fish-Cult., 39: 157-165.

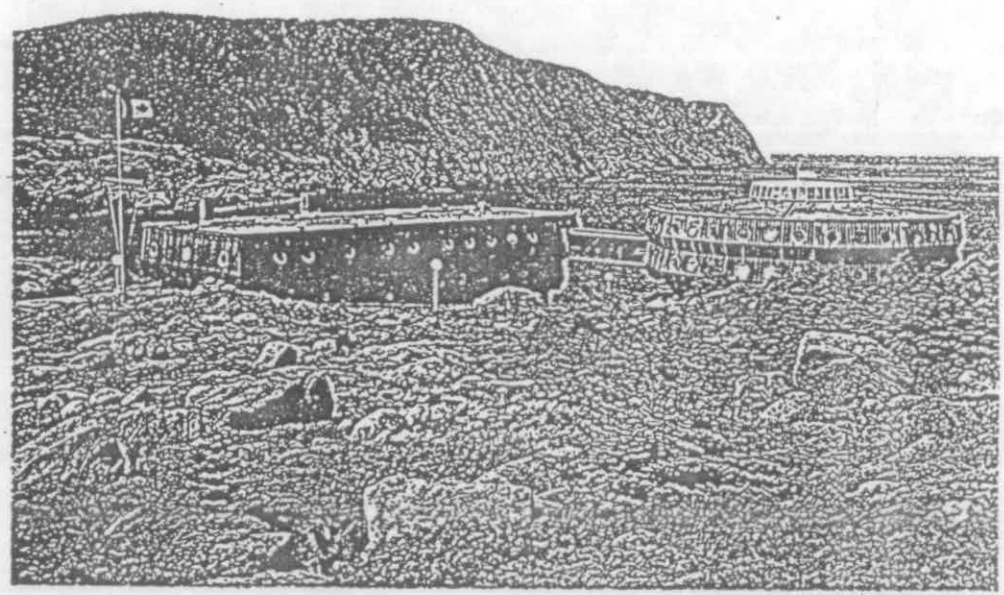
REPRODUCTIVE PHYSIOLOGY OF FISH 1987



ST. JOHN'S, NEWFOUNDLAND CANADA, AUGUST, 1987

Proceedings of the Third International Symposium on the Reproductive Physiology of Fish

St. John's, Newfoundland, Canada, 2-7 August 1937



Marine Sciences Research Laboratory

Memorial University of Newfoundland

Logy Bay, Newfoundland

PROCEEDINGS

PROCEEDINGS OF THE THIRD INTERNATIONAL SYMPOSIUM ON REPRODUCTIVE PHYSIOLOGY OF FISH.
ST. JOHN'S, NEWFOUNDLAND, CANADA. AUGUST 2-7, 1987.

D.R. IDLER, L.W. CRIM AND J.M. WALSH

ISBN 0-9692692-0-X

THIRD FISH SYMPOSIUM 1987
MARINE SCIENCES RESEARCH LABORATORY
MEMORIAL UNIVERSITY OF NEWFOUNDLAND
ST. JOHN'S, NEWFOUNDLAND
CANADA A1C 5S7

FRONT AND BACK WATERCOLORS BY RON FOURNEY
PRINTED IN CANADA

THE INTERACTION OF STRESS AND REPRODUCTION IN TROUT

J.P. Sumpter, J. Carragher, *T.G. Pottinger and *A.D. Pickering

Department of Applied Biology, Brunel University, Uxbridge, Middlesex, UB8 3PH, and *Freshwater Biological Association, The Ferry House, Far Sawrey, Near Ambleside, Cumbria LA22 OLP, U.K.

Summary

Acute handling and confinement stress for 1h elevated plasma ACTH, cortisol, and gonadotrophin (GTH) levels, but suppressed circulating testosterone and 11-ketotestosterone levels in sexually mature, male trout. Chronic confinement for one month caused a significant elevation of plasma cortisol, and again suppressed the levels of both androgens. Basal 17β-oestradiol secretion from cultured ovarian follicles was reduced in the presence of cortisol, but not ACTH. GTH stimulated 17β-oestradiol secretion from cultured follicles, an effect partially inhibited by cortisol, but not by ACTH. These results demonstrate that stress affects the plasma concentrations of the reproductive hormones, and suggest that this effect may be mediated, at least in part, by a direct action of cortisol on the gonads. We have shown also that sexual maturity affects the stress response. Mature and maturing male trout showed a reduced cortisol rise in response to acute stress compared to immature males. Collectively our results suggest that in trout the hypothalamic-pituitary-interrenal axis and the hypothalamic-pituitary-gonadal axis are linked.

Introduction

It is well established that many forms of environmental stress can inhibit reproductive processes in higher vertebrates, and a reasonable amount is known about the mechanisms underlying the phenomenon. In fish, however, little attention has been given to the effects of stress on reproduction. Although there is some circumstantial evidence, obtained from the study of both natural and laboratory populations of fish, that stress might depress the reproductive performance of fish, there is very little direct evidence. Further, the mechanisms whereby stress might affect the reproductive axis of fish are unknown. Billard et al. (1981), reviewing the information available at the time, concluded "there are only a few data in fish showing interactions between gonadotrope and corticotrope systems .... etc." The situation remains largely unaltered, although we have recently shown that acute stress can affect circulating levels of reproductive hormones in brown trout (Pickering et al., 1987). Here we extend those findings to investigate the interactions between stress responses and the reproductive axis of trout.

Materials and Methods

Acute stress

One hundred and twenty 2-year old sexually mature male brown trout were used. Five fish were placed in each of 24 large tanks. After a recovery period of two weeks, the fish in all tanks (with the exception of the 0h control fish) were subjected to an acute stress. This consisted of netting the fish, transferring them to a small confinement tank for a period of 1h, and then returning the fish to their original tank. Duplicate tanks of fish were then sampled at 0h (pre-stress) and at 1, 4, 8, 24 and 48h post-stress. Plasmas were assayed for ACTH, cortisol, GTH, testosterone, and 11-ketotestosterone.

Chronic stress

Thirteen sexually mature male brown trout were confined individually for one month in small glass aquaria, after which they were blood sampled. On the same day blood samples were taken from 27 sexually mature male brown trout, of the same stock, which had been maintained in large rearing tanks. Plasma was assayed for cortisol, testosterone, and 11-ketotestosterone.

Ovarian follicle culture

Follicles at an early stage of vitellogenin sequestration (diameter 0.8 to 1.2 mm) were gently teased from the ovary and cultured for 18h at room temperature in Leibovitz L-15 medium.. They were cultured in groups of 10 in 1ml medium, with six replicates for each treatment. Cortisol, ACTH, or maturational GTH, or combinations of these hormones, were added to the medium at concentrations of 100 ng/ml. At the end of the incubations the media were assayed for 17β-oestradiol.

Effect of reproductive development on the stress response

In experiments carried out to investigate various aspects of the stress response of trout, we have consistently observed that the

degree of sexual maturity of the fish affected the stress response. These experiments have been conducted using male trout in their second year, some of which matured as 2-year old fish, while others did not. All of the experiments involved subjecting the fish to 1h of acute stress, after which the fish were blood sampled and the plasma assayed subsequently for cortisol. The degree of sexual maturity of each fish was recorded, so that the response of immature males could be compared to that of maturing or mature males.

Results

Acute stress caused a marked stimulation of the hypothalamic-pituitary-interrenal (HPI) axis; ACTH and cortisol levels had risen from basal levels of 50 pg/ml and 13 ng/ml, respectively, to 120 pg/ml and 70 ng/ml at the end of the 1h confinement ($P<0.01$ in both cases). Upon return to their large tanks the ACTH level fell rapidly to that of the control fish, and remained there for the following 48 hours. Cortisol levels took longer to return to unstressed values; they were still elevated after 8 hours, but were back to basal by 24 hours (Fig. 1). This expected stimulation of the HPI axis was accompanied by a suppression in plasma androgens. Testosterone levels were significantly depressed, compared to the unstressed fish, after 1h of confinement ($P<0.01$), and remained depressed for at least 8h, before returning to the unstressed level. Plasma 11-ketotestosterone levels were also significantly depressed, compared to the control fish, but only at the 4h sampling point ($P<0.05$). Plasma GTH levels were high at the beginning of the experiment, because the fish were fully mature. The 1h of acute stress caused a significant increase in the blood GTH level, from 2 to 4 ng/ml ($P<0.05$). This elevated level was maintained for a further 3 hours at least, but had fallen back to the level in unstressed, control fish by 8 hours (Fig. 1).

Chronically-confined trout did not acclimate fully to the conditions, and even after one month they still had significantly elevated plasma cortisol levels (14 ng/ml) compared to the unstressed fish (2 ng/ml). The stressed fish also had significantly depressed plasma testosterone ($P<0.05$) and 11-ketotestosterone ($P<0.05$) levels after 1 month of chronic confinement. As observed in the experiment assessing the effects of acute stress, this suppression of plasma androgens was more pronounced for testosterone (>50%) than for 11-ketotestosterone (~25%); the testosterone and 11-ketotestosterone levels were 10 and 50 ng/ml, respectively, in the unstressed trout, and 5 and 38 ng/ml in the confined trout.

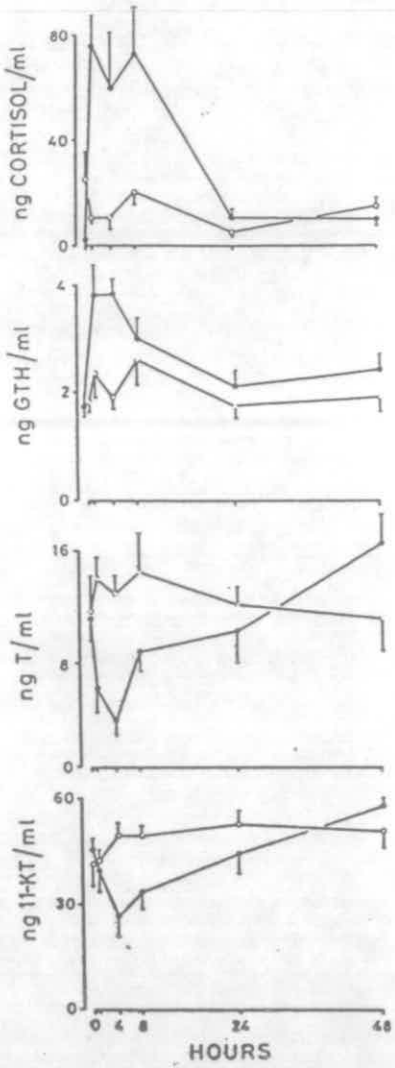


Figure 1. The effects of 1 hour handling and confinement stress (0 to 1 hour) on the plasma levels of cortisol, gonadotrophin, testosterone, and 11-ketotestosterone in sexually mature, male brown trout. The open circles (○) represent unstressed, control fish and the closed circles (●) represent stressed fish.

Cultured ovarian follicles secreted 17β-oestradiol into the medium. ACTH had no effect on the amount of 17β-oestradiol secreted, whereas cortisol alone, or in combination with ACTH, significantly depressed the basal secretion rate (Fig. 2). GTH significantly increased 17β-oestradiol secretion. This enhanced secretory rate was not affected by ACTH, but was depressed by cortisol ($P<0.05$), although not by a combination of ACTH and cortisol (Fig. 2).

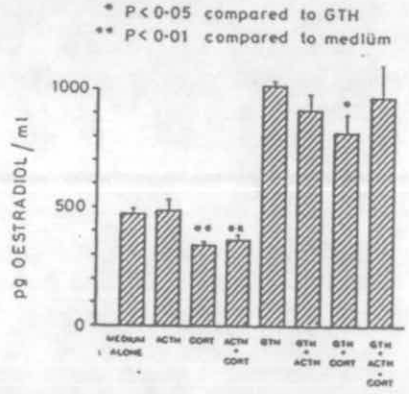


Figure 2. The effects of ACTH, cortisol, and gonadotrophin, alone or in combination, on the secretion of 17β-oestradiol from cultured ovarian follicles. All hormones were tested at a concentration of 100 ng/ml. Results are expressed as mean±SEM of six replicates.

Reproductive maturity had a pronounced effect on the stress response. In three separate experiments, two with brown trout and one with rainbow trout, the maturing or mature males showed a significantly reduced cortisol response to stress compared to the immature fish (Table 1).

Table 1. Effect of reproductive development on the response of trout to environmental stress

	Cortisol (ng/ml)*		Significance
	mature	immature	
Brown trout	11.6±3.6 (n=8)	57.7±10.0 (n=16)	$P<0.001$
Brown trout	34.3±5.7 (n=12)	81.4±5.1 (n=36)	$P<0.001$
Rainbow trout	31.2±3.1 (n=11)	49.0±6.1 (n=37)	$P<0.05$

*Plasma cortisol levels after 1h of acute stress. See Methods for further details.

Discussion

Both acute and chronic confinement stress caused a marked suppression of the concentration of circulating androgens, with testosterone levels being suppressed more markedly than 11-ketotestosterone levels. On the other hand, plasma GTH levels were significantly elevated for at least 4 hours following acute stress. The simultaneous elevation of plasma gonadotrophin and suppression of testosterone levels has also been observed in mammals subjected to acute stress, though gonadotrophin levels decline ultimately if the stress is prolonged. Unfortunately we did not determine the GTH levels in the chronically-stressed trout to know if they had depressed GTH, as well as androgen, levels.

If it is the elevation of stress hormones (ACTH and cortisol) that leads to suppression of the reproductive hormones, the former could be acting directly on the gonads, or at a higher centre, such as the pituitary and/or hypothalamus. Our preliminary results suggest that cortisol can directly inhibit the basal secretion of 17β-oestradiol from cultured follicles. The dose of cortisol tested (100 ng/ml) is well within the physiological range. In contrast, ACTH at 100 ng/ml, a dose well above the physiological range, did not affect the basal secretion rate of 17β-oestradiol. The presence of cortisol may also reduce the enhanced 17β-oestradiol secretion produced by GTH, although the results were not unequivocal. In mammals cortisol can act directly on both the testis and ovary. Further, the action of cortisol is selective; for example, glucocorticoids inhibit FSH-stimulated secretion of oestrogen, but augment the FSH-stimulated secretion of progesterone (Hseuh and Erickson, 1978). Receptors for ACTH are also present in the gonads of mammals, but the action of this peptide on steroidogenesis is unclear presently. Although our results suggest that direct glucocorticoid inhibition could be a factor in the stress-induced suppression of plasma androgen levels we observed, we cannot yet assess the importance of this mechanism. Detailed investigations are necessary on the effects of ACTH and cortisol on both basal and GTH-stimulated secretion of the various steroids (oestrogens, androgens, progesterones) from different-sized follicles. The direct effects of stress hormones on the secretion of sex steroids from the testes also requires investigation.

Besides demonstrating that stress affects the reproductive axis of trout, we have also shown that the reproductive condition of the fish can affect their response to stress. Sexually maturing or mature male trout had lower cortisol levels after 1h of acute

stress compared to immature males. This result can be accounted for simply by a different temporal pattern of cortisol secretion in response to stress in mature and immature trout, or the whole endocrine response to stress may be attenuated in mature fish. Whichever of these two explanations is correct, the mechanism remains unknown.

Acknowledgements

We are grateful to NERC and MAFF for financial support.

References

Billard, R., C. Bry & C. Gillet, 1981. Stress, environment and reproduction in teleost fish. In: A.D. Pickering (Ed.): Stress and fish. Academic Press, London/ New York. p. 185-208.

Hseuh, A.J.W. & G.F. Erickson, 1978. Glucocorticosteroid inhibition of FSH-induced estrogen production in cultured rat granulosa cells. Steroids 32: 639-648.

Pickering, A.D., T.G. Pottinger, J.Carragher & J.P. Sumpter, 1987. The effects of acute and chronic stress on the levels of reproductive hormones in the plasma of mature male brown trout, Salmo trutta L. Gen. Comp. Endocrinol. in press.

J. Fish Biol. (1988) 32, 689–697

Lymphocytopenia and the overwinter survival of Atlantic salmon parr, *Salmo salar* L.

A. D. PICKERING AND T. G. POTTINGER

Freshwater Biological Association, Windermere Laboratory, The Ferry House, Far Sawrey, Ambleside, Cumbria, LA22 0LP, U.K.

(*Received 16 July 1987, Accepted 23 October 1987*)

The overwinter mortality rate of underyearling S2 Atlantic salmon parr (fish that will remain in fresh water for at least two years prior to smoltification) was almost ten times greater than that of S1 parr (fish that will smoltify after 1 year in fresh water) kept under similar conditions. The loss of condition (*K* factor) of the S2 parr was proportionally greater than that of S1 parr during the early winter months and this coincided with a marked lymphocytopenia and thrombocytopenia in the S2 parr. Limited evidence indicates that S2 parr have chronically elevated blood cortisol levels during the winter months. It is suggested that chronic stress resulting (in part) from nutritional deficiency has a debilitating effect on the defence systems of these fish. The ultimate cause of mortality in most cases was bacterial fin-rot and/or fungal (*Saprolegnia*) infection. These findings are discussed in relation to the habitat requirements of overwintering salmonid fish.

I. INTRODUCTION

Compensatory mortality in the juvenile (rather than adult) stages is a major factor in the regulation of fish population size (Ricker, 1954). Thus, an understanding of the mechanisms responsible for juvenile mortality is fundamental to any study of fish population dynamics and the subsequent implementation of management policies. In general, populations of juvenile salmonids are characterized by a high, initially density-dependent, mortality during the first 3 months post-hatching followed by a persistent, but lower, mortality rate for the rest of the year (Latta, 1962; Mortensen, 1977; Egglishaw & Shackley, 1977, 1980; Gee *et al.*, 1978*a*,*b*) although some exceptions to this pattern of mortality do exist (Elliott, 1987). Little is known about the exact causes of death although Mason & Chapman (1965), Hunt (1969) and Gee *et al.* (1978*b*) have all suggested that 'physiological stress' might be a contributing factor.

During the winter months, the growth of underyearling salmonid fishes slows down or even ceases (Egglishaw & Shackley, 1977; Thorpe, 1987) and the fish depend heavily upon energy stores laid down during the previous growing season. Under natural conditions, larger fish have a greater survival capability than smaller fish during these winter months (Hunt, 1969; Peterson, 1982) and it has been shown in juvenile Atlantic salmon, *Salmo salar*, that overwinter mortality is associated with a depletion of the fish's energy stores (Gardiner & Geddes, 1980). Moreover, Gardiner & Geddes also demonstrated that larger individuals had proportionally less water (i.e., proportionally greater energy reserves) than smaller fish. A similar size difference in overwinter mortality of underyearling Atlantic salmon has been reported for hatchery-reared fish (Lindroth, 1965), a difference in mortality rate that was largely density-independent. Interestingly, Lindroth

0022–1112/88/050689+09 $03.00/0

showed that it was the size position within the year class, and not size *per se*, that determined first winter mortality. It is now known that much of the size variation of underyearling Atlantic salmon at the end of the summer growing season is related to a bimodal distribution of potential S1 and S2 smolts (Knutsson & Grav, 1976; Thorpe, 1977). This raises the possibility that the winter survival of potential S1 smolts (fish that will smoltify at 1 year old) is greater than that of potential S2 smolts (fish that will remain in fresh water for at least 2 years before smoltifying).

The present study investigates the overwinter mortality rates of segregated populations of potential S1 and S2 Atlantic salmon smolts (from here on referred to as S1 and S2 parr). In an attempt to assess the degree of stress experienced by these fish during the winter months, differential blood cell counts were also made. Our previous studies with salmonid fish have shown that periods of chronic stress and periods of increased mortality are frequently associated with elevated blood cortisol levels and/or a reduction in the number of circulating white blood cells, particularly the lymphocytes (Pickering & Pottinger, 1985, 1987*a*, *b*).

II. MATERIALS AND METHODS

FISH

Freshly fertilized eggs from wild Atlantic salmon taken on their spawning migration in the River Lune, Lancashire were kindly provided by the North West Water Authority in December 1985. The eggs were incubated in a Heath incubator at the Freshwater Biological Association's hatchery and alevins were transferred to fish-rearing troughs until the yolk sac was fully resorbed and the fish were actively feeding on BP Nutrition 'Mainstream' pellets (size 00). The resultant fry were then transferred to large (2 m dia.), outdoor, fibreglass tanks each supplied with a constant flow of Windermere lake water (35 l min^{-1}). The young salmon were fed with commercial salmon food at the rates recommended by the manufacturers and grown on until November 1986 (the end of the first growing season). At this point all the fish were lightly anaesthetized in phenoxyethanol (0·5 ml l^{-1}) and measured (fork length) to the nearest 0·5 cm. The length/frequency data revealed a clear bimodal distribution (Fig. 1) and the population was segregated into fish longer than 8·5 cm (S1 parr) and fish shorter than 7 cm (S2 parr). These two groups of fish were then used for the subsequent study on haematology and overwinter mortality.

EXPERIMENTAL DESIGN

Twelve large, outdoor, fibreglass tanks were randomly allocated to receive a total of 1800 underyearling salmon so that six tanks each contained 150 S1 parr and six tanks each contained 150 S2 parr. The fish were left for a period of 2 weeks to recover from the effects of handling (Pickering *et al.*, 1982) and were then sampled on a monthly basis from December 1986 to March 1987. Throughout this period the young salmon were fed at the rates recommended by the food manufacturers (exact rate dependent upon water temperature; see Fig. 2(a)) and the tanks were cleaned on a fortnightly basis with the fish *in situ*. Dead or moribund fish were removed daily and cumulative weekly mortality rates (corrected for sampling losses) were calculated.

At each sampling time six fish $tank^{-1}$ were anaesthetized in phenoxyethanol, weighed, measured and examined for gross signs of disease. Blood samples were taken by sectioning the caudal peduncle and collecting the blood in heparinized capillary tubes. Aliquots of 5 µl blood were diluted in trout Ringer solution (final dilution 1:40 000), fixed with Lugol's iodine, and sedimented in polypropylene tubes mounted on glass microscope slides. Total blood cell counts were made on the sedimented blood samples, using an inverted microscope, and differential counts were made from air-dried, methanol-fixed, stained (haematoxylin and eosin) blood smears. Absolute concentrations of erythrocytes, neutrophils, thrombocytes, and lymphocytes were calculated from the total and differential blood cell counts.

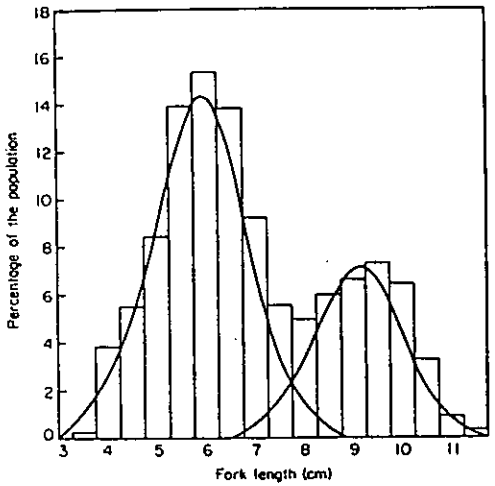


FIG. 1. Length frequency histogram of underyearling Atlantic salmon at the end of their first growing season (November). The bimodal distribution was determined using Cassie's (1954) probability paper technique.

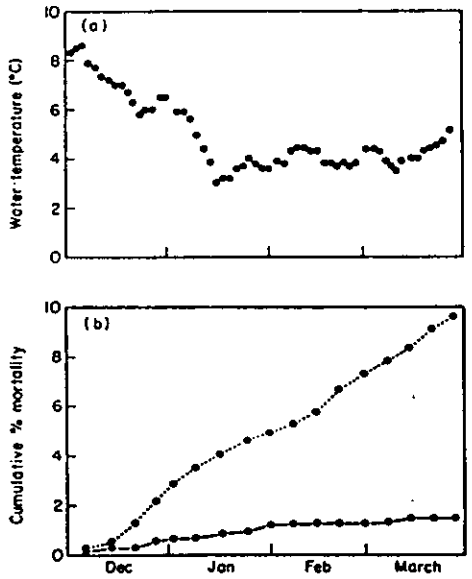


FIG. 2. (a) Change in ambient water temperature during the winter months December–March. (b) Cumulative percent mortality of the S1 parr (———) and the S2 parr (· · ·) during the same period.

STATISTICAL ANALYSES

The data for body weight (W), length (L), coefficient of condition (K factor $= 100\ W/L^3$) and blood cell counts were separately subjected to analysis of variance (ANOVA, Genstat) using parr group (S1 or S2), time and fish number (number 1–6 in the sampling sequence of fish within tanks) as factors. Tank and fish were used as blocking effects to give a nested error structure with which to assess the significance of the factors and their interactions. From a plot of the residuals against fitted values, appropriate transformations ($\sqrt{}$ or log) were selected, where necessary, to improve the homogeneity of variance. The levels of significance given here are derived from these analyses but, for ease of presentation, data are given as arithmetic means $\pm$ S.E.M. A comparison of the total mortality figures between replicate tanks was made by χ^2 analysis, and Cassie's (1954) probability paper method was used to determine the bimodal distribution of length in the underyearling salmon population prior to segregation in November 1986.

III. RESULTS

MORTALITY RATES

χ^2 analysis of the total mortalities within replicate tanks did not reveal any significant tank-to-tank variation for either S1 or S2 parr, so the data from the replicate tanks have been pooled and presented as cumulative mortalities (Fig. 2(b)). It is clear that the mortality rate of the S2 parr was much greater than that of the S1 parr during the winter months. The mean instantaneous daily mortality rate ($Z = \dfrac{\log_e N_T - \log_e N_t}{t - T}$, where N_T and N_t are the numbers of fish at times T and t respectively) of the S2 parr (0·00068) was almost ten times greater than that of the S1 parr (0·000085). Examination of the dead and moribund fish revealed a high incidence of fin-rot with occasional *Saprolegnia* infection.

GROWTH

The changes in weight, length and coefficient of condition of the S1 and S2 parr during the winter months are presented in Fig. 3. The mean weight and length of S1 parr increased from 8·5 to 11 g and from 9·5 to 10·2 cm, respectively. During the same period, the S2 parr increased in weight from 1·4 to 1·9 g and in length from 5·5 to 6·0 cm. Despite the overall size difference between the S1 and S2 parr, it was not possible to demonstrate any significant differences in the rates of increase in weight and length. However, changes in the coefficient of condition were significantly different between the two groups of fish (group × time interaction $P < 0{\cdot}001$). S2 parr had a lower K factor throughout the study and the decrease in condition of these fish during December and January [the period of decreasing water temperature (Fig. 2(a))] was much greater than in the S1 parr (Fig. 3(c)).

HAEMATOLOGY

Changes in the four major blood cell types of S1 and S2 parr are presented in Fig. 4. In both groups of fish the concentration of circulating erythrocytes declined significantly during the course of the investigation ($P < 0{\cdot}001$). At each sampling time the mean erythrocyte count of the S2 parr was lower than that of the S1 parr (Fig. 4(a)) although these differences were not large enough to permit the demonstration of a significant difference in erythrocyte numbers between the two groups. The mean neutrophil count of the S2 parr was always greater than that of the

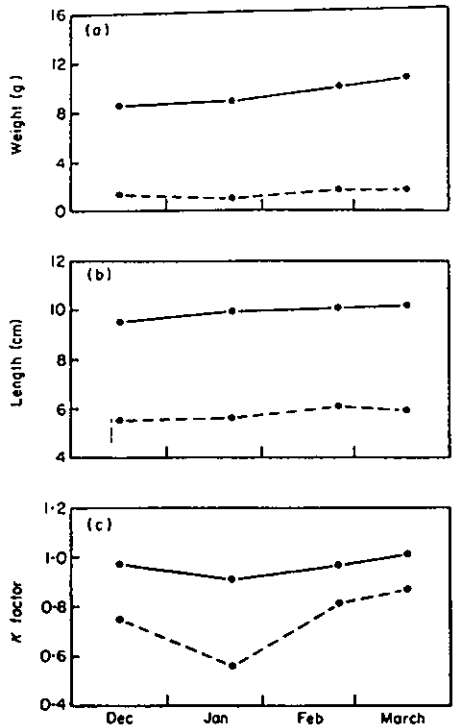


FIG. 3. Changes in weight (a), length (b), and coefficient of condition (c) of S1 parr (———) and S2 parr (– – –) during the winter months. Each point is the arithmetic mean; vertical lines represent ± S.E.M. ($n = 36$). In most cases the S.E.M. is too small to be depicted graphically.

S1 parr (Fig. 4(b)) but the high variability of these data again prevented the demonstration of a significant difference between the two groups.

By contrast, both the thrombocyte and lymphocyte concentrations in the circulating blood showed marked and highly significant group × time interactions ($P < 0{\cdot}005$ and 0·001, respectively). Thrombocyte and lymphocyte counts in the S1 parr were significantly greater than in the S2 parr during December and January (Fig. 4(c), (d)) but by the end of February these differences had disappeared. Thus, in December 1986 the mean blood thrombocyte count of S1 parr was 15 673 cells μl^{-1} compared with 10 668 cells μl^{-1} for the S2 parr. By March 1987 the mean thrombocyte counts for S1 and S2 parr were 16 841 and 16 763 cells μl^{-1}, respectively. Similarly, the mean lymphocyte count of S1 parr at the start of the investigation was 27 462 cells μl^{-1} compared with 16 688 cells μl^{-1} for the S2 parr, but by the end of the study the lymphocyte counts were 22 674 and 25 136 cells μl^{-1}, respectively. In both the S1 and S2 parr, the lymphocyte counts decreased significantly during December and January, stabilized during February and started to increase during March (Fig. 4(d)) thus paralleling, to a large degree, the changes in water temperature (Fig. 2(a)).

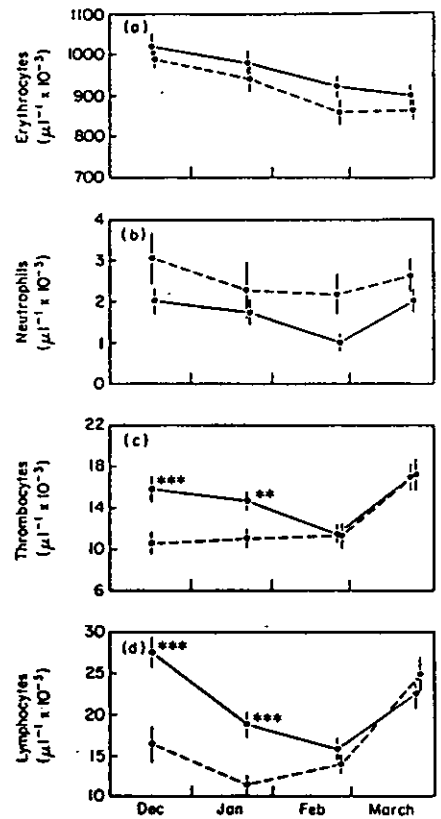


FIG. 4. Changes in the concentration of circulating erythrocytes (a), neutrophils (b), thrombocytes (c), and lymphocytes (d) of S1 parr (———) and S2 parr (– – –) during the winter months. Each point is the arithmetic mean; vertical lines represent ± S.E.M. ($n = 36$).

IV. DISCUSSION

The present investigation has shown that the winter mortality rate of S2 salmon parr (mean weight 1·6 g) is approximately ten times greater than that of S1 parr (mean weight 9·7 g) kept under similar conditions. The initial stocking density was identical for both groups of fish, thus supporting the conclusion of Lindroth (1965) that overwinter mortality in hatchery-reared Atlantic salmon is largely density-independent. Previous studies on natural populations of salmonids have shown that overwinter survival is size-dependent (Hunt, 1969; Peterson, 1982) with larger fish having a greater survival rate than smaller fish. Little is known about the mechanisms responsible for this differential survival, although Peterson (1982) suggested that avian predation was probably the main cause of mortality in overwintering populations of juvenile coho salmon, *Oncorhynchus kisutch*. However, Hunt (1969), working with the brook trout, *Salvelinus fontinalis*, considered that

predation was not a major factor and suggested that size could have influenced overwinter survival by ' modifying thresholds at which physiological stresses were either fatal or directly contributory to other causes of natural mortality '. In the present investigation on hatchery-reared Atlantic salmon, predation was not responsible for the overwinter mortality, but several pieces of evidence suggest that the group of fish with the greatest mortality rate (S2 parr) were suffering from chronic stress (see below).

The growth rate was extremely low in both S1 and S2 parr during the winter months ($\approx 0{\cdot}35\%$ day^{-1}) and the coefficient of condition decreased significantly in both groups of fish during December–January but recovered during February and March. A direct comparison of the K factors of S1 and S2 parr is not strictly appropriate because the length/weight relationship of juvenile salmonid fish normally changes with increasing size (Mortensen, 1977). However, it is clear from our work that the loss of condition of the S2 parr during the early winter months was proportionally greater than the loss of condition of the S1 parr. This conforms with the work of Metcalfe *et al.* (1986) who present evidence to show that the reduced growth of S2 parr is caused by an internal suppression of appetite which may commence as early as mid-summer and which continues throughout the autumn and winter regardless of competition, food supply or water temperature. Moreover, Gardiner & Geddes (1980) have shown that the loss of energy content of Atlantic salmon parr was greatest during October–December and that smaller fish had proportionally smaller energy reserves than the larger fish. Thus, nutritional insufficiency is probably a major stress during overwintering of both hatchery-reared and natural populations of S2 Atlantic salmon parr.

A comparison of the haematological profiles of S1 and S2 parr during the present study revealed a marked, and highly significant, suppression of the concentration of circulating thrombocytes and lymphocytes. This type of leucopenia is a typical response of salmonid fish to a wide range of acute and chronic stresses (Pickering *et al.*, 1982; Pickering & Pottinger, 1987*b*) and evidence is now accumulating that lymphoid tissue and leucocyte activity in fish are strongly suppressed by corticosteroids secreted from the interrenal tissue (Pickering, 1984; Stave & Roberson, 1985; Maule *et al.*, 1986; Pickering *et al.*, 1987). In the present study, the small size of the fish precluded a regular sampling programme to monitor plasma cortisol levels in individual fish at each sampling time. However, in February we were able to pool blood samples from fish within the six replicate tanks for both S1 and S2 parr. Cortisol levels (determined by radioimmunoassay; Pickering *et al.*, 1987*b*) in the pooled plasma samples from S1 and S2 parr were $1{\cdot}5 \pm 0{\cdot}4$ (6) [mean $\pm$ S.E.M. (n)] and $6{\cdot}4 \pm 1{\cdot}9$ ng ml^{-1} respectively ($P < 0{\cdot}05$). This compares favourably with the studies of Simpson & Thorpe (1976) in which it was shown that the blood cortisol levels of S2 Atlantic salmon parr sampled in January were significantly higher than those of S1 parr. Taken together, these limited data suggest that the S2 parr have chronically elevated plasma cortisol levels during the winter months. Thus, S2 parr are characterized by elevated plasma cortisol levels and suppressed white blood cell counts, both indicators of chronic stress. Studies on the brown trout have shown that extended periods of elevated plasma cortisol levels and/or reduced lymphocyte counts are usually associated with increased mortality due to disease (Pickering & Duston, 1983; Pickering & Pottinger, 1985, 1987*a*; Pickering, 1986). It would seem that a similar situation exists in Atlantic salmon parr during the winter months, because most of the moribund fish and many of the S2 parr taken during routine sampling showed signs of bacterial fin-rot and *Saprolegnia* infection.

In some natural populations of Atlantic salmon parr, high overwinter mortality may act as a selective pressure in determining the time of downstream migration. Riddell & Leggett (1981) suggested that an autumnal downstream migration of parr (rather than the more usual spring smolt migration) may be related to the high energy costs of overwintering in a harsh environment. The majority of salmonid populations, however, do not have extensive downstream migrations in the autumn, but there is a marked change in the fishes' behaviour and habitat selection; in general, they move from relatively unsheltered summer stations to winter positions characterized by slower water velocity and a greater degree of overhead cover (Rimmer *et al.*, 1983, 1984; Cunjak & Power, 1986; Swales *et al.*, 1986). In this connection it is interesting to note that the provision of overhead cover, whilst not influencing the mortality rate of hatchery-reared Atlantic salmon parr during the summer growing season, did significantly enhance the growth rate of the fish and alleviate the mild leucopenia observed in those fish without access to overhead cover (Pickering *et al.*, 1987*a*). It is suggested, therefore, that provision of cover during the winter months (the time of year at which the fish would seek natural overhead cover) might reduce the relatively high mortality rates observed in S2 parr during a period of nutritional deficiency and minimal growth. Further studies on the practical application of this research are planned.

The authors are grateful to Mrs J. Pollard and Miss J. M. Fletcher (FBA) for maintaining the experimental fish, to Mr C. Durie, Mr A. P. Atkinson and colleagues (NWWA) for providing the salmon eggs, Mr T. I. Furnass (FBA) for the artwork and to NERC and MAFF (Project NBB 13) for financial support.

References

Cassie, R. M. (1954). Some uses of probability paper in the analysis of size frequency distributions. *Aust. J. Mar. Freshwat. Res.* 5, 513–522.

Cunjak, R. A. & Power, G. (1986). Winter habitat utilization by stream resident brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*). *Can. J. Fish. Aquat. Sci.* 43, 1970–1981.

Egglishaw, H. J. & Shackley, P. E. (1977). Growth, survival and production of juvenile salmon and trout in a Scottish stream, 1966–75. *J. Fish Biol.* 11, 647–672.

Egglishaw, H. J. & Shackley, P. E. (1980). Survival and growth of salmon, *Salmo salar* (L.), planted in a Scottish stream. *J. Fish Biol.* 16, 565–584.

Elliott, J. M. (1987). Population regulation in contrasting populations of trout *Salmo trutta* in two Lake District streams. *J. Anim. Ecol.* 56, 83–98.

Gardiner, W. R. & Geddes, P. (1980). The influence of body composition on the survival of juvenile salmon. *Hydrobiologia* 69, 67–72.

Gee, A. S., Milner, N. J. & Hemsworth, R. J. (1978*a*). The production of juvenile Atlantic salmon, *Salmo salar* in the upper Wye, Wales. *J. Fish Biol.* 13, 439–451.

Gee, A. S., Milner, N. J. & Hemsworth, R. J. (1978*b*). The effect of density on mortality in juvenile Atlantic salmon (*Salmo salar*). *J. Anim. Ecol.* 47, 497–505.

Hunt, R. L. (1969). Overwinter survival of wild fingerling brook trout in Lawrence Creek, Wisconsin. *J. Fish. Res. Bd Can.* 26, 1473–1483.

Knutsson, S. & Grav, T. (1976). Seawater adaptation in Atlantic salmon (*Salmo salar* L.) at different experimental temperatures and photoperiods. *Aquaculture* 8, 169–187.

Latta, W. C. (1962). Periodicity of mortality of brook trout during first summer of life. *Trans. Am. Fish. Soc.* 91, 408–411.

Lindroth, A. (1965). First winter mortality of Atlantic salmon parr in the hatchery. *Can. Fish Cult.* 36, 23–26.

Mason, J. C. & Chapman, D. W. (1965). Significance of early emergence, environmental rearing capacity, and behavioural ecology of juvenile coho salmon in stream channels. *J. Fish. Res. Bd Can.* 22, 173–190.

Maule, A. G., Schreck, C. B. & Kaattari, S. L. (1986). Changes in the immune system of coho salmon (*Oncorhynchus kisutch*) during the parr-to-smolt transformation and after implantation of cortisol. *Can. J. Fish. Aquat. Sci.* 44, 161–166.

Metcalfe, N. B., Huntingford, F. A. & Thorpe, J. E. (1986). Seasonal changes in feeding motivation of juvenile Atlantic salmon (*Salmo salar*). *Can. J. Zool.* 64, 2439–2446.

Mortensen, E. (1977). Population, survival, growth and production of trout *Salmo trutta* in a small Danish stream. *Oikos* 28, 9–15.

Peterson, N. P. (1982). Population characteristics of juvenile coho salmon (*Oncorhynchus kisutch*) overwintering in riverine ponds. *Can. J. Fish. Aquat. Sci.* 39, 1303–1307.

Pickering, A. D. (1984). Cortisol-induced lymphocytopenia in brown trout, *Salmo trutta* L. *Gen. Comp. Endocr.* 53, 252–259.

Pickering, A. D. (1986). Changes in blood cell composition of the brown trout, *Salmo trutta* L., during the spawning season. *J. Fish Biol.* 29, 335–347.

Pickering, A. D. & Duston, J. (1983). Administration of cortisol to brown trout, *Salmo trutta* L., and its effects on the susceptibility to *Saprolegnia* infection and furunculosis. *J. Fish Biol.* 23, 163–175.

Pickering, A. D. & Pottinger, T. G. (1985). Cortisol can increase the susceptibility of brown trout, *Salmo trutta* L., to disease without reducing the white blood cell count. *J. Fish Biol.* 27, 611–619.

Pickering, A. D. & Pottinger, T. G. (1987*a*). Lymphocytopenia and interrenal activity during sexual maturation in the brown trout, *Salmo trutta* L. *J. Fish Biol.* 30, 41–50.

Pickering, A. D. & Pottinger, T. G. (1987*b*). Crowding causes prolonged leucopenia in salmonid fish, despite interrenal acclimation. *J. Fish Biol.* 30, 701–712.

Pickering, A. D., Pottinger, T. G. & Christie, P. (1982). Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: a time-course study. *J. Fish Biol.* 20, 229–244.

Pickering, A. D., Griffiths, R. & Pottinger, T. G. (1987*a*). A comparison of the effects of overhead cover on the growth, survival and haematology of juvenile Atlantic salmon, *Salmo salar* L., brown trout, *Salmo trutta* L., and rainbow trout, *Salmo gairdneri* Richardson. *Aquaculture* 66, 109–124.

Pickering, A. D., Pottinger, T. G. & Sumpter, J. P. (1987*b*). On the use of dexamethasone to block the pituitary-interrenal axis in the brown trout, *Salmo trutta* L. *Gen. Comp. Endocr.* 65, 346–353.

Ricker, W. E. (1954). Stock and recruitment. *J. Fish. Res. Bd Can.* 11, 559–623.

Riddell, B. E. & Leggett, W. C. (1981). Evidence of an adaptive basis for geographic variation in body morphology and time of downstream migration of juvenile Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* 38, 308–320.

Rimmer, D. M., Paim, U. & Sanders, R. L. (1983). Autumnal habitat shift of juvenile Atlantic salmon (*Salmo salar*) in a small river. *Can. J. Fish. Aquat. Sci.* 40, 671–680.

Rimmer, D. M., Paim, U. & Saunders, R. L. (1984). Changes in the selection of microhabitat by juvenile Atlantic salmon (*Salmo salar*) at the summer-autumn transition in a small river. *Can. J. Fish. Aquat. Sci.* 41, 469–475.

Simpson, T. H. & Thorpe, J. E. (1976). Growth bimodality in the Atlantic salmon. *Int. Counc. Explor. Sea C.M. 1976/M:22* 7 pp. (mimeo).

Stave, J. W. & Roberson, B. S. (1985). Hydrocortisone suppresses the chemiluminescent response of striped bass phagocytes. *Dev. comp. Immun.* 9, 77–84.

Swales, S., Lauzier, R. B. & Levings, C. D. (1986). Winter habitat preferences of juvenile salmonids in two interior rivers of British Columbia. *Can. J. Zool.* 64, 1506–1514.

Thorpe, J. E. (1977). Bimodal distribution of length of juvenile Atlantic salmon (*Salmo salar* L.) under artificial rearing conditions. *J. Fish Biol.* 11, 175–184.

Thorpe, J. E. (1987). Environmental regulation of growth patterns in juvenile Atlantic salmon. In *Age and Growth of Fish* (R. C. Summerfelt & G. E. Hall, eds), pp. 463–474. Ames: Iowa State University Press.

1988 Trout News 5, 13-14

CAN OVERHEAD COVER REDUCE THE STRESS OF AQUACULTURE?

Alan D Pickering, Freshwater Biological Association
The Ferry House, Far Sawrey, Ambleside, Cumbria LA22 OLP

In the natural environment, young trout and salmon are attracted to those areas of a stream with plenty of overhead cover. Indeed, the carrying capacity of trout streams can be increased by the construction of artificial covers at strategic points. During the autumn and winter months, this preference for cover is further increased as young salmonids change their habitat from sites adjacent to overhead cover to positions directly under cover, for example under the larger stones on the bed of a stream. Thus, overhead cover is an important feature of the natural environment and one which is extensively used by young salmonid fish.

There are very few published accounts of attempts to exploit this phenomenon for aquacultural purposes by providing floating, overhead covers under which the fish can take refuge. At the Freshwater Biological Association, we have recently compared the effects of overhead cover on the physiology and growth of salmon parr, rainbow trout and brown trout during their first 6 months of life under aquaculture conditions. The fish were reared in standard 2 metre outdoor tanks and fed with commercial diets at the rates recommended by the manufacturers. Provision of floating, annular covers to the tanks markedly increased the growth rate of Atlantic salmon parr and doubled the proportion of potential S1 smolts (identified as the upper mode of a bimodal distribution in length in November of their first growing season). The parr without access to cover showed physiological signs of chronic stress, in the form of reduced white blood cell counts, whereas the fish in the tanks provided with cover were unstressed. Moreover, in a more recent, unpublished study we found that the overwintering mortality of potential S2 smolts was ten times greater than that of potential S1 smolts but that this high mortality rate could be halved by the provision of overhead cover.

We were unable to reproduce the growth-promoting effect on either species of trout reared under identical conditions. Since publishing our initial study, however, a Danish trout farmer has written to me with details of an experiment he is undertaking on the effect of floating overhead cover on young rainbow trout reared in an earth pond system. Preliminary results appeared to show a growth-promoting effect, as we found for salmon parr, but the study is still in its infancy and the initial results require verification with a more rigorous experimental design.

It is not clear, at this stage, whether the differences we found in the response to overhead cover between trout and salmon parr represent true species differences or whether they are a consequence of the past history of the strains of fish used in the study. The Atlantic salmon were first generation fry from wild, adult fish taken on their spawning migration in the River Lune, Lancashire, whereas both species of trout were derived from domesticated strains. It is possible that the beneficial effects of overhead cover may be evident in some, but not all, strains of domesticated trout and may be modified by other factors, such as stocking density. Clearly, more work in this area is needed.

Whatever the final explanation, overhead cover would seem to be an important tool for any unit rearing smolts from wild fish (for stock enhancement) and may, ultimately, prove to be of more general value in reducing aquacultural stress.

I would be interested to hear from any fish farmer who has views on (and, preferably, experience of) the use of floating, overhead cover as a technique for reducing stress and increasing the growth rate of salmonid fish.

1988 Proc. Conf. 3, 35-49. Aqua Nor 1987, Trondheim

Stress Responses and Disease Resistance in Farmed Fish

By

A.D. Pickering

Freshwater Biological Association,
The Ferry House, Far Sawrey,
Ambleside, Cumbria, LA22 OLP, England

A.D. Pickering
Freshwater Biological Association,
The Ferry House, Far Sawrey,
Ambleside, Cumbria, LA22 OLP, England

I. Introduction

It is generally accepted that there is a strong association between deleterious changes in the environment and an increase in the susceptibility of fish to disease (Wedemeyer, 1970; Snieszko, 1974). Under aquaculture conditions such environmental changes, or stresses, include water quality deterioration, sudden temperature changes, social interaction between fish, disturbance, physical handling and confinement. However, much of the evidence is anecdotal or, at best, circumstantial and there are surprisingly few rigorous experimental studies of the links between environmental stress and the onset of disease in fish populations. Disease outbreaks occur when susceptible fish are exposed to potential pathogens under conditions that favour the survival and growth of the infective organism. Changes in the physical and chemical characteristics of the environment can increase the abundance and virulence of pathogenic organisms, a factor which must have an important influence on the outcome of a situation in which fish are challenged by pathogens. However another influence, namely the degree of susceptibility of the host, may also be instrumental in determining whether or not pathogenic challenge results in disease. The aim of this paper is to review the evidence for increased vulnerability of stressed fish to disease and to identify the mechanisms behind this response. In view of the immunosuppressive and anti-inflammatory effects of corticosteroid hormones on the defence systems in mammals and the fact that corticosteroid elevation is an almost invariable response of teleost fish to stress, particular attention will be given to the role of the pituitary-interrenal axis. No attempt will be made to define the optimal aquacultural conditions consistent with maximizing the economic return — this can only be achieved by experienced fish farmers for each particular set of circumstances and is likely to be affected by the species and strain of fish under cultivation, the physical and chemical characteristics of the water supply and by the design of the aquaculture facilities. However, a deeper understanding of the physiological and endocrinological mechanisms behind stress-induced vulnerability to disease will allow us to outline suitable approaches for alleviating this response. Wherever possible, evidence has been selected from studies of salmonid fish although some reference will be made to other groups of fish if evidence for salmonids is limited or lacking.

II. Evidence of stress predisposing fish to disease

Perhaps the most rigorously documented evidence concerns the impact of pollutants on disease outbreaks in fish populations. In general, the incidence of disease is greater in polluted environments than in clean ones (Pippy & Hare, 1969; Brown et al. 1979; Sindermann, 1979; Moller, 1985). Thus, chronic low-level pollution of the water supply is likely to result in an increased incidence of disease in an aquaculture unit. More specifically, Hetrick et al. (1979) and Knittel (1981) have shown that exposure of rainbow trout, **Salmo gairdneri**, to copper increases their susceptibility to infections haematopoietic necrosis virus and to the bacterium responsible for redmouth infection, **Yersinia ruckeri**. Copper has also been shown to predispose channel catfish, Ictalurus punctatus, to experimental infection with white-spot disease, **Ichthyophthirius multifiliis**, (Ewing et al. 1982) and to predispose eels, **Anguilla anguilla**, to vibriosis, Vibrio anguillarum (Rodsaether et al., 1977).

Water quality deterioration as a result of aquacultural processes themselves can also lead to disease. The stress of elevated ammonia and carbon dioxide levels combined with low dissolved oxygen concentrations was sufficient to increase the mortality of channel catfish caused by experimental infection with **Aeromonas hydrophila** (Walters & Plumb, 1980). Elevated nitrite levels were also capable of predisposing this species of fish to bacterial infections (Hanson & Grizzle, 1985). It seems probable that a similar association between water quality and disease resistance exists for salmonid fish. For example, Sodeberg et al. (1983) found that the mortality rate (due to parasite epizootics) of rainbow trout reared in an intensive static water culture system was directly related to the average daily maximum concentration of unionized ammonia. An outbreak of **Pseudomonas fluerescens** in farmed rainbow trout was attributed to high levels of organic matter in the water originating from dusty food (Eiras & Saraiva, 1986). The stress of simulated transportation for 21 hours was sufficient to markedly increase the mortality rate of Atlantic salmon, **Salmo salar**, subsequently exposed to **A. hydrophila** (Johansson & Bergström, 1977) and crowding and handling have been shown to result in significantly higher mortalities in furunculosis-infected brown trout (Hosmer, 1980). Heat stress increased the rate of transmission of **Y. ruckeri** between experimental populations of rainbow trout (Hunter et al., 1980) and the of a viral infection, chronic pancreatic necrosis, appeared to increase the incidence of a bacterial disease, **P. fluorescens** (Roberts & Horne, 1978).

These examples form part of an accumulating body of evidence linking various forms of stress to a subsequent increase in the susceptibility of fish to a wide range of infectious diseases (see also Wedemayer & McLeay, 1981). One might argue that each represents specific response to a particular set of environmental circumstances and that there is no fundamental relationship between these studies. However, it seems much more probable that there are common mechanisms at work which might account for the similarities in the response of the fish to pathogenic challenge. The next section of this paper examines one such mechanism, activation of the hypthalamic-pituitary-interrenal axis, which occurs in response to many different forms of environmental stress (Table 1).

Table 1. Selected examples of environmental stress known to stimulate the HPI axis in salmonid fish.

Type of Stress		Species	Reference
Pollution	Chromium	S. gairdneri	Hall & Fromm (1958)
	Copper	O. nerka	Donaldson & Dye (1973)
	Endrin	S. gairdneri	Grant & Mehrle (1973)
	2-4-D	O. nerka	Donaldson (1981)
	Low pH	S. gairdneri	Brown et al. (1984)
Water Quality	NH_3	O. nerka	Donaldson (1981)
Physical	Handling	S. trutta	Pickering et al. (1982)
	Confinement	O. tshawytscha	Strange et al. (1978)
	Forced Exercise	O. nerka	Fagerlund (1967)
		S. gairdneri	Zeinik & Goldspink (1981)
	Stocking	S. gairdneri	Barton et al. (1980)
Temperature	12 —> 26°C	S.clarki	Strange et al. (1977)
	10 —> 17°C	O. kisutch	Wedemeyer (1980)
	10 —> 3°C	O. kisutch	Wedemeyer (1969)
Osmotic	FW —> SW	S. salar	Nichols & Weisbart (1985)
Social Interaction	Dominance/	S. gairdneri	Noakes & Leatherland (1977)
	Submission	S. gairdneri	Bodolngius (1976)
		O. kisutch	Ejike & Schreck (1980)
	Crowding	O. kisutch	Fagerlund et al. (1981)
Disease	Saprolegnia	S. trutta	Pickering & Christie (1981)
	Mycobacterium	S. gairdneri	Simpson (1975/76)
	BKD	O. gorbuscha	Donaldson (1981)

III. Stress and the hypothalamic-pituitary-interrenal (HPI) axis

The HPI axis is part of the fish's endocrine system and consists of a hierarchy of hormonal pathways (Fig. 1). The release of the hormone corticotropin-releasing factor (CRF), from certain neurosecretory cells in a specialized region of the ventral part of the brain (the hypothalamus), stimulates a group of cells in the pituitary gland to secrete another hormone, adrenocorticotropin (ACTH), into the blood stream. ACTH in turn stimulates certain cells in the anterior region of the fish's kidney (the interrenal tissue) to secrete one or more steroid hormones, the corticosteroids, into the blood. In salmonid fish, cortisol is the major corticosteroid (Idler & Truscott, 1972). The activity of this axis can be controlled and modified by feedback loops acting on the hypothalamus and/or the pituitary and by several hormones from other components of the fish's endocrine system. For further details of the HPI axis in fish the reader is referrend to Donaldson (1981).

As indicated in the previous section, the HPI axis of teleost fish is activated in response to almost all forms of environmental stress, with a resultant elevation of blood corticosteroid levels. This forms part of a complex reaction, the so-called «fight or flight» response, which switches the metabolism of the fish from an anabolic state (the uptake and storage of energy) to a catabolic state (the breakdown of body reserves). Catecholamines, such as adrenaline and nor-adrenaline, also play a vital role in this aspect of the stress response (see Mazeaud & Mazeaud, 1981). Under natural conditions the fish utilizes these changes in its physiological state to avoid or overcome the immediate threat. The precise role(s) of cortisol in the stress response is still the subject of much speculation although some evidence suggests that, as in mammals, it has the ability to stimulate gluconeogenesis (the production of glucose or glycogen fron non-carbohydrate sources, usually protein) (Storer, 1967; Chan & Woo, 1978; Lidman et al., 1979; Leach & Taylor, 1982). Cortisol might, therefore, attenuate the catecholamine-induced breakdown of carbohydrate reserves and, ultimately promote the recovery of carbohydrate reserves in the liver and muscle. The lipolytic activity of cortisol (Lidman et al., 1979; Dave et al., 1979; Sheridan, 1986) may also be an important factor in the mobilization of energy reserves in stressed fish. In addition, several lines of evidence indicate that cortisol has an osmoregulatory role in fish (Balm, 1986; see also Eddy, 1981 and Chester Jones et al., 1969 for earlier references). Consequently, the hormone may accelerate the re-establishment of osmotic and ionic equilibrium during the recovery phase, following stress. However, a great deal more research is needed if we are to fully understand the adaptive significance of elevated blood cortisol levels during periods of environmental stress, particularly in view of the potentially damaging effects of this hormone on the fish's defence systems (see sections IV and V).

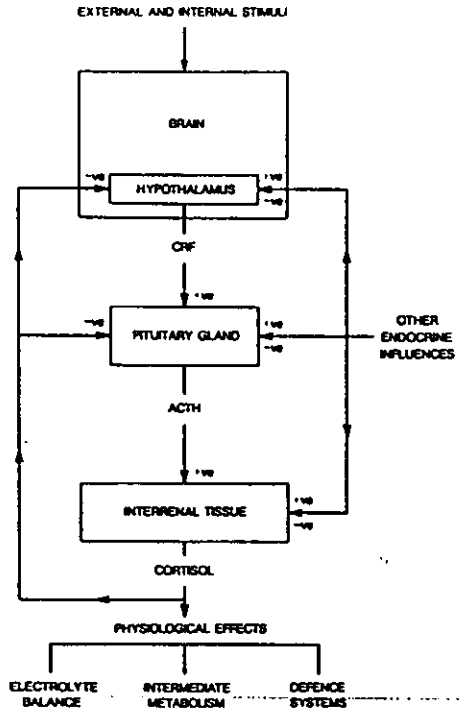


Fig. 1. A simplified, schematic diagram of the hypothalamic-pituitary-interrenal (HPI) axis in salmonid fish.

When a fish is subjected to an acute stress (i.e. a situation in which the stress is removed before physiological compensation has been achieved) blood ACTH levels are generally elevated within 2 minutes of the onset of stress (Sumpter et al., 1986) and cortisol levels rise during the next 5—10 minutes (Sumpter et al., 1985). In most cases, peak cortisol levels are attained within a few hours and this is followed by a recovery to basal levels within 12—24 hours (Fig. 2). Both the magnitude and rate of change of blood cortisol

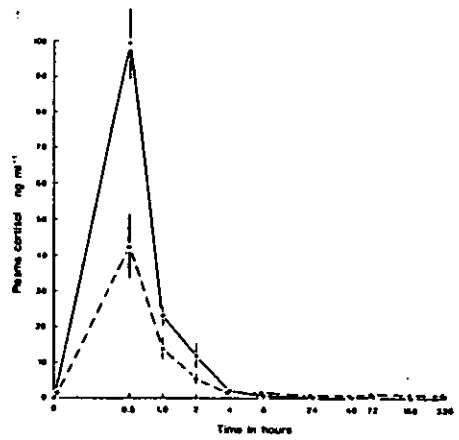


Fig. 2. Changes in the blood cortisol levels of brown trout (continuous line) and rainbow trout (broken line) subjected to an acute emersion stress (30 sec exposure of the fish to air at 0 h). Values are arithmetic means ± SEM (n = 12).

levels in stressed salmonid fish are influenced not only by the nature and severity of the stress but also by the species and strain of fish (Davis & Parker, 1986; A.D. Pickering and T.G. Pottinger, unpublished) and by the environmental temperature (Sumpter et al., 1985; Barton & Schreck, 1987). The cortisol response has been used as a quantitative index of the degree of stress experienced by the fish although its reliability in this context is not absolute. For example, Schreck & Lorz (1978) failed to observe any elevation of corticosteroid levels in rainbow trout exposed to a lethal dose of cadmium although James & Wigham (1986) were unable to verify this result. In general, however, cortisol levels are elevated in response to most forms of stress, including those highlighted in the previous section as predisposing factors for fish diseases. Table I lists some of the stresses that have been shown to stimulate the HPI axis in salmonid fish.

Frequently, environmental stresses occur in combination rather than as isolated factors and under such circumstances the magnitude and duration of the cortisol response are less easy to predict. Barton et al. (1985) reported that the response of rainbow trout to acute handling stress was increased in fish which had been previously exposed to low pH whereas Pickering & Pottinger (1987a) found that poor water quality reduced the cortisol response of both brown trout and rainbow trout to handling and confinement. Schreck (1981) argues convincingly that the cortisol response to stress is largely dependent upon the fish's sense of 'awareness', a case that is supported by the induction of anaesthesia in salmonid fish without any elevation of plasma cortisol (Strange & Schreck, 1978; A.D. Pickering and T.G. Pottinger, unpublished). However, cortisol levels are elevated soon after the fish recover consciousness. A reduction or lack of 'awareness' might account for the absence of a cortisol response to cadmium (Schreck & Lorz, 1978) and for the suppressive effect of poor water quality on the response to handling and confinement (Pickering & Pottinger, 1987a).

When a fish is subjected to a chronic stress (i.e. a continuous form of stress from which there is no escape) the HPI axis is initially stimulated, with a resultant elevation of blood cortisol levels. Ultimately, however, the fish must acclimate (albeit at a reduced level of performance) if they are to survive. Severe crowding and/or reduced water quality are common examples of chronic stress in the aquaculture industry. Under such circumstances, cortisol levels may be elevated for several days but 'ideal' compensation is eventually achieved with plasma corticosteroids returning to basal levels (Schreck, 1981). Picering & Stewart (1984) have shown that cortisol levels may be elevated in chronically-crowded brown trout for as long as 25 days before acclimation occurs although cortisol elevation for a period of 7—10 days is more usual (Schreck, 1981; Pickering & Pottinger, 1987b). A change in the type of aquaculture facility can also constitute an important form of chronic environmental stress to salmonid fish. Simpson (1975/76) found that blood cortisol levels were still significantly elevated in trout 10 days after their transfer from raceways to circular rearing tanks. Thus, a novel environment is sufficient to cause a prolonged stimulation of the HPI axis in salmonid fish.

It has been known for many years that corticosteroids can act as potent anti-inflammatory and immunosuppressive drugs in mammals (see Parrillo & Fauci, 1979; Munch et al., 1984). In view of the fact that most forms of environmental stress not only stimulate the secretion of corticosteroids in fish but also increase the susceptibility of fish to disease, it is reasonable to propose that corticosteroids can limit the ability of stressed fish to resist pathogenic challenge. The next section of this paper examines the evidence for such debilitating effects of corticosteroids on the defence systems of teleost fish.

IV. Stress, cortisol and the defence systems of fish

The defence systems of fish include tissue repair, inflammation, phagocytosis and a multitude of specific and non-specific responses mediated by the lymphoid system (see Ellis, 1981). Most of the experimental studies linking stress with particular components of the defence systems in fish have concentrated on specific immune responses, both cell-mediated and humoral. Heavy metal pollution has been repeatedly shown to suppress the immune response of fish to pathogenic bacteria and viruses as well as to human and sheep red blood cells (Sarot & Perlmutter, 1976; Roales & Perlmutter, 1977; Stevens, 1977; Sugatt, 1980; O'Neill, 1981). Physical stresses such as handling, transport, crowding and captivity have also been shown to be markedly immunosuppressive (Perlmutter et al., 1973; Miller & Tripp, 1982; Eltsaesseer & Clem, 1986) although in none of these studies was the physiological mechanism behind stress-induced immunosuppression investigated. Recently, however, evidence has begun to accumulate which suggests that corticosteroids play a central role in this type of immunosuppression. Experimental administration of ACTH or corticosteroids suppresses the number of circulating

lymphocytes, cells responsible for the fish's immune response, in the blood of salmonid fish (McLeay, 1973a, b; Pickering, 1984) and depletes the lymphocyte populations from the kidney, spleen and thymus (Chilmonczyk, 1982; Ghoneum et al., 1986). Oral administration of a physiological dose of cortisol to brown trout elicited a transient suppression of the number of circulating lymphocytes over a time course very similar to that observed when the fish were stressed by handling (Fig. 3). Thus, the lymphocytopenia

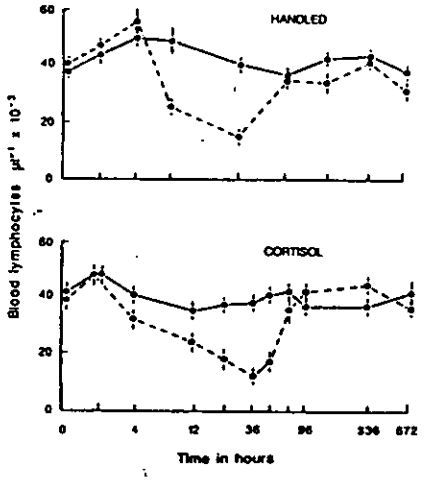


Fig. 3. Changes in the number of circulating lymphocytes in the blood of the brown trout following a single incidence of handling (upper graph) or the oral administration of a physiological dose of cortisol (lower graph). The broken line represents treated fish, the continuous line represents unstressed control fish. Values are arithmetic means ± SEM (n = 12).

observed in acutely stressed fish is almost certainly mediated by changes in blood cortisol levels. Under conditions of chronic stress, however, the situation is less clear. Pickering & Pottinger (1987b) noted that the lymphocytopenia in both brown trout and rainbow trout subjected to a chronic crowding stress outlasted the period of elevated blood cortisol levels. Similarly, Klinger et al. (1983) found a marked leucopenia in chronically stressed channel catfish even though blood cortisol had acclimated to the levels found in unstressed fish. This raises the possibility that factors other than the HPI axis can also suppress lymphoid activity.

It is becoming generally accepted that lymphocyte heterogeneity exists in teleost fish although there is still a great deal of discussion concerning the identity and origin of T-like and B-like cells (Clem et al., 1977; Miller et al., 1985). Thus, gross changes of total lymphocyte numbers in response to corticosteroids must be considered to be a rather crude (albeit useful) estimate of the effect of these hormones on the immune system of fish. However Anderson et al (19821), working with rainbow trout, and Maule et al. (1987), working with coho salmon, have both demonstrated a corticosteroid-induced suppression of the production of specific antibody-secreting lymphocytes in fish injected with V. anguillarum antigen. Antibody-secreting cells were identified by plaque formation when incubated with sheep red blood cells that had been coated with Vibrio antigen. A specific suppression of the immune system was also observed in the striped bass, Morone saxatilis, by Wechsler et al. (1986) who showed that the synthetic corticosteroid, triamcinolone acetonide, delayed the appearance and reduced the levels of circulating virusneutralizing antibodies to infectious pancreatic necrosis virus. Grimm (1985) reported that physiological levels of cortisol were capable of suppressing the mitogenic response of plaice lymphocyte populations. Thus, corticosteroids can not only cause a general lymphocytopenia in teleost fish but can also suppress specific immune responses to both bacterial and virat antigens.

Phagocytosis, the cellular ingestion and digestion of particulate matter, is also an extremely important component of the fish's defence system (MacArthur & Fletcher, 1985). As in mammals the phagocytic cells of fish fall into two categories, the granulocytes and the mononuclear phagocytes (the latter being represented by tissue macrophages and circulating monocytes). Phagocytosis involves the processes of recognition of the foreign material, attachment, engulfment, degranulation, killing (in the case of bacteria) and digestion. All of this is sometimes preceded by a chemically-guided movement of mobile phagocytes towards their target (chemotaxis). Recognition of the target is usually enhanced by the process of opsonisation (coating of the particle with various proteins, predominantly antibodies). Thus, there is a strong interrelation between the production of specific antibodies by cells of the lymphoid system and the phagocytosis of particulate matter by the macrophages of the reticulo-endothelial system.

Weeks et al. (1986) have shown that the chemotactic efficiency (to Escherichia coli) of macrophages isolated from the kidneys of two species of estuarine fish was reduced in fish from polluted water and that this effect could be reversed by holding the fish in clean water for 3 weeks. Using an in vivo approach, MacArthur et al. (1984) demonstrated that cortisol injection caused a significant reduction in the extent of inflammatory cell infiltration (predominantly neutrophils and macrophages) in the peritoneum of the plaice, Pleuronectes platessa, challenged by glycogen or Vibrio alginolyticus. This study was supported by an in vitro demonstration of cortisol-induced suppression of migratory activity in inflammatory neutrophils derived from the peritoneum of glycogen-injected plaice (Fletcher, 1986). Much earlier, Weinreb (1958) had reported a suppressive effect of corticosteroids on chemical and surgically-induced inflammation in the rainbow trout.

Once the target has been located, its attachment to the phagocytic cell membrane initiates a series of metabolic reactions, the respiratory burst. This results in the formation of a number of highly reactive microbicidal oxygen radicals which, in conjunction with lysosomal enzymes, are responsible for bacterial killing by phagocytic cells. The respiratory burst can be measured by monitoring phagocytic cell chemoluminescence during the process of phagocytosis. Stave & Roberson

(1985) found that hydrocortisone acetate reduced the normal ability of phagocytes from the kidney of the striped bass to generate a chemiluminescent response when exposed to bacteria although it must be pointed out that the concentrations of corticosteroids used in this study were much higher than the levels normally found in teleost fish. Nevertheless, when all the data are considered, it seems likely that elevated cortisol levels, as a result of environmental stress, suppress phagocytosis in fish by acting on at least two different mechanisms (chemotaxis and intracellular killing).

The effects of stress and corticosteroids on the wound healing process in fish is a virtually unexplored subject (see Fletcher, 1981) and it is necessary, therefore, to rely heavily on our knowledge of mammalian systems. Morgan & Roberts (1976), in a study on the histopathology of salmon tagging, provide some evidence that stress can have a deleterious effect on the wound healing process. They found that Atlantic salmon parr, stressed by severe exercise at high temperature, developed extensive necrotic lesions at the site of tag insertion. This would seem to be an effect on would healing per se because there were no signs of secondary microbial infection. The most immediate defence to any physical injury involving damage to the vascular system is the clotting process. This is a complex cascade of events which ultimately results in the conversion of a soluble plasma protein, fibrinogen, into an insoluble protein, fibrin, which in turn forms a fibrous network at the site of injury. The clot is completed by the adherence and trapping of some of the cellular components of the blood. Thrombocytes are intimately involved by virtue of their adhesion to the forming clot and by the release of factors which stimulate the enzymatic process of fibrin formation. In fish the clotting process may occur rapidly (within 3 minutes) but the rate of clotting is directly proportional to the concentration of thrombocytes in the blood (Srivastava, 1969).

In previous studies we have been unable to demonstrate any effect of acute stress or corticosteroid administration on the circulating thrombocyte population of brown trout (Pickering et al., 1982; Pickering, 1984). Moreover, McLeay and colleagues could not find any effect of chronic stress (exposure to pollutants) on the thrombocytes of juvenile coho salmon (McLeay 1973c; McLeay & Brown, 1974) and, despite showing that ACTH and dexamethasone (but not cortisol) could cause thrombocytopenia (McLeay, 1973a,b), were unable to correlate seasonal variation in thrombocyte numbers with adenocortical activity (McLeay, 1975). However, chronic crowding stress caused a marked and prolonged reduction in the circulating thrombocyte count of both brown trout and rainbow trout (Pickering & Pottinger, 1987b) thereby suggesting that this type of chronic stress might impair the clotting process in salmonid fish. If so, this differs from the response to acute stress because Casillas & Smith (1977) noted a threefold increase in thrombocyte numbers and a decrease in the clotting time in rainbow trout immediately after an acute stress, a response which appears to be of adaptive significance. Fibrinolysis, the process of clot dissolution, may also be influenced by environmental stress. Thus, Woodward et al. (1979) found that rapid decompression stress accelerated fibrinolysis in fingerling salmon, a process that could, under certain circumstances, antagonise the success of clot formation. In mammals, it is known that adrenal corticosteroids can enhance the rate of fibrinolytic activity (Keele & Neil, 1971) but nothing is known about the control of this process in fish. The somewhat conflicting evidence linking stress with the clotting process in fish emphasizes the need for further research in this area.

Clot formation, an immediate mechanism for reducing blood loss and sealing the wound, is followed by tissue repair. In cases of minor, superficial injury to fish there is a rapid migration of Malpighian cells over the damaged area which, in the plaice, is not accompanied by an immediate increase in epidermal mitotic activity (Bullock et al., 1978). However, in the case of more severe lesions, it is difficult to seee how tissue repair could be affected without some increase in the rate of mitosis of the damaged tissues. The immediate control of epidermal mitosis, by means of an antimitotic chemical messenger (epidermal chalone), would seem to be similar throughout the vertebrate series (Bullough et al., 1967). However, unlike the situation in mammals, little is known about the effects of circulating hormones on this system in fishes. In man, cortisol and related compounds in large doses interfere with collagen formation and wound-healing, but physiological or small therapeutic doses do not impair healing. When applied topically to the skin, corticosteroid derivates may even promote healing in many types of dermatitis by suppressing the inflammatory response. However in other mammals, it has been demonstrated that low concentrations of cortisol, although having little inherent antimitotic activity, can prolong the mitotic depression induced by chalone and adrenaline (Bullough & Lawrence, 1968). Thus, corticosteroids may have an indirect physiological role in the control of epidermal mitosis. As far as the author is aware, no data are available on the effects of corticosteroids on mitotic activity in the fish epidermis and one can only speculate on the possible effects of stress on wound healing. However, it is interesting that in another group of lower vertebrates, the amphibians, an inverse relationship has been found between epidermal mitotic rate and the level of plasma corticosteroids (Garcia-Arce & Mizell, 1972).

In summary, it has been shown in this section that elevated blood cortisol levels can compromise the defence of fish by suppressing immune responses, inhibiting inflammatory reactions and phagocytosis and, possibly, by retarding wound healing processes. To complete the evidence in favour of the hypothesis that the predisposition to disease of stressed fish is largely mediated by elevated blood corticosteroids, it ought to be possible to increase the incidence of disease in fish populations by corticosteroid treatment. The next section examines the effects of the administration of exogenous corticosteroids on the mortality rate and incidence of disease in otherwise unstressed fish.

V. The effect of corticosteroid administration on the incidence of disease

Work in this area is extremely limited and some of the earlier studies must be criticized on the grounds that either the degree of corticosteroid elevation in the blood was not measured or that the doses were obvio-

usly pharmacological rather than physiological. Perhaps the most rigorous of the early studies is that of Robertson et al. (1963). In their study, rainbow trout were given intraperitoneal implants consisting of a mixture of cortisol and cholesterol and it was shown that a prolonged elevation of plasma 17-hydrocorticosteroids (300—2,000 ng ml^{-1}) increased the mortality rate. Fish treated with these high doses lost weight rapidly and developed ichtyopthiriasis (white spot disease) and/or fungal infection. Roth (1972) confirmed the predisposing effect of cortisol on fungal infections (in this case in the white sucker, **Catastomus commersoni commersoni**) but from the predicted levels of corticosteroids in the blood (≈20,000 ng ml^{-1}) this investigation can only be described as pharmacological. McCarthy (1977) used a combination of the synthetic steroid prednisolone acetate and heat stress to identify carrier fish in furunculosis studies although no measurements of plasma corticosteroid levels were made.

Following a series of studies on the brown trout to define the physiological range for plasma cortisol levels in unstressed fish, acutely stressed fish and chronically stressed fish, we initiated an investigation into the effects of chronic elevation of blood cortisol levels, within the physiological range for the species, on the incidence of those diseases common throughout the aquaculture industry (Pickering & Duston, 1983). Fish were given exogenous cortisol either orally (by incorporating the hormone into the normal diet of the fish) or by slow-release intraperitoneal implants and were then exposed naturally to pathogens in the incoming water (untreated lake water). In both cases, cortisol treatment significantly increased the incidence of fungal infection (**Saprolegnia diclina** Type 1 — Pickering & Willoughby, 1982) bacterial fin-rot (Fig. 4) and furunculosis. It was concluded that chronic elevation of blood cortisol levels within the range 30-130 ng ml^{-1}) was sufficient to predispose the fish to disease. Such plasma levels of the hormone are well within the physiological range for salmonid fish (i.e. levels which can be produced and maintained by the activity of the fish's (HPI axis). For example, Brown et al. (1984) found that the blood cortisol levels of rainbow trout

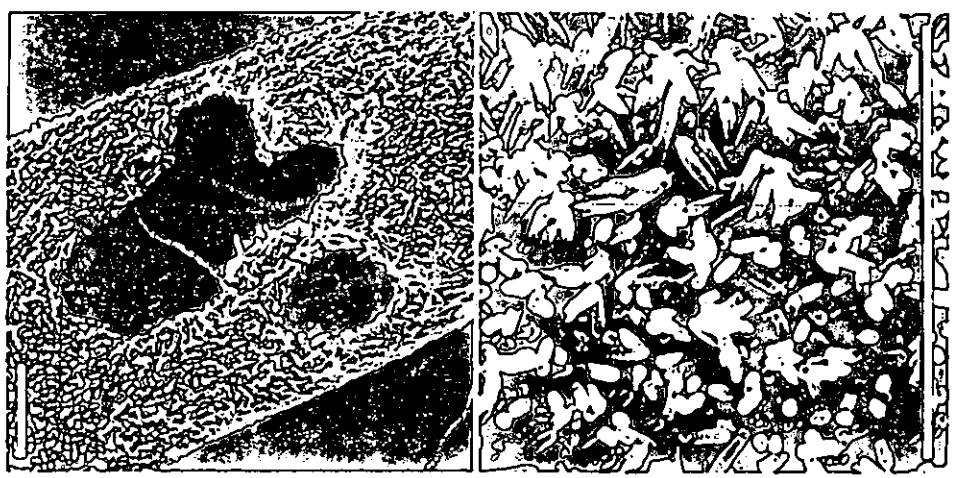


Fig. 4. Scanning electron micrograph of the cartilaginous fin-ray of a brown trout with severe fin rot. Note the damage caused by the large number of adherent bacteria (bar line -10 um). On right, high power view of the mat of rod-shaped bacteria colonizing the fin-ray (bar line -2 um).

exposed to low pH were still ≈ 80 ng ml^{-1}) 3 weeks after the onset of acidification. Indeed, in fungal-infected brown trout blood cortisol levels may exceed 600 ng ml^{-1} (Pickering & Charistie, 1981). Since our initial study on the brown trout, Woo et al. (1987) have confirmed that experimentally-induced elevations of blood cortisol levels of a similar magnitude can predispose rainbow trout to disease. In their study, cortisol implantation increased the mortality of fish experimentally infected with the haemoflagellate, **Cryptobia salmositica**, and they were also able to show that the steroid treatment significantly depressed the circulating antibody titres.

Non-salmonid fish also seem to be sensitive to the effects of corticosteroid treatment. Using intraperitoneal injections of the synthetic corticosteroid, triamcinolone acetonide, Houghton & Matthews (1986) were able to overcome the defence systems of juvenile mirror carp, **Cyprinus carpio**, immunized against ichthyophthiriasis. Steroid-treated, immunized fish showed a marked increase in the mortality rate after exposure to a potentially lethal dose of ichthyophthirius theronts when compared with saline-injected, immunized control fish. Lack of information about the relative potency of triamcinolone acetonide in this respect makes it difficult, however, to comment on the relevance of the dose of steroid used.

In a further study on the brown trout (Pickering & Pottinger, 1985) we were able to show that the chronic elevation of plasma cortisol levels from a base-line of 1-4 ng ml^{-1}) to only 10 ng ml^{-1}) was sufficient to increase the susceptibility of the fish to a range of

naturally-occurring pathogens. Interestingly in this study, as in that of Woo et al. (1987), the dose of cortisol used was not sufficient to depress the concentration of circulating lymphocytes even though it could still predispose some of the fish within the population to disease. Fig. 5 presents the results of several

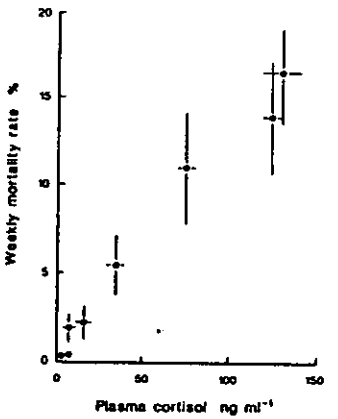


Fig. 5. Effect of chronic elevation of plasma cortisol levels by means of slow-release implants on the mortality rate of brown trout. Mortality rates and cortisol levels were monitored for 5-10 weeks and causes of death included Saprolegnia infection, furunculosis and fin-rot. Values are weekly means ± SEM.

experiments in which we have chronically elevated the blood cortisol levels of brown trout by means of slow-release intraperitoneal implants and shows that the mortality rate (as a result of disease) is positively correlated with the mean blood cortisol level. It is clear from this that, should a group of fish not acclimate to a chronic aquacultural stress, prolonged elevation of blood cortisol levels will inevitably increase the incidence disease within the population. Furthermore, even though fish populations do normally acclimate to chronic environmental stresses (see Section III), the period of elevated blood corticosteroids prior to acclimation is a period of increased vulnerability. Thus, chronic cortisol elevation in stressed salmonid fish could be used as a potential indicator of disease susceptibility.

VI. Other factors capable of predisposing fish to disease

In this penultimate section, two other factors which have a marked influence on the ability of fish to resist pathogenic challenge will be briefly considered. These are the process of sexual maturation in salmonid fish and the state of nutrition.

a) Sexual Maturation

As fish farmers are all too aware, sexually mature salmonid fish are much more susceptible than immature fish to disease, particularly to fungal and parasitic infestations of the integument. This occurs in both sexes although the problem is usually greatest in mature male fish. We have shown that sexually mature male brown trout are more frequently or more severely infested by Ichthyophthirius, Scyphidia, Gyrodactylus and Saprolegnia than are immature fish or sexually mature female fish (Pickering & Christie, 1980). Similarly, Paling (1965) observed a greater incidence of infestation by the monogenean, Discocotyle sagittata, in sexually mature males from a natural population of brown trout and Robertson (1979) has shown that sexual maturation in farmed rainbow trout is associated with an increase in susceptiblity to the flagellate, Ichthybodo (Fig. 6).

Fig. 6. Upper — severe infestation of the epidermis of the brown trout by the flagellate protozoan, Ichthyobodo (bar line 100 um) Lower — high power showing the attachment of two parasites to adjacent epidermal cells (bar line 10 um).

The physiological mechanisms behind this response appear to be twofold. Firstly, the later stages of sexual maturation are associated in both sexes with changes which parallel those exhibited by stressed fish. Thus, there is a prolonged elevation of blood cortisol levels (Pickering & Christie, 1981; Pickering & Pottinger, 1986) coupled with a pronounced lymphocytopenia (Pickering, 1986). From the preceding evidence it would seem that these changes alone are sufficient to compromise both specific and non-specific components of the fish's defence system. Furthermore, Katz & Southward (1950) demonstrated that salmon undergoing spawning stress had considerably lengthened blood-clotting times when compared to prespawners, again indicative of an impaired defence system.

Secondly, sexual maturation in the male fish is accompanied by an androgen-dependent loss of mucus-secreting goblet cells (Pottinger & Pickering, 1985a,b). It is suggested that this exacerbates the existing problems of cortisol-induced changes in disease susceptibility and accounts for much of the observed difference in vulnerability to skin infections between the two sexes. In practical terms, some of these problems of disease susceptiblity in sexually mature trout have been avoided by the increased use of all female or sterile triploid stocks of fish.

b) Nutrition

It has been known for many years that dietary deficiencies can lead to specific types of nutritional disease in fish (Snieszko, 1972). However, evidence is now accumulating which indicates that dietary problems can also lead to an increased susceptibility to infectious disease. Durve & Lovell(1982) found that levels of ascorbic acid (vitamin C) higher than the minimum dietary requirement for normal growth provided an increased resistance in channel catfish fingerlings to the pathogenic bacterium, Edwardsiella tarda. This effect is distinct from the well-documented and specific consequence of ascorbic acid deficiency on wound healing in fish (Ashley et al., 1975). Li & Lovell (1985) extended this work and demonstrated that dietary vitamin C can influence non-specific defence mechanisms, including an effect on the rate of phagocytosis. Blazer & Wolke (1984a) showed that the resident peritoneal macrophages of rainbow trout fed on a diet dificient in a-tocopherol (vitamin E) exhibited significantly decreased phagocytosis of latex beads. In another study (Blazezr & Wolke, 1984b) they found that two groups of rainbow trout fed different diets (one commercial, one laboratory-prepared), although suffering no mortalities and all apparently healthy, had marked differences in the magnitude of their immune response both to sheep red blood cells and to the bacterial pathogen Yersinia ruckeri. Perhaps more topically, Poppe et al. (1986) suggest that Haemorrhagic Syndrome (HS) or 'Hitra Disease', which has been the most important and widespread disease in farmed Atlantic salmon in Norway, is a multifactorial disease complex in which nutritional disorders are an important component.

It seems unlikely that the immunosuppresive effects of nutritional deficiencies are mediated via the HPI axis because all the evidence to date indicates that prolonged starvation (with, presumably, attendant nutritional deficiencies) does not elevate blood corticosteroid levels in teleost fish (Milne et al., 1979; Brown et al., 1984; A.D. Pickering and T.G. Pottinger, unpublished). It is apparent that the association between dietary composition and resistance to infectious disease in an important area for future research.

VII. Summary and conclusions

It has been established that most forms of environmental stress can predispose fish to infectious diseases. Such stresses also activate the hypothalamic-pituitary-interrenal (HPI) axis with a resultant increase in blood corticosteroids (predominantly cortisol in salmonid fish). In the case of acute stresses, blood cortisol levels are temporarily elevated but return to basal levels within 24 hours. This forms part of a marked physiological change which allows the fish to use energy reserves not normally available to it, in its attempts to avoid or overcome the stress. With chronic stresses, however, plasma cortisol levels may be elevated for many days (or even weeks) before acclimation ultimately occurs. Corticosteroids have a debilitating effect on the defence systems of fish by suppressing both cell-mediated and humoral immune responses, by inhibiting the chemotactic response and phagocytic ability of macrophages and, possibly, by retarding the processes of tissue repair. Chronic administration of physiological doses of corticosteroids to otherwise unstressed fish result in a dose-dependent increase in susceptiblity to such aquacultural diseases as Saprolegnia infection, bacterial fin-rot and furunculosis.

From this it concluded that activation of the HPI axis in fish exposed to acute environmental stresses in an adaptive response, the benefits of which (in terms of energy mobilization and, perhaps, osmoregulation) normally outweigh any temporary suppression of the fish's defence systems. Under conditions of chronic stress, however, the debilitating effects of prolonged cortisol elevation result in a marked predisposition to disease. In such cases the fish must ultimately acclimate to the new environmental conditions by reducing the level of circulating cortisol if it is to servive. Sexually mature salmonid fish have several features in common with chronically stressed fish, including prolonged cortisol elevation and lymphocytopenia. This results in an increase in the incidence of disease in both sexes during the spawning season but the situation in the males is exacerbated by androgen-dependant changes in the integument. New evidence is also beginning to emphasize the importance of diet in determining the ability of fish to resist pathogenic challenge.

The main conclusion from this review is that most of the debilitating effects of environmental stress on the fish's defence systems are mediated by excessive or prolonged stimulation of the HPI axis. It follows, therefore, that methods of reducing blood cortisol levels during periods of unavoidable stress ought to ameliorate some of the worst aspects of stress-induced disease. We are adopting two basic approaches to this problem. Firstly, we are investigating the possibility of specifically blocking the cortisol receptors in the target tissues, thereby inhibiting the effects of elevated blood corticosteroid levels. If successful, this approach (by means of drug-treatment) is likely to result in the development of a powerful experimental tool to investigate other aspects of the corticosteroid stress response in fish but it is unlikely to result in any practical aquacultural application. Secondly, the genetic basis of the sensitivity of the HPI axis to environmental stress will be studied. The ultimate aim of this work would be to selectively breed fish with a high threshold to environmental stress (i.e. to accelerate the rate of domestication) in an attempt to avoid, or reduce, the problems of stress-induced predisposition to disease. Similar approaches have already been successful in work with poultry (Edens & Siegel, 1975). In this connection it is important to acknowledge the work of T. Refstie and colleagues on salmonid fish who have already demonstrated significant differences in the stress response between species, between sires and

between dams (Refstie, 1982, 1986). Much more work is now needed in this area if we are to fully benefit from the research effort already expended.

Acknowledgements The author is grateful to the organisers of Aqua Nor 87 for the invitation to present this paper, to T.G. Pottinger (FBA) for his critical comments on the manuscript, to T.I. Furnass (FBA) for the artwork, and to NERC and MAFF for financial support for much of the original research on which this article is based.

References

Anderson, D.P., Roberson, B.S. & Dixon, O.W. (1982) Immuno suppression induced by a corticosteroid or an alkylating agent in rainbow trout (Salmo gairdneri) administered a Yersinia ruckeri bacterin. Dev. Comp. Immunol. 2(Suppl.), 197—204.

Ashley, L.M., Halver, J.E. & Smith, R.R. (1975) Ascorbic acid deficiency in rainbow trout and coho salmon and effects on wound healing. In The Pathology of Fishes (W.E. Ribelin & G. Migaki, eds), pp. 769—786. The University og Wisconsin Press: Madison, Wisconsin.

Balm, P.H.M. (1986). Osmoregulation in teleosts by cortisol and prolactin. Ph. D. Thesis, Department of Zoology, University of Nijmegen, The Netherlands. 176pp.

Barton, B.A., Peter, R.E. & Paulencu, C.R. (1980). Plasma cortisol levels of fingerling rainbow trout (Salmo gairdneri) at rest, and subjected to handling, confinement, transport, and stocking. Can. J. Fish. Aquat. Sci. 37, 805—811.

Barton, B.A. & Schreck, C.B. (1987) Influence of acclimation temperature on interrenal and carbohydrate stress responses in juvenile chinook salmon (Oncorhynchus tshawytscha). Aquaculture 62, (in press).

Barton, B.A. Weiner, G.S. & Schreck, C.B. (1985) Effect of prior acid exposure on physiological responses of juvenile rainbow trout (Salmo gairdneri) to acute handling stress. Can. J. Fish Aquat. Sci. 42, 710—717.

Blazer, V.S. & Wolke, R.E. (1984a). The effects of a-tocopherol on the immune response and non-specific resistance factors of rainbow trout (Salmo gairdneri Richardson). Aquaculture 37, 1—11.

Blazer, V.S. & Wolke, R.E. (1984a). Effect of diet on the immune response of rainbow trout (Salmo gairdneri). Can. J. Fish. Aquat. Sci. 41, 1244—1247.

Boddingius, J. (1976). The influence of social rank on adenohyphysial cell activity in Salmo irideus. Cell Tiss. Res. 170, 383—414.

Brown, E.R., Koch, E., Sinclair, T.F., Spitzer, R & Callaghan, O. (1979). Water pollution and diseases in fish (an epizootiologic survey). J. Env. Pathol. Toxicol. 2, 917—925.

Brown, S.B., Eales, J.G., Evans, R.E. & Hara, T.J. (1984). Interrenal, thyroidal, and carbohydrate response of rainbow trout (Salmo gairdneri) to environmental acidification. Can. J. Fish. Aquat. Sci. 41, 36—45.

Bullock, A.M., Marks, R. & Roberts, R.J. (1978). The cell kinetics of teleost fish epidermis: Epidermal mitotic activity in relation to wound healing at varying temperatures in plaice (Pleuronectes platessa). J. Zool., Lond. 185, 197—204.

Bullough, W.S. & Laurence, E.B. (1968) The role of glucocorticoid hormones in the control of epidermal mitosis. Cell Tiss. Kinet. 1, 5—10.

Bullough, W.S., Laurence, E.B., Iversen, O.H. & Elgjo, K. (1967). The vertebrate epidermal chalone. Nature 214, 578—580.

Casillas, E. & Smith, L.S. (1977). Effect of stress on blood coagulation and hematology in rainbow trout (Salmo gairdneri). J. Fish Biol. 10, 481—491.

Chan, D.K.O., & Woo, N.Y.S. (1978) Effect of cortisol on the metabolism of the eel, Anguilla japonica. Gen. Comp. Endocrinol. 35, 205—215.

Chester-Jones, I., Chan, D.K.O., Henderson, I.W. & Ball, J.N. (1969). The adrenocortical steroids, adrenocorticotropin and the corpuscles of Stannius. In Fish Physiology, Vol. II (W.S. Hoar & D.J. Randall, eds), pp. 321—376. Academic Press: New York/London.

Chilmonczyk, S. (1982) Rainbow trout lymphoid organs: cellular effects of corticosteroids and anti-thymocyte serum. Dev. Comp. Immunol. 6, 217-280.

Clem, L.W., McLean, W.E., Shankey, V.T. & Cuchens, M.A. (1977). Phylogeny of lymphocyte heterogeneity. I Membrane immunoglobulins of teleost lymphocytes. Dev. Comp. Immunol. 1, 105—118.

Dave, G., Johansson-Sjöbeck, M.-L., Larsson, A., Lewander, K. & Lidman, U. (1979). Effects of cortisol on the fatty acid composition of the total blood plasma lipids in the European eel, Anguilla anguilla L. Comp. Biochem. Physiol. 64A, 37—40.

Davis, K.B. & Parker, N.C. (1986). Plasma corticosteroid stress response of fourteen species of warmwater fish to transportation. Trans. Am. Fish. Soc. 115, 495—499.

Donaldson, E.M., (1981) The pituitary-interrenal axis as an indicator of stress in fish. In Stress and Fish (A.D. Pickering, ed.), pp. 11—47. Academic Press: London/New York.

Donaldson, E.M. & Dye, H.M. (1975). Corticosteroid concentrations in sockeye salmon (Oncorhynchus nerka) exposed to low concentrations of copper. J. Fish. Res. Bd. Can. 32, 533—539.

Durve, V.S. & Lovell, R.T. (1982). Vitamin C and disease resistance in channel catfish (Ictalurus punctatus). Can. J. Fish. Aquat. Sci. 39, 948—951.

Eddy, F.B. (1981). Effects of stress on osmotic and ionic regulation in fish. In Stress and Fish (A.D. Pickering, ed.), pp. 77—102. Academic Press: London/New York.

Edens, F.W & Siegel, H.S. (1975). Adrenal responses in high and low ACTH response lines of chickens during acute heat stress. Gen. Comp. Endocrinol. 25, 64—73.

Eiras, J.C. & Saraiva, A. (1986). Nutritional factors as the cause of an outbreak of Pseudomonas fluorescens in rainbow trout, Salmo gairdneri Richardson. Publ. Inst. Zool., Fac. Sci. Porto. 192, 1—8.

Ejike, C. & Schreck, C.B. (1980) Stress and social hierarchy rank in coho salmon. Trans. Am. Fish Soc. 109, 423—426.

Ellis, A.E. (1981). Stress and the modulation of defence mechanisms in fish. In Stress and Fish (A.D. Pickering, ed.), pp. 147—169. Academic Press: London/New York.

Ellsaesser, C.F. & Clem, L.W. (1986). Haematological and immunological changes in channel catfish stressed by handling and transport. J. Fish Biol. 28, 511—521.

Ewing, M.S., Ewing, S.A. & Zimmer, M.A. (1982). Sublethal copper stress and susceptibility of channel catfish to experimental infections with Ichthyophthirius multifiliis. Bull. Environ. Contam. Toxicol. 28, 674—681.

Fagerlund, U.H.M. (1967) Plasma cortisol concentration in relation to stress in adult sockeye salmon during the freshwater stage of their life cycle. Gen. Comp. Endocrinol. 8, 197—207.

Fagerlund, U.H.M., McBride, J.R. & Stone, E.T. (1981). Stress-related effects of hatchery rearing density on coho salmon. Trans. Am. Fish. Soc. 110, 644—649.

Fletcher, T.C. (1981). Non-antibody molecules and the defence mechanisms of fish. In Stress and Fish (A.D. Pickering, ed.), pp. 171—183. Academic Press: London/New York.

Fletcher, T.C. (1986). Modulation of non-specific host defenses in fish. Vet. Immunol. Immunopathol. 12, 59—67.

Garcia-Arec, H. & Mizell, S. (1972). Mitotic activity in dorsal epidermis of Rana pipiens. Comp. Biochem. Physiol. 42A, 501—509.

Ghoneum, M.H., Egami, N., Jiri, K. & Cooper, E.L. (1986). Effect of corticosteroids on the thymus of the fish Oryzius latipes. Dev. Comp. Immunol. 10, 35—45.

Grant, B.F. & Mehrle, P.M. (1973). Endrin toxicosis in rainbow trout (Salmo gairdneri). J. Fish. Res. Bd Can. 30, 31—40.

Grimm, A.S. (1985) Suppression by cortisol of the mitogen-induced proliferation of peripheral blood leucocytes from plaice, Pleuronectes platessa L. In Fish Immunology (M.J. Manning & M.F. Tatner, eds), pp. 263—271. Academic Press: London/New York.

Hanson, L.A. & Grizzle, J.M. (1985). Nitrite-induced predisposition of channel catfish to bacterial diseases. Progve Fish-Cult. 47, 98—101.

Hetrick, F.M., Knittel, M.D. & Fryer, J.L. (1979). Increased suscentibility of rainbow trout to infectious haematopoetic necrosis virus after exposure to copper. Appl. Env. Microbiol. 37, 198—201.

Hill, C.W. & Fromm, P.O. (1968). Response of the interrenal gland of rainbow trout (Salmo gairdneri) to stress. Gen. Comp. Endocrinol. 11, 69—77.

Hosmer, M.J. (1980). Handling as a factor in mortality of trout with or without furunculosis. Progve Fish-Cult. 42, 157—159.

Houghton, G & Matthews, R.A. (1986). Immunosuppression of carp (Cyprinus carpio L.) to ichthyophthiriasis using the corticosteroid triamcinolone acetonide. Vet. Immunol. Immunopath. 12, 413—419.

Hunter, V.A., Knittel, M.D. & Fryer, J.L. (1980). Stress-induced transmission of Yersinia ruckeri infection from carriers to recipient steelhead trout Salmo gairdneri Richardson. J. Fish. Dis. 3, 467—472.

Idler, D.R. & Truscott, B. (1972). Corticosteroids in fish. In Steroids in Nonmammalian Vertebrates (D.R. Idler, ed.), pp. 126—252. Academic Press, London/New York.

James, V.A. & Wigham, T. (1986). The effects of cadmium on prolactin cell activity and plasma cortisol levels in the rainbow trout (Salmo gairdneri). Aquat. Toxicol. 8, 273—280.

Johansson, N. & Bergström, E. (1977). Transportation in tanks of two-year old salmon (Salmo salar L.) as a predisposing factor for infection with Aeromonas hydrophila. Laxforsk Inst. Meddn. 3, 1—12.

Katz, M. & Southward, M. (1950). The blood clotting time in spent silver salmon, Oncorhynchus kisutch. Copeia 2, 150.

Keele, C.A. & Neil, E. (1971). Sampson Wright's Applied Physiology 12th Edition. Oxford University Press: London. 576pp.

Klinger, H., Delventhal, H. & Hilge, V. (1983). Water quality and stocking density as stressors of channel catfish (Ictalurus punctatus Raf.). Aquaculture 30, 263—272.

Knittel, M.D. (1981). Susceptibility of steelhead trout Salmo gairdneri Richardson to redmouth infection Yersinia ruckeri following exposure to copper. J. Fish. Dis. 4, 33—40.

Leach, G.J. & Taylor, M.H. (1982). The effects of cortisol treatment on carbohydrate and protein metabolism in Fundulus heteroclitus. Gen. Comp. Endocrinol. 48, 76—83.

Li, Y. & Lovell, R.T. (1985). Elevated levels of dietary ascorbic acid increase immune responses in channel catfish. J. Nutr. 115, 123—131.

Lidman, U., Dave, G., Johansson-Sjöbeck, M.L., Larsson, A. & Lewander, K. (1979). Metabolic effects of cortisol in the European eel, Angullla anguilla (L.). Comp. Biochem. Physiol. 63A, 339—344.

MacArthur, J.I. & Fletcher, T.C. (1985). Phagocytosis in fish. In Fish Immunology (M.J. Manning & Tatner, eds), pp. 29—46. Academic Press: London/New York.

MacArthur, J.I., Fletcher, T.C., Pirie, B.J.S., Davidson, R.J.L. & Thompson, A.W. (1984). Peritoneal inflammatory cells in plaice, Pleuronectes platessa L.: Effects of stress and endotoxin. J. Fish Biol. 25, 69—81.

Maule, A.G., Schreck, C.B. & Kaattari, S.L. (1987). Changes in the immune system of coho salmon (Oncorhynchus kisutch) during the parr-to-smolt transformation and after implantation of cortisol. Can. J. Fish. Aquat. Sci. 44, 161—166.

Mazeaud, M.M. & Mazeaud, F. (1981) Adrenergic responses to stress in fish. In Stress and Fish (A.D. Pickering, ed.), pp 49—75. Academic Press: London/New York.

McCarthy, D.H. (1977). Some ecological aspects of the bacterial fish pathogen — Aeromonas salmonicida. Symp. Soc. Appl. Bact. 6, 299—324.

McLeay, D.J. (1973a). Effects of ACTH on the pituitary-interrenal axis and abundance of white blood cell-types in juvenile coho salmon, Oncorhynchus kisutch. Gen. Comp. Endocrinol. 21, 431—440.

McLeay, D.J. (1973b). Effects of cortisol and dexamethasone on the pituitary-interrenal axis and abundance of white blood cell types in juvenile coho salmon, Oncorhynchus kisutch. Gen. Comp. Endocrinol. 21, 441—450.

McLeay, D.J. (1973c) Effects of a 12-hr and 25-day exposure to kraft pulp mill effluent on the blood and tissues of juvenile coho salmon (Oncorhynchus kisutch). J. Fish. Res. Bd Can. 30, 395—400.

McLeay, D.J. (1975). Variations in the pituitary-interrenal axis and the abundance of circulating blodd-cell types in juvenile coho salmon, Oncorhynchus kisutch, during stream residence. Can. J. Zool. 53, 1882—1891.

McLeay, D.J. & Brown, D.A. (1974). Growth stimulation and biochemical changes in juvenile coho salmon (Oncorhynchus kisutch) exposed to a bleached kraft pulpmill effluent for 200 days. J. Fish. Res. Bd Can. 31, 1043—1049.

Miller, N.W., Sizemore, R.C. & Clem, L.W. (1985). Phylogeny of lymphocyate heterogeneity: The celiular requirements for in vitro antibody responses of channel catfish leukocytes. J. Immunol. 134, 2884—2888.

Miller, N.W. & Tripp, M.R. (1982). The effect of captivity on the immune response of the killifish, Fundulus heteroclitus L. J. Fish Biol. 20, 301—308.

Milne, R.S., Leatherland, J.F. & Holub, B.J. (1979). Changes in plasma thyroxine, triodothyronine and cortisol associated with starvation in rainbow trout (Salmo gairdneri). Environ. Biol. Fishes 4, 185—190.

Mollier, H. (1985). A critical review on the role of pollution as a cause of fish diseases. In Fish and Shellfish Pathology (A.E. Ellis, ed.), pp. 169—182. Academic Press: London/New York.

Morgan, R.I.G. & Roberts, R.J. (1976). The histopathology of salmon tagging IV. The effect of severe exercise on the induced tagging lesion in salmon parr sat two temperatures. J. Fish Biol. 8, 289—292.

Munck, A., Gruyre, P.M. & Hobrook, N.J. (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev. 5, 25—44.

Nichols, D.J. & Weisbart, M. (1985). Cortisol dynamics during seawater adaption of Atlantic salmon Salmo salar. Am. J. Physiol. 248, R651—R659.

Noakes, D.L.G. & Leatherland, J.F. (1977). Social dominance and interrenal cell activity in rainbow trout, Salmo gairdneri (Pisces, Salmonidae). Env. Biol. Fish 2, 131—136.

O'Neill, J.G. (1981). Effects of intraperitoneal lead and cadmium on the humoral immune response of Salmo trutta. Bull. Environ. Contam. Toxicol. 27, 42—48.

Paling, J.E. (1965). The population dynamics of the monogenean gill parasite Discocotyle sagittata Leuckart on Windermere trout, Salmo trutta L. Parasitology 55, 667—694.

Parrillo, J.E. & Fauci, A.S. (1979). Mechanisms of glucocorticoid action in immune processes. Ann. Rev. Pharmacol. Toxicol. 19, 179—201.

Perlmutter, A., Sarot, D.A. Yu, M.-L.,Filazzola, R.J. & Seeley, R.J. (1973). The effect of crowding on the immune response of the blue gourami. Trichogaster trichopterus, to infectious pancreatic necrosis (IPN) virus. Life Sci. 13, 363—375.

Pickering, A.D. (1984). Cortisol-induced lymphocytopenia in brown trout, Salmo trutta L. Gen. Comp. Endocrinol. 53, 252—259.

Pickering, A.D. (1986). Changes in blood cell composition of the brown trout, Salmo trutta L., during the spawning season. J. Fish Biol. 29, 335—347.

Pickering, A.D. & Christie, P. (1980). Sexual differences in the incidence and severity of the brown trout, Salmo trutta L. J. Fish Biol. 16, 669—683.

Pickering, A.D. & Christie, P. (1981). Changes in the concentrations of plasma cortisol and thyroxine during sexual maturation of the hatchery-reared brown trout, Salmo trutta L. Gen. Comp. Endocrinol. 44, 487—496.

Pickering, A.D. & Duston, J. (1983). Administration of cortisol to brown trout, Salmo trutta L., and its effects on the susceptibility to Saprolegnia infection and furunculosis. J. Fish Biol. 23, 163—175.

Pickering, A.D. & Pottinger, T.G. (1985). Cortisol can increase the susceptibility of brown trout, Salmo trutta L., to disease without reducing the white blood cell count. J. Fish Biol. 27, 611-619.

Pickering, A.D. & Pottinger, T.G. (1986). Lymphocytopenia and interrenal activity during sexual maturation in the brown trout. J. Fish Biol. 30, 41—50.

Pickering, A.D. & Pottinger, T.G. (1987a). Poor water quality suppresses the cortisol response of salmonid fish to handling and confinement. J. Fish Biol. 30, 363—374.

Pickering, A.D. & Pottinger, T.G. (1987b). Crowding causes prolonged leucopenia in salmond fish, despite interrenal acclimation. J. Fish Biol. 30, 701—712.

Pickering, A.D., Pottinger, T.G. & Christie, P. (1982). Recovery of the brown trout, Salmo trutta L., from acute handling stress : a time-course study. J. Fish Biol. 20, 229-244.

Pickering, A.D. & Stewart, A. (1984). Acclimation of the interrenal tissue of the brown trout, Salmo trutta L., to chronic crowding stress. J. Fish Biol. 24, 731-740.

Pickering, A.D. & Willoughby, L.G. (1982). Saprolegnia infections of salmonid fish. In Microbial Diseases of Fish (R.J. Roberts, ed.), pp. 271—279. Academic Press: London/New York.

Pippy, J.H.C. & Hare, G.M. (1969). Relationship of river pollution to bacterial infection in salmon (Salmo salar) and suckers (Catostomus commersoni). Trans. Am. Fish. Soc. 98, 685—690.

Poppe, T.T., Håstein, T., Froslie, A., Koppang, N. & Norheim, G. (1986). Nutritional aspects of haemorrhagic syndrome ('Hitra Disease') in farmed Atlantic salmon Salmo salar. Dis. Aquat. Org. 1, 155—162.

Pottinger, T.G. & 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. & 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.

Refstie, T. (1982). Preliminary results: differences between rainbow trout families in resistance against vibriosis and stress. In Developmental and Comparative Immunology (W.B. van Muiswinkel, ed.). Supplement 2, pp. 205—209. Pergamon Press: New York.

Refstie, T. (1986) Genetic differences in stress response in Atlantic salmon and rainbow trout. (Abstract). Aquaculture 57, 374.

Roales, R.R. & Perlmutter, A. (1977). The effects of sub-lethal doses of methyl mercury and copper, applied singly and jointly, on the immune response of the blue gourami (Trichogaster trichopterus) to viral and bacterial antigens. Arch. Environ. Contam. Toxicol. 5, 325—331.

Roberts, R.J. & Horne, M.T. (1978). Bacterial meningitis in farmed rainbow trout Salmo gairdneri Richardson affected with chronic pancreatic necrosis. J. Fish Dis. 1, 157—164.

Robertson, D. (1979). Host parasite interactions between Ichtypbodo necator (Henneguy 1883) and farmed salmonids. J. Fish Dis. 2, 481—493.

Robertson, O.H., Hane, S., Wexler, B.C. & Rinfret, A.P. (1963). The effect of hydrocortisone on immature rainbow trout (Salmo gairdneri). Gen. Comp. Endocrinol. 3. 422—436.

Rodsaether, M.C., Olafsen, J., Raa, J., Myhre, K. & Steen, J.B. (1977). Copper as an initiating factor of vibriosis (Vibrio angullarum) in eel (Anguilla anguilla). J. Fish Biol. 10, 17-21.

Roth, R.R. (1972). Some factors contributing to the development of fungus infection in freshwater fish. J. Wildl. Dis. 8, 24—28.

Sarot, D.A. & Perlmutter, A. (1976). The toxicity of zinc to the immune response of the zebrafish, Brachydanio rerio, injected with viral and bacterial antigens. Trans. Am. Fish. Soc. 105, 456—459.

Schreck, C.B. (1981). Stress and compensation in teleostean fishes : Response to social and physical factors. In Stress and Fish (A.D. Pickering, ed.), pp. 295—321. Academic Press: London/New York.

Schreck, C.H. & Lorz, H.W. (1978). Stress response of coho salmon (Oncorhynchus kisutch) elicited by cadmium and copper and potential use of cortisol as an indicator of stress. J. Fish. Res. Bd. Can. 35, 1124—1129.

Sheridan, M.A. (1986). Effects of thyroxin, cortisol, growth hormone, and prolactin on lipid metabolism of coho salmon, Oncorhynshus kisutch, during smoltification. Gen. Comp. Endocrinol. 64, 220—238

Simpson, T.H. (1975/76) Endocrine aspect of salmonid culture. Proc. Roy. Soc. Edinb. (B) 75, 241—252.

Sindermann, C.J. (1979). Pollution-associated diseases and abnormalities of fish and shellfish : A review. Fish. Bull., U.S. Fish Wildl. Serv. 76, 717—749.

Snieszko, S.F. (1972). Nutritional fish diseases. In Fish Nutrition (J.E. Halver, ed.), pp. 403—437. Academic Press : New York/London.

Snieszko, S.F. (1974). The effects of environmental stress on outbreaks of infectious diseases of fishes. J. Fish Biol. 6, 197—208.

Soderberg, R.W., Flynn, J.B. & Schmittou, H.R. (1983). Effects of ammonia on growth and survival of rainbow trout in intensive static-water culture. Trans. Am. Fish. Soc. 112, 448—451.

Srivastava, A.K. (1969). Studies on the haematology of certain freshwater teleosts — V. Thrombocytes and the clotting of blood. Anat, Anz. 124, 368—374.

Stave, J.W. & Roberson, B.S. (1985). Hydrocortisone suppresses the chemiluminescent response of striped bass phagocytes. Dev. Comp. Immunol. 9, 77—84.

Stave, J.W. & Roberson, B.S. (1985). Hydrocortisone suppresses the chemiluminescent response of striped bass phagocytes. Dev. Comp. Immunol. 9, 77—84.

Stevens, D.G. (1977). Survival and immune response of coho salmon exposed to copper. Ecol. Res. Ser. U.S. Environ. Protect. Agency EPA — 600/3 3—77—031.

Strange, R.J. & Schreck, C.B. (197). Anaesthetic and handling stress on survival and cortisol concentration in yearling chinook salmon (Oncorhynchus tshawytscha). J. Fish. Res. Bd. Can. 35, 345—349.

Strange, R.J., Schreck, C.B. & Ewing, R.D. (1978). Cortisol concentrations in confined juvenile chinook salmon (Oncorhynchus tshawytscha). Trans. Am. Fish. Soc. 107, 812—819.

Strange, R.J., Schreck, C.B. & Golden, J.T. (1977). Corticoid stress responses to handling and temperature in salmonids. Trans, Am. Fish. Soc. 106, 213—218.

Storer, H.H. (1967) Starvation and the effects of cortisol in the goldfish. Comp. Biochem. Physiol. 20, 939—948.

Sugatt, R.H. (1980) Effects of sodium dichromate exposure on the immune response of juvenile coho salmon (Oncorhynchus kisutch) against Vibrio angullarum. Arch. Environ. Contam. Toxicol. 9, 207—216.

Sumpter, J.P., Pickering, A.D. & Pottinger, T.G. (1985). Stress-induced elevation of plasma a-MSH and endorphin in brown trout, Salmo trutta L. Gen. Comp. Endocrinol. 59, 257—265.

Sumpter, J.P., dye, H.M. & Benfey, T.J. (1986). The effect of stress on plasma ACTH, a-MSH, and cortisol levels in salmonid fishes. Gen. Comp. Endocrinol. 62, 377—385.

Walters, G.R. & Plumb, J.A. (1980). Environmental stress and bacterial infection in channel catfish, Ictalurus punctatus Rafinesque. J. Fish Biol.

Wechsler, S.J., McAllister, P.E., Hetrick, F.M. & Anderson, D.P. (1986). Effects of exogenous corticosteroids on circulating virus and neutralizing antibodies in striped bass (**Morone saxatilis**) infected with infectious pancreatic necrosis virus. In **Fish Immunology** (J.S. Stolen, D.P. Anderson & W. van Muiswinkel, eds), pp. 305—311. Elsevier: Amsterdam.

Wedemeyer, G. (1969). Stress-induced ascorbic acid depletion and cortisol production in two salmonid fishes. **Comp. Biochem. Physiol.** 29, 1247—1251.

Wedemeyer, G.A. (1970). The role of stress in the disease resistance of fishes. **Spec. Publs. Am. Fish. Soc.** 5, 30—35.

Wedemeyer, G.A. & McLeay, D.J. (1981). Methods for determining the tolerance of fishes to environmental stressors. In **Stress and Fish** (A.D. Pickering, ed.), pp. 248—275. Academic Press: London/New York.

Weeks, B.A., Warinner, J.E., Mason, P.L. & McGinnis, D.S. (1986). Influence of toxic chemicals on the chemotactic response of fish macrophages. **J. Fish Biol.** 28, 653—659.

Weinreb, E.L. (1958). Studies on the histology and histopathology of the rainbow trout, **Salmo gairdneri irideus**. 1. Hematology: under normal and experimental conditions of inflammation. **Zoologica, N.Y.** 43, 145—154.

Woo, P.T.K., Leatherland, J.F. & Lee, M.S. (1987). **Cryptobia salmositica**: cortisol increases the susceptibility of **Salmo gairdneri** Richardson to experimental cryptobiosis. **J. Fish Dis.** 10, 75—83.

Woodward, J.J., Casillas, E., & D'Aoust, B.G. (1979). Rapid decompression stress accelerates fibrinolysis in fingerling salmon. **J. Fish. Res. Bd. Can.** 36, 592—594.

Zetnik, P.R. & Goldspink, G. (1981). The effect of exercise on plasma cortisol and blood sugar levels in the rainbow trout, **Salmo gairdneri** Richardson. **J. Fish Biol.** 19, 37—43.

GENERAL AND COMPARATIVE ENDOCRINOLOGY 76, 310–321 (1989)

The Deleterious Effects of Cortisol Implantation on Reproductive Function in Two Species of Trout, *Salmo trutta* L. and *Salmo gairdneri* Richardson

J. F. CARRAGHER, J. P. SUMPTER, T. G. POTTINGER,* AND A. D. PICKERING*

*Department of Biology and Biochemistry, Brunel University, Uxbridge, Middlesex, UB8 3PH, United Kingdom, and *Institute of Freshwater Ecology, Ferry House, Far Sawrey, Nr Ambleside, Cumbria, LA22 0LP, United Kingdom*

Accepted February 17, 1989

Implantation of a cortisol-releasing pellet (60 mg kg^{-1} fish) into the peritoneal cavity of brown trout, *Salmo trutta* L. (sexually maturing males and females), and rainbow trout, *Salmo gairdneri* Richardson (maturing males and immature fish of both sexes), significantly elevated their plasma cortisol level. At 18 days postimplantation, cortisol-implanted sexually maturing male brown trout had smaller gonads, a lower plasma testosterone level, and less gonadotropin in their pituitary gland than control fish. Plasma levels of 11-ketotestosterone and gonadotropin were not significantly affected. Cortisol-implanted sexually maturing female brown trout had smaller gonads, reduced plasma levels of 17β-oestradiol, testosterone, and vitellogenin, and a lower pituitary gland gonadotropin content than control fish. The plasma gonadotropin level was unaffected. At 36 days postimplantation, cortisol treatment of maturing male rainbow trout significantly suppressed plasma gonadotropin levels. Plasma levels of testosterone, 11-ketotestosterone, and 17α,20β-dihydroxy-4-pregnen-3-one, pituitary gonadotropin content, and gonad size were not significantly affected. In sexually immature female rainbow trout, cortisol administration suppressed the level of vitellogenin in the plasma, compared to control-implanted fish. The 17β-oestradiol level was not affected. Cortisol implantation did not affect the plasma testosterone level in sexually immature male trout. These results suggest that prolonged elevation of plasma cortisol, to levels well within physiological range, can affect a wide range of reproductive parameters in both brown and rainbow trout. Further, some effects are manifest in immature as well as in mature fish. These findings are discussed in relation to the effects of cortisol treatment on the state of health of the treated fish.

Stress is a well-recognised suppressor of reproductive function in mammals (Stephens, 1980; Moberg, 1985; Armstrong, 1986). There is considerable evidence from administration studies that corticosteroids can mediate this phenomenon. Corticosteroids have been found to have effects at the level of the hypothalamus (Thibier and Rolland, 1976), the pituitary gland (Welsh and Johnson, 1981; Mann *et al.*, 1982), and the gonads themselves (Bambino and Hsueh, 1981; Sapolsky, 1985). In general, the effect of corticosteroid is to suppress the secretion of the various releasing factors or hormones. Stress-induced suppressions of reproductive functions have also been observed in birds (Siegel, 1980), reptiles (Lance and Elsey, 1986), and amphibians (Licht *et al.*, 1983, Moore and Zoeller, 1985). The effect of corticosteroid administration on reproductive parameters in two of these groups has also been described: birds (Wilson and Follett, 1976; Deviche *et al.*, 1979; Petitte and Etches, 1988) and amphibians (Moore and Zoeller, 1985; Kupwaide and Saidapur, 1987). In all of these studies, administration of corticosteroid had a significant deleterious effect on reproductive function.

There is presently only very limited published information on the effects of either stress or corticosteroids on reproductive function in teleosts (see Billard *et al.*, 1981; Pickering *et al.*, 1987a; Sumpter *et al.*, 1987; Safford and Thomas, 1987). Our previous work (Pickering *et al.*, 1987a) showed that chronically stressed sexually mature male brown trout had elevated plasma cortisol levels and that this was accompanied by a suppression of plasma testosterone and 11-ketotestosterone levels. Furthermore, we also demonstrated that an acute stress (duration 1 hr) elevated plasma ACTH and cortisol levels, but temporarily suppressed the plasma levels of both androgens. However, the plasma gonadotropin level was temporarily increased. Exogenous administration of cortisol to brown trout, *Salmo trutta*, is known to increase the susceptibility of the fish to disease (Pickering and Duston, 1983), but its effects on reproduction are not known.

The present study was designed to investigate whether elevated plasma cortisol levels could have been responsible for the effects on reproductive endocrinology observed in our previous studies. To this end, sexually maturing brown and rainbow trout were given cortisol-releasing implants, and, after a period of time, a wide range of reproductive parameters was assessed.

MATERIALS AND METHODS

Experimental fish. The brown trout used for this study were of the Dunsop Bridge strain and had been kept at the Windermere hatchery since being brought in as 1+ fish the previous year. The rainbow trout (1+ fish, Cloan strain) were raised on site from eyed ova. The brown trout sampled were all maturing fish, sex ratio (♂:♀) of 1.4:1. The rainbow trout were a year younger, and the population consisted of mature male fish and immature fish of both sexes, with a sex ratio of 1.75:1. Sixty-five percent of male rainbow trout were maturing.

Fish were kept outside in 1500-liter circular tanks supplied with 35 liters min^{-1} Windermere lake water (temperature range 11.7–16.5°) and were fed daily at recommended rate. Eighty brown trout were divided among four tanks (two tanks control, two tanks cortisol-treated). Rainbow trout were similarly distributed among four tanks but at a density of 50 fish per tank. Two weeks were allowed for the fish to recover from handling stress and acclimate to the conditions (Pickering *et al.*, 1982) before the experiment commenced.

Implantation. Fish were anaesthetized using 2-phenoxyethanol (Sigma, 1:2000). Control fish received 0.5 ml molten (40°) cocoa butter as an intraperitoneal injection, cortisol-implanted fish received a similar injection but containing cortisol at a concentration of 60 mg kg^{-1} fish. The solidified cocoa butter pellet acts as a slow release implant over several weeks (see Pickering and Duston (1983) for details).

Sampling. Immediately after netting, the fish were anaesthetized and blood was taken from the caudal vessels using a heparinized syringe and kept on ice until centrifugation. Fish were weighed and measured and then killed by spinal section. Gonads from mature fish were excised and weighed, and the pituitary gland was removed and frozen. Plasma was stored frozen prior to radioimmunoassay (RIA).

Radioimmunoassays. Most hormones were measured using established, previously validated RIAs, the details of which have been published. Cortisol was determined according to Pickering *et al.* (1987b). Testosterone and 11-ketotestosterone were measured as described in Pottinger and Pickering (1985), but using the antisera mentioned in Pickering *et al.* (1987a). 17α,20β-Dihydroxy-4-pregnen-3-one (17α,20β-P) was determined as described by Scott *et al.* (1982), except that ethyl acetate extractions of plasmas were made. 17β-Oestradiol was measured using the same basic methodology as adopted for the other steroid assays. Steroids were extracted from plasma using ethyl acetate, duplicate aliquots of which were evaporated in assay tubes before being redissolved in 100 μl assay buffer. Label (approximately 10,000 dpm 2,4,6,7,16, 17-[^{3}H] oestradiol, Amersham International) and antibody, in separate 100-μl aliquots, were then added. Tubes were incubated overnight at 4° and separation of free and bound radiolabel was achieved with dextran-coated charcoal. The antibody was kindly provided by Dr. Z. Yaron (Tel Aviv, Israel). The detection limit of the assay (taken as 10% depression of maximum binding) was 8.6 ± 1.7 pg ml^{-1} (n = 5). Ethyl acetate extractions of plasmas diluted parallel to the standard.

The gonadotropin assay is fully described in Pickering *et al.* (1987a) and Sumpter and Scott (1989); the assay preferentially detects the gonadotropin referred to as either GTH II or maturational and ovulatory gonadotropin (see Sumpter and Scott (1989) for full details). Plasmas were diluted 1 in 2 before assay. Pituitary glands were homogenized in assay buffer and diluted appropriately to determine pituitary gonadotropin content. Rainbow trout vitellogenin was measured as described in Sumpter (1985) and Copeland *et al.* (1986) and brown trout vitellogenin was measured using the homologous assay described in Norberg and Haux (1988). We thank Dr. B. Norberg (Austevoll Ma-

rine Aquaculture Station, Norway) for the gift of the reagents.

Statistics. The effect of cortisol treatment on the parameters examined was assessed by analysis of variance (Genstat). From a plot of the residuals against fitted values, appropriate transformations (square root or log) were selected, where necessary, to improve homogeneity of variance. The levels of significance given in the paper are derived from these analyses but, for ease of presentation, data are given as arithmetic means ± SEM. The effect of cortisol implantation on the weight of the gonads from mature fish was analysed by linear regression of log gonad weight against log body length because of the allometric relationship between gonad size and body size.

RESULTS

All brown and rainbow trout recovered from the implantation procedure. Five days postimplantation, mortalities (due to *Saprolegnia* infection and presumptive furunculosis) began to occur amongst cortisol-implanted brown trout and by 18 days post-implantation, 30% of these fish had died. The remaining brown trout were sampled at this point. Rainbow trout showed a low mortality rate (less than 6%), and the fish were sampled after 36 days.

Brown Trout

Cortisol-implanted maturing male brown trout had a mean plasma cortisol level of 35 ng ml^{-1} compared to a control fish level of 2 ng ml^{-1} ($P < 0.001$, Fig. 1a). Cortisol implantation had no significant effect on either the weight or the length of fish (Figs. 1b and 1c). However, the weights of testes from cortisol-implanted fish were less than those of control fish ($P < 0.05$, Fig. 1d). The plasma level of testosterone in cortisol-implanted fish was reduced by 50% relative to the control fish ($P < 0.05$, Fig. 1e). However, plasma levels of 11-ketotestosterone were not significantly different between the two groups (Fig. 1f). An assay for 17α,20β-P was performed on these samples but levels were found to be very low and near the detection limit of the assay (about 50 pg ml^{-1}). The plasma level of gonadotropin in cortisol-implanted fish was not significantly different from the control fish (Fig. 1g), but the pituitary gland content o gonadotropin was significantly reduced b cortisol administration ($P < 0.05$, Fig. 1h) Analysis of the testes weights of this grou of fish in relation to body length revealed that cortisol implantation significantly re duced testes weight in a body size independent manner ($P < 0.05$, Fig. 2).

Cortisol-implanted sexually maturing fe male brown trout had a mean plasma corti sol level of 80 ng ml^{-1}, compared to th level in control fish of 5 ng ml^{-1} ($P < 0.001$ Fig. 3a). Cortisol administration had no sig nificant effect on either the weight or th length of fish (Figs. 3b and 3c), but th weights of ovaries from cortisol-implante fish were significantly lower than those o control fish ($P < 0.05$, Fig. 3d). The level of two sex steroids determined were signif icantly reduced by cortisol administration with 17β-oestradiol and testosterone at 3 and 16% of respective control levels ($P <$ 0.05, Figs. 3e and 3f). Cortisol implantatio did not significantly affect the plasma go nadotropin level (Fig. 3g). However, th gonadotropin content of the pituitary glan was significantly reduced by cortisol treat ment ($P < 0.05$, Fig. 3h). The plasma leve of the yolk precursor, vitellogenin, was als suppressed by cortisol, to only 30% of th level in control fish ($P < 0.01$, Fig. 3i).

Rainbow Trout

Cortisol-implanted maturing male rain bow trout had a mean plasma cortisol leve of 17 ng ml^{-1}, whereas control fish had level of 1 ng ml^{-1} ($P < 0.001$, Fig. 4a) Cortisol implantation had no effect on ei ther body weight and length or teste weight (Figs. 4b–4d). The plasma levels o three sex steroids (testosterone, 11 ketotestosterone, and 17α,20β-P) were de termined in these fish and, in all thre cases, the level of hormone in cortisol implanted fish was lower the controls However, none of these reductions wer statistically significant (Figs. 4e–4g). Th plasma level of gonadotropin was signifi cantly suppressed in cortisol-implanted fis

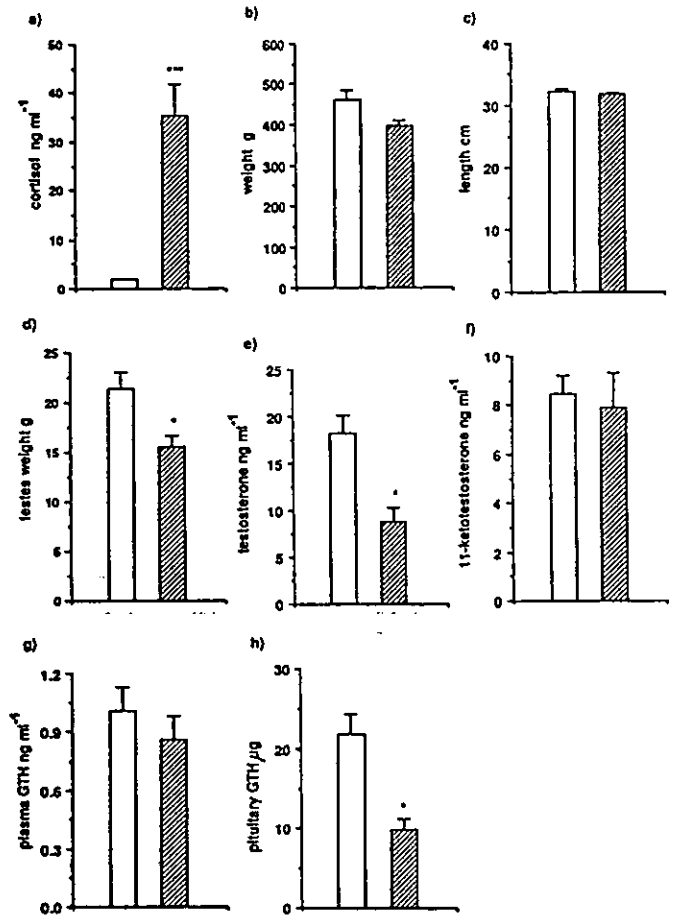


FIG. 1. The effect of cortisol implantation on sexually maturing male brown trout; (a) plasma cortisol, (b) body weight, (c) body length, (d) testes weight, (e) plasma testosterone, (f) plasma 11-ketotestosterone, (g) plasma gonadotropin (GTH), and (h) pituitary gland gonadotropin (GTH) content. Open columns represent control fish ($n = 18$), hatched columns represent cortisol-implanted fish ($n = 19$). (*$P < 0.05$, ***$P < 0.001$).

($P < 0.05$, Fig. 4h), although the pituitary gland gonadotropin content did not differ significantly (Fig. 4i).

The level of cortisol in the plasma of cortisol-implanted sexually immature female rainbow trout was 30 ng ml^{-1}, compared to control fish with a level of 1 ng ml^{-1} ($P <$ 0.001, Fig. 5a). Body length did not differ between the two groups (Fig. 5c), although the weight of cortisol-implanted fish was less than controls ($P < 0.05$, Fig. 5b). Two reproductive parameters were determined in immature female fish. The level of 17β-oestradiol was low and no significant effect of cortisol was detected (Fig. 5d), but cortisol did suppress the plasma vitellogenin levels in implanted fish ($P < 0.05$, Fig. 5e).

Cortisol-implanted sexually immature male rainbow trout had a plasma cortisol level of 28 ng ml^{-1}, compared to control fish with a level of 1 ng ml^{-1} ($P < 0.001$,

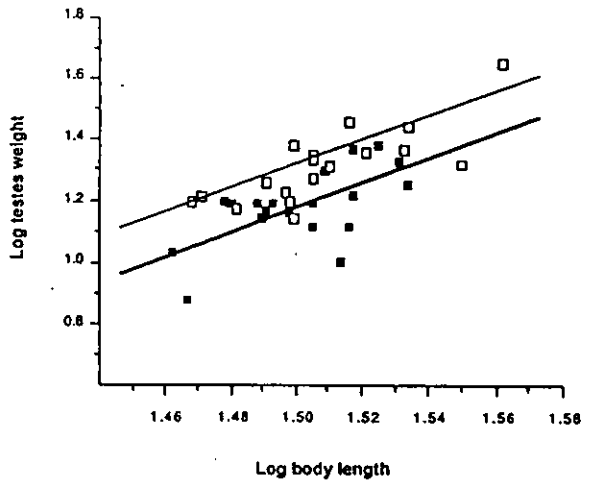


FIG. 2. The relationship between testes weight and body length in control (□) and cortisol-implanted (■) mature male brown trout. The two sets of data can be described by the regressions ($\log_{10}$ testes weight = 3.84 $\log_{10}$ fish length − 4.48; control fish) and ($\log_{10}$ testes weight = 3.74 $\log_{10}$ fish length − 4.43; cortisol-implanted fish). An F test for parallelism in the two lines showed that a common gradient ($P > 0.05$) was adequate to describe the two data sets but that a separate intercept was required ($P < 0.005$).

Fig. 6a). Body weight and length were not affected by cortisol (Fig. 6b and 6c), and neither was the one reproductive parameter determined, plasma testosterone (Fig. 6d). The pituitary glands of immature fish of both sexes were not analysed for gonadotropin because levels are extremely low (Sumpter and Scott, 1989).

DISCUSSION

This study shows that administration of cortisol to both brown and rainbow trout leads to reductions in a number of reproductive parameters. Such studies are open to the criticism that these deleterious effects of cortisol administration are a secondary consequence of the poor health of such fish. It is well established that elevated plasma cortisol levels lead to reduced disease resistance and hence an increase in the mortality rate of fish (Pickering and Duston, 1983). It is also well established that elevated plasma cortisol levels are associated with reduced growth (Pickering and Stewart, 1984), impaired reproduction (Pickering *et al.*, 1987a; Sumpter *et al.*, 1987), and probably other regulatory systems that have not been investigated as yet. What is presently unknown are the interrelationships between these different systems. In the present study we do not attribute the reduction in reproductive function to a direct effect of cortisol on the hypothalamic–pituitary–gonadal axis, but neither do we dismiss this possibility.

Although it is possible that the reproductive effects are secondary to the reduced health of the fish, there is some evidence available suggesting that cortisol can act directly to modify hormone output from reproductive tissues. For example, cortisol suppresses sex steroid secretion from cultured rainbow trout ovarian follicles (Sumpter *et al.*, 1987) and gonadotropin secretion from cultured pituitary glands (unpublished observations). Such studies in fish are strongly supported by studies using mammalian tissues (Bambino and Hsueh, 1981; Padmanabhan *et al.*, 1983). Further

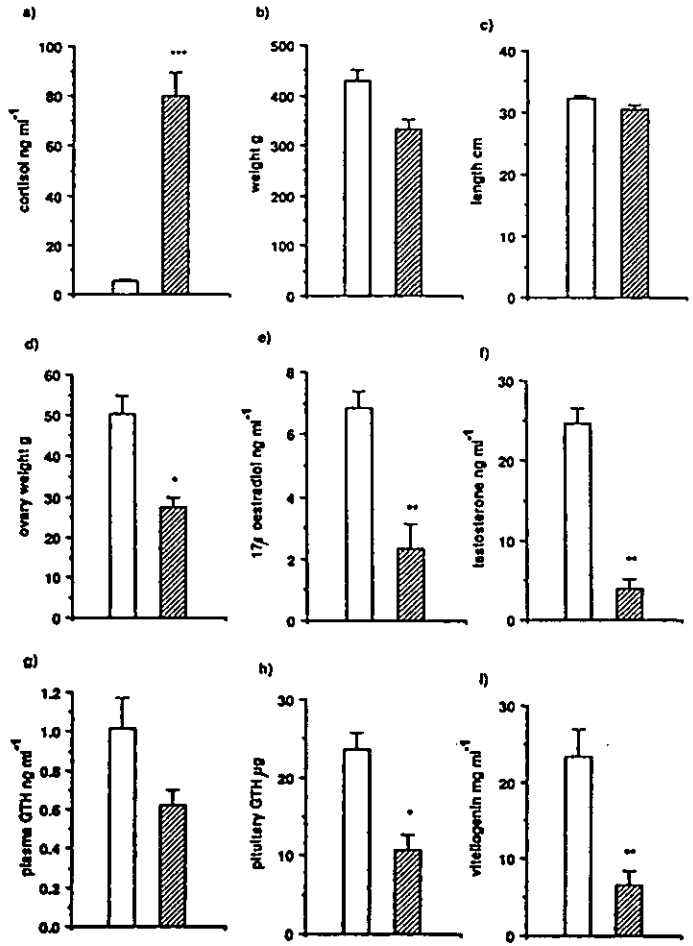


FIG. 3. The effect of cortisol implantation on sexually maturing female brown trout; (a) plasma cortisol, (b) body weight, (c) body length, (d) ovary weight, (e) plasma 17β-oestradiol, (f) plasma testosterone, (g) plasma gonadotropin (GTH), (h) pituitary gland gonadotropin (GTH) content, and (i) plasma vitellogenin. Open columns represent control fish (n = 19), hatched columns represent cortisol-implanted fish (n = 8). ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

support for this hypothesis can be obtained from the present study, by examining in more detail the results from the cortisol-implanted brown trout. For example, of the 19 male brown trout sampled, 11 had visible signs of disease, whereas 8 did not. The diseased fish were characterized by the first signs of *Saprolegnia* infection along the margin of the fins (a common feature in sexually mature male brown trout; see Richards and Pickering (1978)). It has been shown that the degree of morbidity is directly related to the extent of fungal colonization (Richards and Pickering, 1979) and in no way could the fish sampled during the present study be described as severely dis-

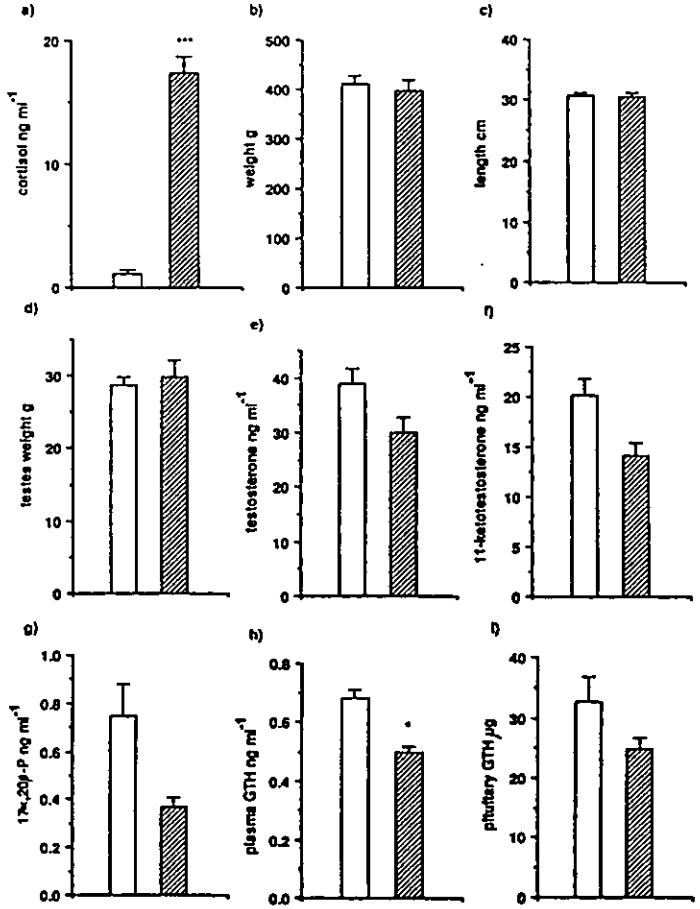


FIG. 4. The effect of cortisol implantation on sexually maturing male rainbow trout; (a) plasma cortisol, (b) body weight, (c) body length, (d) testes weight, (e) plasma testosterone, (f) plasma 11-ketotestosterone, (g) plasma 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-P), (h) plasma gonadotropin (GTH), and (i) pituitary gland gonadotropin (GTH) content. Open columns represent control fish (n = 18), hatched columns represent cortisol-implanted fish (n = 18). ($^*P < 0.05$, $^{***}P < 0.001$).

eased. Moreover, when the data were separated on the basis of presence or absence of *Saprolegnia* and the two groups were compared, no significant differences were found (Table 1). This table presents the results of three reproductive parameters that were significantly affected by cortisol implantation when cortisol-implanted fish were considered as a single group (see Fig. 1). It can be seen from Table 1 that both the nondiseased and diseased groups showed similarly suppressed testes weights, plasma testosterone levels, and pituitary gland gonadotropin contents, compared to the controls.

Previous studies have shown that incorporating cortisol into intraperitoneal cocoa butter implants gives a constant, stable,

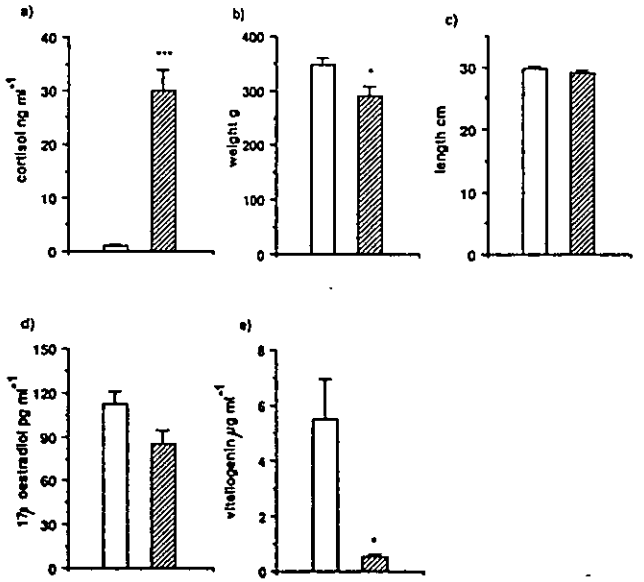


FIG. 5. The effect of cortisol implantation on sexually immature female rainbow trout; (a) plasma cortisol, (b) body weight, (c) body length, (d) plasma 17β-oestradiol, and (e) plasma vitellogenin. Open columns represent control fish (n = 17), hatched columns represent cortisol-implanted fish (n = 15). ($^*P < 0.05$, $^{***}P < 0.001$).

low-level release of the steroid. Plasma cortisol levels are elevated for several weeks using this procedure (Pickering and Duston, 1983; Pickering and Pottinger, 1985). Although differences in plasma cortisol levels were found among cortisol-implanted fish, values were 17–80 ng ml^{-1}, well within the range reported for chronically stressed fish (Donaldson, 1981). For this reason we believe that the doses of cortisol given to the fish in this experiment are not pharmacological and hence the effects on reproductive function are likely to occur to chronically stressed fish.

It became obvious a few days postimplantation that there was a difference in the sensitivity of the two trout species, with respect to disease resistance, to cortisol. This resulted in the death, due to furunculosis and/or *Saprolegnia* infection, of 30% of the cortisol-implanted brown trout between Days 5 and 18 postimplantation. No control fish died during the same period. Rainbow trout with cortisol implants suffered only 5% mortality over 36 days, and again, no control fish died.

This apparent interspecific difference in sensitivity to cortisol was accompanied by a marked variation in plasma cortisol levels in implanted fish. Cortisol-implanted maturing male brown trout had a mean plasma cortisol level of 35 ng ml^{-1}, less than half the level of identically treated maturing female fish ($P < 0.001$). In addition to sex, the state of maturity also appeared to affect the level of cortisol in the plasma of cortisol-implanted fish. The plasma cortisol level of maturing male rainbow trout was significantly lower than that of immature fish of both sexes with identical implants (17 cf. 30 ng ml^{-1}, $P < 0.001$). It is of interest that mature male trout stressed for 1 hr had a lower plasma cortisol level than similarly treated immature fish (Sumpter *et al.*,

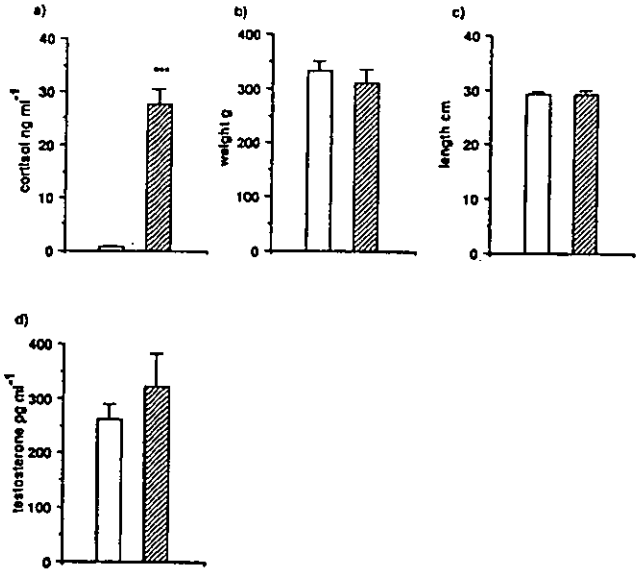


FIG. 6. The effect of cortisol implantation on sexually immature male rainbow trout; (a) plasma cortisol, (b) body weight, (c) body length, and (d) plasma testosterone. Open columns represent control fish (n = 7), hatched columns represent cortisol-implanted fish (n = 13). (***$P < 0.001$).

1987). The reason for these differences is not known, but work on the metabolic clearance rate of the hormone during sexual maturation may be useful in answering these questions.

Despite these differences among the various groups of fish, it is obvious that chronic low-level cortisol elevation had a markedly suppressive effect on a wide range of reproductive parameters in brown trout of both sexes. Thus, pituitary gonadotropin content, plasma testosterone level, and testes weight were suppressed in male fish. Similarly, pituitary gonadotropin content, plasma 17β-oestradiol, testosterone, and vitellogenin levels, and ovary weight were suppressed in the females. Although sex steroid and vitellogenin levels, which are thought to be regulated by gonadotropin, were suppressed, plasma gonadotropin levels were not. In both males and females, plasma gonadotropin levels were lower in

TABLE 1
A COMPARISON OF SOME REPRODUCTIVE PARAMETERS BETWEEN VISIBLY DISEASED AND NONDISEASED MATURE MALE BROWN TROUT IMPLANTED WITH CORTISOL

	Nondiseased (n = 8)	Diseased (n = 11)	P value	Controls (n = 18)
Testes weight (g)	13.8 ± 1.2	16.9 ± 1.3	0.11	21.4 ± 1.7
Testosterone (ng ml^{-1})	10.9 ± 2.5	7.2 ± 1.8	0.23	18.2 ± 2.0
Pituitary gonadotropin (μg)	10.3 ± 2.5	9.6 ± 1.4	0.90	21.9 ± 2.4

Note. Levels in control-implanted fish are included for comparison. Note that there are no significant differences between the visibly diseased and nondiseased trout in any of these parameters, although they were significantly suppressed compared to control-implanted fish.

cortisol-implanted fish than in the control fish, but the differences were not significant. Because the plasma gonadotropin levels were similar in the two sexes at this stage of sexual maturity (see also Sumpter and Scott, 1989), we felt justified in combining data from the two sexes. When this was done there was a significant suppressive effect of cortisol on the plasma gonadotropin level ($P < 0.05$).

When maturing male rainbow trout were implanted with cortisol, only one reproductive parameter, the plasma gonadotropin level, was found to be significantly affected. This further suggests that rainbow trout are less sensitive than brown trout to chronic cortisol elevation, although again this conclusion is complicated because plasma cortisol levels differed between the two groups.

Although cortisol implantation affected most reproductive parameters in maturing brown trout including plasma testosterone levels, plasma levels of the other androgen, 11-ketotestosterone showed no such change. This suggests that the enzymes of the steroidogenic pathway may have varying sensitivities to cortisol. It is interesting to note that acute and chronic stresses affected plasma testosterone levels to a greater degree than 11-ketotestosterone levels in mature male brown trout (Pickering *et al.*, 1987a).

The present study also revealed that the detrimental effects of chronic cortisol elevation on reproductive function are not restricted to fish in the later stages of maturity, but can also occur in immature fish. Female rainbow trout are known to begin preparing for spawning over 12 months before the event (Sumpter *et al.*, 1984). A very early indication of this is a rise in the plasma level of vitellogenin, the precursor molecule for yolk. This study shows that vitellogenin production can be suppressed by low plasma levels of cortisol in sexually immature fish.

This study reveals that *chronic* cortisol elevation, to within the range typical for chronically stressed salmonid fish (Donaldson, 1981), reduced the level of gonadotropin in the plasma of maturing male rainbow trout and brown trout (combined data). Interestingly, *acute* stress elevated the plasma gonadotropin level in mature trout (Pickering *et al.*, 1987a). This differential response of the gonadotropes to acute or chronic stresses is similar to the pattern found in some mammals (Gray *et al.*, 1978; Ducharme *et al.*, 1982), but the mechanism is still unknown.

The notion that stress detrimentally affects reproduction in fishes is not new. What has been lacking prior to this and our earlier studies (Pickering *et al.*, 1987a; Sumpter *et al.*, 1987) is anything more than fragmentary or circumstantial evidence that the two are linked (see Bry and Zohar, 1980; Zohar, 1980; Gillet *et al.*, 1981). In the present study we have demonstrated that cortisol elevation is, at least partly, responsible for the suppressive effects of acute and chronic stress on the reproductive endocrinology of trout. Work is now needed to identify the precise mechanisms through which these effects are mediated and to examine the biological consequences, in terms of fecundity, gamete quality, offspring viability, and overall reproductive success, of incidences of stress during sexual maturation.

ACKNOWLEDGMENTS

The authors thank Dr. P. A. Copeland (Port Aransas Marine Laboratory, Texas) and Dr. B. Breton (INRA, Rennes) for their gifts of ingredients for the gonadotropin RIA, Dr. H. M. Dye (Department of Fisheries and Oceans, West Vancouver) for the 11-ketotestosterone antiserum, Dr. A. P. Scott (MAFF, Lowestoft) for the antiserum and label to measure 17α,20β-dihydroxy-4-pregnen-3-one, Dr. Z. Yaron (Tel Aviv) for the 17β-oestradiol antiserum, Dr. B. Norberg (Austevoll Marine Aquaculture Station, Norway) for the brown trout vitellogenin reagents, Mrs. J. Pollard and Miss J. M. Fletcher (IFE) for care and maintenance of the fish, and Mrs. M. A. Hurley (IFE)

for statistical advice. We are grateful to NERC and MAFF for financial support.

REFERENCES

Armstrong, D. T. (1986). Environmental stress and ovarian function. *Biol. Reprod.* 34, 29–39.

Bambino, T. H., and Hsueh, A. J. W. (1981). Direct inhibitory effect of glucocorticoids upon testicular luteinizing hormone receptor and steroidogenesis *in vivo* and *in vitro*. *Endocrinology* 108, 2142–2148.

Billard, R., Bry, C., and Gillet, C. (1981). Stress, environment and reproduction in teleost fish. *In* "Stress and Fish" (A. D. Pickering, Ed.), pp. 185–208. Academic Press, New York/London.

Bry, C., and Zohar, Y. (1980). Dorsal aorta catheterization in rainbow trout (*Salmo gairdneri*). Glucocorticoid levels, haematological data and resumption of feeding during five days after surgery. *Reprod. Nutr. Dev.* 20, 1825–1834.

Copeland, P. A., Sumpter, J. P., Walker, T. K., and Croft, M. (1986). Vitellogenin levels in male and female rainbow trout (*Salmo gairdneri* Rich.) at various stages of the reproductive cycle. *Comp. Biochem. Physiol. B* 83, 487–493.

Deviche, P., Heyns, W., Balthazart, J., and Hendrick, J. C. (1979). Inhibition of LH plasma levels by corticosterone administration in the male duckling (*Anas platyrhynchus*). *IRCS Med. Sci.* 7, 622.

Donaldson, E. M. (1981). The pituitary–interrenal axis as an indicator of stress in fish. *In* "Stress and Fish" (A. D. Pickering, Ed.), pp. 11–47. Academic Press, New York/London.

Ducharme, J. R., Tache, Y., Charpenet, G., and Collu, R. (1982). Effects of stress on the hypothalamic-pituitary-testicular function in rats. *In* "Brain Neuropeptides and Hormones" (R. Collu *et al.*, Eds.), pp. 305–317. Raven Press, New York.

Gillet, C., Billard, R., and Breton, B. (1981). La reproduction du poisson rouge *Carassius auratus* élevé à 30°C. Effet de la photopériode, de l'alimentation et de l'oxygénation. *Cah. Lab. Montereau* 11, 49–56.

Gray, G. D., Smith, E. R., Damassa, D. A., Ehrenkranz, J. R. L., and Davidson, J. M. (1978). Neuroendocrine mechanisms mediating the suppression of circulating testosterone levels associated with chronic stress in male rats. *Neuroendocrinology* 25, 247–256.

Kupwaide, V. A., and Saidapur, S. K. (1987). Effect of dexamethasone and ACTH on oocyte growth and recruitment in the frog *Rana cyanophlyctis* during the prebreeding vitellogenic phase. *Gen. Comp. Endocrinol.* 65, 394–398.

Lance, V. A., and Elsey, R. M. (1986). Stress-induced suppression of testosterone secretion in male alligators. *J. Exp. Zool.* 239, 241–246.

Licht, P., McCreery, B. R., Barnes, R., and Pang, P. (1983). Seasonal and stress related changes in plasma gonadotropins, sex steroids, and corticosterone in the bullfrog, *Rana catesbeiana*. *Gen. Comp. Endocrinol.* 50, 124–145.

Mann, D. R., Jackson, G. G., and Blank, M. S. (1982). Influence of adrenocorticotropin and adrenalectomy on gonadotropin secretion in immature rats. *Neuroendocrinology* 34, 20–26.

Moberg, G. P. (1985). Influence of stress on reproduction: Measure of well-being. *In* "Animal Stress" (G. P. Moberg, Ed.), pp. 245–267. American Physiol. Soc., Bethesda, MD.

Moore, F. L., and Zoeller, R. T. (1985). Stress-induced inhibition of sexual reproduction: Evidence of suppressed secretion of LH-RH in an amphibian. *Gen. Comp. Endocrinol.* 60, 252–258.

Norberg, B. and Haux, C. (1988). A homologous radioimmunoassay for brown trout (*Salmo trutta*) vitellogenin. *Fish Physiol. Biochem.* 5, 59–68.

Padmanabhan, V., Keech, C., and Convey, E. M. (1983). Cortisol inhibits and adrenocorticotropin has no effect on luteinizing hormone-releasing hormone-induced release of luteinizing hormone from bovine pituitary cells *in vitro*. *Endocrinology* 112, 1782–1787.

Petitte, J. N., and Etches, R. J. (1988). The effect of corticosterone on the photoperiodic response of immature hens. *Gen. Comp. Endocrinol.* 69, 424–430.

Pickering, A. D., and Duston, J. (1983). Administration of cortisol to brown trout, *Salmo trutta* L., and its effects on the susceptibility to *Saprolegnia* infection and furunculosis. *J. Fish Biol.* 23, 163–175.

Pickering, A. D., and Pottinger, T. G. (1985). Cortisol can increase the susceptibility of brown trout, *Salmo trutta* L., to disease without reducing the white blood cell count. *J. Fish Biol.* 27, 611–619.

Pickering, A. D., Pottinger, T. G., Carragher, J. F., and Sumpter, J. P. (1987a). The effects of acute and chronic stress on the levels of reproductive hormones in the plasma of mature male brown trout, *Salmo trutta* L. *Gen. Comp. Endocrinol.* 68, 249–259.

Pickering, A. D., Pottinger, T. G., and Christie, P. 1982. Recovery of brown trout, *Salmo trutta* L., from acute handling stress: A time course study. *J. Fish Biol.* 20, 229–244.

Pickering, A. D., Pottinger, T. G., and Sumpter, J. P. (1987b). On the use of dexamethasone to block the pituitary-interrenal axis in the brown trout, *Salmo trutta* L. *Gen. Comp. Endocrinol.* 65, 346–353.

Pickering, A. D., and Stewart, A. (1984). Acclimation

of the interrenal tissue of the brown trout, *Salmo trutta* L., to chronic crowding stress. *J. Fish Biol.* 24, 731–740.

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

Richards, R. H., and Pickering, A. D. (1978). Frequency and distribution patterns of *Saprolegnia* infection in wild and hatchery-reared brown trout, *Salmo trutta* L., and char, *Salvelinus alpinus* (L.). *J. Fish Dis.* 1, 69–82.

Richards, R. H., and Pickering, A. D. (1979). Changes in serum parameters of *Saprolegnia*-infected brown trout, *Salmo trutta* L. *J. Fish Dis.* 2, 197–206.

Safford, S. E., and Thomas, P. (1987). Effects of capture and handling on the circulating levels of gonadal steroids and cortisol in the spotted seatrout, *Cynoscion nebulosus*. *In* "Reproductive Physiology of Fish" (D. R. Idler, L. W. Crim, and J. M. Walsh, Eds.), p. 312. Memorial University Press, St. John's.

Sapolsky, R. M. (1985). Stress-induced suppression of testicular function in the wild baboon: Role of glucocorticoids. *Endocrinology* 116, 2273–2278.

Scott, A. P., Sheldrick, E. L., and Flint, A. P. F. (1982). Measurement of 17α,20β-dihydroxy-4-pregnen-3-one in plasma of trout (*Salmo gairdneri* Richardson): Seasonal changes and response to salmon pituitary extract. *Gen. Comp. Endocrinol.* 46, 444–451.

Siegel, H. S. (1980). Physiological stress in birds. *BioScience* 30, 529–534.

Stephens, D. B. (1980). Stress and its measurement in domestic animals: A review of behavioural and physiological studies under field and laboratory conditions. *Adv. Vet. Sci. Comp. Med.* 24, 179–210.

Sumpter, J. P. (1985). The purification, radioimmunoassay and plasma levels of vitellogenin from the rainbow trout, *Salmo gairdneri*. *In* "Current Trends in Comparative Endocrinology" (B. Lofts and W. N. Holmes, Eds.), pp. 355–357. Hong Kong Univ. Press, Hong Kong.

Sumpter, J. P., Carragher, J. F., Pottinger, T. G., and Pickering, A. D. (1987). The interaction of stress and reproduction in trout. *In* "Reproductive Physiology of Fish" (D. R. Idler, L. W. Crim, and J. M. Walsh, Eds.), pp. 299–302. Memorial University Press, St. John's.

Sumpter, J. P., and Scott, A. P. (1989). Seasonal variations in plasma and pituitary levels of gonadotropin in males and females of two strains of rainbow trout (*Salmo gairdneri*). *Gen. Comp. Endocrinol.* 75, 376–388.

Sumpter, J. P., Scott, A. P., Baynes, S. M., and Witthames, P. R. (1984). Early stages of the reproductive cycle in virgin female rainbow trout (*Salmo gairdneri* Richardson). *Aquaculture* 43, 235–242.

Thibier, M., and Rolland, A. (1976). The effect of dexamethasone (DXM) on circulating testosterone (T) and luteinizing hormone (LH) in young postpubertal bulls. *Theriogenology* 5, 53–59.

Welsh, T. H., Jr., and Johnson, B. H. (1981). Stress-induced alterations in secretion of corticosteroids, progesterone, luteinizing hormone and testosterone in bulls. *Endocrinology* 109, 185–190.

Wilson, F. E., and Follett, B. K. (1976). Corticosterone-induced gonadosuppression in photostimulated tree sparrows. *Life Sci.* 17, 1451–1456.

Zohar, Y. (1980). Dorsal aorta catheterization in rainbow trout (*Salmo gairdneri*): Its validity in the study of blood gonadotropin patterns. *Reprod. Nutr. Dev.* 20, 1811–1823.

Freshwater Biology (1989) 21, 47–55

Environmental stress and the survival of brown trout, *Salmo trutta*

A. D. PICKERING Freshwater Biological Association, Windermere Laboratory, England

SUMMARY. 1. The hypothalamic–pituitary–interrenal axis of the brown trout, *Salmo trutta*, is activated in response to most forms of environmental stress. This results in an elevation of blood cortisol levels.

2. Experimental elevation of blood cortisol levels in otherwise unstressed brown trout caused a dose-dependent increase in mortality rate due to disease. In our studies, *Saprolegnia*-infection, furunculosis and bacterial fin-rot were the principal diseases.

3. Chronic cortisol elevation also suppressed several of the endocrine processes controlling sexual maturation, resulting in a significant reduction in the size of the gonad in both male and female fish.

4. It is argued that many of the deleterious effects of sublethal pollution (including acidification) on natural trout populations can be attributed to chronically-elevated blood cortisol levels and that a knowledge of such physiological changes would allow an assessment of the impact of pollution events and act as an early warning of potential disease and recruitment problems.

Introduction

Sudden fish kills, as a result of rapid, deleterious changes in the aquatic environment, are all too familiar to fishery biologists. However, a more gradual deterioration of environmental conditions may go undetected even when the fishery is in serious decline. Under such circumstances, environmental stresses, although not directly lethal themselves, may reduce the probability of survival of fish stocks by increasing their susceptibility to disease (Wedemeyer, 1970; Snieszko, 1974) and by reducing reproductive success (June, 1977).

Until recently, little was known of the physiological and endocrinological changes responsible for this decrease in disease resistance; even less was known about the mechanisms inhibiting reproduction (see Billard, Bry & Gillet, 1981). However, during the past decade, evidence has accumulated which shows that excessive or prolonged stimulation of the hypothalamic–pituitary–interrenal (HPI) axis is a major factor in the mediation of such changes. The present paper reviews some of the recent literature in this field and draws heavily on our own experimental studies with the brown trout, *Salmo trutta* L. Much of the original work emphasizes the aquacultural aspects of both acute and chronic stress but the physiological and endocrinological changes that occur in stressed trout appear to have several common components, irrespective of the nature of the stress. Consequently, such studies have important implications for understanding how environmental stresses may influence natural fish populations.

Correspondence: Dr A. D. Pickering, Freshwater Biological Association, The Ferry House, Ambleside, Cumbria LA22 0LP.

Stress and the hypothalamic–pituitary–interrenal (HPI) axis

The HPI axis, a central component of the fish's endocrine system, consists of a hierarchy of hormonal pathways controlling the secretion of corticosteroids into the blood stream (see Donaldson, 1981, for details). Corticotropin releasing factor (CRF) from the hypothalamus stimulates the release of adrenocorticotropin (ACTH) from the pituitary gland which, in turn, promotes the synthesis and release of corticosteroids from the interrenal tissue (Fryer & Peter, 1977). Cortisol is the principal corticosteroid in salmonid fish (Henderson & Garland, 1980) and is released in response to most, if not all, forms of environmental stress (Donaldson, 1981). Corticosteroids are catabolic hormones capable of promoting gluconeogenesis (Storer, 1967; Chan & Woo, 1978; Leach & Taylor, 1982) and stimulating lipolysis (Lidman *et al.*, 1979; Dave *et al.*, 1979; Sheridan, 1986) and it seems likely that such energy-mobilizing properties are of adaptive value as the fish attempts to avoid or overcome the immediate threat. However, *direct* evidence of the advantage of elevated blood cortisol levels in stressed fish has yet to be produced.

Blood cortisol levels in brown trout are rapidly elevated in response to physical stresses such as handling and confinement (Pickering, Pottinger & Christie, 1982), to the chemical stresses of malachite green and formalin treatment (Pickering & Pottinger, 1984, 1985a) and to the stress of overcrowding (Pickering & Stewart, 1984; Pickering & Pottinger, 1987c). In general, the cortisol response of trout to an acute stress (i.e. a stress of short duration in which the time-course of the response of the fish is much greater than that of the stress itself) occurs over a few hours, with a return to basal levels (<2 ng ml^{-1}) within 24–48 h. By comparison, the elevation of blood cortisol levels in response to a chronic stress (i.e. a continuous form of stress from which there may be no escape) may last for several days, or even weeks, before acclimation occurs (Pickering & Stewart, 1984). In most cases, however, blood cortisol levels eventually return to normal despite the continued presence of the stress (Schreck, 1981). Under aquaculture conditions handling and disturbance are examples of acute stresses, water quality deterioration and overcrowding are examples of chronic stresses. In the natural environment, predator avoidance and territorial defence would be classed as acute stresses, sublethal pollution (including acidification) would constitute a chronic stress.

The magnitude and duration of the cortisol response to environmental stress is dependent not only upon the nature of the stress itself, but also on the species and strain of fish (Pickering & Pottinger, 1989), the fish's age and sex (Sumpter *et al.*, 1987), temperature (Sumpter, Pickering & Pottinger, 1985; Barton & Schreck, 1987) and even, under certain circumstances, on the chemical composition of the water (Pickering & Pottinger, 1987b). Normally, however, the blood cortisol levels of acutely-stressed brown trout are temporarily elevated in excess of 100 ng ml^{-1} (Pickering *et al.*, 1982), cortisol levels in chronically-stressed brown trout (including the stress of spawning) are in the range 10–20 ng ml^{-1} (Pickering & Stewart, 1984; Pickering & Pottinger, 1987a).

Corticosteroids and disease resistance

It has been known for many years that corticosteroids are markedly immunosuppressive in mammals (see Parrillo & Fauci, 1979; Munck, Gruyre & Holbrook, 1984) and evidence has accumulated which shows that these steroids can also suppress the defence systems of fish. For example, we have shown that the blood lymphocyte count of the brown trout is significantly reduced 36 h after an acute stress (Pickering *et al.*, 1982) and that this response can be reproduced, almost exactly, by the administration of a physiological dose of cortisol in the diet of otherwise unstressed fish (Pickering, 1984). In other studies with teleost fish it has been shown that corticosteroids cause involution of lymphoid tissues such as the thymus (Chilmonczyk, 1982; Ghoneum *et al.*, 1986), inhibit inflammatory responses (MacArthur *et al.*, 1984), suppress phagocyte activity (Stave & Roberson, 1985), block lymphocyte mitogen responsiveness (Grimm, 1985; Ellsaesser & Clem, 1987) and reduce specific antibody pro-

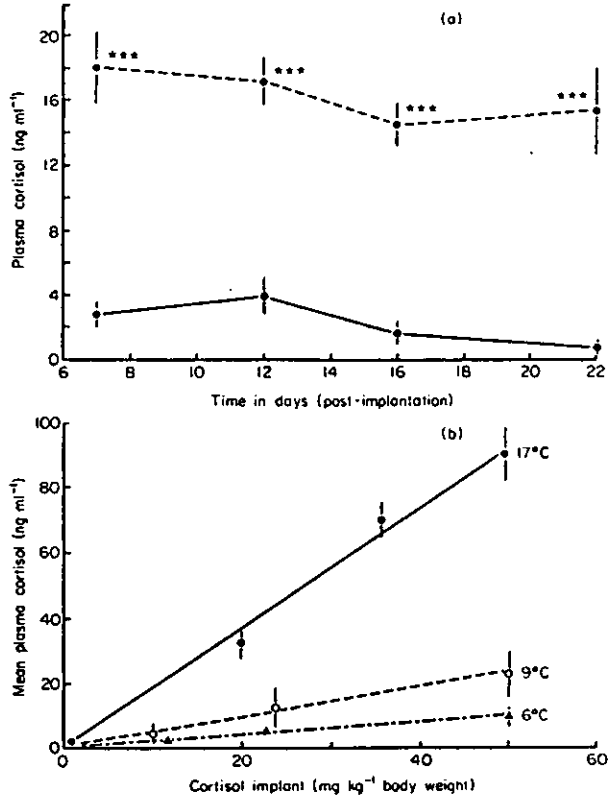


FIG. 1. (a) Chronic elevation of plasma cortisol levels in immature brown trout given a slow-release intraperitoneal implant of cortisol (30 mg kg^{-1}). Cortisol-implanted fish are represented by the broken line, sham-implanted control fish by the continuous line. Each point represents the arithmetic mean ±SE (n=10), ***P<0.001. (b) Relationship between implant size, environmental temperature and mean plasma cortisol levels of cortisol-treated sexually immature brown trout. Each point represents the arithmetic mean ±SE (n=8–16).

duction (Anderson, Roberson & Dixon, 1982; Maule, Schreck & Kaattari, 1987).

It is presumed that all, or combinations of some, of these factors are responsible for the observed increase in susceptibility to disease of stressed fish. However, with one or two notable exceptions, much of the earlier work in this field has involved the use of excessively high doses of corticosteroids, or synthetic steroids of unknown or unmeasured potency in teleost fish. In many cases, no measurements have been made of the levels of circulating steroids in treated fish and it is difficult, therefore, to evaluate the physiological significance of much of the data.

In a series of studies, we have examined the sensitivity of brown trout to experimentally elevated plasma cortisol levels using doses of the hormone well within the physiological range for this species (Pickering & Duston, 1983; Pickering, 1984; Pickering & Pottinger, 1985b). It is possible, by means of slow-release intraperitoneal implants of cortisol (see Pickering & Duston, 1983, for details), to elevate the blood cortisol levels of otherwise unstressed fish for a period of several weeks (Fig. 1a). The concentration of hormone appearing in the blood stream is directly proportional to the implant size, although this relationship is extremely temperature-sensitive (Fig. 1b). Using this technique to elevate the cortisol levels of brown trout in a predictable manner we have studied the relationship between the mean plasma cortisol levels and mortality rate, due to disease, in otherwise unstressed fish (Pickering & Duston, 1983; Pickering & Pottinger, 1985b). Cortisol-treated and sham-implanted control brown trout were subjected to the natural pathogenic challenge of an untreated lake water supply to the FBA's fish hatchery. Mortalities occurred only in the cortisol-implanted group and the main causes of disease were *Saprolegnia*-infection, furunculosis and bacterial fin-rot. A strong positive correlation was found between the mortality rate due to disease within the experimental fish populations and their mean blood cortisol levels (Fig. 2). A significant increase in mortality rate was observed when blood cortisol levels were elevated for a period of 2–4 weeks from basal values to only 10 ng ml^{-1} (Pickering & Pottinger, 1985b). This degree of cortisol elevation is well within the physiological range for brown trout and typical of fish subjected to chronic forms of environmental stress (see previous section). Thus, these studies have demonstrated that prolonged elevation of blood cortisol levels is a major factor responsible for the predisposition of chronically-stressed brown trout to infection by common fungal and bacterial pathogens. Clearly, the defence systems of brown trout are extremely sensitive to relatively low concentrations of circulating cortisol and it is of interest, therefore, that a similar degree of sensitivity has recently been demonstrated by Maule *et al.* (1987) and by Tripp *et al.* (1987) for the defence systems of another salmonid fish, the coho salmon, *Oncorhynchus kisutch*.

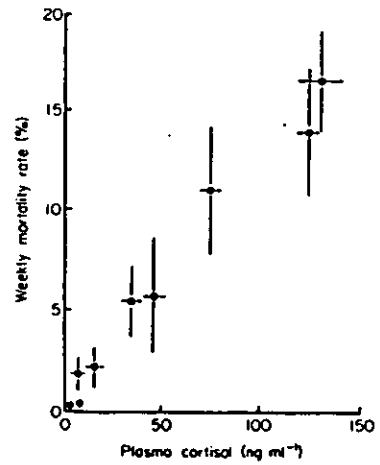


FIG. 2. Correlation between the mean plasma cortisol level of cortisol-implanted brown trout and the weekly mortality rate due to disease, during 2–10 weeks post-implantation, within the experimental fish populations. Recalculated from original data in Pickering & Duston (1983), Pickering & Pottinger (1985b) and J. F. Carragher, J. P. Sumpter, T. G. Pottinger and A. D. Pickering (unpublished).

Corticosteroids and reproduction

The synchronization of sexual development in salmonid populations, culminating in sexual maturation at a specific time of year, is under the control of a variety of hormones (see Scott & Sumpter, 1983, for review). Both stimulatory and suppressive neurosecretions from the brain/hypothalamus control the activity of the pituitary gonadotropes (Peter, 1983) which, in turn, secrete the proteinaceous hormone gonadotropin (GTH). Indeed, the latest evidence suggests that, as in higher vertebrates, there are two gonadotropins in teleost fish (Suzuki, Kawauchi & Nagahama, 1988). Circulating gonadotropins then stimulate the gonad to synthesize and secrete a range of steroid hormones (Fostier *et al.*, 1983) which control gonadal development, stimulate the appearance of secondary sexual characters and regulate the activity of this pituitary–gonadal axis by feedback mechanisms located in the brain and the pituitary gland. In female fish, the gonadal steroid oestradiol also stimulates the liver to synthesize and secrete vitellogenin, a yolk precursor, which is then incorporated into the developing oocytes (Ng & Idler, 1983).

There is good evidence in the literature that environmental stress can markedly suppress reproductive activity in mammals (Moberg, 1985); the evidence for such a suppression in fish is much more fragmentary (Billard *et al.*, 1981). However, we have shown that both acute and chronic forms of stresss can cause a significant reduction in the concentration of the androgens

testosterone and 11-ketotestosterone in the blood of sexually maturing brown trout (Pickering *et al.*, 1987). In this study it was also observed that the blood cortisol levels of the chronically-stressed fish (stressed by confinement) were still significantly elevated 1 month after the onset of the stress, thus raising the possibility that the pituitary–interrenal axis may be instrumental in suppressing sexual development in these fish.

In an attempt to resolve this question, we have recently undertaken a study to examine the effects of chronic cortisol elevation (by means of slow-release intraperitoneal implants) on the reproductive endocrinology of sexually maturing brown trout and rainbow trout, *Salmo gairdneri*, of both sexes (J. F. Carragher, J. P. Sumpter, T. G. Pottinger and A. D. Pickering, unpublished). The full details of this experiment will be published elsewhere but a summary of the main effects of cortisol elevation on the reproductive endocrinology of brown trout is presented in Figs. 3 and 4. At 18 days post-implantation there was a significant suppression of pituitary GTH and blood testosterone levels in the maturing male fish (Fig. 3b, c) and a reduction in the weight of the testes (Fig. 3e).

In the sexually maturing female brown trout, cortisol-implantation also raised blood cortisol levels and suppressed the pituitary GTH content (Fig. 4a, b). Moreover, the plasma levels of testosterone and oestradiol were markedly suppressed in the cortisol-treated fish (Fig. 4c, d). The reduction in oestradiol levels fitted well with a suppression of the concentration of yolk precursers in transit from the liver to the ovary (Fig. 4e) and, again, the overall effect of these changes was a significant reduction in gonad weight (Fig. 4f).

Thus, the effects of chronic stress on sexual maturation in brown trout can be mimicked by chronic cortisol elevation in otherwise unstressed fish and it seems most likely, therefore, that the deleterious effects of stress on the pituitary–gonadal axis are largely mediated by corticosteroids released as a consequence of activation of the hypothalamic–pituitary–interrenal axis. Similar functional links between the two axes, resulting in the suppression of many reproductive characteristics, have been demonstrated in mammals (see Moberg, 1985).

Clearly, much more work is now needed on the precise mechanisms involved in this cortisol-mediated suppression of reproductive endocrinology in fish, and on the consequences, in terms of gamete quality and quantity, for reproductive success. However, our data strongly suggest that incidences of chronic stress in the prespawning period will have deleterious effects on sexual maturation and reproductive success. Thus, environmental stresses which, although not directly lethal themselves, may

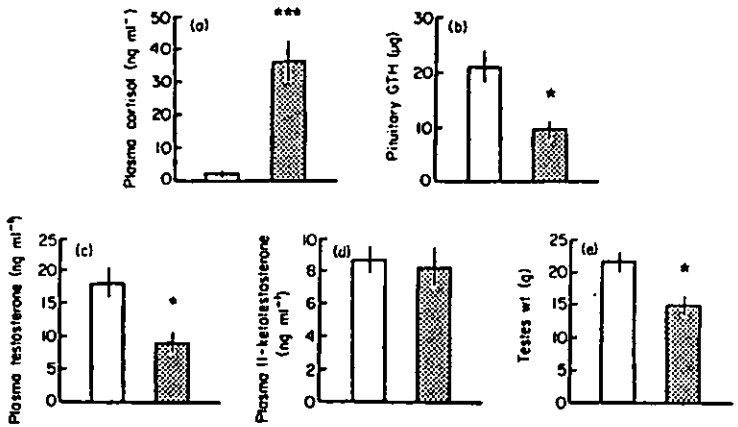


FIG. 3. The effect of 18 days cortisol implantation (60 mg kg^{-1}) on reproductive endocrinology and gonad weight of sexually maturing male brown trout. Each value is the arithmetic mean ±SE. Open columns represent the sham-implanted control fish (n=18), shaded columns the cortisol-implanted fish (n=19). $^{*}P<0.05$, $^{***}P<0.001$.

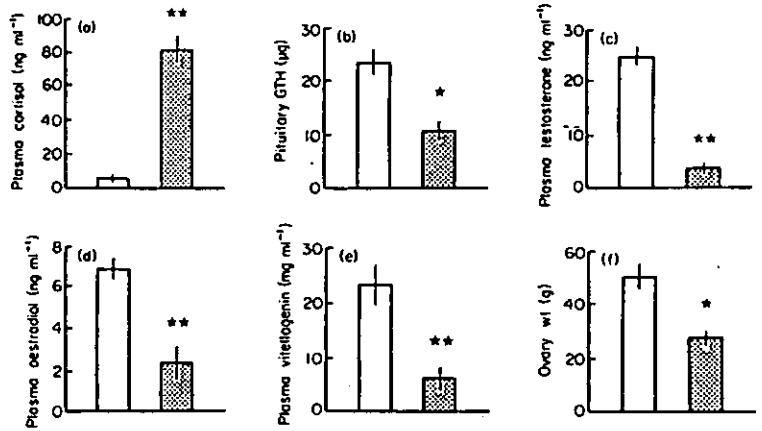


FIG. 4. The effect of 18 days cortisol implantation (60 mg kg^{-1}) on reproductive endocrinology and gonad weight of sexually maturing female brown trout. Each value is the arithmetic mean ±SE. Open columns represent the sham-implanted control fish (n=19), shaded columns the cortisol-implanted fish (n=8). $^{*}P<0.05$, $^{**}P<0.01$.

reduce the chances of survival of brown trout not only by predisposing the fish to disease (see previous section) but also by inhibiting successful reproduction.

Chronic stress and the natural environment

The present paper has shown that elevation of blood cortisol levels, as a result of environmental stress, can have debilitating effects on the defence systems and on the reproductive endocrinology of brown trout. Most of the examples used to illustrate the phenomenon of environmental stress have been related to aquacultural conditions (handling, crowding, confinement, prophylactic treatment, etc.) and it has been demonstrated that the elevation of blood cortisol levels from a basal concentration of <2 ng ml^{-1} to only 10 ng ml^{-1} is sufficient to predispose brown trout to disease and that a chronic cortisol level of 30–40 ng ml^{-1} is sufficient to suppress sexual development. It should be recognized, however, that these data were obtained using domesticated strains of brown trout under intensive culture conditions and that we have little information concerning the physiology of natural trout populations. This is clearly an area which requires urgent attention, provided that the problems of sampling stress can be overcome. The limited available evidence shows that first generation fish from wild stocks are more sensitive, with regard to their cortisol response, than are domesticated strains (Woodward & Strange, 1987). This is not surprising, but it emphasizes that the damaging effects of stress, demonstrated in our own studies, are probably minimal compared to the potential effects on wild fish.

The bulk of the evidence presented so far indicates that chronic, or continuous, forms of stress have the most serious consequences for survival and it has been shown that under such circumstances the fish may acclimate to the stress after several days or even weeks, with a resultant reduction in blood cortisol levels despite the continued presence of the stress. Indeed if the process of acclimation did not occur, the fish's chance of survival would be greatly reduced.

Man's deleterious influence on the aquatic environment has created conditions of chronic stress, in the form of sublethal pollution, which might be expected to increase disease susceptibility and reduce reproductive success in natural fish populations. Indeed, several examples of exactly such debilitating effects have been reported. Thus, the incidence of fish dis-

eases is greater in polluted environments than in clean ones (Pippy & Hare, 1969; Brown *et al.*, 1979; Sindermann, 1979; Moller, 1985). More specifically, Hetrick, Knittel & Fryer (1979) and Knittel (1981) have shown that exposure of rainbow trout to copper increases the fish's susceptibility to viral and bacterial infections. Copper is a potent stimulator of the HPI axis in salmonid fish (Donaldson & Dye, 1975). Furthermore, exposure of fish to low pH can cause a suppression of plasma androgen levels (Freeman *et al.*, 1983), a reduction in yolk deposition (Ruby, Aczel & Craig, 1977), a delay in ovulation (Tam & Payson, 1986) and a loss of egg viability (Menendez, 1976; Craig & Baski, 1977; Lee & Gerking, 1980). Similarly, Macek (1968) found that sublethal concentrations of DDT could suppress gamete quality in both male and female brook trout, *Salvelinus fontinalis*.

On the basis of our work with brown trout, it is suggested that chronic activation of the HPI axis is a common mechanism that could explain all these examples. This hypothesis is further supported by the observation that sublethal pollution, in the form of low pH, caused a 2-week elevation of blood cortisol levels in the char, *Salvelinus alpinus* (Jones, Brown & Hara, 1987), a 3-week elevation of blood cortisol levels (in excess of 150 ng ml^{-1}) in the rainbow trout (Brown *et al.*, 1984) and a 6-month elevation of blood cortisol levels in the brook trout (Tam *et al.*, 1987). Other forms of sublethal pollution can also provoke a similar response. Thus, Bennett & Wolke (1987) reported that exposure of rainbow trout to sublethal concentrations of the pesticide, endrin, caused a chronic elevation of blood cortisol levels in excess of 60 days. Clearly, sublethal pollution can cause a prolonged stimulation of the HPI axis in salmonid fish; it is also possible that the fish may not be able to acclimate fully to this form of stress. The magnitude and duration of such interrenal activation in the above examples would be more than enough to both predispose brown trout to disease and to suppress sexual reproduction.

In conclusion, the current evidence strongly suggests that chronic interrenal activation, as a result of various forms of environmental stress, has deleterious consequences for the survival of brown trout (and salmonid fish in general) both under aquaculture conditions and in the wild. A knowledge of the physiological and endocrinological changes in natural trout populations could provide an early warning of potential disease and recruitment problems within the population.

Acknowledgments

The author is grateful to Mrs J. Pollard, Miss J. M. Fletcher and Miss K. M. Atkinson (FBA) for care and maintenance of the experimental fish, to Dr J. M. Elliott (FBA) for the invitation to present this paper, to Dr T. G. Pottinger (FBA) for his critical comments on the manuscript, to Mr T. I. Furnass (FBA) for the artwork and to Dr J. P. Sumpter and J. F. Carragher (Brunel University) for access to unpublished data. Much of the original research on which this review is based was financed by NERC and MAFF.

References

Anderson D.P., Roberson B.S. & Dixon O.W. (1982) Immunosuppression induced by a corticosteroid or an alkylating agent in rainbow trout (*Salmo gairdneri*) administered a *Yersinia ruckeri* bacterin. *Developmental and Comparative Immunology*, 2 (Suppl.), 197–204.

Barton B.A. & Schreck C.B. (1987) Influence of acclimation temperature on interrenal and carbohydrate stress responses in juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture*, 62, 299–310.

Bennett R.O. & Wolke R.E. (1987) The effect of sublethal endrin exposure on rainbow trout *Salmo gairdneri* Richardson. I. Evaluation of serum cortisol concentrations and immune responsiveness. *Journal of Fish Biology*, 31, 375–385.

Billard R., Bry C. & Gillet C. (1981) Stress, environment and reproduction in teleost fish. *Stress and Fish* (Ed. A. D. Pickering), pp. 185–208. Academic Press, London.

Brown E.R., Koch E., Sinclair T.F., Spitzer R. & Callaghan O. (1979) Water pollution and diseases in fish (an epizootiologic survey). *Journal of Environmental Pathology and Toxicology*, 2, 917–925.

Brown S.B., Eales J.G., Evans R.E. & Hara T.J. (1984) Interrenal, thyroidal, and carbohydrate responses of rainbow trout (*Salmo gairdneri*) to environmental acidification. *Canadian Journal of Fisheries and Aquatic Sciences*, 41, 36–45.

Chan D.K.O. & Woo N.Y.S. (1978) Effect of cortisol on the metabolism of the eel, *Anguilla japonica*. *General and Comparative Endocrinology*, 35, 205–215.

Chilmonczyk S. (1982) Rainbow trout lymphoid organs: Cellular effects of corticosteroids and anti-

thymocyte serum. *Developmental and Comparative Immunology*, 6, 271–280.

Craig G.R. & Baski W.F. (1977) The effects of depressed pH on flagfish reproduction, growth and survival. *Water Research*, 11, 621–626.

Dave G., Johansson-Sjöbeck M.-L., Larsson A., Lewander K. & Lidman U. (1979) Effects of cortisol on the fatty acid composition of the total blood plasma lipids in the European eel. *Anguilla anguilla* L. *Comparative Biochemistry and Physiology*, 64A, 37–40.

Donaldson E.M. (1981) The pituitary-interrenal axis as an indicator of stress in fish. *Stress and Fish* (Ed. A. D. Pickering), pp. 11–47. Academic Press, London.

Donaldson E.M. & Dye H.M. (1975) Corticosteroid concentrations in sockeye salmon (*Oncorhynchus nerka*) exposed to low concentrations of copper. *Journal of the Fisheries Research Board of Canada*, 32, 533–539.

Ellsaesser C.F. & Clem L.W. (1987) Cortisol-induced hematologic and immunologic changes in channel catfish (*Ictalurus punctatus*). *Comparative Biochemistry and Physiology*, 87A, 405–408.

Fostier A., Jalabert B., Billard R., Breton B. & Zohar Y. (1983) The gonadal steroids. *Fish Physiology*, Vol. IXA (Eds W. S. Hoar, D. J. Randall and E. M. Donaldson), pp. 277–372. Academic Press, New York.

Freeman H.C., Sangalang G.B., Burns G. & McMenemy M. (1983) The blood sex hormone levels in sexually mature male Atlantic salmon (*Salmo salar*) in the Westfield River (pH 4.7) and the Medway River (pH 5.2), Nova Scotia. *The Science of the Total Environment*, 32, 87–91.

Fryer J.N. & Peter R.E. (1977) Hypothalamic control of ACTH in goldfish. I. Corticotrophin-releasing factor activity in teleost brain tissue extracts. *General and Comparative Endocrinology*, 33, 196–201.

Ghoneum M.H., Egami N., Jiri K. & Cooper E.L. (1986) Effect of corticosteroids on the thymus of the fish *Oryzias latipes*. *Developmental and Comparative Immunology*, 10, 35–45.

Grimm A.S. (1985) Suppression by cortisol of the mitogen-induced proliferation of peripheral blood leucocytes from plaice, *Pleuronectes platessa* L. *Fish Immunology* (Eds M. J. Manning and M. F. Tatner), pp. 263–271. Academic Press, London.

Henderson I.W. & Garland H.O. (1980) The interrenal gland in pisces. Part 2. Physiology. *General, Comparative and Clinical Endocrinology of the Adrenal Cortex*, Vol. 3 (Eds I. Chester Jones and I. W. Henderson), pp. 473–523. Academic Press, London.

Hetrick F.M., Knittel M.D. & Fryer J.L. (1979) Increased susceptibility of rainbow trout to infectious hematopoietic necrosis virus after exposure to copper. *Applied and Environmental Microbiology*, 37, 198–201.

Jones K.A., Brown S.B. & Hara T.J. (1987) Behavioural and biochemical studies of onset and recovery from acid stress in Arctic char (*Salvelinus alpinus*). *Canadian Journal of Fisheries and Aquatic Sciences*, 44, 373–381.

June F.C. (1977) Reproductive patterns in seventeen species of warmwater fishes in a Missouri River reservoir. *Environmental Biology of Fishes*, 2, 285–296.

Knittel M.D. (1981) Susceptibility of steelhead trout *Salmo gairdneri* Richardson to redmouth infection *Yersinia ruckeri* following exposure to copper. *Journal of Fish Diseases*, 4, 33–40.

Leach G.J. & Taylor M.H. (1982) The effects of cortisol treatment on carbohydrate and protein metabolism in *Fundulus heteroclitus*. *General and Comparative Endocrinology*, 48, 76–83.

Lee R.M. & Gerking S.D. (1980) Survival and reproductive performance of the desert pupfish, *Cyprinodon n. nevadensis* (Eigenmann and Eigenmann), in acid waters. *Journal of Fish Biology*, 17, 507–515.

Lidman U., Dave G., Johansson-Sjöbeck M.-L., Larsson A. & Lewander K. (1979) Metabolic effects of cortisol in the European eel, *Anguilla anguilla* (L.). *Comparative Biochemistry and Physiology*, 63A, 339–344.

MacArthur J.I., Fletcher T.C., Pirie B.J.S., Davidson R.J.L. & Thompson A.W. (1984) Peritoneal inflammatory cells in plaice, *Pleuronectes platessa* L.: effects of stress and endotoxin. *Journal of Fish Biology*, 25, 69–81.

Macek K.J. (1968) Reproduction in brook trout (*Salvelinus fontinalis*) fed sublethal concentrations of DDT. *Journal of the Fisheries Research Board of Canada*, 25, 1787–1796.

Maule, A.G., Schreck, C.B. & Kaattari S.L. (1987) Changes in the immune system of coho salmon (*Oncorhynchus kisuich*) during the parr-to-smolt transformation and after implantation of cortisol. *Canadian Journal of Fisheries and Aquatic Sciences*, 44, 161–166.

Menendez R. (1976) Chronic effects of reduced pH on brook trout (*Salvelinus fontinalis*). *Journal of the Fisheries Research Board of Canada*, 33, 118–123.

Moberg G.P. (1985) Influence of stress on reproduction: Measure of well-being. *Animal Stress* (Ed. G. P. Moberg), pp. 245–267. American Physiological Society, Bethesda.

Moller H. (1985) A critical review on the role of pollution as a cause of fish diseases. *Fish and Shellfish Pathology* (Ed. A. E. Ellis), pp. 169–182. Academic Press, London.

Munck A., Gruyre P.M. & Holbrook N.J. (1984) Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocrine Reviews*, 5, 25–44.

Ng T.B. & Idler D.R. (1983) Yolk formation and differentiation in teleost fishes. *Fish Physiology*, Vol. IXA (Eds W. S. Hoar, D. J. Randall and E. M. Donaldson), pp. 373–404. Academic Press, New York.

Parillo J.E. & Fauci A.S. (1979) Mechanisms of glucocorticoid action in immune processes. *Annual Review of Pharmacology and Toxicology*, 19, 179–201.

Peter R.E. (1983) The brain and neurohormones in teleost reproduction. *Fish Physiology*, Vol. IXA (Eds W. S. Hoar, D. J. Randall and E. M. Donaldson), pp. 97–135. Academic Press, New York.

Pickering A.D. (1984) Cortisol-induced lymphocytopenia in brown trout, *Salmo trutta* L. *General and Comparative Endocrinology*, **53**, 252–259.

Pickering A.D. & Duston J. (1983) Administration of cortisol to brown trout, *Salmo trutta* L., and its effects on the susceptibility to *Saprolegnia* infection and furunculosis. *Journal of Fish Biology*, **23**, 163–175.

Pickering A.D. & Pottinger T.G. (1984) Acclimation of the brown trout, *Salmo trutta* L., to the stress of daily malachite green treatment. *Aquaculture*, **44**, 145–152.

Pickering A.D. & Pottinger T.G. (1985a) Factors influencing blood cortisol levels in brown trout under intensive culture conditions. *Current Trends in Comparative Endocrinology* (Eds B. Lofts and W. N. Holmes), pp. 1239–1242. Hong Kong University Press, Hong Kong.

Pickering A.D. & Pottinger T.G. (1985b) Cortisol can increase the susceptibility of brown trout, *Salmo trutta* L., to disease without reducing the white blood cell count. *Journal of Fish Biology*, **27**, 611–619.

Pickering A.D. & Pottinger T.G. (1987a) Lymphocytopenia and interrenal activity during sexual maturation in the brown trout, *Salmo trutta* L. *Journal of Fish Biology*, **30**, 41–50.

Pickering A.D. & Pottinger T.G. (1987b) Poor water quality suppresses the cortisol response of salmonid fish to handling and confinement. *Journal of Fish Biology*, **30**, 363–374.

Pickering A.D. & Pottinger T.G. (1987c) Crowding causes prolonged leucopenia in salmonid fish, despite interrenal acclimation. *Journal of Fish Biology*, **30**, 701–712.

Pickering A.D. & Pottinger T.G. (1989) Stress responses and disease resistance in salmonid fish: effects of chronic elevation of plasma cortisol. *Fish Physiology and Biochemistry* (in press).

Pickering A.D., Pottinger T.G., Carragher J.F. & Sumpter J.P. (1987) The effects of acute and chronic stress on the levels of reproductive hormones in the plasma of mature male brown trout, *Salmo trutta* L. *General and Comparative Endocrinology*, **68**, 249–259.

Pickering A.D., Pottinger T.G. & Christie P. (1982) Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: a time-course study. *Journal of Fish Biology*, **20**, 229–244.

Pickering A.D. & Stewart A. (1984) Acclimation of the interrenal tissue of the brown trout, *Salmo trutta* L., to chronic crowding stress. *Journal of Fish Biology*, **24**, 731–740.

Pippy J.H.C. & Hare G.M. (1969) Relationship of river pollution to bacterial infection in salmon (*Salmo salar*) and suckers (*Catostomus commersoni*). *Transactions of the American Fisheries Society*, **98**, 685–690.

Ruby S.M., Aczel J. & Craig G.R. (1977) The effects of depressed pH on oogenesis in flagfish, *Jordanella floridae*. *Water Research*, **11**, 757–762.

Schreck C.B. (1981) Stress and compensation in teleostean fishes: Response to social and physical factors. *Stress and Fish* (Ed. A. D. Pickering), pp. 295–321. Academic Press, London.

Scott A.P. & Sumpter J.P. (1983) The control of trout reproduction: Basic and applied research on hormones. *Control Processes in Fish Physiology* (Eds J. C. Rankin, T. J. Pitcher and R. Duggan), pp. 200–220. Croom Helm, London.

Sheridan M.A. (1986) Effects of thyroxin, cortisol, growth hormone, and prolactin on lipid metabolism of coho salmon, *Oncorhynchus kisutch*, during smoltification. *General and Comparative Endocrinology*, **64**, 220–238.

Sindermann C.J. (1979) Pollution-associated diseases and abnormalities of fish and shellfish: A review. *Fishery Bulletin, Seattle*, **76**, 717–751.

Snieszko S.F. (1974) The effects of environmental stress on outbreaks of infectious disease of fishes. *Journal of Fish Biology*, **6**, 197–208.

Stave J.W. & Roberson B.S. (1985) Hydrocortisone suppresses the chemiluminescent response of striped bass phagocytes. *Developmental and Comparative Immunology*, **9**, 77–84.

Storer J.H. (1967) Starvation and the effects of cortisol in the goldfish. *Comparative Biochemistry and Physiology*, **20**, 939–948.

Sumpter J.P., Carragher J., Pottinger T.G. & Pickering A.D. (1987) The interaction of stress and reproduction in trout. *Reproductive Physiology of Fish* (Eds D. R. Idler, L. W. Crim and J. M. Walsh), pp. 299–302. Memorial University of Newfoundland, St. Johns.

Sumpter J.P., Pickering A.D. & Pottinger T.G. (1985) Stress-induced elevation of plasma α-MSH and endorphin in brown trout, *Salmo trutta* L. *General and Comparative Endocrinology*, **59**, 257–265.

Suzuki K., Kawauchi H. & Nagahama Y. (1988) Isolation and characterization of two distinct gonadotropins from chum salmon pituitary glands. *General and Comparative Endocrinology*, **71**, 292–302.

Tam W.H., Birkett L., Makaran R., Payson P.D., Whitney D.K. & Yu C.K.-C. (1987) Modification of carbohydrate metabolism and liver vitellogenic function in brook trout (*Salvelinus fontinalis*) by exposure to low pH. *Canadian Journal of Fisheries and Aquatic Sciences*, **44**, 630–635.

Tam W.H. & Payson P.D. (1986) Effects of chronic exposure to sublethal pH on growth, egg production, and ovulation in brook trout, *Salvelinus fontinalis*. *Canadian Journal of Fisheries and Aquatic Sciences*, **43**, 275–280.

Tripp R.A., Maule A.G., Schreck C.B. & Kaattari S.L. (1987) Cortisol mediated suppression of salmonid lymphocyte responses *in vitro*. *Developmental and Comparative Immunology*, **11**, 565–576.

Wedemeyer G.A. (1970) The role of stress in the disease resistance of fishes. *Special Publications of the American Fisheries Society*, **5**, 30–35.

Woodward C.C. & Strange R.J. (1987) Physiological stress responses in wild and hatchery-reared rainbow trout. *Transactions of the American Fisheries Society*, **116**, 574–579.

J. Fish Biol. (1989) 34, 757–768

Differences in the sensitivity of brown trout, *Salmo trutta* L., and rainbow trout, *Salmo gairdneri* Richardson, to physiological doses of cortisol

A. D. PICKERING, T. G. POTTINGER AND J. F. CARRAGHER*

*Institute of Freshwater Ecology, Windermere Laboratory, The Ferry House, Far Sawrey, Nr. Ambleside, Cumbria LA22 0LP, U.K. and *Department of Applied Biology, Brunel University, Uxbridge, Middlesex UB8 3PH, U.K.*

(*Received 22 September 1988, Accepted 14 November 1988*)

Interspecific differences in the stress response of fish may be due, in part, to differences in the sensitivity of target tissues to cortisol. The relative response of brown and rainbow trout to a standardized dose of cortisol was assessed by monitoring condition (*K* factor), the number of circulating lymphocytes and mortality due to disease, following cortisol treatment. Cortisol implantation resulted in a significant decline in *K* factor and number of circulating lymphocytes in immature brown trout, but not in immature rainbow trout, despite plasma cortisol levels being similar in both cases. Cortisol implantation in mature brown and rainbow trout significantly increased the mortality rate due to bacterial and fungal infection compared with control fish. Furthermore, the mortality rate due to disease was significantly greater in brown trout than rainbow trout, despite both groups receiving similar doses of steroid.

I. INTRODUCTION

Corticosteroid release from the adrenal cortex (or its homologue) is a characteristic response of most vertebrates to stressful situations. The catabolic effects of corticosteroid hormones (lipolysis and gluconeogenesis) complement the glycolytic action of catecholamines to give the animal access to energy reserves not normally available to it, energy which the animal can then use to avoid or overcome the stress (Moberg, 1985).

Salmonid fish are sensitive to many forms of environmental stress (Donaldson, 1981; Schreck, 1981) and respond by increasing the rate of secretion of cortisol, the predominant corticosteroid in teleost fish (Henderson & Garland, 1980). However, prolonged elevation of blood cortisol levels has been shown to predispose some species of salmonid fish to infectious diseases (Pickering & Duston, 1983; Pickering & Pottinger, 1985; Woo *et al.*, 1987), an effect almost certainly mediated by the suppressive action of corticosteroids on the fish's defence systems (Anderson *et al.*, 1982; MacArthur *et al.*, 1984; Grimm, 1985; Maule *et al.*, 1987). There is now an accumulating body of evidence linking various forms of stress to a subsequent increase in the susceptibility of fish to a wide range of infectious diseases (Johansson & Bergstrom, 1977; Hetrick *et al.*, 1979; Hanson & Grizzle, 1985; see also Wedemeyer & McLeay, 1981) and we believe that elevated cortisol levels play a major role in this phenomenon. In general, rainbow trout are more amenable than brown trout to aquaculture conditions and, in our experience, show fewer problems of stress-related diseases. In a series of studies, G. A. Wedemeyer has demonstrated marked interspecific differences in the response of salmonid

0022–1112/89/050757 + 12 $03.00/0

TABLE I. The species, strain and age of the fish used in the present study

Experiment no.	Date	Species	Strain	Hatching date
1	August 1986	Brown trout	Dunsop Bridge 2	February 1985
		Rainbow trout	Annandale I	May 1985
2	September–October 1987	Brown trout	Dunsop Bridge 2a	February 1985
		Rainbow trout	Cloan	January 1986

fish to the common aquacultural stresses of handling, crowding, sudden temperature change and formalin treatment (Wedemeyer, 1971, 1972, 1973, 1976). These differences were manifest in a wide range of physiological processes including ionic regulation, carbohydrate and sterol metabolism, acid/base balance, oxygen consumption and interrenal ascorbate metabolism. Such differences between species with regard to their sensitivity to stress may be reflected not only by quantitative differences in the levels of circulating stress hormones, such as cortisol (Wedemeyer & Yasutake, 1974), but also by differences in the sensitivity of the target tissues to the hormone.

It has been shown previously that cortisol treatment causes a reduction in the coefficient of condition (*K* factor) of salmonid fish (Pickering & Duston, 1983) and that the steroid can also depress the number of circulating lymphocytes (McLeay, 1973; Pickering, 1984). The present study investigates the possibility that interspecific differences in the stress response may be caused, at least in part, by differences in the sensitivity of the target tissues to cortisol, by comparing the sensitivity of two species of trout, the brown trout, *Salmo trutta* L., and the rainbow trout, *S. gairdneri* Richardson, to cortisol administration. Care was taken to ensure that the steroid was administered at similar physiological doses to each species, and the responses of the fish were monitored in terms of changes in the coefficient of condition, circulating lymphocyte counts and mortality rate due to disease.

II. MATERIALS AND METHODS

FISH

Details of the species, strain and age of the fish used in this study are given in Table I. All the fish, with the exception of the brown trout used in experiment 2 (Dunsop Bridge 2a), were obtained as eyed-ova and hatched at the I.F.E.'s Windermere hatchery. The fish were reared, via fry-troughs, in large (1500 l), circular, outdoor, fibreglass tanks each supplied with a constant flow of Windermere lake water (35 l min^{-1}) and fed, once daily, with commercial trout pellets at a rate of 1–2% body weight day^{-1} (exact rate dependent upon temperature). The brown trout for experiment 2 were brought in as 1-year-old fish and then reared as described above.

EXPERIMENT 1: EFFECTS OF CORTISOL IMPLANTATION (30 mg kg^{-1}) IN IMMATURE FISH ON THE COEFFICIENT OF CONDITION AND ON LYMPHOCYTE LEVELS

One hundred and sixty sexually immature 1+ brown trout (mean weight 170 g) were evenly distributed into four rearing tanks and left for a period of 2 weeks to recover from the handling stress. Similarly, 160 sexually immature, 1+ rainbow trout (mean weight 340 g) were distributed into four rearing tanks and left for a period of 2 weeks. For each species, the fish from two of the tanks were then netted, lightly anaesthetized (2-phenoxyethanol

1:2000) and given an intraperitoneal implant of cortisol suspended in molten (40° C) cocoa butter (10 mg ml^{-1}) to give a final dose of 30 mg cortisol kg^{-1} fish. Fish from the remaining two tanks were given a similar implant of cocoa-butter only (sham-implanted controls). The fish were then returned to their rearing tanks and samples of five fish per tank were taken at 7, 12, 16 and 22 days post-implantation. During this sampling period the water temperature varied between 13·6 and 16·4° C and the fish were given a daily prophylactic treatment with malachite green (2 ppm) to prevent fungal infection. At each sampling time fish were lightly anaesthetized, weighed and measured and a blood sample was taken from the caudal vessels by means of an heparinized syringe. A 50-µl aliquot of blood was diluted with trout Ringer solution (final dilution 1:40 000), fixed with Lugol's iodine and sedimented on to a glass microscope slide for total blood cell counts. Differential blood cell counts were made on air-dried, methanol-fixed, stained (haematoxylin and eosin) smears and the absolute lymphocyte concentration was calculated from the total and differential cell counts. Blood plasma was stored at −70° C until assayed for cortisol by means of a fully-validated radioimmunoassay (see Pickering *et al.*, 1987*b* for details). At the end of the experiment (22 days post-implantation) the remaining fish in each tank were then weighed and measured.

EXPERIMENT 2: EFFECTS OF CORTISOL IMPLANTATION (60 mg kg^{-1}) IN SEXUALLY MATURE FISH ON THE COEFFICIENT OF CONDITION AND ON DISEASE RESISTANCE

Eighty sexually mature 2+ brown trout (mean weight 404 g; sex ratio, ♂:♀, 1·32) were evenly distributed into four outdoor rearing tanks and left for 2 weeks to acclimate to the new conditions. The fish from two tanks were then given an intraperitoneal implant of cortisol suspended in molten cocoa butter (60 mg cortisol kg^{-1} fish), the two remaining tanks serving as sham-implanted controls (cocoa butter only). No prophylactic treatments were given and the mortality rate following implantation was monitored on a daily basis. In view of the high mortality rate in the cortisol-treated fish, this experiment was terminated 18 days after implantation and the surviving fish were anaesthetized, weighed and measured. A blood sample was taken from the caudal vessels of each fish for subsequent cortisol radioimmunoassay. The fish were then killed by a blow to the head and the sex and state of maturity determined.

A similar experiment with 1+ rainbow trout (mean weight 346 g) was run in parallel to the brown trout study, but in this case each tank contained 50 fish and, in view of the low mortality rate, the experiment was allowed to run for a period of 36 days post-implantation. At the end of this period the surviving fish were sampled as above. The sex ratio (♂:♀) within the rainbow trout population was 1·42, and 65% of the males were undergoing sexual maturation. None of the females showed any signs of maturation. The water temperature during experiment 2 ranged between 11·7 and 16·5° C.

STATISTICAL ANALYSES

Plasma cortisol levels, *K* factor and lymphocyte counts were analysed by one-way or multifactorial analysis of variance (ANOVA, Genstat) with treatment, species and time as factors. Tank and fish were used as blocking effects to give a nested error structure with which to assess the significance of the factors and their interactions. From a plot of the residuals against fitted values, appropriate transformations were selected, where necessary, to improve homogeneity of variance. The levels of significance given are derived from these values but, for ease of presentation, data are given as arithmetic means ± S.E.M. Exact probability tests or 2 × 2 contingency tables (χ^2 analysis) were used to estimate the significance of the differences in mortality and incidence of disease between the various groups of fish in experiment 2.

III. RESULTS

EXPERIMENT 1: IMPLANTATION OF CORTISOL (30 mg kg^{-1}) IN IMMATURE FISH

Cortisol implantation resulted in a significant and stable elevation of plasma cortisol levels in both brown trout and rainbow trout ($P<0·001$ in each case)

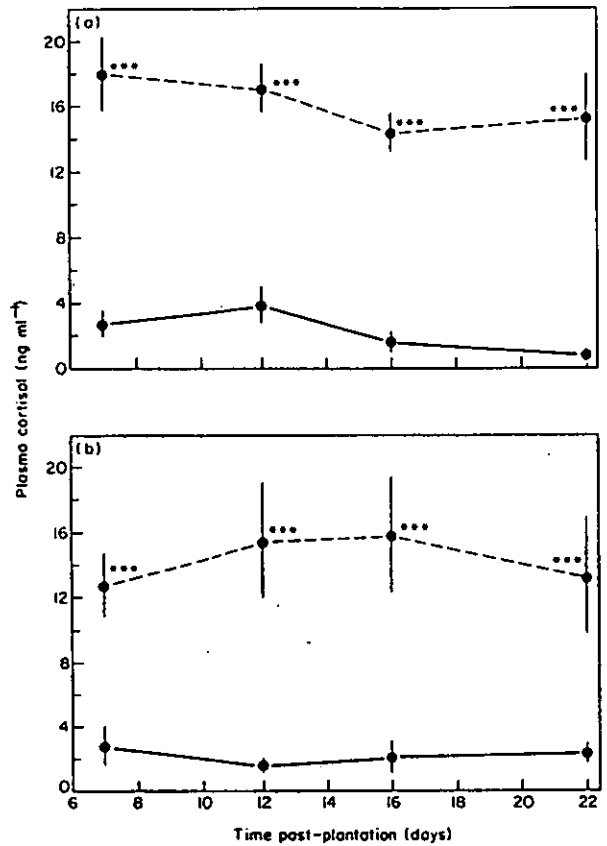


FIG. 1. The effect of cortisol implantation (30 mg kg^{-1}) on plasma cortisol levels of (a) immature brown trout and (b) rainbow trout. – – –, Cortisol-implanted fish; ———, cocoa-butter-implanted control fish. Each point represents the arithmetic mean ± S.E.M. ($n=10$); ***, $P<0·001$.

during the 22-day post-implantation period (Fig. 1). Implanted fish had mean plasma cortisol levels of 16·3 ± 1·0 and 14·3 ± 1·6 ng ml^{-1} for brown trout and rainbow trout, respectively, compared with 2·3 ± 0·4 and 2·2 ± 0·4 ng ml^{-1} for the cocoa-butter-implanted control fish ($n=40$ in each case). No significant differences in cortisol levels were detected between the two species at any time, with the single exception of the control brown trout which had slightly, but significantly, elevated plasma cortisol levels at 12 days post-implantation (3·9 ± 1·0 ng ml^{-1}, $P<0·05$).

During the course of this experiment, no mortalities were recorded for either species. However, cortisol-implantation resulted in a significant suppression of the coefficient of condition (*K* factor = 100 W/L^3) of brown trout at both 7 and 22 days post-implantation ($P<0·01$ in each case) but no such response was observed in the rainbow trout [Fig. 2(a)]. Similarly, cortisol-treatment caused a significant reduction in the number of circulating lymphocytes at 22 days post-implantation in the

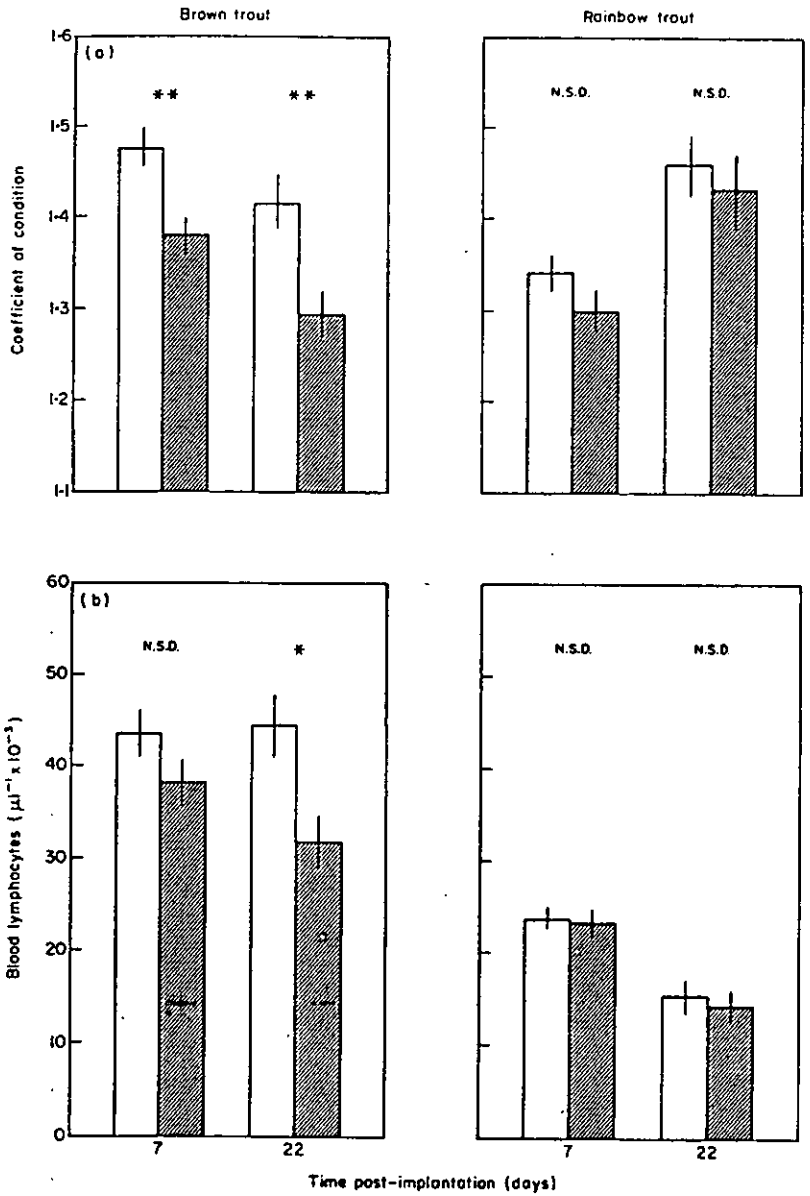


FIG. 2. The effect of cortisol implantation (30 mg kg^{-1}) on (a) the coefficient of condition and (b) the blood lymphocyte count of immature brown trout and rainbow trout at 7 and 22 days post-implantation. □, Cocoa-butter-implanted control fish, ▨, cortisol-implanted fish. Values are arithmetic means ± S.E.M. ($n = 10$ in all cases except for the coefficient of condition at 22 days post-implantation where $n = 50$); *, $P < 0.05$; **, $P < 0.01$.

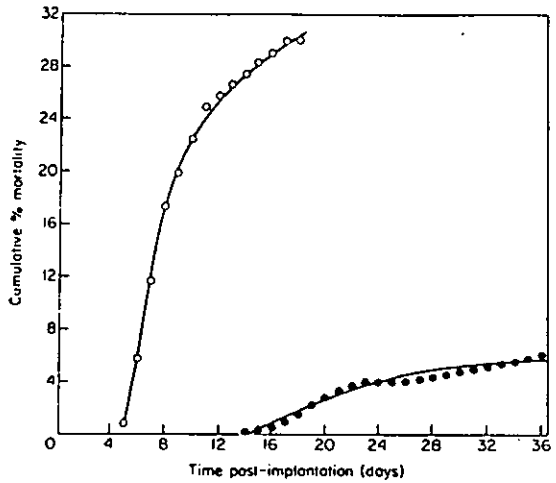


FIG. 3. The effect of cortisol implantation (60 mg kg^{-1}) on the mortality rate of mature brown trout, (○) and rainbow trout, (●). The causes of death were diagnosed as furunculosis and/or severe *Saprolegnia* infection. No mortalities occurred in cocoa-butter-implanted control fish of either species.

brown trout ($P < 0.05$) but not in the rainbow trout [Fig. 2(b)]. The difference in lymphocyte numbers between control brown trout and rainbow trout ($P < 0.001$) is a species difference which we have consistently observed in different strains of hatchery-reared brown trout and rainbow trout (e.g., Pickering *et al.*, 1987*a*). However, we have no explanation for the decrease in lymphocyte counts between 7 days and 22 days post-implantation in both cortisol-treated and control rainbow trout.

EXPERIMENT 2: IMPLANTATION OF CORTISOL (60 mg kg^{-1}) IN SEXUALLY MATURE FISH

Cortisol implantation significantly increased the mortality rate of both brown trout and rainbow trout (Fig. 3; $P < 0.001$ for each species). The presumptive cause of death was identified as furunculosis (characterized by internal haemorrhage, a bloody exudate from the vent and occasional furuncles on the flank of the fish) combined with severe fungal infection in the brown trout but not in the rainbow trout. Both sexes were affected in the brown trout population; the dead rainbow trout included both mature males and sexually immature fish. Because of the high mortality in the cortisol-treated brown trout population, this part of the experiment was terminated at 18 days post-implantation. Of the surviving brown trout, more than 50% of the cortisol-treated fish had visible signs of fungal infection, compared with less than 10% of the cocoa-butter-implanted control fish ($P < 0.005$). No mortalities occurred in the control groups of either species during the course of the experiment and no signs of disease were observed in any of the control rainbow trout. The rainbow trout study was allowed to continue for a period of 36 days post-implantation.

Blood cortisol levels were significantly elevated in all groups of cortisol-implanted fish [Fig. 4(a); $P < 0.001$ in all cases]. However, highly significant differences in

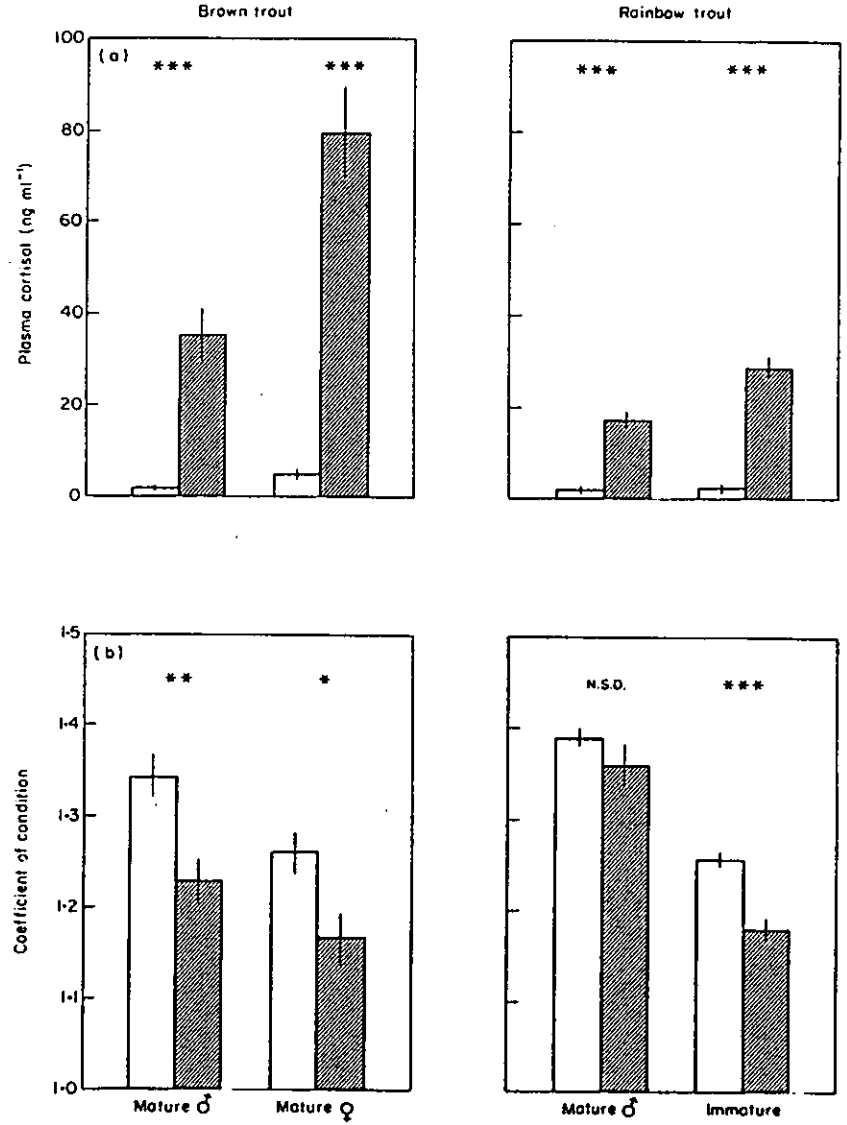


FIG. 4. The effect of cortisol implantation (60 mg kg^{-1}) on (a) blood cortisol levels and (b) the coefficient of condition of mature brown trout and rainbow trout at 18 and 36 days post-implantation, respectively. □, Cocoa-butter-implanted control fish; ▩, cortisol-implanted fish. Values are arithmetic means ± S.E.M. (n=8–28); *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

cortisol levels were observed between the various groups of implanted fish, despite the fact that the initial implantation dose was similar for all groups. Thus, cortisol-implanted, sexually mature, female brown trout had a mean plasma cortisol level of

79·9 ± 9·7 ng ml^{-1} (n=8) compared with 35·4 ± 6·4 ng ml^{-1} (n=19) for the cortisol-implanted mature male brown trout at 18 days post-implantation ($P<0.001$). Similarly, in the rainbow trout population at 36 days post-implantation the cortisol-treated sexually immature fish had significantly higher blood cortisol levels [28·6 ± 2·3 ng ml^{-1} (n=28)] than the cortisol-treated mature males [17·3 ± 1·4 ng ml^{-1} (n=18), $P<0.001$]. In the cocoa-butter-implanted control fish, sexually mature female brown trout had significantly higher blood cortisol levels than sexually mature males (5·3 ± 0·8 cf. 1·9 ± 0·2 ng ml^{-1}, $P<0.001$). No significant differences in blood cortisol levels were found between mature male and immature control rainbow trout.

As in experiment 1, cortisol implantation significantly decreased the coefficient of condition of brown trout, an effect evident in both sexes [Fig. 4(b)]. In the rainbow trout, however, cortisol treatment had no significant effect on the K factor of mature male fish although it did significantly reduce that of sexually immature fish at 36 days post-implantation ($P<0.001$).

IV. DISCUSSION

Cortisol implantation (30 mg kg^{-1}) produced a stable elevation of blood cortisol levels in both immature brown trout and rainbow trout over a period of 18 days post-implantation (experiment 1). The levels achieved by this technique (≈ 15 ng ml^{-1}) were well within the physiological range for salmonid fish (Donaldson, 1981). The plasma cortisol levels from the present investigation were not significantly different for the two species and were similar to those found by Pickering & Duston (1983) in a previous study on cortisol-implanted brown trout. However, the chronic elevation of blood cortisol levels in experiment 1 had no significant effect on either the K factor or the concentration of circulating lymphocytes in the rainbow trout whereas, in the brown trout, it caused a significant reduction in both these parameters. We have previously shown that cortisol causes a loss of condition and a lymphocytopenia in the brown trout (Pickering & Duston, 1983; Pickering, 1984). In the present study the differences in the response of the two species to a similar physiological dose of cortisol appear to be quantitative (rather than qualitative) differences because, although a similar chronic elevation of the mean plasma cortisol level in experiment 2 (17·3 ng ml^{-1}) also failed to reduce the K factor in mature male cortisol-implanted rainbow trout, a higher level (29·0 ng ml^{-1}) *was* sufficient to significantly reduce the K factor of immature rainbow trout. Moreover, it has been known for many years that rainbow trout lymphocytes are corticosteroid-sensitive (Weinreb, 1958) but, unfortunately, at the time of this early study, techniques were not available for the measurement of circulating corticosteroids in the blood of treated fish.

In the present investigation, the differences in the response of brown trout and rainbow trout to similar levels of cortisol suggest that the sensitivity of the target tissues for the hormone may differ between the two species. The catabolic effects of cortisol in teleost fish are well-documented (Storer, 1967; Chan & Woo, 1978; Lidman *et al.*, 1979; Dave *et al.*, 1979; Leach & Taylor, 1982; Sheridan, 1986) and are responsible for the reduction in the condition factor of the fish. However, whether the species difference in cortisol-sensitivity represents catabolic effects at the tissue level, or differences in the levels of food intake and utilization (or a

combination of both), remains to be determined. Certainly, rainbow trout feed more readily than many other species of salmonid fish following an incidence of stress (Wedemeyer, 1976; J. Pollard, pers. comm.) and an increase in food uptake may well explain the significant increase in *K* factor of both cortisol-implanted and sham-implanted rainbow trout during the course of experiment 1. By comparison, both groups of brown trout showed an overall decrease in *K* factor. The effects of steroid hormones, such as cortisol, are mediated by specific intracellular receptors (Baulieu, 1979) and cortisol receptors have been identified in the intestine and gills of teleost fish (Di Battista *et al.*, 1983, 1984; Sandor *et al.*, 1984; Chakraborti *et al.*, 1987). The characterization and quantification of such receptors in other potential target tissues (muscle, liver, adipose tissue) should increase our understanding of the relative sensitivity of such tissues to cortisol, both within and between species.

The drop in the number of circulating lymphocytes in cortisol-treated brown trout reflects a general suppressive effect of corticosteroids on fish lymphoid tissues (Chilmonczyk, 1982; Ellsaesser & Clem, 1987; Ghoneum *et al.*, 1986). Moreover, corticosteroids have been shown to be severely immunosuppressive in fish (Anderson *et al.*, 1982; Grimm, 1985; Wechsler *et al.*, 1986; Maule *et al.*, 1987) and can result in a marked predisposition to disease (Robertson *et al.*, 1963; Pickering & Duston, 1983; Pickering & Pottinger, 1985; Woo *et al.*, 1987). Thus, it might be suspected that the greater responsiveness of the brown trout lymphocyte population to cortisol treatment would result in a more severe predisposition of this species to disease. This appears to be strongly supported by the results of experiment 2, in which both the incidence of disease (furunculosis and *Saprolegnia* infection) and the mortality due to disease were significantly greater in cortisol-treated brown trout when compared with rainbow trout given a similar dose of the steroid (60 mg kg^{-1}). Thus, at 18 days post-implantation (the time at which the experiment was terminated for the brown trout population) the cumulative mortality for the brown trout was 30% compared with only 2% for the cortisol-implanted rainbow trout. However, interpretation of this result is complicated by the fact that the plasma cortisol levels achieved by intraperitoneal implantation (60 mg kg^{-1}) varied significantly between the groups. These differences are clearly related to the sex and state of sexual maturation of the fish. In the immature rainbow trout, which were given an implant of 60 mg kg^{-1} (experiment 2), the mean blood cortisol level at 36 days post-implantation was 29·0 ng ml^{-1}, twice that of immature rainbow trout given a cortisol implant of 30 mg kg^{-1} (14·3 ng ml^{-1}, experiment 1). The mean plasma cortisol level of implanted, sexually mature, male rainbow trout was almost 50% lower than that of the implanted immature fish. Similarly, the plasma cortisol levels of implanted, mature, male brown trout were 50% lower than those of implanted, mature, female brown trout. This suggests that the rate of clearance of the hormone differs significantly between the groups. In this context, it is interesting to note that Sumpter *et al.* (1987) reported that sexually mature male brown trout and rainbow trout had a significantly reduced cortisol response to a standardized, short-term (1 h) handling and confinement stress when compared with sexually immature fish from the same population.

These differences in circulating cortisol levels make it more difficult to correlate differences in lymphocyte sensitivity to cortisol (experiment 1) with the observed differences in susceptibility to disease seen in experiment 2. However, the dramatic increase in disease seen in cortisol-treated brown trout (compared with the rainbow trout) strongly suggest that the greater sensitivity of the circulating lymphocyte population in the brown trout is at least partly responsible for the increased susceptibility to disease. Thus, when attempting to assess, by means of physiological and endocrinological changes, the sensitivity of fish populations to environmental stress, not only must one take into account species differences in the magnitude of the measurable cortisol response but also potential differences between species in the sensitivity of the target tissues to the steroid.

The authors are grateful to Dr V. J. Bye (MAFF) for providing the cloan strain of rainbow trout, Mrs J. Pollard and Miss J. M. Fletcher (IFE) for care and maintenance of the experimental fish, Mrs M. A. Hurley (IFE) for statistical advice, and Mr T. I. Furnass (IFE) for the artwork. Financial support for this work was provided by NERC and MAFF (Project NBB 13).

References

Anderson, D. P., Roberson, B. S. & Dixon, O. W. (1982). Immunosuppression induced by a corticosteroid or an alkylating agent in rainbow trout (*Salmo gairdneri*) administered a *Yersinia ruckeri* bacterin. *Dev. Comp. Immun.* 2(Suppl.), 197–204.

Baulieu, E.-E. (1979). Aspects of steroid hormone-target cell interactions. *Adv. Exp. Med. Biol.* 117, 377–399.

Chakraborti, P. K., Weisbart, M. & Chakraborti, A. (1987). The presence of corticosteroid receptor activity in the gills of the brook trout, *Salvelinus fontinalis*. *Gen. Comp. Endocr.* 66, 323–332.

Chan, D. K. O. & Woo, N. Y. S. (1978). Effect of cortisol on the metabolism of the eel, *Anguilla japonica*. *Gen. Comp. Endocr.* 35, 205–215.

Chilmonczyk, S. (1982). Rainbow trout lymphoid organs: cellular effects of corticosteroids and anti-thymocyte serum. *Dev. Comp. Immun.* 6, 271–280.

Dave, G., Johansson-Sjöbeck, M.-L., Larsson, A., Lewander, K. & Lidman, U. (1979). Effects of cortisol on the fatty acid composition of the total blood plasma lipids in the European eel, *Anguilla anguilla* L. *Comp. Biochem. Physiol.* 64A, 37–40.

DiBattista, J. A., Mehdi, A. Z. & Sandor, T. (1983). Intestinal triamcinolone acetonide receptors of the eel (*Anguilla rostrata*). *Gen. Comp. Endocr.* 51, 228–238.

DiBattista, J. A., Mehdi, A. Z. & Sandor, T. (1984). A detailed investigation of the cytoplasmic cortisol-binding receptor of North American eel (*Anguilla rostrata*) tissues. *Can. J. Biochem. Cell Biol.* 62, 991–997.

Donaldson, E. M. (1981). The pituitary-interrenal axis as an indicator of stress in fish. In *Stress and Fish* (A. D. Pickering, ed.), pp. 11–47. London: Academic Press.

Ellsaesser, C. F. & Clem, L. W. (1987). Cortisol-induced haematologic and immunologic changes in channel catfish (*Ictalurus punctatus*). *Comp. Biochem. Physiol.* 87A, 405–408.

Ghoneum, M. H., Egami, N., Jiri, K. & Cooper, E. L. (1986). Effect of corticosteroids on the thymus of the fish *Oryzius latipes*. *Dev. Comp. Immun.* 10, 35–45.

Grimm, A. S. (1985). Suppression by cortisol of the mitogen-induced proliferation of peripheral blood leucocytes from plaice, *Pleuronectes platessa* L. In *Fish Immunology* (M. J. Manning & M. F. Tatner, eds), pp. 263–271. London: Academic Press.

Hanson, L. A. & Grizzle, J. M. (1985). Nitrite-induced predisposition of channel catfish to bacterial diseases. *Progve Fish Cult.* 47, 98–101.

Henderson, I. W. & Garland, H. O. (1980). The interrenal gland in Pisces. Part 2. Physiology. In *General, Comparative and Clinical Endocrinology of the Adrenal Cortex* (I. Chester Jones & I. W. Henderson, eds), Vol. 3, pp. 473–523. London: Academic Press.

Hetrick, F. M., Knittel, M. D. & Fryer, J. L. (1979). Increased susceptibility of rainbow trout to infectious haematopoetic necrosis virus after exposure to copper. *Appl. Env. Microbiol.* 37, 198–201.

Johansson, N. & Bergström, E. (1977). Transportation in tanks of two-year-old salmon (*Salmo salar* L.) as a predisposing factor for infection with *Aeromonas hydrophila*. *Laxforsk. Inst. Meddn.* 3, 1–12.

Leach, G. J. & Taylor, M. H. (1982). The effects of cortisol treatment on carbohydrate and protein metabolism in *Fundulus heteroclitus*. *Gen. Comp. Endocr.* 48, 76–83.

Lidman, U., Dave, G., Johansson-Sjöbeck, M.-L., Larsson, A. & Lewander, K. (1979). Metabolic effects of cortisol in the European eel, *Anguilla anguilla* (L.). *Comp. Biochem. Physiol.* 63A, 339–344.

MacArthur, J. I., Fletcher, T. C., Pirie, B. J. S., Davidson, R. J. L. & Thompson, A. W. (1984). Peritoneal inflammatory cells in plaice, *Pleuronectes platessa* L.: effects of stress and endotoxin. *J. Fish Biol.* 25, 69–81.

Maule, A. G., Schreck, C. B. & Kaattari, S. L. (1987). Changes in the immune system of coho salmon (*Oncorhynchus kisutch*) during the parr-to-smolt transformation and after implantation of cortisol. *Can. J. Fish. Aquat. Sci.* 44, 161–166.

McLeay, D. J. (1973). Effects of cortisol and dexamethasone on the pituitary-interrenal axis and abundance of white blood cell types in juvenile coho salmon, *Oncorhynchus kisutch*. *Gen. Comp. Endocr.* 21, 441–450.

Moberg, G. (1985). Biological responses to stress: key to assessment of animal well-being? In *Animal Stress* (G. Moberg, ed.), pp. 24–29. Bethseda: American Physiological Society.

Pickering, A. D. (1984). Cortisol-induced lymphocytopenia in brown trout, *Salmo trutta* L. *Gen. Comp. Endocr.* 53, 252–259.

Pickering, A. D. & Duston, J. (1983). Administration of cortisol to brown trout, *Salmo trutta* L., and its effects on the susceptibility to *Saprolegnia* infection and furunculosis. *J. Fish Biol.* 23, 163–175.

Pickering, A. D. & Pottinger, T. G. (1985). Cortisol can increase the susceptibility of brown trout, *Salmo trutta* L., to disease without reducing the white blood cell count. *J. Fish Biol.* 27, 611–619.

Pickering, A. D., Griffiths, R. & Pottinger, T. G. (1987*a*). A comparison of the effects of overhead cover on the growth, survival and haematology of juvenile Atlantic salmon, *Salmo salar* L., brown trout, *Salmo trutta* L., and rainbow trout, *Salmo gairdneri* Richardson. *Aquaculture* 66, 109–124.

Pickering, A. D., Pottinger, T. G. & Sumpter, J. P. (1987*b*). On the use of dexamethasone to block the pituitary-interrenal axis in the brown trout, *Salmo trutta* L. *Gen. Comp. Endocr.* 65, 346–353.

Robertson, O. H., Hane, S., Wexler, B. C. & Rinfret, A. P. (1963). The effect of hydrocortisone on immature rainbow trout (*Salmo gairdneri*). *Gen. Comp. Endocr.* 3, 422–436.

Sandor, T., DiBattista, J. A. & Mehdi, A. Z. (1984). Glucocorticoid receptors in the gill tissue of fish. *Gen. Comp. Endocr.* 53, 353–364.

Schreck, C. B. (1981). Stress and compensation in teleostean fishes: response to social and physical factors. In *Stress and Fish* (A. D. Pickering, ed.), pp. 295–321. London: Academic Press.

Sheridan, M. A. (1986). Effects of thyroxin, cortisol, growth hormone, and prolactin on lipid metabolism of coho salmon, *Oncorhynchus kisutch*, during smoltification. *Gen. Comp. Endocr.* 64, 220–238.

Storer, J. H. (1967). Starvation and the effects of cortisol in the goldfish. *Comp. Biochem. Physiol.* 20, 939–948.

Sumpter, J. P., Carragher, J., Pottinger, T. G. & Pickering, A. D. (1987). The interaction of stress and reproduction in trout. *Proc. Third Int. Symp. Reprod. Physiol. Fish*, pp. 299–302.

Wechsler, S. J., McAllister, P. E., Hetrick, F. M. & Anderson, D. P. (1986). Effects of exogenous corticosteroids on circulating virus and neutralizing antibodies in striped bass (*Morone saxatilis*) infected with infectious pancreatic necrosis virus. In *Fish Immunology* (J. S. Stolen, D. P. Anderson & W. van Muiswinkel, eds), pp. 305–311. Amsterdam: Elsevier.

Wedemeyer, G. (1971). The stress of formalin treatment in rainbow trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). *J. Fish. Res. Bd Can.* 28, 1899–1904.

Wedemeyer, G. (1972). Some physiological consequences of handling stress in the juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*). *J. Fish. Res. Bd Can.* 29, 1780–1783.

Wedemeyer, G. (1973). Some physiological aspects of sublethal heat stress in the juvenile steelhead trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). *J. Fish. Res. Bd Can.* 30, 831–834.

Wedemeyer, G. A. (1976). Physiological response of juvenile coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Salmo gairdneri*) to handling and crowding stress in intensive fish culture. *J. Fish. Res. Bd Can.* 33, 2699–2702.

Wedemeyer, G. A. & McLeay, D. J. (1981). Methods for determining the tolerance of fishes to environmental stressors. In *Stress and Fish* (A. D. Pickering, ed.), pp. 248–275. London: Academic Press.

Wedemeyer, G. & Yasutake, W. T. (1974). Stress of formalin treatment in juvenile spring chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Salmo gairdneri*). *J. Fish. Res. Bd Can.* 31, 179–184.

Weinreb, E. L. (1958). Studies on the histology and histopathology of the rainbow trout, *Salmo gairdneri irideus*. 1. Hematology: under normal and experimental conditions of inflammation. *Zoologica, N.Y.* 43, 145–154.

Woo, P. T. K., Leatherland, J. F. & Lee, M. S. (1987). *Crytobia salmositica:* cortisol increases the susceptibility of *Salmo gairdneri* Richardson to experimental cryptobiosis. *J. Fish Dis.* 10, 75–83.

Fish Physiology and Biochemistry vol. 7 no. 1-4 pp 253-258 (1989)
Kugler Publications, Amsterdam/Berkeley

Stress responses and disease resistance in salmonid fish: Effects of chronic elevation of plasma cortisol

A.D. Pickering and T.G. Pottinger
Freshwater Biological Association, The Ferry House, Far Sawrey, Nr Ambleside, Cumbria, LA22 OLP, UK

Keywords: stress response, pituitary-interrenal axis, cortisol elevation, Salmonidae, disease resistance, immunosuppression

Abstract

Basal levels of plasma cortisol in unstressed salmonid fish are normally in the range 0–5 ng ml^{-1}. An acute stress such as handling or 1 h confinement caused a temporary elevation of the plasma cortisol levels of both brown trout, *Salmo trutta* L., and rainbow trout, *Salmo gairdneri* Richardson, in the range 40–200 ng ml^{-1} with a return to basal levels within 24–48 h. The extent of the cortisol elevation in response to an acute stress was dependent upon both the species and strain of trout. Chronic stresses, such as prolonged confinement or crowding, resulted in an elevation of plasma cortisol levels to approximately 10 ng ml^{-1}. Under these circumstances, blood cortisol levels remained elevated for periods of up to 4 weeks before acclimation finally occurred.

It is shown, by means of intraperitoneal implantation of cortisol, that chronic elevation of plasma cortisol levels in the brown trout results in a dose-dependent increase in mortality due to common bacterial and fungal diseases. This effect is apparent at plasma cortisol levels as low as 10 ng ml^{-1}, levels below those often reported as being representative of 'unstressed' fish. These findings are discussed in relation to the known immunosuppressive effects of corticosteroids in teleost fish.

Introduction

Activation of the hypothalamic-pituitary-interrenal axis is a central component of the response of teleost fish to most forms of environmental stress (Donaldson 1981). It has been presumed, although by no means convincingly demonstrated, that the energy-mobilizing properties of the secreted corticosteroids are of adaptive value in the fish's attempts to avoid or overcome the immediate threat. Paradoxically, corticosteroids are also known to have suppressive effects on the defence systems of fish (see Ellsaesser and Clem 1987; Maule *et al.* 1987; Tripp *et al.* 1987 for key references), effects which are believed to be responsible for the observed increase in susceptibility to disease during or after periods of environmental stress (Wedemeyer and McLeay 1981). Thus, there must be a balance between the adaptive and maladaptive effects of this component of the fish's stress response.

Some of the earlier studies on the effects of corticosteroids on the defence systems and disease resistance of teleost fish used pharmacologically high hormone doses (*e.g.* Robertson *et al.* 1963; Roth 1972; Chilmonczyk 1982), others have used synthetic corticosteroids of unknown or unmeasured potency in teleost fish (Bullock and Stuckey 1975; Anderson *et al.* 1982; Houghton and Matthews 1986; Wechsler *et al.* 1986) or have not measured the concentration of circulating corticosteroids following experimental administration (Ghoneum *et al.* 1986). This makes it difficult to assess the physiological significance of these studies and emphasizes the need for more detailed information on the levels of circulating corticosteroids in both unstressed and stressed fish if we are to understand how the balance between adaptive and maladaptive effects of elevated corticosteroids levels is achieved in stressed fish.

The present study examines the magnitude and duration of the cortisol response of brown trout, *Salmo trutta* L., and rainbow trout, *Salmo gairdneri* Richardson, to both acute and chronic stresses and, in the light of this information, investigates the effects of physiological doses of cortisol on the resistance of brown trout to the natural challenge of common fungal and bacterial pathogens.

Materials and methods

Experimental fish

All the fish used in this investigation were reared at the FBA's experimental fish hatchery in large (1500 l), outdoor, fibreglass tanks each supplied with a constant flow of Windermere lake water (35 l min^{-1}). The fish were fed with commercial trout pellets at the rates recommended by the manufacturers (exact rate dependent upon water temperature – annual range 3–18°C).

Response to acute stress

828 2+ brown trout (FBA strain, mean body weight 330 g) were divided equally into 18 rearing tanks during early April and left for a period of two weeks to recover from the effects of handling (Pickering *et al.* 1982). The fish from each tank were then stressed by transfer to small (80 × 40 × 20 cm) tanks, each supplied with a constant flow of lake water (20 l min^{-1}, 6°C), for a period of 1 h before being returned to their original rearing tanks. Samples of 6 fish were taken from duplicate tanks at 0, 0.5, 1, 2, 4, 8, 24, 48 and 96 h post-stress so that no tank was repeatedly sampled. At each sampling time the fish were rapidly anaesthetized in phenoxyethanol (1:2000) and blood samples taken by means of a heparinized syringe from the caudal vessels. Aliquots of blood plasma were then stored at −70°C until assayed for cortisol. The experiment was then repeated with 1+ rainbow trout (Home strain, mean body weight 207 g, water temperature 9°C).

A further comparison of the effects of acute stress on brown trout and rainbow trout was performed in a subsequent study during July. A population of 660 1+ brown trout (Dunsop Bridge strain) was divided equally into 22 rearing tanks and left for a period of 2 weeks to recover from the handling stress. The water in each tank was then drained and the fish were exposed to the air for a period of 30 seconds (emersion stress) before the tanks were refilled. Thus, the stress was of shorter duration than in the first experiments (see above) and did not include the stress of handling. Water temperature during this study was in the range 11–14°C. Blood samples were taken at regular intervals up to 336 h post-stress from duplicate tanks (6 fish $tank^{-1}$). A parallel study with 1+ rainbow trout (Stirling strain) was undertaken and again, no tank was sampled more than once.

Strain differences in response to acute stress

Five strains of 1+ rainbow trout (A Caribou, B New Zealand, C Butley, D Home, E Cloan) and three strains of 1+ brown trout (F Dunsop Bridge, G FBA, H Hungerford) were subjected to a period of 1 h confinement stress (see above) at four different times of the year (October, January, March, July). Blood samples were taken from 10 fish of each strain at the end of each confinement period for subsequent plasma cortisol determination. In addition, blood samples were taken from ten unstressed fish of each strain at the start of the study for the determination of basal plasma cortisol levels.

Response to chronic stress

a) Confinement

In July, ten 1+ rainbow trout (Butley strain, mean body weight 250 g) were individually confined in

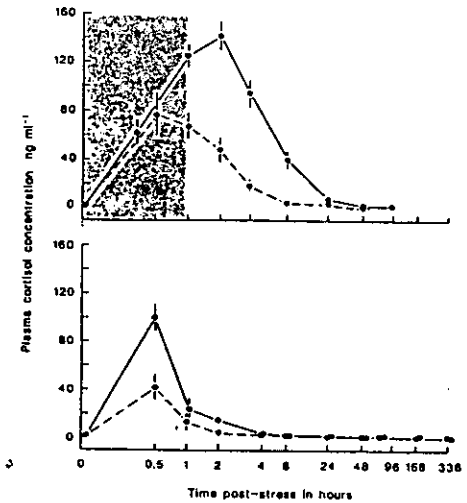


Fig. 1. Examples of the magnitude and duration of the cortisol response of trout to acute stress. Upper graph – the response to and subsequent recovery from handling/1 h confinement (indicated by the shaded area). Lower graph – the response to and recovery from 30 sec emersion stress. Continuous lines represent the brown trout, broken lines the rainbow trout. Each point is the arithmetic mean ± SEM (n = 12).

small tanks (50 l), each supplied with a constant flow of lake water (20 l min^{-1}, 13–17°C), and fed at a rate of 1% body weight day^{-1}. Fish were anaesthetized (phenoxyethanol, 1:2000) and blood samples (300 µl) were taken from the sinus venosus of each fish at 0, 1, 2, 4 and 6 weeks post-confinement. Thus in this experiment, the stress consisted of chronic confinement plus repeated blood sampling. Fish of the same stock but maintained in large rearing tanks served as controls (each control fish was terminally sampled).

b) Crowding

Replicate rearing tanks were stocked with 1+ rainbow trout (Stirling strain) at a density of 120 g l^{-1}, control tanks at 20 g l^{-1} (water flow 35 l min^{-1} in each case, temperature range 12–15°C). Sixteen fish from each stocking density were taken at 0, 2, 6, 10, 14 and 21 days post-crowding (September-October), anaesthetized and blood-sampled from the caudal vessels. In a further study with 1+

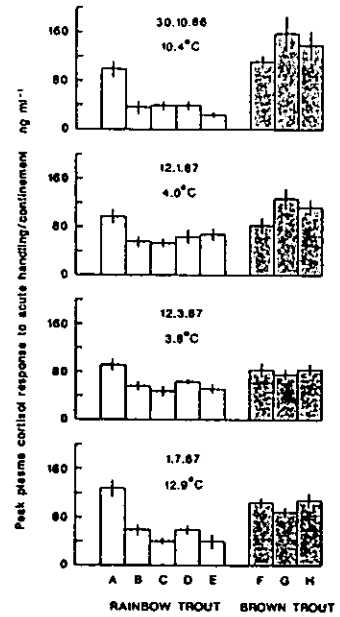


Fig. 2. A comparison of the mean plasma cortisol levels of five strains of rainbow trout and three strains of brown trout immediately after 1 h confinement (see Materials and methods for details of strains). The magnitude of the cortisol response of each strain was measured at four different times of the year. Each value is the arithmetic mean ± SEM (n = 10).

brown trout (FBA strain), similar stock densities were maintained but, in view of the time taken for acclimation of blood cortisol levels (Fig. 3), the sampling programme was extended to 120 days (April-August, water temperature 8–16°C).

Chronic cortisol implantation

Stocks of 1+ and 2+ brown trout (FBA strain) were implanted with cortisol suspended in molten cocoa-butter (see Pickering and Duston 1983 for details) at doses within the range 0–100 mg kg^{-1} during the period February-September (temperature range 4–18°C). The majority of the 2+ fish and approximately 50% of the 1+ males showed signs of sexual maturation during the later stages of the study. Mortalities due to disease within each ex-

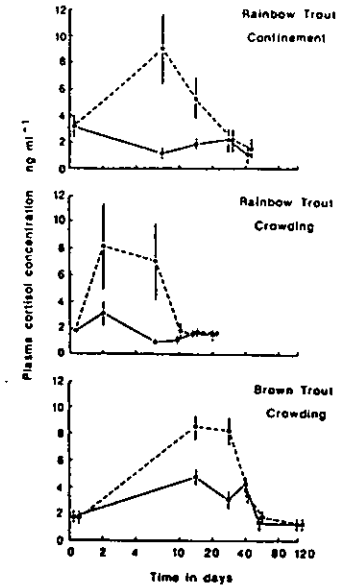


Fig. 3. Examples of the cortisol response of trout to confinement stress and to crowding stress (see Materials and methods for details). Stressed fish are represented by the broken lines, unstressed controls by the continuous lines. Each value is the arithmetic mean ± SEM (n = 10–16).

perimental population were monitored on a daily basis and weekly blood samples were taken from a minimum of eight healthy fish per treatment. The duration of the monitoring period for each experimental population was inversely related to the mortality rate and varied between 2–10 weeks.

Plasma cortisol determination

Plasma cortisol levels were measured using a validated and characterized radioimmunoassay (see Pickering *et al.* 1987 for details). The efficiency of the ethyl acetate extraction stage was greater than 99% for both species of trout. The cortisol data for each experiment were analysed by analysis of variance (Genstat) with prior transformation (√ or log), where necessary, to improve homogeneity of variance. However, for ease of presentation, the cortisol data in this paper are given as the arithmetic mean ± SEM.

Results

The mean basal plasma cortisol levels in unstressed trout of both species were always less than 5 ng ml^{-1} and usually in the range 1–2 ng ml^{-1}. Both acute confinement and brief emersion stress caused a transitory elevation of plasma cortisol levels with a return to control values within 24–48 h (Fig. 1). Peak cortisol levels were 100–150 ng ml^{-1} in the brown trout, 40–80 ng ml^{-1} in the rainbow trout. Analysis of variance of the cortisol response of different strains of the two species to 1 h confinement revealed marked and highly significant ($p < 0.001$) differences in the magnitude of the response between different strains of rainbow trout. A comparison of means (Fig. 2) showed that one strain of rainbow trout (Strain A) had consistently higher plasma cortisol levels than the rest ($p < 0.01$ in each case); levels, in fact, which were similar to those of the brown trout (80–130 ng ml^{-1}). The mean plasma cortisol levels of unstressed fish were all less than 5 ng ml^{-1} although some significant variation was apparent among strains (Rainbow trout: A Caribou 3.2 ± 0.6 ng ml^{-1}, B New Zealand 2.8 ± 0.4 ng ml^{-1}, C Butley 2.7 ± 0.8 ng ml^{-1}, Home 4.0 ± 1.3 ng ml^{-1}, E Cloan 0.5 ± 0.1 ng ml^{-1}. Brown trout: F Dunsop Bridge 0.8 ± 0.2 ng ml^{-1}, G FBA 3.3 ± 1.0 ng ml^{-1}, Hungerford 1.1 ± 0.3 ng ml^{-1}). Chronic confinement and crowding produced a more prolonged elevation of plasma cortisol levels to ≈ 10 ng ml^{-1} (Fig. 3). Blood cortisol concentrations eventually returned to basal levels, despite the continued stress, but this type of acclimation took more than 4 weeks in the case of brown trout subjected to the stress of overcrowding.

Chronic elevation of plasma cortisol levels by means of intraperitoneal implantation of the hormone caused a dose-dependent increase in the mortality rate of brown trout (Fig. 4). Even relatively low cortisol levels (10 ng ml^{-1}) resulted in a significant ($p < 0.05$) elevation of the mean instantaneous mortality rate to 0.2% day^{-1}. Causes of death were diagnosed as a combination of *Saprolegnia-*

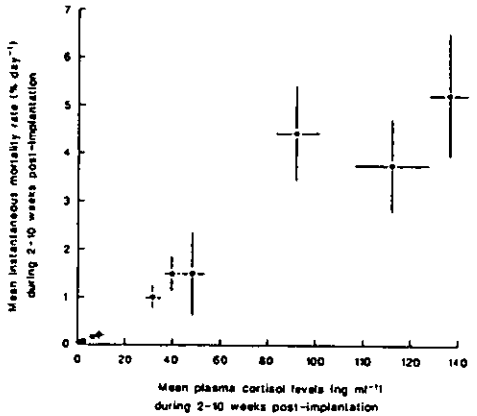


Fig.4. Correlation between the mean plasma cortisol levels of stocks of cortisol-implanted brown trout and the mean instantaneous mortality rate ($Z = \frac{\log_e N_T - \log_e N_t}{t-T}$, where N_T and N_t are the numbers of surviving fish at times T and t respectively) within each experimental population. The major causes of death in the cortisol-treated fish were diagnosed as *Saprolegnia* infection, bacterial fin-rot and furunculosis.

Linear regression $y = 0.05 + 0.034x$, $r^2 = 93.3\%$, $p < 0.001$.

infection, severe bacterial fin-rot and furunculosis, all well known stress-related diseases (see Wedemeyer and McLeay 1981).

Discussion

This paper summarizes a series of studies on the magnitude and duration of the cortisol response of trout to environmental stress and demonstrates that chronic, experimental elevation of plasma cortisol levels in the brown trout, from basal values of 1–2 ng ml^{-1} to only 10 ng ml^{-1} is sufficient to predispose some of the fish within the population to common bacterial and fungal pathogens. This degree of cortisol elevation is similar to that observed when trout are subjected to prolonged confinement or overcrowding and is also similar to that found in brown trout during the spawning season (Pickering and Pottinger 1987), another period of increased susceptibility to disease (Pickering and Christie 1980). However, as the present study shows, the magnitude of the stress-induced cortisol response may differ significantly not only between species but also between strains of the same species.

It is difficult to equate these findings with many of the previous studies in this field because of the use of excessively high doses of natural corticosteroids or the use of synthetic corticosteroids of unknown potency (see Introduction). However, in a recent study Maule *et al.* (1987) demonstrated that chronic elevation of plasma cortisol levels in the coho salmon, *Oncorhynchus kisutch*, from basal values of 0.5 ng ml^{-1} to only 14 ng ml^{-1} was sufficient to reduce the number of antibody-secreting cells in fish immunized with *Vibrio anguillarum* antigen. Moreover, in an *in vitro* study Tripp *et al.* (1987) also showed that 10 ng ml^{-1} cortisol suppressed the mitogenic response of coho salmon lymphocytes. Thus, the coho salmon would seem to be as sensitive to chronically elevated cortisol levels as the brown trout. Circumstantial evidence indicates that the defence systems of Atlantic salmon parr, *Salmo salar*, are also very sensitive to corticosteroid elevation (Pickering and Pottinger 1988) although other evidence suggests that rainbow trout may be less sensitive than brown trout to corticosteroid elevation (A.D. Pickering, T.G. Pottinger and J.F. Carragher, unpublished).

The levels of cortisol capable of suppressing the defence systems of these fish are lower than many of the reported plasma cortisol levels from 'unstressed' fish. Indeed in a literature survey of this field, of 82 publications on salmonid fish we found that the majority (46 papers) reported basal cortisol levels of salmonid fish in excess of 10 ng ml^{-1}. Undoubtedly species and strain differences in basal plasma cortisol levels exist (see also Woodward and Strange 1987), although we have consistently measured basal cortisol levels in three *Salmo* species (*S. trutta*, *S. gairdneri* and *S. salar*) and two species of *Salvelinus* (*S. alpinus* and *S. fontinalis*) within the range 0–5 ng ml^{-1} (A.D. Pickering and T.G. Pottinger, unpublished), and further comparative studies within the Salmonidae are needed. If, however, the degree of sensitivity of the defence systems of the brown trout to chronic elevation of plasma cortisol levels proves to be characteristic of many

members of this group of teleosts, considerable attention must be given to experimental design in order to avoid problems of chronic stress in control fish.

Acknowledgements

The authors are grateful to the organizing committee for the invitation to participate in the First International Symposium on Fish Endocrinology, to Miss K.M. Atkinson and Miss J.M. Fletcher (FBA) for maintaining the experimental fish, to Mr T.I. Furnass (FBA) for the artwork and to NERC and MAFF for financial support.

References cited

Anderson, D.P., Roberson, B.S. and Dixon, O.W. 1982. Immunosuppression induced by a corticosteroid or an alkylating agent in rainbow trout (*Salmo gairdneri*) administered a *Yersinia ruckeri* bacterin. Dev. Comp. Immunol. Suppl. 2: 197–204.

Bullock, G.L. and Stuckey, H.M. 1975. *Aeromonas salmonicida*: Detection of asymptomatically infected trout. Progve Fish-Cult. 37: 237–239.

Chilmonczyk, S. 1982. Rainbow trout lymphoid organs: Cellular effects of corticosteroids and anti-thymocyte serum. Dev. Comp. Immunol. 6: 271–280.

Donaldson, E.M. 1981. The pituitary-interrenal axis as an indicator of stress in fish. *In* Stress and Fish. pp. 11–47. Edited by A.D. Pickering. Academic Press, London.

Ellsaesser, C.F. and Clem, L.W. 1987. Cortisol-induced haematologic and immunologic changes in channel catfish (*Ictalurus punctatus*). Comp. Biochem. Physiol. 87A: 405–408.

Ghoneum, M.H., Egami, N., Ijiri, K. and Cooper, E.L. 1986. Effects of corticosteroids on the thymus of the fish *Oryzias latipes*. Dev. Comp. Immunol. 10: 35–44.

Houghton, G. and Matthews, R.A. 1986. Immunosuppression of carp (*Cyprinus carpio* L.) to ichthyophthiriasis using the corticosteroid triamcinolone acetonide. Vet. Immunol. Immunopathol. 12: 413–419.

Maule, A.G., Schreck, C.B. and Kaattari, S.L. 1987. Changes in the immune system of coho salmon (*Oncorhynchus kisutch*) during the parr-to-smolt transformation and after implantation of cortisol. Can. J. Fish. Aquat. Sci. 44: 161–166.

Pickering, A.D. and Christie, P. 1980. Sexual differences in the incidence and severity of ectoparasitic infestation of the brown trout, *Salmo trutta* L. J. Fish Biol. 16: 669–683.

Pickering, A.D. and Duston, J. 1983. Administration of cortisol to brown trout, *Salmo trutta* L., and its effects on the susceptibility to *Saprolegnia* infection and furunculosis. J. Fish Biol. 23: 163–175.

Pickering, A.D. and Pottinger, T.G. 1987. Lymphocytopenia and interrenal activity during sexual maturation in the brown trout, *Salmo trutta* L. J. Fish Biol. 30: 41–50.

Pickering, A.D. and Pottinger, T.G. 1988. Lymphocytopenia and the overwinter survival of Atlantic salmon parr, *Salmo salar* L. J. Fish Biol. 32: 689–697.

Pickering, A.D., Pottinger, T.G. and Christie, P. 1982. Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: A time-course study. J. Fish Biol. 20: 229–244.

Pickering, A.D., Pottinger, T.G. and Sumpter, J.P. 1987. On the use of dexamethasone to block the pituitary-interrenal axis in the brown trout, *Salmo trutta* L. Gen. Comp. Endocrinol. 65: 346–353.

Robertson, O.H., Hane, S., Wexler, B.C. and Rinfret, A.P. 1963. The effect of hydrocortisone on immature rainbow trout (*Salmo gairdneri*). Gen. Comp. Endocrinol. 3: 422–436.

Roth, R.R. 1972. Some factors contributing to the development of fungus infection in freshwater fish. J. Wildl. Dis. 8: 24–28.

Tripp, R.A., Maule, A.G., Schreck, C.B. and Kaattari, S.L. 1987. Cortisol mediated suppression of salmonid lymphocyte responses *in vitro*. Dev. Comp. Immunol. 11: 565–576.

Wechsler, S.J., McAllister, P.E., Hetrick, F.M. and Anderson, D.P. 1986. Effect of exogenous corticosteroids on circulating virus and neutralizing antibodies in striped bass (*Morone saxatilis*) infected with infectious pancreatic virus. Vet. Immunol. Immunopathol. 12: 305–311.

Wedemeyer, G.A. and McLeay, D.J. 1981. Methods for determining the tolerance of fishes to environmental stressors. *In* Stress and Fish. pp. 247–275. Edited by A.D. Pickering. Academic Press, London.

Woodward, C.C. and Strange, R.J. 1987. Physiological stress responses in wild and hatchery-reared rainbow trout. Trans. Am. Fish Soc. 116: 574–579.

1989 Trout News 8, 12-14

FISH HUSBANDRY AND STRESS

A D Pickering, Institute of Freshwater Ecology, The Ferry House, Far Sawrey, Near Ambleside, Cumbria LA22 OLP

Introduction

At a recent Coordinator of Fisheries Research and Development (CFRD) Working Group on Trout Cultivation it was minuted that "there had been significant improvement with diseases on farms through greater attention to stress on the fish, but that it had been achieved by intuition rather than through scientific advice or understanding". A recommendation was made, therefore, to improve understanding and provide such advice by means of an article in Trout News.

The Stress Response

When a fish is alarmed in any way, or subjected to adverse environmental conditions, it initiates a series of physiological changes which switch its metabolism from a state of feeding and growth to one in which the body reserves are broken down and used. In the natural environment, the increased available energy obtained by this mechanism is used by the fish as it attempts to avoid or overcome the immediate threat. If the fish cannot avoid or overcome the stress, as is usually the case under farm conditions, the stress response will be both prolonged and exaggerated and the fish's state of health will be impaired.

Damaging Effects of Stress

In its simplest terms, the life of a fish can be described by 3 essential features: survival, growth and reproduction. The primary aim of trout farming is to maximise these characteristics under intensive rearing conditions.

a) Survival Stressful conditions can decrease survival rates by several mechanisms. Stressed fish have a greatly increased rate of oxygen consumption and are, therefore, much more liable to deplete the dissolved oxygen to dangerously low levels during confinement (e.g. long-distance transport). This problem is exacerbated at high water temperatures. Stressed fish also lose salts in freshwater and can die because the salt concentration in the blood drops to lethal levels. The third, and perhaps most far-reaching, consequence of stress is the ability of the fish to resist disease. Most aspects of the fish's defence systems are so severely suppressed that the fish become extremely vulnerable to a wide range of common pathogens (viral, bacterial, fungal and parasitic). A related consequence of this type of damage is a marked reduction in the effectiveness of vaccines in stressed fish (see article by P D Smith, Trout News No 4, 1987, 11-13).

b) Growth One of the first, and most useful, indications of stress is a refusal of the fish to feed. This, together with the hormonal changes that cause a stressed fish to break down its own body tissues, is responsible for reduced growth rate, increased food conversion ratio and reduced condition factor. From an economic viewpoint, loss of production via growth suppression may be as damaging to a fish farm as stress-related disease problems.

c) Reproduction Recent evidence has linked the suppression of reproductive processes in fish to stressful environmental conditions. However, much more work is needed on the relationships between this effect of stress and the differences between various rainbow trout strains on reproductive success (see article by J Springate and N Bromage, Trout News No. 3, 1987, 6-7, for a

discussion of egg quality in the industry).

d) Product Quality Fish should be captured and slaughtered as quickly and carefully as possible in order to avoid the stress-induced depletion of glucose reserves in the muscle. This improves the keeping quality and flavour (see BTA Quality Assurance Code of Practice*). Furthermore, stress reduces pigment deposition in fish destined for the table (see article by R Simnott, Trout News No 7, 1988, 8-11) and may reduce the firmness of the flesh by inhibiting the normal development of connective tissue between the muscle fibres.

Minimising Stress

So far, this article has outlined the nature of the stress response in fish and emphasised the damage to fish production but little has been said about the factors causing stress and methods of minimising damage. It is the convention to describe stresses as either acute or chronic, a distinction which does not necessarily reflect the degree of severity but which describes the time-course of the stress. An acute stress is one of short duration (minutes or hours) and one in which the time-course of the response of the fish far outlasts that of the stress. In the aquaculture industry, netting, grading, handling, vaccination, flush prophylactic treatments, transport, sudden temperature shock (in either direction) would all be included in the category of acute stress. A chronic stress is a continual stress from which there is no escape. Overcrowding, poor water quality, social interaction between fish and exposure to a novel environment (e.g. transfer of fish from earth ponds to circular tanks) are all examples of chronic stress associated with fish farming.

It is clear that, during normal aquaculture operations, it will be impossible to avoid many of the procedures known to induce stress responses in fish. Netting, grading and transport are integral components of the fish-farming routine and, at best, all that the fish farmer can do is to minimise the effects of this type of stress. The choice of techniques, however, can influence the degree of stress. For example, some recent Scandinavian work shows that moving fish by means of nets caused greater growth suppression than that of piping the fish. Some chronic stresses are avoidable and must be evaded if the fish are to remain healthy, attain their full growth potential and produce eggs and sperm of the highest quality. Under conditions of chronic stress, the fish will make certain physiological adjustments to minimise damage as it attempts to acclimate to the conditions, but this process is rarely complete and performance will invariably be impaired. It is not the purpose of this article to define optimal environmental conditions - indeed these vary with the species and size of fish and most farmers are fully familiar with appropriate guidelines for stocking density, water flow, feeding rates etc. It is the purpose of this article to convince the farmer of the damaging consequences of stress on survival, growth and reproduction if these guidelines are ignored.

In those circumstances where stresses (usually acute) cannot be avoided, there are still some tactics that the farmer can adopt to protect his fish:

1. In general, the longer the period of stress the greater the damage. Thus, reducing the time taken to net, grade and transport fish will encourage a more rapid recovery from stress. However, it must be realised that some of the effects of acute stress of only 1 or 2 minutes may last for a considerable period of time. The defence systems may be suppressed for several days and farmers should be particularly vigilant in their watch for disease outbreaks

---

* Published by the British Trout Association Ltd., P O Box 189, London SW6 5LY

during the week following any disturbance of the fish. In our experimental work, we routinely use a recovery period of 2 weeks to be confident that the physiology of the fish has returned to normal.

2. The higher the water temperature, the greater the stress response. On those farms in which the water temperature varies seasonally, it is safer to carry out potentially stressful procedures during the winter months. Note: One possible exception to this principle is vaccination, because the time taken to develop protective immunity is much longer at low water temperatures. Respiratory stress is particularly damaging at high water temperatures because the oxygen requirements of the fish are elevated at a time when the oxygen carrying capacity of the water is reduced.

3. The effects of multiple stresses may be additive or even synergistic (i.e. they produce a greatly exaggerated stress response). If repeated stresses are unavoidable, there is an advantage in allowing a sufficient recovery period between stresses. (In the case of minor disturbances such as tank cleaning, the fish may acclimate to the routine and reduce their own stress response.) Simultaneous, multiple stresses such as sudden temperature change during or immediately after transport (a combination of confinement stress, handling, thermal shock and possible respiratory stress) should be avoided as far as possible because the damage to the fish may far exceed that caused by any of the stresses on its own.

4. The use of dilute salt solutions (approx. 5 g l^{-1} NaCl) during severe stresses such as hauling has been shown to be effective in reducing the stress response, limiting salt loss and reducing stress-associated mortality in freshwater.

5. Withdrawal of food 2 to 3 days prior to any operation involving fish movement or confinement not only prevents fouling of the water with faecal material and regurgitated food but, more importantly, it reduces the fish's oxygen requirements thereby ameliorating respiratory stress.

6. Mild anaesthesia, using any of the conventional fish anaesthetics, can be used with effect to promote survival during severe stress. Anaesthesia has been shown to reduce the metabolic demands of the fish, inhibit certain aspects of the stress response and increase the survival of fish subsequently exposed to a second stress.

Future Developments

By comparison with other forms of agriculture, the trout farming industry is still in its infancy and there is considerable scope for further modification, by means of artificial selection, of the genetic composition of the fish under cultivation. The available evidence indicates that the magnitude of the stress response is genetically controlled and it ought to be responsive, therefore, to a selective breeding programme. We are currently involved in research to develop strains of rainbow trout with reduced stress responses to common forms of aquacultural stress. The performance capacity of these fish will then be tested under different conditions to determine their tolerance to intensive aquaculture. This type of acceleration of the rate of domestication has been successfully adopted by the poultry industry and has resulted in significantly improved rates of production. It is in the interests of fish farming that we should exploit ideas generated in other forms of agriculture and apply them to specific aquacultural problems.

STRESS AND REPRODUCTION IN TROUT

John Carragher*, John Sumpter*,
Alan Pickering** and John Pottinger**

1989 Trout News 9, 8-9

*Department of Biology and Biochemistry,
Brunel University, Uxbridge, Middlesex UB8 3PH.
**Institute of Freshwater Ecology, Windermere Laboratory,
Ambleside, Cumbria LA22 0LP.

In this article, we will review all of the available information on the subject of stress and reproduction in fish. Fortunately (for the authors), there isn't that much hard fact to review; and fortunately (for the editor of this publication), most of the experiments in this field have been carried out on trout.

We are reminded constantly, by popular science in the media, that too much stress is bad for you. This is certainly true; but what is stress? We can all imagine situations which we would describe as being stressful, however, most examples that we think of are conditions of emotional stress (work, relationships, etc). These cannot, however, be relevant to a trout. What a trout finds stressful are changes to its environment, whether they be due to natural causes (temperature, dissolved oxygen, etc.), or to the actions of man (pollution, poor water quality, etc.). The mechanisms whereby both we ourselves and trout try to cope with these stressful situations are, however, the same. Initially, the impulse is to move away from the stress; but, if this isn't possible or successful, then we try to acclimate to the stress. To do this we have a number of different hormones, of which cortisol appears to be the most important, to modify other bodily systems, enabling us (and trout) to cope. The amounts of these 'stress hormones' in the blood is dependent on the degree and persistence of the stress - generally, the more stressful the situation, the higher the blood levels of the stress hormones. Generally, cortisol has a suppressive effect on other bodily systems. Hence, parameters such as growth, disease resistance and reproduction are all detrimentally affected by stress. This article deals with the latter.

Reproductive success or ability, like stress, is a hard thing to define and quantify. What parameters can be measured to give us an indication of the state of the reproductive system? In the work that has been carried out to date, the 'handle' on the reproductive system that has most often been used is the blood level of various hormones which are involved in the trouts' sexual development (again, the trout is not too dissimilar from us, it has many of the same hormones that we have). Thus, the growth of the gonads (both testes and ovaries) of trout is controlled by hormones. As the levels of these hormones in the blood increase during the summer and autumn, the gonads are stimulated to grow. By determining the blood levels of these hormones, we can assess how successfully the gonads are growing.

In our initial studies, we looked at the effects of the simplest form of stress that we could subject fish to. This involved netting the fish from their large 'home' tank and transferring them to a very much smaller tank for a period of 1 hour, after which they were returned to their home tank. We took blood samples from the fish over the following 2 days. The blood levels of cortisol and various reproductive hormones were then determined. What we found was that this simple 1 hour of confinement to stress affected the levels of reproductive hormones in the trouts' bloodstream for at least the next 48 hours. For example, the stressed fish had lower testosterone levels than unstressed fish; this was accompanied by an elevated cortisol level (the blood cortisol level is a very good indicator of the degree of stress which the fish is experiencing

Our next experiment was designed to assess the effects of long-term stress on the levels of the same sex hormones. To do this we put some maturing male trout into small aquaria (1 fish per

tank) for a month, a situation which trout find fairly stressful. After this time, the fishes' blood was sampled and hormones were measured as before. The levels of the sex hormones measured in these fish were suppressed, compared to others which had been in the large tanks throughout the experiment. This suppression was accompanied by an elevation in their blood cortisol levels, indicating that they were stressed by their restricted environment.

By this time, we were fairly sure that it was the high cortisol level in the blood of stressed fish which was responsible for the suppression of the reproductive hormones. To test this hypothesis, we put a pellet which slowly released cortisol into the gut cavity of the fish (both brown trout and rainbow trout were used). After a few weeks, the fish were sampled. Again, several sex hormones were measured in the blood of each fish, but in addition, other things, like the weights of the gonads, were determined. Some of the results are shown in Figure 1. This figure shows that the blood levels of two reproductive hormones, oestradiol and testosterone (B and C, respectively), were much lower in fish that received a cortisol-releasing pellet compared to those which only received a "dummy" pellet. Further, the ovaries of the fish with artificially elevated cortisol levels in their blood were smaller than the ovaries in control fish (A). These effects were noticed after only 3 weeks. Thus, the bad news is that, in most cases, the effect of cortisol-implantation on the reproductive parameter was suppressive. The good news (for you) though, is that brown trout were affected far more than rainbow trout.

Presently, the hard evidence that stress affects reproduction in trout is limited to these few studies; (the even fewer studies on other species suggest that the same also occurs there). However, we consider that these studies demonstrate convincingly that stress can reduce the reproductive capacity of trout. We are now trying to assess the importance of these suppressed levels of reproductive hormones on the sizes and quantity of the gametes (eggs and milt). On the brighter side, it seems likely that the domestication of rainbow trout, which has occurred over a long period, has raised the tolerance of this species to stressful procedures. However, we recommend that any preventable stressing of broodstock ought to be minimised, not only just prior to spawning, but also in the period well before spawning, because we have evidence that the reproductive cycle of fish at an early stage of gonadal growth is also adversely affected by stress.

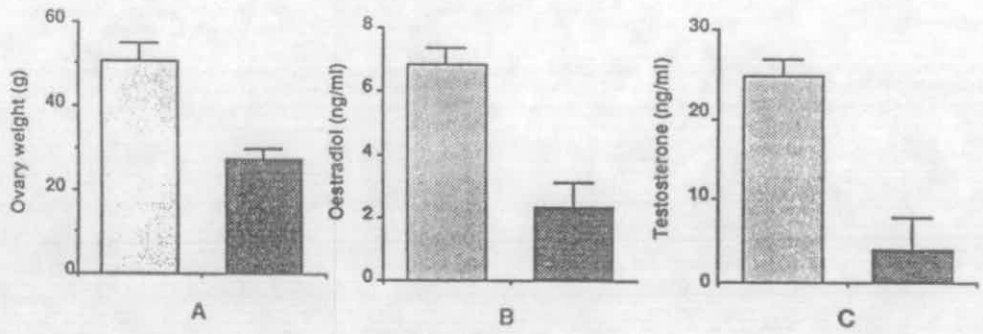
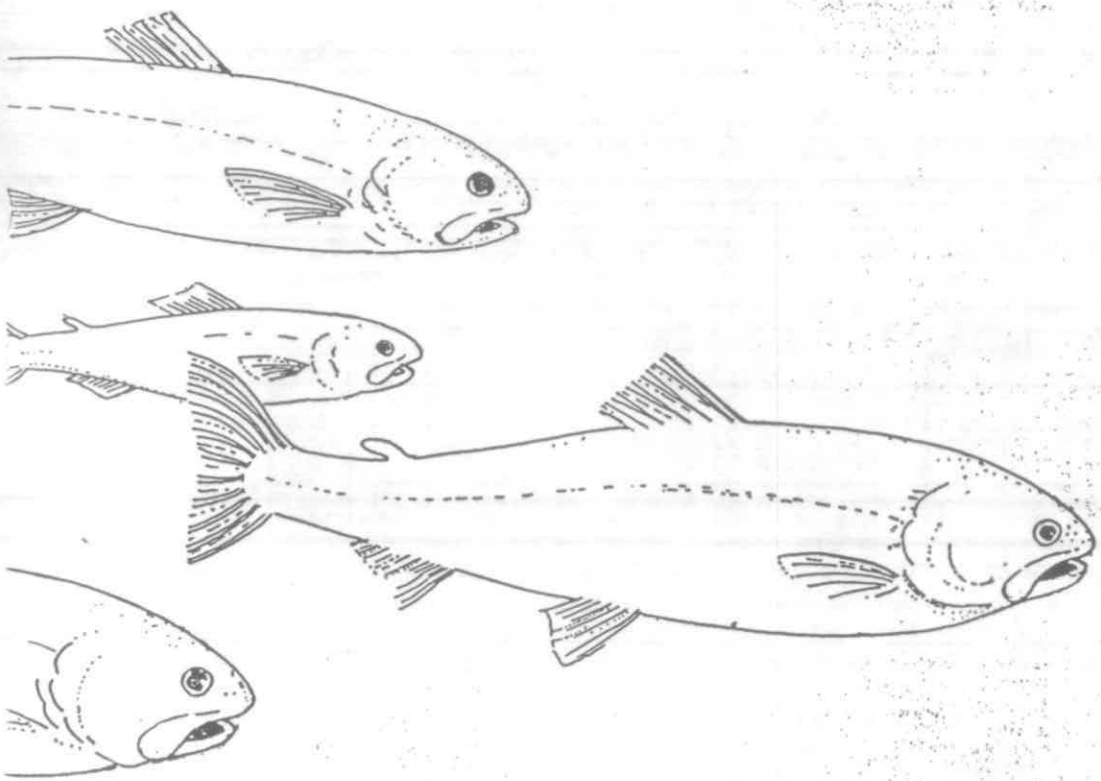


Figure 1. The effects of cortisol implantation on: (A) ovary weight; (B) blood oestradiol; and (C) blood testosterone levels in mature female brown trout. The control fish values are lightly shaded, the values for the fish implanted with cortisol are darkly shaded.

Trout News

Number 9 December 1989



Directorate of Fisheries Research, Lowestoft, Suffolk

Reprint from *Rep. Freshwater biol. Ass.* (1989), No. 57, 61–80

FACTORS AFFECTING THE SUSCEPTIBILITY OF SALMONID FISH TO DISEASE

A. D. PICKERING

Institute of Freshwater Ecology, The Windermere Laboratory, Far Sawrey, Ambleside, Cumbria LA22 0LP, England

Introduction

Outbreaks of disease in fish populations occur when susceptible fish are exposed to potential pathogens under conditions which favour the survival and growth of the infective organism. Changes in the physical and chemical characteristics of the environment can increase the abundance and virulence of pathogenic organisms as can genetic mutation, factors which must have an important influence on the outcome of a situation in which fish are challenged by pathogens in the water. However another influence, namely the *degree of susceptibility* of the host, may also be instrumental in determining whether or not pathogenic challenge results in disease. This aspect of the equation forms the subject for the present review.

Like all vertebrates, fish possess a wide array of defence systems to protect themselves against colonization by disease-causing organisms. Under favourable conditions these systems control pathogen-loading of the fish to such an extent that disease (i.e. an impairment of the normal physiological functioning of the whole, or part, of the body) is absent. However under conditions of stress (Pickering 1981), the defence systems can breakdown and disease may be caused by organisms which, under normal circumstances, are relatively harmless. Stress may take the form of a deleterious change in the environment, which then causes a disturbance of the normal homeostatic mechanisms within the fish, or it may be caused by endogenous physiological processes such as those associated with sexual maturation. Examples of both types of stress are given in this paper.

Studies of the effects of stress on disease resistance are of importance with regard to salmonids because of the value of these fish to man. Salmon and trout require water of high quality and are reared in Britain, almost to the exclusion of all other species, in an expanding aquaculture industry. The

extreme sensitivity of salmonid fish to environmental stresses, such as overcrowding and water quality deterioration, conflicts with the constant economic pressure to rear such fish under increasingly intensive conditions. A successful fish farmer must balance these two opposing factors to produce high quality fish as cheaply as possible. In the natural environment salmonids are usually the first group of fish to react adversely to deleterious changes in the environment and, therefore, are widely used as biological monitors of water quality. Thus, the study of the effects of stress on disease resistance in salmonid fish has major implications for fisheries management and for the aquaculture industry.

This review summarizes a decade of work on this subject at the Windermere Laboratory of the Freshwater Biological Association and suggests possible directions for future research. Initially, much attention was given to the brown trout, *Salmo trutta* L., the dominant native salmonid fish in this area, although more recently the studies have also included the rainbow trout, *Salmo gairdneri* Richardson, economically the most important species of trout under intensive cultivation. The objectives of the research are fourfold:

1. To develop techniques capable of measuring physiological stress without the fish themselves responding to the process of experimentation.
2. To elucidate the links between the immediate physiological and endocrinological changes that occur in response to stress and the subsequent increased susceptibility to disease.
3. To assess the value of physiological changes as predictive indicators of long-term survival.
4. To invesitgate the possibility of controlling stress responses by means of hormonal manipulation or by selecting, for breeding purposes, fish with low sensitivities to environmental stress.

Experimental Design

With a sensitive species such as the brown trout, very careful consideration must be given to experimental design (Objective 1) because the physiology of the fish can be easily altered as a direct result of the processes of experimentation themselves, i.e. the normal systems change simply because they are being studied. This can occur in response to repeated disturbance of the fish during sampling, to any incidence of handling and to the effects of simple procedures such as anaesthesia (see Pickering et al. 1982; Pickering & Pottinger 1985b). Furthermore, the time taken for some of these changes to occur may only be a matter of one or two minutes (even less in the case of certain hormones). In many experiments it may be impossible to avoid handling the fish at some stage but a sufficiently long recovery period must be allowed for the fish to return to normal. For salmonid fish we routinely use

FIG. 1. The FBA's experimental fish hatchery on the shores of Windermere. Each tank is supplied with a constant flow of lake water.

a recovery period of two weeks following even the briefest case of physical handling. Problems of disturbance can be overcome by avoiding repeated sampling of any one tank of fish during a single study. However, this means that relatively large numbers of tanks must be used in even the most basic investigation because separate tanks have to be used at each sampling time. Moreover, it is imperative that replicate tanks are used at each sample time if tank to tank variation (which will inevitably occur in any relatively long-term experiment) is to be distinguised from true variation in time. All this imposes severe restrictions on experimental design. Furthermore, extensive fish-rearing facilities, with adequate tank replication, are essential for valid research. We are fortunate at the FBA to have such facilities (Fig. 1), without which much of the following progress could not have been made.

Effects of Environmental Stress

(a) Acute Stress

An acute stress is one in which the duration of the stress (usually a matter of minutes or, at most, a few hours) is considerably shorter than the physiological stress response, components of which may last for more than a

week (Pickering et al. 1982). Examples of this type of stress are readily seen in the aquaculture industry where fish are regularly subjected to handling, grading and transport. Within the natural environment successful avoidance of a predator, an incidence of intraspecific confrontation associated with territoriality and capture/release by an angler would all come under the general heading of acute stress. Very broadly, the physiological response of a fish to such a stress consists of a series of changes which switch the metabolism from an anabolic state (the uptake and storage of energy) to a catabolic state (the breakdown of body reserves), the so-called "flight or fight" response (see Pickering 1981). An important component of this acute stress response is an activation of the hypothalamic-pituitary-interrenal (HPI) axis (Fig. 2). The release of a hormone, corticotropin-releasing factor (CRF) from certain neurosecretory cells in a specialized region of the ventral part of the brain (the hypothalamus) stimulates a group of cells in the pituitary gland to secrete another hormone, adrenocorticotropin (ACTH), into the blood stream. ACTH in turn stimulates certain cells in the anterior region of the fish's kidney (the interrenal tissue) to secrete a further hormone, cortisol, into the blood. Cortisol (a steroid hormone) has many physiological effects, including metabolic changes which allow the fish to use energy reserves not normally available to it, a selective advantage in situations of acute stress.

We have shown that the HPI axis of salmonid fish is stimulated in response to handling and disturbance (Pickering et al. 1982), to prophylactic treatment with fungicides (Pickering & Pottinger 1985a), to sudden temperature changes (Sumpter et al. 1985; Pickering et al. 1986) as well as to chronic stresses (see below) such as crowding (Pickering & Stewart 1984). However, identification of stress responses from the activity of the HPI axis alone is complicated by diel rhythms of plasma cortisol levels at certain times of the year (Pickering & Pottinger 1983), by increases in cortisol levels during sexual maturation (Pickering & Christie 1981; Pickering & Pottinger 1987a) and by possible suppressive effects of one form of stress on the response to another stress (Pickering & Pottinger 1987b).

In salmonid fish, blood cortisol levels are normally elevated within five minutes of the onset of an acute stress and may remain elevated for a period of several hours. This is followed by many adjustments to the fish's physiology including changes in the protein, lipid and carbohydrate metabolism. Approximately one to two days after an incidence of acute stress such as handling, changes occur in the composition of circulating white blood cell types. This is characterized by a dramatic reduction in the number of circulating lymphocytes which are essential components of the fish's defence systems. As in higher vertebrates, fish lymphocytes are concerned with the production of specific antibodies to foreign material and act, in concert with other types of white blood cells, to neutralize and eliminate invading microorganisms. Thus, the decrease in numbers of circulating lymphocytes (lymphocytopenia), following acute stress, is the first indication of a link between stress and

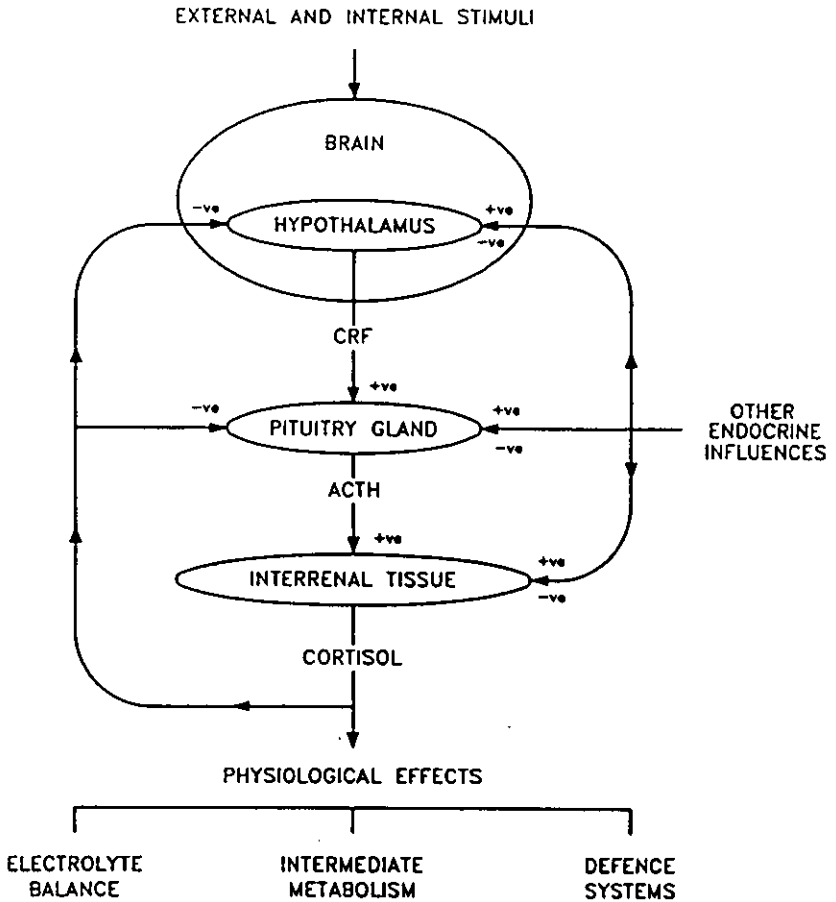


FIG. 2. A simplified, schematic diagram of the hypothalamic-pituitary-interrenal (HPI) axis in salmonid fish.

disease resistance in salmonid fish. It is tempting to suggest that the lymphocytopenia occurs as a direct result of the elevated cortisol levels one day earlier but temporal relationships such as this are no evidence of cause and effect. In an effort to resolve this question, we administered physiological levels of cortisol to brown trout by incorporating the hormone in the fish's normal diet (Pickering 1984). The fish were in no way stressed yet their blood cortisol had been temporarily elevated and this resulted in an almost identical

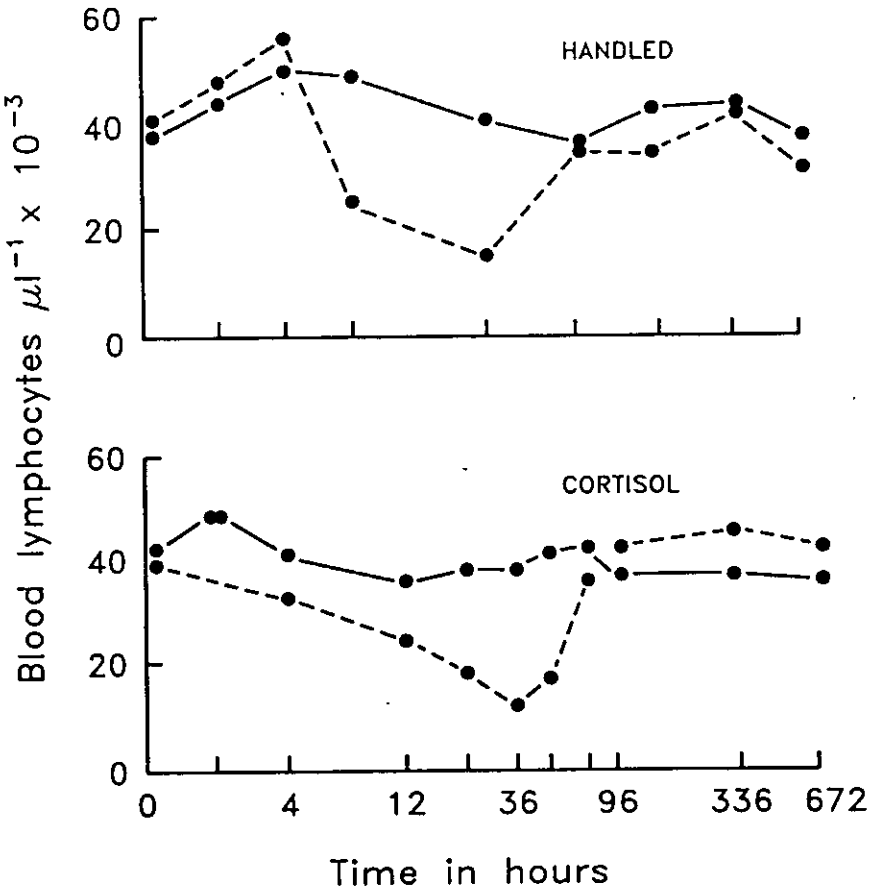


FIG. 3. Changes in the number of circulating lymphocytes in the blood of the brown trout following a single incidence of handling (upper graph) or the oral administration of a physiological dose of cortisol (lower graph). The broken line represents treated fish, the continuous line represents unstressed control fish.

lymphocytopenia to that observed when fish were subjected to an acute handling and confinement stress (Fig. 3). It is clear from this experiment that, following acute stress, elevated cortisol levels cause, either directly or indirectly, a reduction in the number of circulating lymphocytes (Objective 2). In view of this conclusion, we have concentrated on the links between elevated cortisol and disease resistance in salmonid fish, paying particular attention to those circumstances in which the stress is of a continuous (chronic) nature and in which blood cortisol levels are elevated for prolonged periods (days or weeks rather than hours).

(b) Chronic stress

Chronic or continuous stresses are those from which the fish cannot escape (thus making the stress response not only ineffective but also potentially dangerous) and to which the fish must ultimately acclimate, albeit at a reduced performance capacity, if they are to survive. In the aquaculture industry overcrowding and water quality deterioration are prime examples of chronic stresses. Deterioration of water quality may also occur in the natural environment as a result of drought, nutrient enrichment or pollution (including acidification, a topical issue). Under these circumstances the HPI axis is initially activated but, in many cases, the fish will acclimate to the new environmental conditions with plasma cortisol levels returning to normal despite the continuous presence of the stress. We have shown, that the HPI

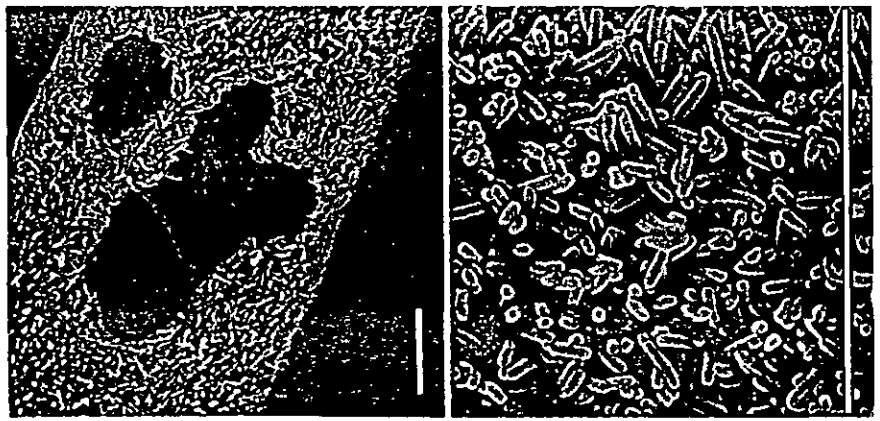


FIG. 4. Scanning electron micrograph of the cartilaginous fin-ray of a brown trout with severe fin rot.

Note the damage caused by the large number of adherent bacteria (bar line 10 μm). On right, high power view of the mat of rod-shaped bacteria colonizing the fin-ray (bar line 2 μm).

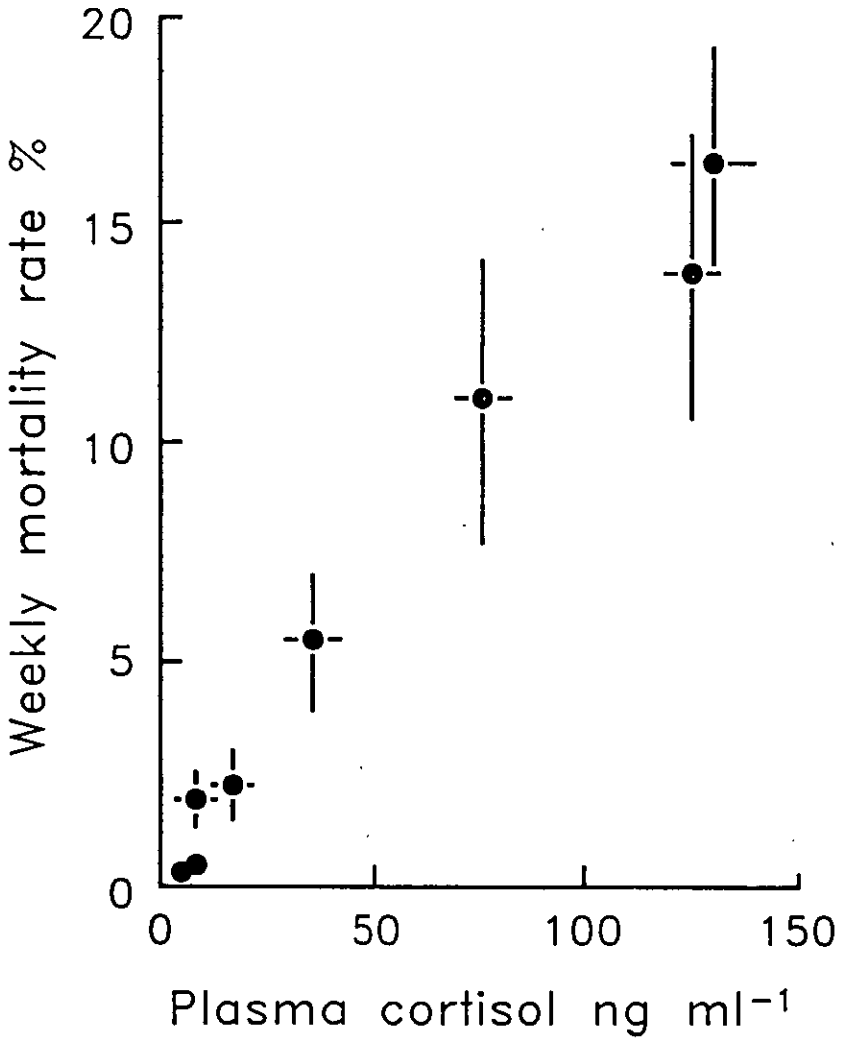


FIG. 5. Effect of chronic elevation of plasma cortisol levels by means of slow-release implants on the mortality rate of brown trout.

Mortality rates and cortisol levels were monitored for 5–10 weeks and causes of death included *Saprolegnia* infection, furunculosis and fin-rot. Values are weekly means ± SEM.

axis of the trout will ultimately acclimate to the stress of overcrowding (Pickering & Pottinger 1987c) although sometimes this may take several weeks to accomplish (Pickering & Stewart 1984), and that it will also acclimate to the repeated stress of routine administration of the fish fungicide, malachite green (Pickering & Pottinger 1985a). If the cortisol levels of chronically stressed fish remain high, outbreaks of disease are likely. We have demonstrated this fact by administering cortisol in the form of slow-release implants so that blood cortisol levels in otherwise unstressed fish remain elevated, within the physiological range, for a period of several weeks (Pickering & Duston 1983; Pickering & Pottinger 1985c). These fish then showed an increase in mortality rate as a result of the bacterial disease, furunculosis, of bacterial fin-rot (Fig. 4) and infection by the parasitic fungus, *Saprolegnia* (see Pickering et al. 1979; Pickering & Willoughby 1982a,b). Fig. 5 illustrates the relationship between the mean weekly mortality rate of these fish and their plasma cortisol levels. It is clear from this that chronically elevated cortisol levels can be used as predictive indicators of the probability of survival (Objective 3). A chronic elevation of plasma cortisol from basal values of 1–2 ng ml^{-1} to only 10 ng ml^{-1} is sufficient to increase the mortality due to disease of brown trout (Pickering & Pottinger 1985c). Many workers have previously considered plasma cortisol levels of 10 ng ml^{-1} to be typical of unstressed fish, a view which now needs to be revised. At these relatively low cortisol levels it is not always possible with immature fish to demonstrate a link between plasma cortisol and white blood cell count but work from other laboratories is now beginning to show that corticosteroids can exert effects on the *activity* of white blood cells as well as on their number and it seems likely that such mechanisms operate in chronically stressed salmonid fish.

Even if the HPI axis of chronically stressed fish does acclimate so that plasma cortisol returns to true basal levels, the performance capacity of the fish may still be impaired. The growth rate of crowded trout under intensive aquaculture conditions is still suppressed despite interrenal acclimation (Pickering & Stewart 1984) and white blood cell counts may remain low long after blood cortisol levels have come down (Pickering & Pottinger 1987c). Thus, although the HPI axis plays a major role in the response of salmonid fish to chronic stress, other factors must almost certainly operate to influence both growth and disease resistance. Further studies in this area should prove rewarding.

Disease itself can also act as a form of severe stress, producing complications in the form of secondary infections. The debilitating effects of *Saprolegnia*, itself often considered to be a secondary colonist (see Pickering & Willoughby 1977; Bucke et al. 1979), are caused by damage to the fish's osmoregulatory mechanisms (Richards & Pickering 1979). This results in a massive and prolonged release of cortisol from the interrenal tissue (one of the known roles of cortisol is as an osmoregulatory hormone) with plasma levels in excess of 1000 ng ml^{-1} being recorded (Pickering & Christie 1981). It is clear

from Fig. 5 that this must result in an enormous increase in the probability of further colonization by the fungus or by other potential pathogens in the water. This type of positive feedback is unusual in natural systems and can be catastrophic with regard to the subsequent survival of fish with overt signs of disease.

Sexual Maturation

It is well-known that sexual maturation in both sexes is associated with an increase in susceptibility to disease (Richards & Pickering 1978; Pickering & Christie 1980; Pickering & Richards 1980). Many of the diseases involved are ectoparasitic infestations, i.e. fungi, protozoa and metazoa, which colonize the fish's epidermis, the outermost layer of living tissue. As a consequence, it is logical to examine some of the changes that occur in the epidermis of salmonid fish during sexual maturation before going on to consider possible effects of HPI activation at this time.

(a) Changes in the Epidermis

The normal epidermis of sexually immature salmonid fish is a multilayered tissue overlying the scales and composed predominantly of Malpighian cells and goblet cells (Pickering 1974, 1977; Pickering & Macey 1977; Blackstock & Pickering 1982). The Malpighian cells contain bundles of filaments which, together with the numerous desmosomal attachments between adjacent cells, are responsible for the tensile strength of the epidermis. The goblet cells, which differentiate and develop deep in the epidermis, secrete mucus on to the surface of the fish (Pickering 1976). The constant renewal of this layer of mucus protects the fish by removing adherent particles from the body surface, including potential pathogens such as fungal spores (Willoughby & Pickering 1977; Pickering & Willoughby 1982a,b). Other cell types are occasionally found, including white cells (leucocytes) derived from the skin's blood supply.

During sexual maturation, the epidermis and underlying dermal tissue of both sexes increase in thickness in response to steroid hormones (androgens) secreted by the gonads and the number of mucus-secreting goblet cells decreases in the males (Pickering 1977, 1978; Pottinger & Pickering 1985a,b). It is believed that this represents an adaptive response to the requirements for a physically tough skin during the potentially traumatic events of upstream migration, territorial defence and redd-building. Coincident with these changes is an increase in the incidence of fungal infection (Richards & Pickering 1978) and infestation by the protozoan parasites *Ichthyophthirius* (the organism that causes white-spot) and *Scyphidia* (Fig. 6) and by the monogenic trematode *Cyrodactylus* (Pickering & Christie 1980). The situation is complicated, somewhat, by marked host-parasite specificities (Pickering et al. 1985) and by pathological responses in the epidermis (thickening and loss

FIG. 6. A small colony of *Scyphidia* on the epidermis of the brown trout.
These ciliated protozoans attach to the superficial epidermal cells of the fish and filter-feed on bacteria in the surrounding water (bar line 100 μm).

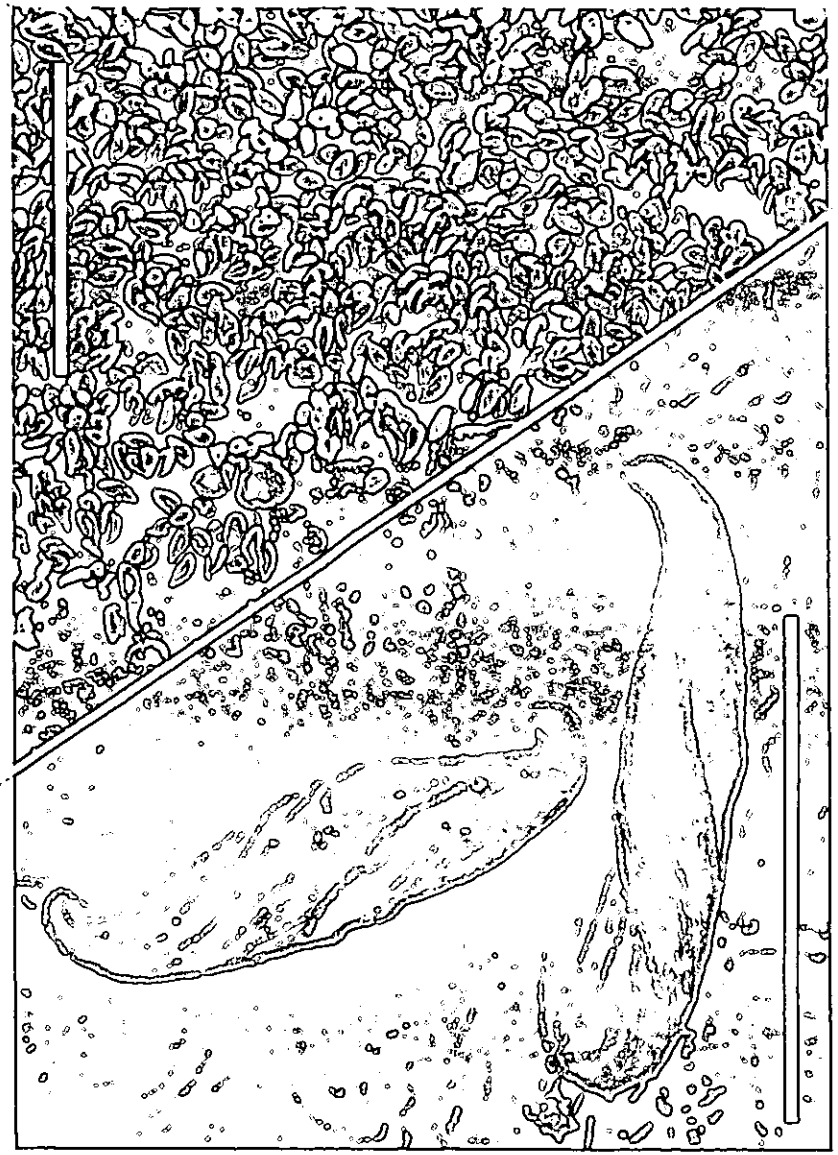


FIG. 7. Upper–severe infestation of the epidermis of the brown trout by the flagellate protozoan, *Ichthyobodo* (bar line 100 μm).
Lower–high power showing the attachment of two parasites to adjacent epidermal cells (bar line 10 μm).

of mucous cells) which, at first inspection, appear similar to those induced by androgen secretion from the gonad (Pottinger et al. 1984). The facts that demucification occurs in sexually mature males but not in mature females (Pickering 1977; Pickering & Richards 1980) and that sexually mature male fish are significantly more frequently or severely infected with ectoparasites than are the mature females (Richards & Pickering 1978; Pickering & Christie 1980) would, apparently, lend support to the argument that in addition to its other roles, the secretion of mucus from the goblet cells is a protective mechanism to prevent colonization by microorganisms (Pickering & Willoughby 1982a). However, Pickering & Christie (1980) note that the sexual differences in ectoparasitic loading occurred before the androgen-induced demucification and concluded that the loss of goblet cells simply exacerbates existing parasitic infestations. Thus, other factors must also influence the susceptibility of sexually mature salmonid fish to disease.

The epidermis of salmonid fish contains cell-types other than the filament-containing Malpighian cells and the mucus-secreting goblet cells. Acidophilic granular cells (Blackstock & Pickering 1980) and sacciform cells (Pickering & Fletcher 1987) have been described although their functions await elucidation. Both cell-types contain an acidophilic, proteinaceous material (as compared with the glycoprotein nature of the goblet cell secretion), which appears to be released on to the epidermal surface. Moreover, the number of epidermal sacciform cells in the brown trout increases dramatically during the chronic stages of infestation by the ectoparasitic flagellate, *Ichthyobodo* (Fig. 7) suggesting that they may play some role in protecting the fish against the parasite (Pickering & Fletcher 1987). Such speculation is even more tempting when one considers the fact that during sexual maturation of both the brown trout and the Arctic charr, *Salvelinus alpinus* (L.), the number of sacciform cells in the epidermis decreases. This is particularly apparent in the mature males, so that during the spawning season the male fish have significantly fewer sacciform cells than have the females (Pickering & Fletcher 1987). If the role of the cell is to secrete some form of protective proteinaceous secretion into the mucus layer, this sexual dimorphism may also contribute to the observed sexual differences in susceptibility to ectoparasitic infestation (see above).

(b) Activity of the HPI axis

Sexual maturation is associated with an increase in the incidence of skin infections and with internal diseases, such as those caused by systemic bacterial infections. It seems likely, therefore, that aspects of the defence systems other than those associated with the skin are also compromised during the spawning season. In view of the deleterious effects of prolonged activation of the HPI axis (see above), we examined sexually mature fish for elevated blood cortisol levels. Studies over several spawning seasons have now established that, in sexually mature male and female brown trout, blood

cortisol levels are elevated for a period of 2–3 months (Pickering & Pottinger 1987a). The precise timing of this elevation varies somewhat from year to year and may be slightly different for the two sexes. On some occasions we have found the highest cortisol levels in the female fish (Pickering & Christie 1981), on others the highest levels were in the males or there were no differences in peak levels but the elevation occurred in the males before the females (see Pickering & Pottinger 1987a). Despite this variation, it is clear that sexual maturation in the brown trout is always associated with chronically elevated blood cortisol levels. Levels may be as high as 50 ng ml^{-1} in ovulated females (Pickering & Christie 1981) although normally the peak elevation is in the range 10–15 ng ml^{-1}. We have seen from the preceding section on chronic environmental stress that prolonged elevation of plasma cortisol from basal values (1–2 ng ml^{-1}) to only 10 ng ml^{-1} is sufficient to predispose immature brown trout to bacterial and fungal infections. Our studies on spawning fish suggest that a similar mechanism operates during sexual maturation. Moreover, we have shown that the blood cell composition of sexually mature fish changes dramatically, with a marked and prolonged reduction in the number of circulating lymphocytes during the spawning period (Pickering 1986; Pottinger & Pickering 1987). This prolonged lymphocytopenia is reminiscent of that observed when sexually immature fish are stressed by overcrowding (Pickering & Pottinger 1987c). In the case of sexual maturation, however, crowding is not a contributory factor because sexually immature fish kept together with the mature fish (i.e. in the same tanks) do not show such changes. The lymphocytopenia parallels the changes in plasma cortisol so closely that it is difficult to avoid the conclusion that the two phenomena are functionally related (Pickering & Pottinger 1987a). However, chronic administration of low doses of cortisol to sexually immature fish was ineffective in changing the white blood cell count even though it still resulted in a predisposition to disease (Pickering & Pottinger 1985c). Further work on possible differences in corticosteroid-sensitivity of the lymphoid tissues (tissues responsible for the production of white blood cells) between mature and immature fish is needed if this problem is to be resolved. Despite this, sexual maturation in salmonid fish is accompanied by physiological changes (interrenal stimulation, lymphocytopenia) that are similar to those found in chronically stressed fish. In both cases the fish are more susceptible to disease.

Research Areas for Future Development

It is clear from our studies that the prolonged elevation of blood cortisol levels predisposes salmonid fish to a wide range of diseases. This occurs in response to chronic stress, in the period before the fish acclimate to the changed environmental circumstances, and also during the natural process of sexual maturation. If we are fully to understand the mechanisms behind this response and if we are to control the fish's physiology under conditions of

stress (Objective 4), it will be necessary to devise procedures for blocking the HPI axis.

Surgical removal of the cortisol-secreting interrenal gland is impossible because of the diffuse nature of the tissue and because of its location in the highly vascularized head kidney around the posterior cardinal veins. In theory it ought to be possible to block the HPI axis by inhibiting the enzymes responsible for the synthesis of the various hormones in the system. However, it would be extremely difficult to restrict the effects of enzyme inhibitors to the HPI axis alone. An alternative approach would be to take advantage of some of the inherent specificity within the system. For any hormone to exert its effects it must combine with highly specific receptors in the target tissues (see Pottinger 1986, 1987). This opens up the possibility of blocking the receptors by the administration of a molecule sufficiently similar to the hormone to form a complex with the receptor, effectively inactivating it to the natural hormone circulating in the blood. This is not without its problems, however, because the blocking agent itself may well have hormone-like effects. We have shown, for example, that the synthetic steroid, dexamethasone, will combine readily with specific cortisol receptors and stop the secretion of ACTH and cortisol (Pickering et al. 1987). However, dexamethasone also has effects on other corticosteroid sensitive cells, such as the leucocytes in the lymphoid tissues. As a consequence, dexamethasone treatment causes a marked lymphocytopenia and, presumably, predisposes the fish to disease. However, new synthetic steroids have now been developed that can effectively block corticosteroid receptors in mammalian tissue, but do not have other cortisol-like effects. Application of these steroids to salmonid fish could greatly increase our current understanding of the presumed adaptive role of the HPI axis in stress responses.

The administration of drugs to suppress the activity of the HPI axis might be a useful experimental tool but it is not necessarily a suitable approach for aquacultural purposes. In this context it may be possible to select, for breeding stock, fish with low sensitivities to environmental stress. We are constantly impressed with the marked individual variation in the response of salmonid fish to a simple acute stress such as handling. Indeed, our experimental designs and subsequent data analyses have to take such variation into account. Some of our current research at the FBA is concerned with identifying strains of fish, or individual fish within a strain, that show consistently low cortisol levels under conditions of environmental stress. Such fish have now been identified and they will be used for breeding purposes to see whether this characteristic is genetically determined. If it proves possible to breed fish with reduced physiological responses to common forms of aquacultural stress, i.e. to accelerate the rate of domestication, further studies will be needed to compare the ability of such fish to resist pathogenic challenge under aquaculture conditions. However, domesticated fish may be ill-suited for stocking into natural environments where a

high sensitivity to an acute stress may well be an advantage (see below) and, ultimately, it may be advisable to develop different strains for intensive aquaculture and for restocking purposes.

Evolutionary Considerations

There can be little doubt from our studies that prolonged elevation of blood cortisol levels during chronic stress significantly increases the susceptibility of salmonid fish to disease. It seems likely that this is mediated, in part at least, by a suppressive effect on the lymphoid tissues. It is difficult to see how this part of the stress response can be adaptive for the individual (although some workers in the mammalian field have speculated about a possible protective role in auto-immune reactions, i.e. damage caused by the animals own defence systems) and one is left with the problem of explaining how such a potentially dangerous mechanism could have evolved.

The adaptive role of cortisol secretion seems to lie in its ability to promote gluconeogenesis (the production of carbohydrates from non-carbohydrate sources, usually protein). This reaction forms part of a complex response to environmental stress by which the fish can utilize energy reserves not normally available to it. Catecholamines, such as adrenaline and noradrenaline, also play an extremely important role in this aspect of the stress response. Under natural conditions the fish utilizes these changes in its physiological state to avoid or overcome the immediate threat ("flight or fight"). The osmoregulatory role of cortisol may also be important during the recovery phase by promoting the re-establishment of osmotic and ionic equilibrium. These advantages may well outweigh, in an evolutionary sense, any disadvantages associated with a temporary impairment of the fish's defence systems. However, under chronic conditions where the environmental stress cannot be avoided or overcome, the disadvantageous effects of chronic interrenal stimulation may eventually show up as an increase in the incidence of disease in the fish population.

With the possible exceptions of prolonged drought (usually associated with elevated temperatures) and overcrowding, it is difficult to find examples of chronic stress in a truly natural environment. The examples given at the beginning of this paper (acidification, nutrient enrichment, pollution) are all effects of man's influences on the aquatic environment, stresses which the fish are incapable of overcoming. At best, the fish can acclimate by reducing the long-term activity of the HPI axis thereby avoiding the worst problems of disease susceptibility. However, other aspects of the fish's biology such as growth rate and reproductive success are usually reduced under such conditions. Thus, physiological stress responses appear to have evolved in salmonids to cope with acute environmental stress (in its many forms) but appear to be ineffective or even mal-adaptive when the fish are faced with a chronic stress. Such responses could be important in density-dependent

mechanisms for controlling population size. Under crowded conditions, increased mortality rates and reduced reproductive success as a result of physiological stress responses, would act to reduce the population density to a level where such responses were minimal.

Sexual maturation is a special case of prolonged interrenal activation that has developed, quite naturally, during the course of evolution. The advantages of this response in terms of the fish's energy requirements during a period of virtual starvation may be greater than the disadvantages of a demonstrable increase in susceptibility to disease. Support for this line of thought can be obtained from the extent of interrenal activity in different species of salmonid fish during the spawning season. In general, those fish with the longest spawning migrations (and, therefore, the greatest energy requirements) have the greatest amount of interrenal tissue and the highest cortisol levels. This line of evolution is taken to its limit with the Pacific salmon (genus *Oncorhynchus*) in which the fish die of exhaustion and disease after a single spawning (semelparity). Under the unnatural conditions of intensive aquaculture, the increase in susceptibility to disease of sexually mature salmonids is a serious problem because diseased fish act as a potential source of infection for the other fish within the unit.

Summary

(a) A central component of the physiological response of salmonid fish to any form of environmental stress is an activation of the hypothalamic-pituitary-interrenal (HPI) axis. This results in an elevation of blood cortisol levels.

(b) Cortisol is of adaptive significance during acute stress responses by enabling the fish to utilize energy reserves in order to avoid or overcome the stress.

(c) In responses to chronic stress, however, prolonged elevation of blood cortisol levels can predispose the fish to disease by suppressing the defence systems. Eventually, the HPI axis may acclimate with a return of blood cortisol levels to normal.

(d) Sexual maturation is also associated with an increase in susceptibility to disease. A prolonged elevation of cortisol levels together with androgen-dependent changes in the skin are responsible for such changes in disease resistance in sexually mature fish.

(e) These findings are discussed in relation to the evolution and adaptive significance of stress responses and to the development of semelparity in salmonid fish.

Acknowledgements

The work described in this paper could not have been undertaken without the cooperation of many colleagues within the FBA and the collaboration of several university departments. Specific acknowledgements have been made in the original publications on which this review is based. The author is also grateful to Mr T. I. Furnass for the artwork and to NERC and MAFF for financial support.

References

Blackstock, N. & Pickering, A. D. (1980). Acidophilic granular cells in the epidermis of the brown trout, *Salmo trutta* L. *Cell Tissue Res.* 210, 359–69.

Blackstock, N. & Pickering, A. D. (1982). Changes in the concentration and histochemistry of epidermal mucous cells during the alevin and fry stages of the brown trout *Salmo trutta*. *J. Zool., Lond.* 197, 463–71.

Bucke, D., Cawley, G. D., Craig, J. F., Pickering, A. D. & Willoughby, L. G. (1979). Further studies of an epizootic of perch *Perca fluviatilis* L., of uncertain aetiology. *J. Fish Dis.* 2, 297–311.

Pickering, A. D. (1974). The distribution of mucous cells in the epidermis of the brown trout *Salmo trutta* (L.) and the char *Salvelinus alpinus* (L.). *J. Fish Biol.* 6, 111–8.

Pickering, A. D. (1976). Synthesis of n-acetyl neuraminic acid from [^{14}C] glucose by the epidermis of the brown trout, *Salmo trutta* L. *Comp. Biochem. Physiol.* 54B, 325–8.

Pickering, A. D. (1977). Seasonal changes in the epidermis of the brown trout *Salmo trutta* (L.). *J. Fish Biol.* 10, 561–6.

Pickering, A. D. (1978). A note on the failure of vitamin A to influence the epidermis of the brown trout, *Salmo trutta* L. *J. Fish Biol.* 12, 441–7.

Pickering, A. D. (ed.) (1981). *Stress and Fish.* London and New York. Academic Press. 367 pp.

Pickering, A. D. (1984). Cortisol-induced lymphocytopenia in brown *Salmo trutta* L. *Gen. comp. Endocrinol.* 53, 252–9.

Pickering, A. D. (1986). Changes in blood cell composition of the brown trout, *Salmo trutta* L., during the spawning season. *J. Fish Biol.* 29, 335–47.

Pickering, A. D. & Christie, P. (1980). Sexual differences in the incidence and severity of ectoparasitic infestation of the brown trout, *Salmo trutta* L. *J. Fish Biol.* 16, 669–83.

Pickering, A. D. & Christie, P. (1981). Changes in the concentrations of plasma cortisol and thyroxine during sexual maturation of the hatchery-reared brown trout, *Salmo trutta* L. *Gen. comp. Endocrinol.* 44, 487–96.

Pickering, A. D. & Duston, J. (1983). Administration of cortisol to brown trout, *Salmo trutta* L., and its effects on the susceptibility to *Saprolegnia* infection and furunculosis. *J. Fish Biol.* 23, 163–75.

Pickering, A. D. & Fletcher, J. M. (1987). Sacciform cells in the epidermis of the brown trout, *Salmo trutta*, and the Arctic char, *Salvelinus alpinus*. *Cell Tissue Res.* 247, 259–65.

Pickering, A. D. & Macey, D. J. (1977). Structure, histochemistry and the effect of handling on the mucous cells of the epidermis of the char *Salvelinus alpinus* (L.). *J. Fish Biol.* 10, 505–12.

Pickering, A. D. & Pottinger, T. G. (1983). Seasonal and diel changes in plasma cortisol levels of the brown trout, *Salmo trutta* L. *Gen. comp. Endocrinol.* 49, 232–9.

Pickering, A. D. & Pottinger, T. G. (1985a). Acclimation of the brown trout, *Salmo trutta* L., to the stress of daily exposure to malachite green. *Aquaculture* 44, 145–52.

Pickering, A. D. & Pottinger, T. G. (1985b). Factors influencing blood cortisol levels of brown trout under intensive culture conditions. In *Current Trends in Comparative Endocrinology* Vol. 2 (eds B. Lofts & W. N. Holmes), 1239–43. Hong Kong. Hong Kong University Press.

Pickering, A. D. & Pottinger, T. G. (1985c). Cortisol can increase the susceptibility of brown trout, *Salmo trutta* L., to disease without reducing the white blood cell count. *J. Fish Biol.* 27, 611–9.

Pickering, A. D. & Pottinger, T. G. (1987a). Lymphocytopenia and interrenal activity during sexual maturation in the brown trout, *Salmo trutta* L. *J. Fish Biol.* 30, 41–50.

Pickering, A. D. & Pottinger, T. G. (1987b). Poor water quality suppresses the cortisol response of salmonid fish to handling and confinement. *J. Fish Biol.* 30, 363–74.

Pickering, A. D. & Pottinger, T. G. (1987c). Crowding causes prolonged leucopenia in salmonid fish, despite interrenal acclimation. *J. Fish Biol.* 30, 701–2.

Pickering, A. D., Pottinger, T. G. & Christie, P. (1982). Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: a time-course study. *J. Fish Biol.* 20, 229–44.

Pickering, A. D., Pottinger, T. G. & Sumpter, J. P. (1986). Independence of the pituitary-interrenal axis and melanotroph activity in the brown trout, *Salmo trutta* L., under conditions of environmental stress. *Gen. comp. Endocrinol.* 64, 206–11.

Pickering, A. D., Pottinger, T. G. & Sumpter, J. P. (1987). On the uses of dexamethasone to block the pituitary-interrenal axis in the brown trout, *Salmo trutta* L. *Gen. comp. Endocrinol.* 65, 346–53.

Pickering, A. D. & Richards, R. H. (1980). Factors influencing the structure, function and biota of the salmonid epidermis. *Proc. R. Soc. Edinb.* 79B, 93–104.

Pickering, A. D. & Stewart, A. (1984). Acclimation of the interrenal tissue of the brown trout, *Salmo trutta* L., to chronic crowding stress. *J. Fish Biol.* 24, 731–40.

Pickering, A. D., Strong, A. J. & Pollard, J. (1985). Differences in the susceptibility of brown trout, *Salmo trutta* L., and American brook trout, *Salvelinus fontinalis* (Mitchill), to infestation by the peritrich ciliate, *Scyphidia* sp. *J. Fish Biol.* 26, 201–8.

Pickering, A. D. & Willoughby, L. G. (1977). Epidermal lesions and fungal infection on the perch, *Perca fluviatilis* L., in Windermere. *J. Fish Biol.* 11, 349–54.

Pickering, A. D. & Willoughby, L. G. (1982a). *Saprolegnia* infections of salmonid fish. In *Microbial Diseases of Fish* (ed. R. J. Roberts), 271–97. London and New York. Academic Press.

Pickering, A. D. & Willoughby, L. G. (1982b). *Saprolegnia* infections of salmonid fish. *Ann. Rep. Freshwat. biol. Ass.* 50, 38–48.

Pottinger, T. G. (1986). Estrogen-binding sites in the liver of sexually mature male and female brown trout, *Salmo trutta* L. *Gen. comp. Endocrinol.* 61, 120–6.

Pottinger, T. G. (1987). Androgen binding in the skin of mature male brown trout, *Salmo trutta* L. *Gen. comp. Endocrinol.* 66, 224–32.

Pottinger, T. G. & 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, 745–53.

Pottinger, T. G. & 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, 335–42.

Pottinger, T. G. & Pickering, A. D. (1987). Androgen levels and erythrocytosis in maturing brown trout. *Fish Physiol. Biochem.* 3, 121–6.

Pottinger, T. G., Pickering, A. D. & Blackstock, N. (1984). Ectoparasite induced changes in epidermal mucification of the brown trout, *Salmo trutta* L. *J. Fish Biol.* 25, 123–8.

Richards, R. H. & Pickering, A. D. (1978). Frequency and distribution patterns of *Saprolegnia* infection in wild and hatchery-reared brown trout *Salmo trutta* L. and char *Salvelinus alpinus* (L.). *J. Fish Dis.* 1, 69–82.

Richards, R. H. & Pickering, A. D. (1979). Changes in serum parameters of *Saprolegnia*-infected brown trout, *Salmo trutta* L. *J. Fish Dis.* 2, 197–206.

Sumpter, J. P., Pickering, A. D. & Pottinger, T. G. (1985). Stress-induced elevation of plasma α–MSH and endorphin in brown trout, *Salmo trutta* L. *Gen. comp. Endocrinol.* 59, 257–65.

Willoughby, L. G. & Pickering, A. D. (1977). Viable saprolegniaceae spores on the epidermis of the salmonid fish *Salmo trutta* and *Salvelinus alpinus*. *Trans Br. mycol. Soc.* 68, 91–5.

GENERAL AND COMPARATIVE ENDOCRINOLOGY 78, 000–000 (1990)

The Effect of Stress and Exogenous Cortisol on Receptor-like Binding of Cortisol in the Liver of Rainbow Trout, ~~Salmo gairdneri~~ Richardson

Oncorhynchus mykiss Walbaum

T. G. POTTINGER

The Institute of Freshwater Ecology, The Ferry House, Far Sawrey, Ambleside, Cumbria LA22 OLP, United Kingdom

Accepted June 20, 1989

Cortisol binding has been identified in cytosols prepared from rainbow trout liver. Binding is of high affinity (k_D = 5.1 ± 0.2 n*M*, n = 23) low capacity (N_{max} = 197 ± 12 fmol mg^{-1} protein, n = 23), and high specificity, only dexamethasone, cortisol, and Ru38486 being efficient in displacing bound [^{3}H]cortisol. Binding is not due to contamination by blood because plasma displayed no affinity for cortisol under the assay regime employed here and, although whole blood cytosol does specifically bind cortisol, the degree of contamination is demonstrably too low to account for the levels of binding detected in liver cytosol. No specific binding of [^{3}H]cortisol could be detected in liver nuclear extracts, although the simultaneous assay for nuclear estradiol-binding sites was positive. Rainbow trout stressed by confinement displayed a significant reduction in cytosolic [^{3}H]cortisol-binding capacity (with no concomitant detectable appearance of binding within nuclear extracts), 96-hr confinement eliciting a 40% depression in binding capacity relative to unstressed fish. The administration of cortisol via intraperitoneal implants also reduced, significantly, the number of hepatic-binding sites. The results are discussed with reference to anomalies in reported characteristics of teleost glucocorticoid receptors and the phenomenon of down-regulation observed in some mammalian systems.

Activation of the salmonid pituitary–interrenal axis under conditions of stress has been extensively characterized (Pickering, 1989) and the deleterious consequences of chronic cortisol elevation are becoming equally well understood. These include predisposition to disease (Pickering and Pottinger, 1989), suppression of reproductive function (Carragher *et al.*, 1989), and reduction in growth rate (Barton *et al.*, 1987). Investigations so far have concentrated on measurement of the primary stress response (plasma ACTH and cortisol levels) and subsequent effects in terms of a secondary response (e.g., plasma metabolite levels, lymphocyte abundance, levels of reproductive hormones). No information is yet available on the role of target tissues themselves in modulation of the stress response. The transduction of the hormone signal to target tissue response occurs via a specific hormone receptor, and studies in mammalian systems suggest that tissues can control their sensitivity to glucocorticoids by altering the number of receptors present within the cell (Danielsen and Stallcup, 1984). To date, putative cortisol receptor proteins have been characterized in detail in only two salmonid tissues, gill and liver (Sandor *et al.*, 1984; Chakraborti and Weisbart, 1987; Chakraborti *et al.*, 1987). Many studies show that administration of exogenous cortisol to teleosts produces alterations in hepatic metabolism (Freeman and Idler, 1973; Chan and Woo, 1978; Davis *et al.*, 1985; Inui and Yokote, 1975; Whiting and Wiggs, 1977; Foster and Moon, 1986) and it is likely therefore that stress-induced perturbations in intermediary metabolism (Leach and Taylor, 1980; Ellsaesser and Clem, 1987) are at least partially mediated by cortisol and hence require a hepatic cortisol receptor. The aim of the present study was, first, to determine whether cortisol-binding sites could be identified within the liver of rainbow trout, and second, to assess whether the nature of such sites was modified by chronic stress.

0016-6480/90 $1.50

MATERIALS AND METHODS

Fish. Two-year-old rainbow trout (Stirling strain) were maintained at a density of 50 fish per tank (mean weight, $\bar{x}$ = 508 ± 17 g, SEM) in 1500-liter outdoor fibreglass tanks, each supplied with a constant flow of lake water (35 liters min^{-1}). The fish were fed once daily with commercial feed at a rate of 1% body weight day^{-1}. To determine the effects of confinement stress on levels of hepatic cortisol binding, four groups of 8 fish (each group comprising 2 fish from each of four stock tanks) were confined to 50-liter polypropylene tanks with a constant flow of lake water (15 liters min^{-1}). The fish were then sampled 24, 48, 72, and 96 hr after the onset of confinement (one group of fish at each time interval). At each sample, a further 8, unstressed, fish (2 from each of four undisturbed stock tanks) were removed, to provide controls. Fish were immediately anaesthetized in in 2-phenoxyethanol (1:2000) and a blood sample was removed using a heparinized syringe for cortisol determination. Fish were then killed by a sharp blow to the head, weighed, measured, and sexed and the livers were removed and treated as described below. To determine the effect of exogenous cortisol on levels of hepatic cortisol binding, the following procedure was employed. Eighty 2-year-old rainbow trout (Annan strain mean weight, $\bar{x}$ = 1056 ± 53 g, SEM) were divided equally among four 1500-liter outdoor fibreglass tanks, maintained as above. The fish from two tanks received an intraperitoneal injection of molten cocoa butter (0.5 ml) containing sufficient cortisol to provide a dose of 60.0 mg kg^{-1} body weight. Fish from the remaining two tanks received cocoa butter vehicle only. After 2 weeks, 8 fish from each tank were sampled and processed as described above (16 fish per treatment).

Tissue preparation. Livers were placed in homogenization buffer (50 m*M* Tris–HCl, 12 m*M* monothioglycerol, 1.0 m*M* EDTA, 10.0 m*M* sodium molybdate, and 20% glycerol) on ice. After rinsing in buffer, livers were weighed and fresh homogenization buffer was added in the ratio of 2.5:1 (vol:weight). Tissue was minced finely with scissors and homogenized, on ice, using an IKA-Ultra Turrax (T18/10) for 10-sec bursts with cooling between bursts. Homogenates were decanted into centrifuge tubes and spun at 1000*g* for 30 min at 4° to prepare a crude nuclear pellet. Supernatant (crude cytosol) was aspirated into a clean tube. The pellet was washed three times in 20.0 ml buffer by resuspension and recentrifugation, then 25.0 ml extraction buffer (0.7 *M* KCl in homogenization buffer) was added to the pellet which was resuspended and incubated at 4°, with frequent mixing for 1 hr. Dextran-coated charcoal suspension (DCC, 1.25% activated charcoal, 0.125% dextran in homogenization buffer) was added to crude cytosol, to remove endogenous steroids, in the ratio of 1:1 (vol:vol), mixed, and incubated at 4° for 45 min and then spun at 50,000*g* for 1 hr at 4°. The resulting clear supernatant was aspirated into clear tubes and stored at −70° until required for assay. Nuclear extracts were similarly treated with DCC, spun down, and stored at −70°.

Quantification of steroid binding. The binding of [^{3}H]cortisol to liver cytosol and nuclear extract was quantified by saturation analysis. Cytosol containing 2.0–5.0 mg protein ml^{-1} was incubated in duplicate 200-μl aliquots with 100 μl homogenization buffer containing 1.5–18.0 n*M* [1, 2, 6, 7 - ^{3}H]cortisol (86 Ci mmol^{-1}, Amersham International) both with and without a 1000-fold excess of inert cortisol. After incubation for 4 hr at 4°, unbound steroid was removed by the addition to each tube of 200 μl DCC, followed by incubation on ice for 10 min (determined by prior experimentation to be the optimal incubation period) and centrifugation at 1000*g* for 5 min at 4°. Aliquots of supernatant were removed, added to 5.0 ml Unisolve 1 scintillant (Koch-Light) in a vial, and counted under standard tritium conditions. Specific binding was calculated from total and nonspecific binding and the equilibrium dissociation constant (k_D) and maximum number of binding sites (N_{max}) were calculated according to Scatchard (1949). The assay procedure was identical for nuclear extracts. Plasma binding was assessed using the same protocol but first diluting plasma 1:10 with homogenization buffer. Estradiol binding was also measured using this procedure, but substituting [1, 2, 6, 7 - ^{3}H]estradiol (100 Ci mmol^{-1}, Amersham International) and inert estradiol.

Protein determination. Protein concentration in the various preparations was determined by the method of Ohnishi and Barr (1978).

Cortisol radioimmunoassay. Plasma cortisol levels were determined according to methodology in Pickering *et al.* (1987).

Effect of proteolytic enzymes on binding of [^{3}H]cortisol to liver cytosol. Ten replicate tubes containing 200 μl liver cytosol and 1 pmol [^{3}H]cortisol, with or without excess inert cortisol, were incubated with 200 μl homogenization buffer containing 500 μg bacterial protease (Sigma, 5.6 U/mg^{-1}). A further 10 tubes were incubated with 200 μl buffer only. Total and nonspecific binding were determined as above.

Specificity of binding of [^{3}H]cortisol to liver cytosol. Aliquots (200 μl) of cytosol were incubated in triplicate together with 1 pmol [^{3}H]cortisol and 0, 1, 10,

100, and 1000 pmol of competitor, for 4 hr at 4°. Specific binding was determined as above.

Time course of binding of [^{3}H]cortisol to liver cytosol. Aliquots (200 µl) of cytosol were incubated together with 1 pmol [^{3}H]cortisol for 15 min, 30 min, 1 hr, 2 hr, 3 hr, 4 hr, 5 hr, 6 hr, 18 hr, and 24 hr at 4°, both in the presence and absence of a 1000-fold excess of inert cortisol. At each sample time, total and nonspecific binding was determined as described above.

Assessment of metabolism during incubation. Ten aliquots of cytosol and 10 of distilled water (200 µl) were incubated together with 1 pmol [^{3}H]cortisol for 4 hr at 4°. The incubates were extracted with 1.5 ml ethyl acetate, and 500 µl of each extract was placed in a scintillation vial with 5.0 ml Unisolve 1 and counted. The remainder of each extract was evaporated under vacuum at 40°, redissolved in 50 µl dichloromethane, and applied to TLC plates (silica gel 60, indicator free, Merck) and run in chloroform:methanol:water (188:12:1). The position of cortisol on the plates was determined by running inert cortisol in three lanes on each plate and visualizing these by spraying with 0.5% potassium permanganate in distilled water. Areas of each lane corresponding to authentic cortisol were removed and eluted in 1.0 ml methanol, 500 µl of which was counted as above.

Separation of bound and unbound [^{3}H]cortisol by Sephadex LH-20 column. Sephadex LH-20-100 gel was swollen in homogenization buffer overnight at 4°. Two milliliters of slurry was loaded into plastic columns (7.0 × 0.7 cm). After settling, 200 µl of either cytosol or nuclear extract, preequilibrated with [^{3}H]cortisol in the presence or absence of excess inert cortisol, was added to the columns. After 10 min at 4°, columns were eluted with 1.0 ml buffer. Eluate was collected in scintillation vials and counted under standard tritium conditions.

Preparation of whole blood cytosol. Twelve rainbow trout (3 years old, Annan strain) were removed from an undisturbed stock tank as described above. In addition to liver tissue, 4.0–5.0 ml blood was removed from each fish. Liver was processed as above. Blood samples were centrifuged at 1000*g* for 15 min at 4°, plasma was removed, and cells were washed twice with homogenization buffer. These samples were then treated exactly as liver samples to produce a whole blood cytosol. This was stored at −70° until assayed.

Colorimetric determination of relative concentrations of haemoglobin in whole blood cytosol and liver cytosol. Haemoglobin absorbs maximally at 540 nm (Snell and Snell, 1954). The absorbance of a series of dilutions of whole blood cytosol (1 in 50 to 1 in 800) was determined to provide a standard curve relating to Abs_{540} to a relative concentration of whole blood cytosol. Liver cytosol at a dilution of 1 in 50 was also measured to provide an estimate of the relative haemoglobin content and thus an estimate of the degree of blood contamination of liver cytosol.

Statistical analysis. The concentration of binding sites in the livers of stressed and unstressed fish and respective plasma cortisol levels were analyzed by a two-way analysis of variance (ANOVA, genstat) with treatment (stressed, unstressed) and time as factors. From a plot of the residuals against fitted values, appropriate transformations were selected (√ or log) to improve homogeneity of variance. The levels of significance given are derived from this analysis, but for ease of presentation, data are given as arithmetic means ± SEM.

RESULTS

Rainbow trout liver cytosol was found to bind [^{3}H]cortisol in a specific and saturable fashion, k_D = 5.1 ± 0.2 n*M*, N_{max} = 197 ± 12 fmol mg^{-1} protein ($\bar{x}$ ± SEM, n = 23). The data from which these values are derived are presented graphically in Fig. 1. Maximum specific binding of [^{3}H]cortisol to liver cytosol was achieved after 2 hr of incubation at 4° (Fig. 2), but declined notably between 6 and 18 hr. Storage of cytosol at −70° completely abolished specific binding; however, binding was maintained at prestorage levels by the inclusion of 20% glycerol in the homogenization buffer. Incubation with protease also completely abolished specific binding. Data on the specificity of the cytosol-binding sites are presented in Fig. 3, the hierarchy of displacement may be summarized as dexamethasone > cortisol > Ru38486 > corticosterone > estradiol > 11-deoxycortisol > cortisone > 17α -hydroxyprogesterone > 11-ketotestosterone > testosterone.

During the processing of liver for cytosol preparation, it was not possible to avoid contamination of the homogenate by whole blood. Plasma was not found to bind [^{3}H]cortisol under the assay conditions employed here, but whole blood cytosol did display specific, saturable, binding of [^{3}H]cortisol. However, both affinity and capacity were significantly different from those of liver cytosol (k_D liver = 6.4 ± 0.5 n*M*, blood = 2.3 ± 0.4 n*M*, $P < 0.001$, t test; N_{max} liver = 171 ± 17 fmol mg^{-1} protein, blood = 40 ± 6 fmol mg^{-1} protein, P < 0.001, t test, n = 12 in both cases). Based on colorimetric measurement, the concentration of haemoglobin in liver cytosol was found to be 1/30 that in whole blood cytosol. Finally, when a regression analysis was carried out on the estimated haemoglobin concentration and a number of specific binding sites, no significant relationship could be demonstrated.

In none of the nuclear extracts prepared and assayed throughout the investigation was reproducible binding of [^{3}H]cortisol apparent. To determine whether this was a true reflection of an absence of binding sites as opposed to a preparation artefact,

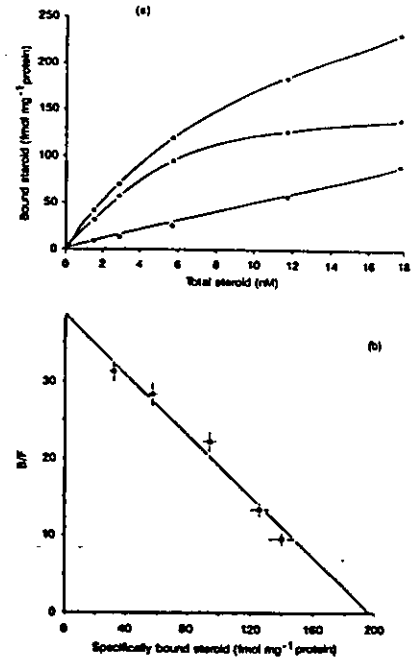


FIG. 1. (a) Saturation curve derived from assays of liver cytosols from 23 individual rainbow trout. Upper line, B_T; middle line, B_S; lower line, B_{NS}. Standard errors of the means were too small to be displayed. (b) Scatchard plot of the above data. Each point is the mean of 23 ± SEM. k_D = 5.1 n*M*, N_{max} = 197 fmol mg^{-1} protein.

samples were assayed simultaneously for [^{3}H]cortisol and [^{3}H]estradiol binding. Estradiol binding has been demonstrated in salmonid liver previously (Lazier *et al.*, 1985; McPherson *et al.*, 1988; Pottinger, 1986). Single point assays were carried out in quadruplicate on cytosol, nuclear pellet washes 1–3, and salt-extracted nuclear pellet. Specific estradiol binding was measurable in all fractions, including nuclear extract. Specific cortisol binding was apparent only in cytosol (Fig. 4). Microscopic examination of nuclear pellets, prior to extraction, under phase-contrast illumination revealed numerous intact nuclei.

To ensure that separation of the bound and unbound steroid in nuclear extracts by DCC, in the presence of high salt concentrations, was providing a valid estimate of specifically bound steroid, a group of samples were separated using LH-20 columns. No specific binding was detectable in the nuclear fraction (cytosol B_s = 23,786 ± 9091 dpm; nuclear extract B_s = 0, n = 5).

No evidence for the conjugation of [^{3}H]cortisol to a solvent-insoluble derivative during incubation with liver cytosol was obtained. Extraction of liver cytosol and distilled water incubates produced recoveries of 224,873 ± 768 dpm and 230,008 ± 551 dpm (n = 10, NSD), respectively. After separation on TLC, extractions of cytosol and distilled water incubates gave recoveries of 51,565 ± 636 dpm and 54,363 ± 764 dpm, respectively (n = 10, $P < 0.05$, t test).

The effects of confinement on plasma cortisol levels and on the measurable binding of [^{3}H]cortisol to liver cytosol are presented in Fig. 5. After 24 hr of confinement, plasma cortisol levels in confined fish were 55 ng ml^{-1} as opposed to 14 ng ml^{-1} in unconfined fish (n = 8, $P < 0.001$; Fig. 5a). Cortisol levels remained high in confined fish for the following 72 hr, declining slightly to 40 ng ml^{-1} after 96 hr of confinement; still significantly elevated compared to control levels of 4 ng ml^{-1} ($P < 0.001$).

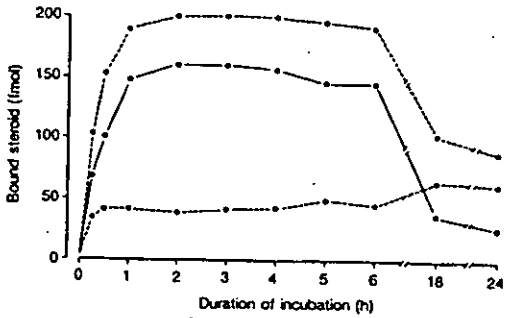


FIG. 2. The time course of binding of [^{3}H]cortisol to rainbow trout liver cytosol at 4°. Solid line, specifically bound steroid; upper broken line, total bound steroid; lower broken line, nonspecifically bound steroid.

ANOVA revealed a significant treatment effect and treatment/time interaction in the number of hepatic cortisol-binding sites. This was resolved as no significant difference at 24 hr in the number of cytosolic-binding sites in stressed and unstressed fish (142 ± 14 vs 161 ± 8, $n = 8$) but significantly fewer sites in the liver of confined, as opposed to unconfined fish (all $P < 0.001$) in the remaining three samples (Fig. 5b).

Cortisol implantation significantly elevated plasma cortisol levels in treated fish relative to the sham-implanted fish (sham-implanted $\bar{x} = 4.2 \pm 0.9$ ng ml^{-1}; cortisol implanted $\bar{x} = 16.3 \pm 2.1$ ng ml^{-1}; $P < 0.001$, $n = 16$, t test). The number of cortisol-binding sites in the liver of cortisol-treated fish was depressed relative to control fish (sham-implanted $\bar{x} = 123 \pm 7$ fmol mg^{-1} protein; cortisol-implanted $\bar{x} = 99 \pm 7$ fmol mg^{-1} protein; $n = 16$, $P < 0.05$, t test) and there was a significant relationship between the plasma cortisol level at the time of sample and the number of hepatic-binding sites (Fig. 6).

DISCUSSION

Rainbow trout liver cytosol binds [^{3}H]-

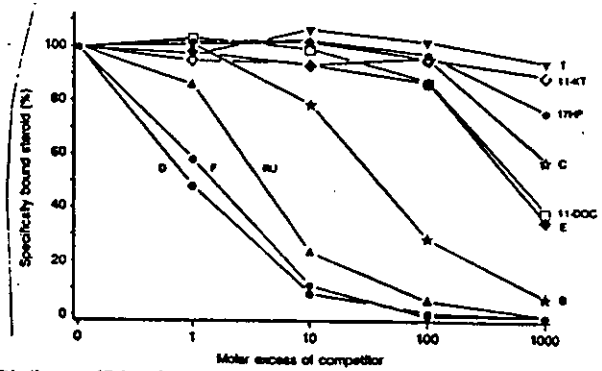


FIG. 3. Binding specificity of rainbow trout liver cytosol. Data are expressed as the percentage of [^{3}H]cortisol bound at the given concentration of unlabeled competitor. D, dexamethasone; F, cortisol; Ru, Ru38486; C, cortisone; B, corticosterone; E, estradiol-17β; 11-DOC, 11-deoxycortisol; 17HP, 17α-hydroxyprogesterone; 11KT, 11-ketotestosterone; T, testosterone.

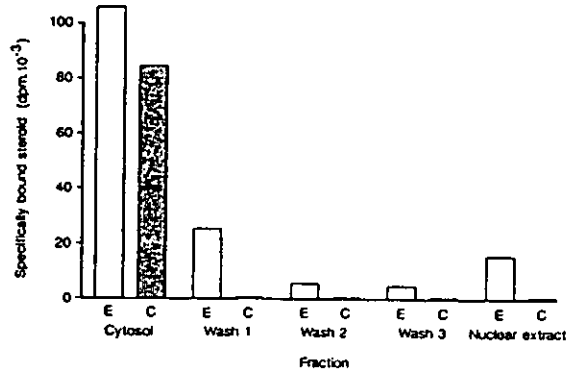


FIG. 4. The presence of specific binding sites for [^{3}H]cortisol and [^{3}H]estradiol in rainbow trout liver cytosol, nuclear pellet washes, and nuclear extract. Each point is the mean of four determinations. E, specific estradiol binding; C, specific cortisol binding.

cortisol with an affinity, specificity, and capacity suggestive of the presence of a specific steroid receptor protein. Binding to cytosol is protease sensitive and displays an equilibrium dissociation constant (k_D) of 5.0 nM, close to that reported for cortisol binding in the liver of brook trout ($k_D = 5.6$ nM; Chakraborti and Weisbart, 1987) and not dissimilar from that reported for brook trout gill cytosol ($k_D = 3.2$ nM; Chakraborti *et al.*, 1987), eel and rainbow trout gill cytosol ($k_D = 2.8$ and 1.4 nM, respectively; triamcinolone acetonide as ligand; Sandor *et al.*, 1984), and eel gut mucosal cytosol ($k_D = 5.2$ nM; DiBattista *et al.*, 1984; $k_D = 2.3$ nM, TA as ligand; DiBattista *et al.*, 1983). Similarly, the maximal binding capacity determined for rainbow trout liver cytosol in this investigation, 200 fmol mg^{-1} protein, is of the same order as that determined in brook trout liver cytosol (167 fmol mg^{-1} protein; Chakraborti and Weisbart, 1987), brook trout gill cytosol (224 fmol mg^{-1} protein; Chakraborti *et al.*, 1987), and rainbow trout gill cytosol (271 fmol mg^{-1} protein; Sandor *et al.*, 1984). Furthermore, the hierarchy of the ability of various steroids to displace [^{3}H]cortisol from its binding sites, dexamethasone > cortisol > Ru38486 > corticosterone > 11-deoxycortisol > cortisone, is similar to those described for brook trout liver (Chakraborti and Weisbart, 1987), eel gill (Sandor *et al.*, 1984), and eel gut (DiBattista *et al.*, 1983).

The receptor-like binding of cortisol observed in rainbow trout liver in the present investigation is likely to be hepatic in origin rather than due to contamination by blood since plasma was not found to bind [^{3}H]cortisol under the assay conditions employed here, and whole blood, although containing high affinity cortisol-binding sites, has a k_D for cortisol significantly different to that of liver (2.3 cf. 5.1). Also, whole blood cytosol has too few binding sites to account for binding within liver cytosol at the level of contamination measured (1 in 30) and no correlation was observed between haemoglobin concentration and the number of binding sites in liver cytosol. In addition, no evidence that cortisol was metabolized to a significant extent during incubation was found.

It was not found possible, during the present investigation, to demonstrate specific binding of cortisol to nuclear extracts. Specific cortisol binding was absent from all preparative fractions except cytosol. Specific estradiol binding *was* detected in the same nuclear extracts, however, sug-

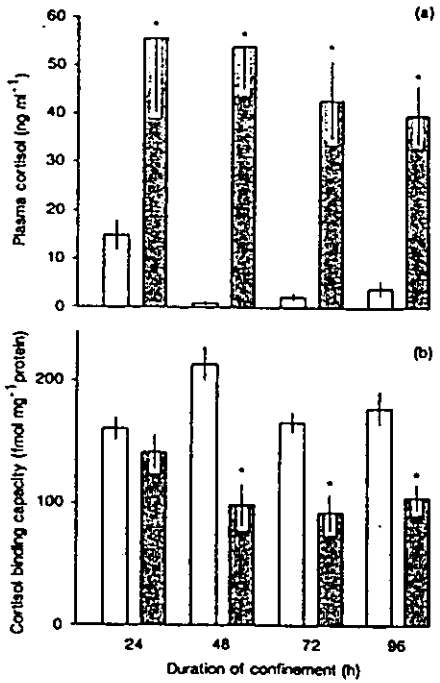


FIG. 5. (a) Plasma cortisol levels in confined (hatched bars) and unconfined (open bars) rainbow trout at each of four sample times. Each value is the mean ± SEM, $n = 8$. (b) Maximum cytosolic cortisol-binding capacity in livers from confined and unconfined rainbow trout ($\bar{x}$ ± SEM, $n = 8$).

gesting that preparative procedures were adequate to isolate binding proteins, were they present. Separation of bound and unbound steroid by LH-20 columns confirmed that the absence of specific nuclear binding was not due simply to the use of DCC coupled with high salt conditions. Currently, it is believed that steroid receptors appear in cytosolic fractions due to "leaching" from nuclei during tissue preparation (King, 1987). Since occupied receptors are believed to have greater affinity for the nucleus (Moudgil, 1987), it might be argued that in fish with very low levels of circulating cortisol a correspondingly low level of receptor occupancy and hence nuclear localisation would be expected. However, nuclear binding could not be demonstrated even in those fish from the confinement experiment, with elevated plasma cortisol levels. DiBattista *et al.* (1983) demonstrated translocation of glucocorticoid receptor–ligand complex to homologous nuclei *in vitro*, and nuclear binding in both gill and liver of brook trout has been reported (Chakraborti *et al.*, 1987; Chakraborti and Weisbart, 1987). However, in the latter two instances affinity of the nuclear "receptor" for cortisol is considerably lower than that of cytosolic binding (liver cytosol $k_D = 5.6$ n*M*; nuclear extract, $k_D = 30.3$ n*M*; gill cytosol $k_D = 3.2$ n*M*, gill nuclear extract $k_D = 50.0$ n*M*). In both cases the affinities of the nuclear elements resemble those typical of a nonreceptor-binding protein. An inability to detect any nuclear binding of cortisol in liver and intestinal mucosa of rainbow trout has been reported by Porthe-Nibelle and Lahlou (1984), but unfortunately these authors did not simultaneously measure cytosolic binding so the significance of their observations is not clear. These reported differences obviously require resolution. It is possible that some characteristic of the cortisol-binding protein leads to a major redistribution of steroid-binding moieties during tissue preparation. However, if this is the case, it is not common to all putative fish receptors since in this and other studies (Lazier *et al.*, 1985) estrogen binding and androgen binding (Pottinger, 1987, 1988) have been localized in the nuclear fraction. A further qualitative difference between the cortisol binding observed in fish tissues is the requirement for a cryoprotectant in the homogenization buffer. In the absence of glycerol (20% by volume), freezing at −70° completely abolished measurable cortisol binding in liver cytosol. Estrogen and androgen binding showed no loss of activity following storage at low temperature (Pottinger, 1986, 1987).

Prolonged confinement stress and administration of exogenous cortisol both caused a marked reduction in the number of binding sites measurable in liver cortisol (Fig. 5b and Fig. 6). Weisbart *et al.* (1987) demonstrated a decline in the number of gill cytosol glucocorticoid-binding sites after both injection of cortisol and elevation of endogenous plasma cortisol by seawater transfer. In both cases, cortisol-binding sites in nuclear extracts rose as cytosol binding declined, the data being interpreted as indicating an increased proportion of occupied receptor resulting in an increased association of receptor with the nucleus. The results of the present investigation do not show this pattern. At no point during confinement or following treatment with cortisol, when cortisol levels were elevated and cytosolic-binding sites were depressed, was binding detectable in the nucleus. Therefore, in this case, the loss of binding sites from cytosol, which if as discussed above, accommodates the total population of binding sites available, may represent a "down-regulation" of such sites. Down-regulation of glucocorticoid receptors by stress or exogenous steroids has been described for various mammalian systems. Both repeated stress and administration of exogenous corticosterone down-regulated glucocorticoid receptors in rat brain (Sapolsky *et al.*, 1984) and total cell content of glucocorticoid receptors was reduced in human lymphocytes by dexamethasone administration (Shipman *et al.*, 1983). More recently, dexamethasone has been shown to reduce the number of hepatic glucocorticoid receptors in mice (Svec, 1988). In functional terms, down-regulation is suggested to provide a means to modulate the effects of exposure to high glucocorticoid levels (Shipman *et al.*, 1983).

In conclusion, a cortisol-binding component of rainbow trout liver cytosol resembles putative glucocorticoid receptors identified in other salmonid tissues in terms of affinity, specificity, and capacity. Chronic stress and prolonged elevation of plasma cortisol levels by implantation significantly reduce the number of binding sites, possibly indicating a mechanism which offers protection against overresponse by the tissue when under conditions of stress. Further work on the anomalies associated with

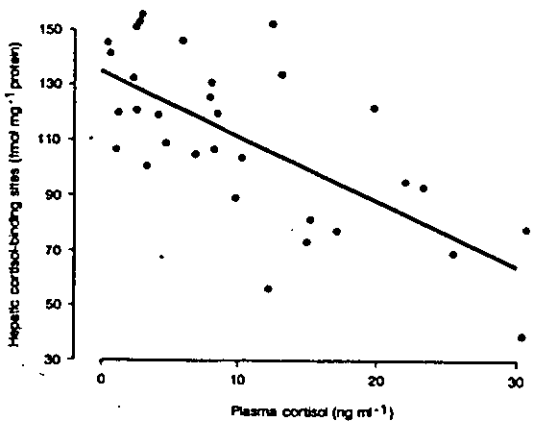


FIG. 6. The relationship between plasma cortisol and the number of hepatic cortisol-binding sites in sham- and cortisol-implanted rainbow trout. The regression, $Y = 135 - 2.4x$, is significant ($P < 0.001$, $n = 32$).

nuclear binding are needed before the functional significance of hepatic cortisol binding can be determined.

ACKNOWLEDGMENT

The author thanks Dr. A. D. Pickering for helpful discussions during the course of this work; Janice Fletcher, Kathleen Atkinson, and Arthur Young for maintaining the experimental fish; and Arthur Young for assistance during sampling. The work was funded by the NERC and MAFF.

REFERENCES

Barton, B. A., Schreck, C. B., and Barton, L. D. (1987). Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout. *Dis. Aquat. Org.* 2, 173–185.

Carragher, J. F., Sumpter, J. P., Pottinger, T. G., and Pickering, A. D. (1989). The deleterious effects of cortisol implantation on reproductive function in two species of trout, *Salmo trutta* L. and *Salmo gairdneri* Richardson. *Gen. Comp. Endocrinol.* 76, 310–321.

Chakraborti, P. K., and Weisbart, M. (1987). High-affinity cortisol receptor activity in the liver of the brook trout, *Salvelinus fontinalis* (Mitchill). *Canad. J. Zool.* 65, 2498–2503.

Chakraborti, P. K., Weisbart, M., and Chakraborti, A. (1987). The presence of corticosteroid receptor activity in the gills of the brook trout, *Salvelinus fontinalis*. *Gen. Comp. Endocrinol.* 66, 323–332.

Chan, D. K. O., and Woo, N. Y. S. (1978). Effect of hypophysectomy on the chemical composition and intermediary metabolism of the Japanese eel, *Anguilla japonica*. *Gen. Comp. Endocrinol.* 35, 169–178.

Danielson, M., and Stallcup, M. R. (1984). Down-regulation of glucocorticoid receptors in mouse lymphoma cell variants. *Mol. Cell. Biol.* 4, 449–453.

Davis, K. B., Torrance, P., Parker, N. C., and Suttle, M. A. (1985). Growth, body composition and hepatic tyrosine aminotransferase activity in cortisol-fed channel catfish, *Ictalurus punctatus* Rafinesque. *J. Fish Biol.* 27, 177–184.

DiBattista, J. A., Mehdi, A. Z., and Sandor, T. (1983). Intestinal triamcinolone acetonide receptors of the eel (*Anguilla rostrata*). *Gen. Comp. Endocrinol.* 51, 228–238.

DiBattista, J. A., Mehdi, A. Z., and Sandor, T. (1984). A detailed investigation of the cytoplasmic cortisol-binding receptor of North American eel (*Anguilla rostrata*) tissues. *Canad. J. Biochem. Cell Biol.* 62, 991–997.

Ellsaesser, C. F., and Clem, L. W. (1987). Blood serum chemistry measurements of normal and acutely stressed channel catfish. *Comp. Biochem. Physiol. A* 88, 589–594.

Foster, G. D., and Moon, T. W. (1986). Cortisol and liver metabolism of immature American eels, *Anguilla rostrata* (LeSueur). *Fish Physiol. Biochem.* 1, 113–124.

Freeman, H. C., and Idler, D. R. (1973). Effects of corticosteroids on liver transaminases in two salmonids, the rainbow trout (*Salmo gairdneri*) and the brook trout (*Salvelinus fontinalis*). *Gen. Comp. Endocrinol.* 20, 69–75.

Inui, Y., and Yokote, M. (1975). Gluconeogenesis in the eel. IV. Gluconeogenesis in the hydrocortisone administered eel. *Bull. Japan. Soc. Sci. Fish* 41, 973–981.

King, R. J. B. (1987). Structure and function of steroid receptors. *J. Endocrinol.* 114, 341–349.

Lazier, C. B., Lonergan, K., and Mommsen, T. P. (1985). Hepatic estrogen receptors and plasma estrogen-binding activity in the Atlantic salmon. *Gen. Comp. Endocrinol.* 57, 234–245.

Leach, G. J., and Taylor, M. H. (1980). The role of cortisol in stress-induced metabolic changes in *Fundulus heteroclitus*. *Gen. Comp. Endocrinol.* 42, 219–227.

McPherson, R., Hannum, J., and Greco, T. (1988). An investigation of an estrogen-binding component in the liver and plasma of brook char, *Salvelinus fontinalis*. *Comp. Biochem. Physiol. A* 89, 615–619.

Moudgil, V. K. (1987). Steroid hormone receptors: Recent advances. *In* "Recent Advances in Steroid Hormone Action" (V. K. Moudgil, Ed.), pp. 1–57. de Gruyter, New York.

Ohnishi, S. T., and Barr, J. K. (1978). A simplified method of quantitating protein using the biuret and phenol reagents. *Anal. Biochem.* 86, 193–200.

Pickering, A. D. (1989). Stress responses and disease resistance in farmed fish: Aqua Nor 1987, Trondheim. *Proc. Conf.* 3, 35–49.

Pickering, A. D., and Pottinger, T. G. (1989). Stress responses and disease resistance in salmonid fish: Effects of chronic elevation of plasma cortisol. *Fish Physiol. Biochem.*, in press. 7, 253-258.

Pickering, A. D., Pottinger, T. G., and Sumpter, J. P. (1987). On the use of dexamethasone to block the pituitary–interrenal axis in the brown trout, *Salmo trutta* L. *Gen. Comp. Endocrinol.* 65, 346–353.

Porthe-Nibelle, J., and Lahlou, B. (1984). Nuclear binding of cortisol in intestinal mucosa and liver of a teleost fish (*Salmo gairdneri irideus*). *Steroids* 43, 385–392.

Pottinger, T. G. (1986). Estrogen-binding sites in the liver of sexually mature male and female brown trout, *Salmo trutta* L. *Gen. Comp. Endocrinol.* 61, 120–126.

Pottinger, T. G. (1987). Androgen binding in the skin of mature male brown trout, *Salmo trutta* L. *Gen. Comp. Endocrinol.* 66, 224–232.

Pottinger, T. G. (1988). Seasonal variation in specific plasma- and target-tissue binding of androgens, relative to plasma steroid levels, in the brown trout, *Salmo trutta* L. *Gen. Comp. Endocrinol.* 70, 334–344.

Sandor, T., DiBattista, J. A., and Mehdi, A. Z. (1984). Glucocorticoid receptors in the gill tissue of fish. *Gen. Comp. Endocrinol.* 53, 353–364.

Sapolsky, R. M., Krey, L. C., and McEwen, B. S. (1984). Stress down-regulates corticosterone receptors in a site-specific manner in the brain. *Endocrinology* 114, 287–292.

Scatchard, G. (1949). The attractions of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.* 51, 660–672.

Shipman, G. F., Bloomfield, C. D., Gajl-Peczalska, K. J., Munck, A. U., and Smith, K. A. (1983). Glucocorticoids and lymphocytes. III. Effects of glucocorticoid administration on lymphocyte glucocorticoid receptors. *Blood* 61, 1086–1090.

Snell, F. D., and Snell, C. T. (1954). "Colorimetric Methods of Analysis," pp. 558–563. Van Nostrand, New York.

Svec, F. (1988). The biopotency of dexamethasone at causing hepatic glucocorticoid receptor down-regulation in the intact mouse. *Biochim. Biophys. Acta* 970, 90–95.

Weisbart, M., Chakraborti, P. K., Gallivan, G., and Eales, J. G. (1987). Dynamics of cortisol receptor activity in the gills of the brook trout, *Salvelinus fontinalis*, during seawater adaptation. *Gen. Comp. Endocrinol.* 68, 440–448.

Whiting, S. J., and Wiggs, A. J. (1977). Effect of nutritional factors and cortisol on tyrosine aminotransferase activity in liver of brook trout, *Salvelinus fontinalis* Mitchill. *Comp. Biochem. Physiol. B* 58, 189–193.

1990 Gen. Comp. Endocrinol. (submitted)

The effect of cortisol administration on hepatic and plasma estradiol-binding capacity in immature female rainbow trout (Oncorhynchus mykiss).

T.G. Pottinger and A.D. Pickering.

Institute of Freshwater Ecology,
The Ferry House,
Far Sawrey,
Ambleside,
Cumbria LA22 OLP
U.K.

Implantation of a cortisol-containing pellet into the peritoneal cavity of immature female rainbow trout raised plasma cortisol levels within the range commonly observed in chronically stressed fish. In cortisol-implanted fish there was a significant decline in the concentration of hepatic estradiol-binding sites relative to sham-implanted controls. This consisted of a 35% drop in cytosolic binding sites, and a 29% reduction in the number of nuclear estradiol-binding sites, by 4 weeks post-implantation. Plasma estradiol-binding capacity was also influenced by cortisol treatment. After 2 weeks there was a 33% increase in plasma estradiol-binding capacity of cortisol implanted fish. Plasma estradiol levels were unaffected by cortisol implantation suggesting that the effects of cortisol on estradiol-binding sites were not mediated by altering the rate of estradiol secretion. The results indicate a possible mechanism by which environmental stress may suppress vitellogenesis.

Both chronic and acute environmental stress have been shown to adversely affect aspects of the reproductive system in fish (Freeman et al., 1983; Pickering et al., 1987; Safford and Thomas, 1987; Truscott et al., 1983) and the administration of cortisol to salmonids, simulating the effect of chronic stress on plasma cortisol levels, results in the suppression of a wide range of reproductive indices. These include a reduction in the levels of circulating estradiol and vitellogenin (Carragher et al., 1989). In fish, the period of vitellogenesis, during which yolk proteins are synthesized in the liver and sequestered by the developing oocytes, is crucial for the reproductive success of the individual. An understanding of the mechanisms by which stress can disrupt the normal functioning of the vitellogenic cycle is, therefore, important.

The synthesis and secretion of the yolk precursor, vitellogenin by the liver is stimulated by the ovarian steroid estradiol. (Ng and Idler, 1983). Recent work has demonstrated that exposure of isolated rainbow trout ovarian follicles to physiological levels of cortisol reduces their secretion of estradiol (Carragher and Sumpter, 1989) indicating one possible mechanism by which stress may compromise vitellogenic processes. The aim of the present study is to determine whether cortisol, at levels similar to those occurring during stress, is capable of impeding the detection and transduction of estrogenic signals in the liver by influencing the abundance of specific, hepatic estradiol receptors.

MATERIALS AND METHODS

Fish. Eighty, 2-year-old, immature rainbow trout (Annan strain, all female, mean weight = 1056 ± 53g) were divided equally between 4 1500 liter outdoor fibreglass tanks, each supplied with a constant flow of lake water (35 liters min^{-1}, temperature during the experimental period 8-10 °c). The fish were fed daily with commercial feed at a rate of 1% body weight day^{-1}. After 2

weeks acclimation to these conditions the fish from 2 tanks received an intraperitoneal injection of molten cocoa butter (0.5 ml) containing sufficient cortisol to provide a dose of 60.0 mg kg^{-1} body weight. Fish from the remaining two tanks received cocoa butter vehicle only.

Two weeks after injection the fish were sampled. Eight fish per tank were removed to give a total of 16 treated and 16 control fish. Fish were immediately anaesthetized in 2-phenoxyethanol (1:2000) and a blood sample was taken from the candal vessels by means of a heparinized syringe. Fish were then killed by a sharp blow to the head, weighed, measured and sexed and the livers were removed and treated as described below. A second sample of fish was taken 4 weeks after implantation, the procedure being identical to that outlined above.

Tissue preparation. Livers were placed in homogenization buffer (50 mM Tris-Hcl, 12 mM monothioglycerol, 1.0 mM EDTA, 10.0 mM sodium molybdate and 20% glycerol) on ice. After rinsing in buffer, livers were weighed and fresh homogenization buffer was added in the ratio 2.5 : 1 (vol : weight). Tissue was minced finely with scissors and homogenized, on ice, using an IKA-Ultra Turrax (T18/10) for 10 second bursts, with cooling between bursts. Homogenates were decanted into centrifuge tubes and spun at 1000 xg for 30 min at 4°C, to prepare a crude nuclear pellet. Supernatant (crude cytosol) was aspirated into a clean tube. The pellet was washed 3 times in 20.0 ml buffer by resuspension and recentrifugation, then 25.0 ml extraction buffer (0.7 M KCL in homogenization buffer) was added to the pellet which was resuspended and incubated at 4°C, with frequent mixing for 1 h. Dextran-coated charcoal suspension (DCC, 1.25% activated charcoal, 0.125% dextran in homogenization buffer) was added to crude cytosol, to remove endogenous steroids, in the ratio 1 : 1 (vol : vol), mixed and incubated at 4°C for 45 min, and then spun at 50,000 g for 1 h at 4°C. The resulting clear supernatant was aspirated into clean tubes and stored at -70°C until required for assay. Nuclear extracts

were similarly treated with DCC, spun down, and stored at -70°C.

Quantification of steroid binding. The binding of [^{3}H] estradiol to liver cytosol and nuclear extract was quantified by saturation analysis. Cytosol containing 2.0-5.0 mg protein ml^{-1} was incubated in duplicate 200 µl aliquots with 100 µl homogenization buffer containing 1.5 - 18.0 nM [1, 2, 6, 7 - ^{3}H] estradiol (106 Ci mmol^{-1}, Amersham International) both with and without a 1000-fold excess of inert cortisol. After incubation for 3 h at 4°C, unbound steroid was removed by the addition to each tube of 200 µl DCC, followed by incubation on ice for 15 min (determined by prior experimentation to be the optimal incubation period) and centrifugation at 1000 g for 5 min at 4°C. Aliquots of supernatant were removed, added to 5.0 ml Unisolve 1 scintillant (Koch-Light) in a vial and counted under standard tritium conditions. Specific binding was calculated from total and non-specific binding and the equilibrium dissociation constant (k_D) and maximum number of binding sites (N_{max}) were calculated according to Scatchard (1949). The assay procedure was identical for nuclear extracts. Plasma binding was assessed using the same protocol but first diluting plasma 1 : 10 with homogenization buffer. During the assay samples were incubated for 2 h at 4°C and for 10 min with DCC.

Protein determination. Protein concentration in the various preparations was determined by the method of Ohnishi and Barr (1978).

Cortisol radioimmunoassay. Plasma cortisol levels were determined according to methodology in Pickering et al. (1987).

Estradiol radioimmunoassay. Estradiol levels in plasma were determined using the assay protocol described by Pottinger and Pickering (1985) for androgens, but omitting the TLC stage and employing an antiserum supplied by Dr. Z. Yaron (Tel Aviv). For details of cross-reactivities see Bogomolnaya and Yaron (1984).

Specificity of binding of [^{3}H] estradiol. Aliquots (200 µl) of cytosol were incubated in triplicate together with 1 pmol [^{3}H] estradiol and 0, 1, 10, 100

and 1000 pmols of competitor, for 3 h at 4°C. Specific binding was determined as above. A similar procedure was carried out for nuclear extract and plasma.

Time-course of binding of [^{3}H] estradiol. Aliquots (200 µl) of cytosol or diluted plasma were incubated together with 1 pmol [^{3}H] estradiol for 15 min, 30 min, 45 min, 1h, 2h, 3h, 4h, 5h, 6h, 18h, 24h and 48h at 4°C both in the presence and absence of a 1000-fold excess of inert estradiol. At each time, total and non-specific binding was determined as described above.

Statistical analyses.

Significance of the results was assessed using an unpooled t-test (Minitab). Results are expressed as mean ± standard error of the mean (SEM)

RESULTS

Rainbow trout liver nuclear extract, cytosol and rainbow trout plasma were all found to bind [^{3}H] estradiol with high affinity and limited capacity (nuclear extract, k_D = 2.2 ± 0.5 nM, N_{max} = 54 ± 9 fmol mg protein $^{-1}$; cytosol, k_D = 4.2 ± 0.8, N_{max} = 83 ± 9; plasma, k_D = 16.8 ± 1.3, N_{max} = 2136 ± 138; n = 4 - 8). Typical Scatchard plots for each fraction are presented in Fig. 1.. Binding of estradiol to nuclear extract and cytosol showed identical specificity, [^{3}H] estradiol being displaced only by estradiol and estrone but not by testosterone, 11-ketotestosterone, cortisol or 17a, 20β - dihydroxy progesterone (Fig. 2a, b). Specificity of plasma binding was notably different. Bound [^{3}H] estradiol was displaced by estradiol, testosterone and 11-ketotestosterone (Fig. 2c). The stability of specifically bound [^{3}H] estradiol also varied. Binding of [^{3}H] estradiol to cytosol was relatively stable for 24h, declining only gradually between 24 - 48h (Fig. 3a) whereas binding of [^{3}H] estradiol to plasma was less stable, declining within 2h of the onset of incubation (Fig. 1b).

Plasma cortisol levels in implanted fish were significantly higher than those in sham-implanted fish at 2 weeks (16.3 ± 2.1 v. 4.2 ± 0.9 ng ml^{-1}, p <0.001) though by 4 weeks the difference was no longer significant (Fig. 4). The concentration of hepatic cytosol estradiol-binding sites was significantly lower in cortisol implanted fish than control fish at both 2 weeks (112 ± 6 v. 91 ± 4 fmol mg^{-1} protein, p <0.01) and 4 weeks post-implantation (116 ± 7 v. 75 ± 5 fmol mg^{-1} protein, p <0.001) (Fig. 5). There was no significant difference in the number of nuclear estradiol-binding sites at 2 weeks post-implantation but after 4 weeks there were significantly fewer in the cortisol implanted fish (64 ± 6 fmol mg^{-1} protein) than control fish (90 ± 7 fmol mg^{-1} protein, p <0.01) (Fig. 5). Regression analysis of all the data revealed a significant (p <0.001) inverse relationship between plasma cortisol level and the number of cytosolic estradiol-binding sites (Fig. 6). Cortisol treatment also significantly <u>increased</u> the number of plasma estradiol-binding

sites at 2 weeks post-implantation (437 ± 21 v. 540 ± 20 fmol mg^{-1} protein, p <0.01) although no difference was apparent at 4 weeks (Fig. 7a). Plasma protein concentration was also increased significantly in cortisol-treated fish at 2 weeks post-implantation (59.9 ± 1.6 v. 64.7 ± 0.8 mg ml^{-1}, p <0.05), however, this difference had disappeared by 4 weeks post-implantation. The increase in concentration of plasma binding sites coupled with elevation in the plasma protein levels resulted in a significant increase in the total plasma estradiol-binding capacity at 2 weeks post- implantation (26.3 ± 1.5 v. 34.9 ± 1.9 pmol ml^{-1}, p <0.001) (Fig. 7b).

Plasma estradiol levels did not differ significantly between control and implanted fish at 2 weeks (1.0 ± 0.1 v. 1.2 ± 0.1 ng ml^{-1}) or 4 weeks post-implantation (1.1 ± 0.2 v. 0.9 ± 0.1 ng ml^{-1}).

DISCUSSION

Estrogen receptors have been identified and characterized in a range of teleost species including the winter flounder, Pseudopleuronectes Americanus (Sloop et al, 1984), black goby, Gobius niger (Le Menn et al, 1980), Atlantic salmon, Salmo salar (Lazier et al, 1985), brown trout, Salmo trutta (Pottinger, 1986) and brook trout, Salvelinus fontinalis (McPherson et al., 1988). The binding of estradiol to components of rainbow trout liver cytosol and nuclear extract is similar in character to that reported for other species. Estradiol is bound with high affinity to cytosol ($k_D \sim 4$ nM) and nuclear extract ($k_D \sim 2$ nM) and with limited capacity in both cases ($N_{max} \sim 50 - 100$ fmol mg^{-1} protein). Binding was highly specific for estradiol in both cytosol and nuclear extract. Of the steroids tested only estrone competed to any degree. To ensure that the binding sites measured in liver preparations were hepatic in origin, estradiol binding in plasma was also partially characterized. The equilibrium dissociation constant ($k_D \sim 17$ nM) and binding capacity (0.5 - 2.0 pmol mg^{-1} protein) determined for estradiol in rainbow trout plasma also resemble those previously reported for salmonids (Lazier et al, 1985; Pottinger, 1986). Furthermore, the fact that the androgens testosterone and 11-ketotestosterone compete successfully with estradiol for binding sites in plasma, in contrast to the liver preparations, suggests that the binding of estradiol measured in plasma can be attributed to the distinct sex hormone binding protein previously identified in rainbow trout (Fostier and Breton, 1975). Plasma binding was found to be considerably less stable than cytosolic binding. Instability of steroid-protein interaction has also been observed in goldfish plasma (Pasmanik and Callard, 1986).

Implantation with cortisol-containing cocoa butter pellets was successful in maintaining elevated levels of cortisol in the plasma of treated fish for between 2 and 4 weeks. Although low (~ 16.0 ng ml^{-1}) these levels are similar to those observed in chronically stressed salmonids (Pickering et al, 1987; Pickering and Pottinger, 1989) and closely resemble the levels observed

by Carragher et al. (1989) in rainbow trout implanted at a similar dosage. Differences between the treated and untreated fish, in terms of both hepatic (cytosol and nuclear) and plasma estradiol-binding capacity, were observed. The number of cytosolic binding sites was significantly reduced at both 2 and 4 weeks post-implantation, and significantly fewer binding sites were present in nuclear extracts of fish treated with cortisol for 4 weeks than in the corresponding controls. Overall, there was a significant inverse relationship between plasma cortisol levels and the number of cytosolic binding sites. Since cortisol does not compete with estradiol for binding-sites (see Fig. 2) and, furthermore, all fractions were treated with DCC prior to assay, the results strongly suggest that prolonged elevation of plasma cortisol levels is capable of causing a reduction in the number of estradiol receptors within the liver of rainbow trout. A positive relationship is known to exist between estradiol level and number of hepatic binding sites in salmonids both in vivo (Lazier et al., 1985) and in vitro (Mommsen and Lazier, 1986). Cortisol is known to suppress plasma estradiol levels in mature female salmonids (Carragher et al., 1989) and, therefore the present study was carried out with immature female trout, with low plasma estradiol levels, to avoid a potentially confounding effect of cortisol on ovarian steroid secretion. No significant difference in plasma estradiol levels could be demonstrated between groups of fish in the present study. Whether these results indicated that chronic stress impairs the ability of the liver to respond to estradiol remains to be seen, but it is of interest that we have previously observed a suppression of plasma vitellogenin levels in immature female rainbow trout, treated with cortisol, despite there being no discernible effect on plasma estradiol levels (Carragher et al., 1989). It might be postulated that the low levels of estradiol present in immature fish are still sufficient to promote vitellogenesis, albeit at a very low level, and that a cortisol-induced reduction in hepatic receptor complement is reflected in the reduced plasma vitellogenin levels. The results reported here are not without precedent. In rats, injection of the

corticosteriod agonist dexamethasome significantly reduced the number of uterine nuclear estradiol receptors (Campbell, 1978), and cortisol treatment also significantly reduced the number of cytosolic estrogen receptors in the uterus of ewes, while adrenalectomy increased receptor number (Atkinson and Adams, 1988).

In addition to an effect on hepatic estradiol receptor number, cortisol administration also caused a significant increase in plasma estradiol-binding capacity. This difference in binding capacity was apparent after 2 weeks of cortisol treatment but was lost by 4 weeks, possibly reflecting a decline in the efficiency of the implant over this latter period. The increase arose because of an elevation in the number of binding sites per unit of protein, potentiated by a rise in plasma protein concentration. Although plasma sex-hormone binding proteins have been identified in a number of teleost species (Callard and Callard, 1987) there is little information on natural changes in abundance, or regulation, of such proteins. In goldfish, serum sex hormone binding protein levels show no seasonal differences (Pasmanik and Callard, 1986) whereas in brown trout a distinct seasonal pattern in plasma androgen-binding capacity has been reported (Pottinger, 1988). Such proteins are believed to be involved in the transport of steroids, and possibly their protection from clearance (Martin, 1980), but may also have a regulatory function. For example, the induction of maturation in trout oocytes by 17α, 20β - dihydroxyprogesterone in vitro is suppressed by the presence of plasma binding protein (Fostier and Breton, 1975) and serum binding proteins antagonize the inhibition of pituitary luteinizing hormone release by estradiol in rats (Moll and Rosenfield, 1968). It may be argued, therefore, that the cortisol-induced elevation of plasma estradiol-binding capacity observed in the present study might further limit the effectiveness of circulating estradiol in inducing vitellogenin synthesis by the liver.

In summary, treatment of immature female rainbow trout with cortisol resulted in the elevation of plasma cortisol in treated fish to levels commonly

found in chronically stressed fish. In cortisol-treated fish the number of hepatic estradiol receptors was significantly reduced and the plasma estradiol-binding capacity was significantly increased. These results indicate a further mechanism by which components of the reproductive system in fish may be suppressed during periods of stress.

ACKNOWLEDGEMENTS

The authors would like to thank Miss J.M. Fletcher, Miss K.M. Atkinson and Mr. H.A. Young for care and maintenance of the fish used in this study. The work received financial support from MAFF and NERC.

REFERENCES

Atkinson, S and Adams, N.R. (1988). Adrenal glands alter the concentration of oestradiol - 17β and its receptor in the uterus of ovariectomized ewes. J. Endocr. 118, 375-380.

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.

Callard, I.P. and Callard, G.V. (1987). Sex steroid receptors and non-receptor binding proteins. In "Hormones and reproduction in fishes, amphibions and reptiles" (D.O. Norris and R.E. Jones, eds), pp. 355-384. Plenum Press, New York.

Campbell, P.S. (1978). The mechanism of the inhibition of uterotrophic responses by acute dexamethasome pretreatment. Endocrinology 103, 716-723.

Carragher, J.F. and Sumpter, J.P. (1989). The effect of cortisol on the secretion of sex steroids from cultured ovarian follicles of rainbow trout. Gen. comp. Endocrinol. (In press).

Carragher, J.F., Sumpter, J.P., Pottinger, T.G. and Pickering, A.D. (1989). The deleterious effects of cortisol implantation on reproductive function in two species of trout, Salmo trutta L. and Salmo gairdneri Richardson. Gen. comp. Endocrinol. (In press).

Fostier, A., and Breton, B. (1975). Binding of steroids by plasma of a teleost : The rainbow trout, Salmo gairdneri. J. Steroid Biochem. 6, 345-351.

Freeman, H.C., Sangalang, G.B., Burns, G. and McMenemy, M. (1983). The blood sex hormone levels in sexually mature male Atlantic salmon (Salmo salar) in the Westfield river (pH 4.7) and the Medway river (pH 5.6), Nova Scotia. Sci. Total Environ. 32, 87-91.

Lazier, C.B., Lonergan, K. and Mommsen, T.P. (1985). Hepatic estrogen receptors and plasma estrogen-binding activity in the Atlantic salmon. Gen. comp. Endocrinol. 57, 234-245.

Le Menn, F., Rochefort, H. and Garcia, M. (1980). Effect of androgen mediated by the estrogen receptor of fish liver : Vitellogenin accumulation. Steroids 35, 315-328.

Martin, B. (1980). Steroid-protein interactions in nonmammalian vertebrates : Distribution, origin, regulation, and physiological significance of plasma steroid binding proteins. In "Steroids and their mechanism of action in nonmammalian vertebrates" (G. Delrio and J. Brachet, Eds) pp. 63-73. Raven Press, New York.

McPherson, R., Hannum, J. and Greco, T. (1988). An investigation of an estrogen-binding component in the liver and plasma of brook char, Salvelinus fontinalis. Comp. Biochem. Physiol. A 89, 615-619.

Moll, G.W. and Rosenfield, R.L. (1986). Estradiol inhibition of pituitary luteinizing hormone release is antagonized by serum proteins. J. Steroid Biochem. 25, 309-314.

Mommsen, T.P. and Lazier, C.B. (1986). Stimulation of estrogen receptor accumulation by estradiol in primary cultures of salmon hepatocytes. FEBS letters. 195, 269-271.

Ng, T.B. and Idler, D.R. (1983). Yolk formation and differentiation in teleost fishes. In "Fish Physiology" (W.S. Hoar, D.J. Randall and E.M. Donaldson, Eds), Vol IXA, pp 373-404. Academic Press, New York.

Ohnishi, S.T. and Barr, J.K. (1978). A simplified method of quantitating protein using the biuret and phenol reagents. Anal. Biochem. 86, 193-200.

Pasmanik, M. and Callard, G. (1986). Characteristics of a testosterone-estradiol binding globulin (TEBG) in goldfish serum. Biol Reprod. 35, 838-845.

Pickering, A.D. and Pottinger, T.G. (1989). Stress responses and disease resistance in salmonid fish : effects of chronic elevation of plasma cortisol. Fish Physiol. Biochem. 7, 253-258.

Pickering, A.D., Pottinger, T.G., Carragher, J. and Sumpter, J.P. (1987). The effects of acute and chronic stress on the levels of reproductive hormones

in the plasma of mature male brown trout, Salmo trutta L.. Gen. Comp. Endocrinol. 68, 249-259.

Pottinger, T.G. (1986). Estrogen-binding sites in the liver of sexually mature and female brown trout, Salmo trutta L.. Gen. Comp. Endocrinol. 61, 120-126.

Pottinger, T.G. (1988). Seasonal variation in specific plasma - and target - tissue binding of androgens, relative to plasma steroid levels, in the brown trout, Salmo trutta L.. Gen. Comp. Endocrinol. 70, 334-344.

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

Safford, S.E. and Thomas, P. (1987). Effects of capture and handling on the circulating levels of gonadal steroids and cortisol in the spotted sea trout, Cynoscion nebulosus. In "Reproductive Physiology of fish 1987" (D.R. Idler, L.W. Crim and J.M. Walsh, Eds) p. 311. Memorial University of Newfoundland : St Johns.

Scatchard, G. (1949). The attractions of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. 51, 660-672.

Sloop, T.C., Sherman, B. and Lucier, G.W. (1984). Characterization of a cytosolic steroid-binding protein in the liver of the winter flounder, Pseudopleuronectes americanus. Gen. comp. Endocrinol. 55, 157-162.

Truscott, B., Walsh, J.M., Burton, M.P., Payne, J.F. and Idler, D.R. (1983). Effect of acute exposure to crude petroleum on some reproductive hormones in salmon and flounder. Comp. Biochem. Physiol. C 75, 121-130.

Fig. 1. Representative Scatchard plots derived from saturation analysis of (a) rainbow trout cytosol ($y = 22.2 - 0.26x$, n_{max} = 85 fmol mg^{-1} protein, k_D = 3.9 nM) (b) nuclear extract ($y = 47.1 - 0.61x$, N_{max} = 77 fmol mg^{-1} protein, k_D = 1.6 nM) and (c) plasma ($y = 128 - 0.05x$, N_{max} = 2560 fmol mg^{-1} protein, k_D = 20 nM).

Fig. 2. Binding speciticity of (a) rainbow trout liver nuclear extract (b) cytosol and (c) plasma. Data are expressed as the percentage of [^{3}H] estradiol specifically bound at the given concentration of unlabelled competitor. Each point is the mean of 3 determinations.

● estradiol, ■ estrone, □ testosterone, △ 11-ketotestosterone,

○ cortisol, ▲17α, 20β-dihydroxyprogesterone.

Fig. 3. The time-course for binding of [^{3}H] estradiol to immature female rainbow trout cytosol (a) and plasma (b).

□ Total bound steroid, ● Specifically bound steroid, △ Non-specifically bound steroid.

Each point is the mean of 5 values. Standard errors were too small to be depicted.

Fig. 4. Plasma cortisol levels in control (unshaded bars) and cortisol-implanted (shaded bars) immature female rainbow trout 2 weeks and 4 weeks post-implantation. *** - significantly different from control, $p < 0.001$, ($\bar{x} \pm$ SEM, $n = 16$).

Fig. 5. The concentration of estradiol-binding sites in immature female rainbow trout liver cytosol and nuclear extract from control (unshaded bars) and cortisol-implanted fish (shaded bars) 2 weeks and 4 weeks after implantation. Values significantly different from control values are denoted :

** , p <0.01, *** p <0.001 (x ± SEM, n = 16).

Fig. 6. The relationship between plasma cortisol levels and the number of cytosol estradiol-binding sites in control and cortisol-implanted immature female rainbow trout. (y = 115 - 1.62x, p <0.001, n = 64).

Fig. 7. The concentration of estradiol-binding sites in plasma (a) and total estradiol-binding capacity of plasma (b) from control (unshaded bars) and cortisol-implanted (shaded bars) immature female rainbow trout at 2 and 4 weeks post-implantation. Values significantly different from controls are denoted : ** p <0.01, *** p <0.001 (X ± SEM, n = 16).

Fig.1

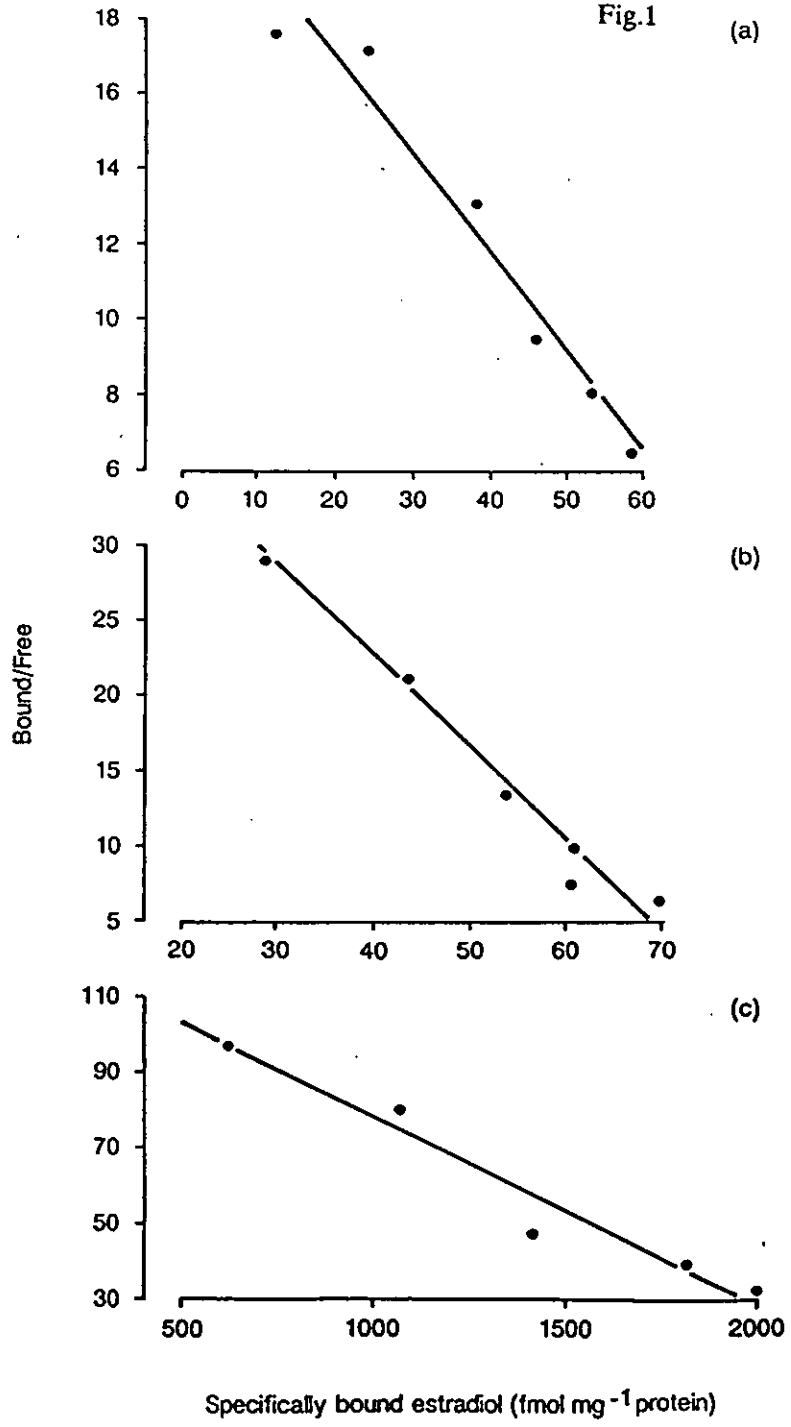


Fig.2

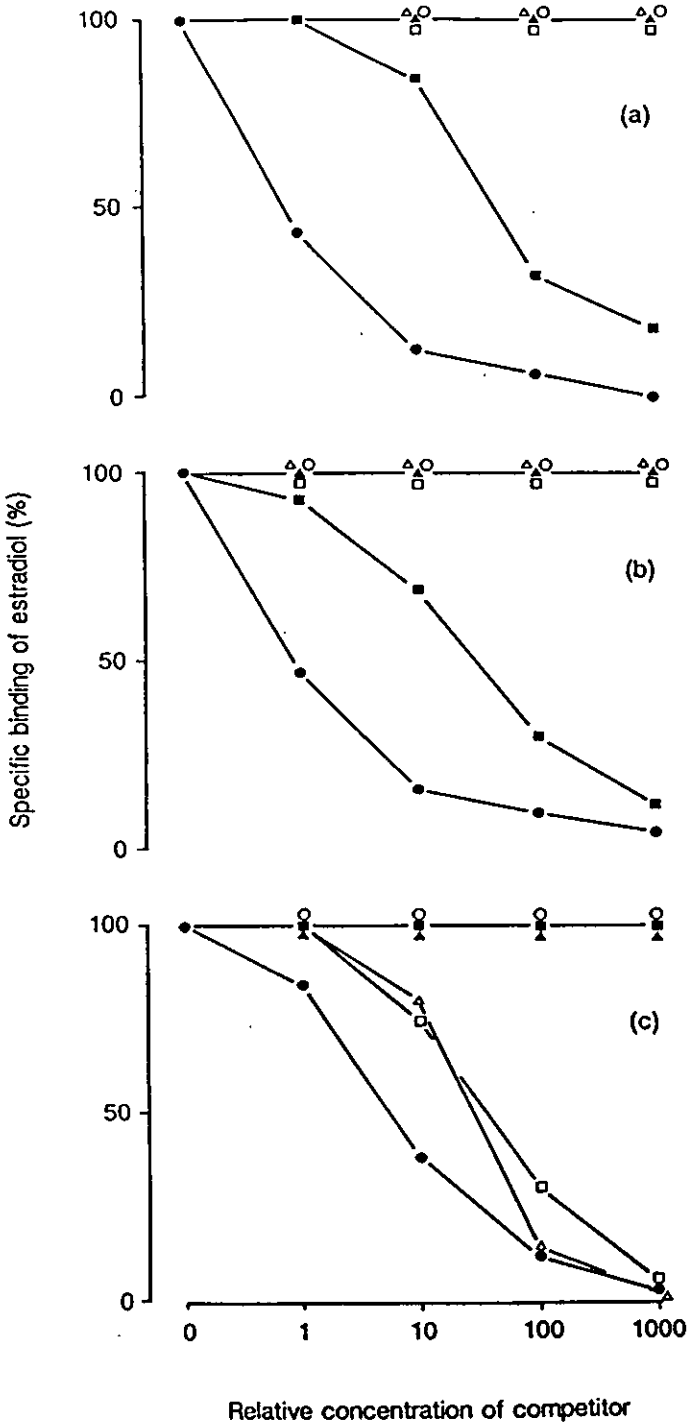
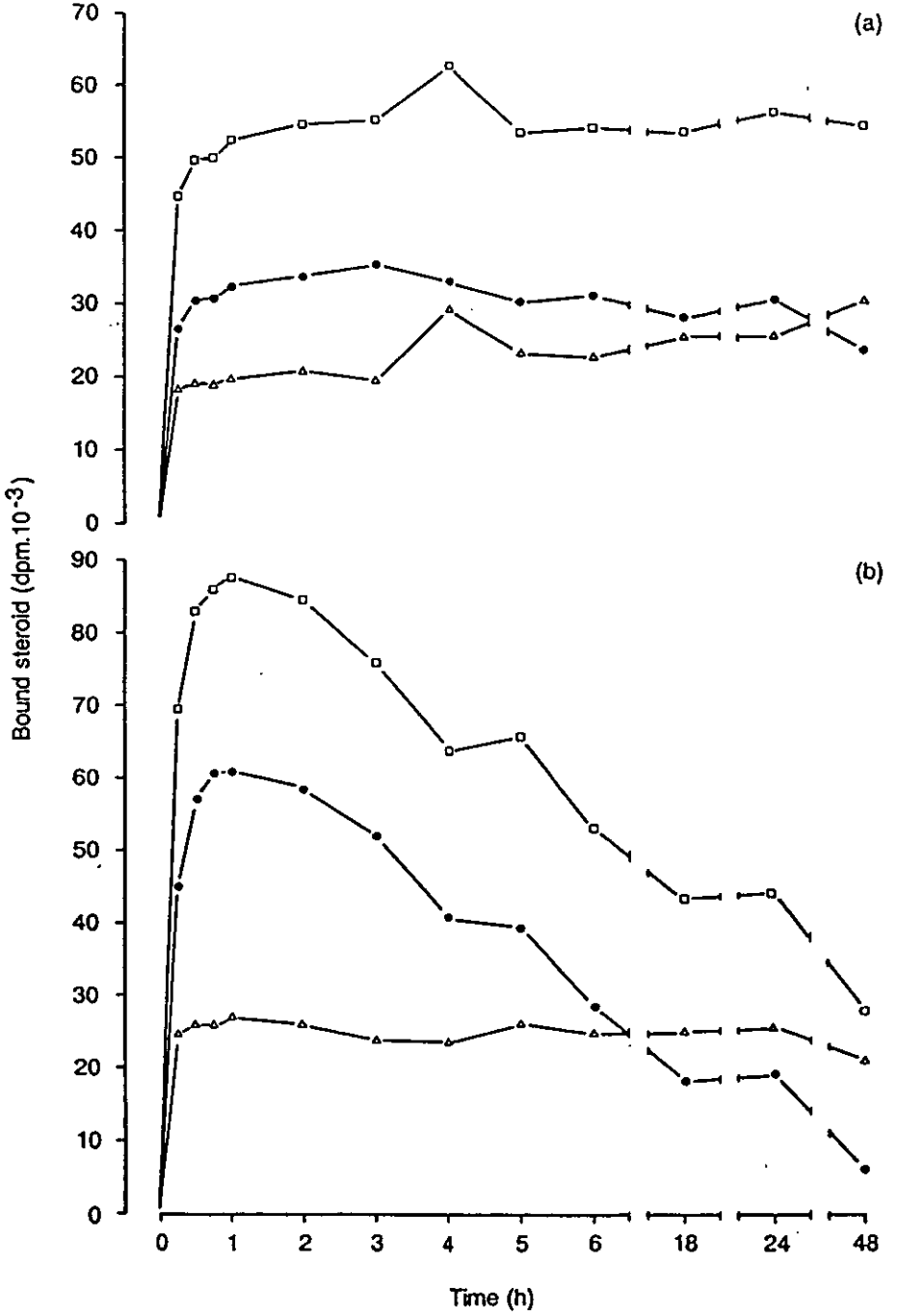

100
50
0
(a)
(b)
(c)
Specific binding of estradiol (%)
0
1
10
100
1000
Relative concentration of competitor

Fig.3


(a)
(b)
Bound steroid (dpm.10-3)
0
10
20
30
40
50
60
70
80
90
0
1
2
3
4
5
6
18
24
48
Time (h)

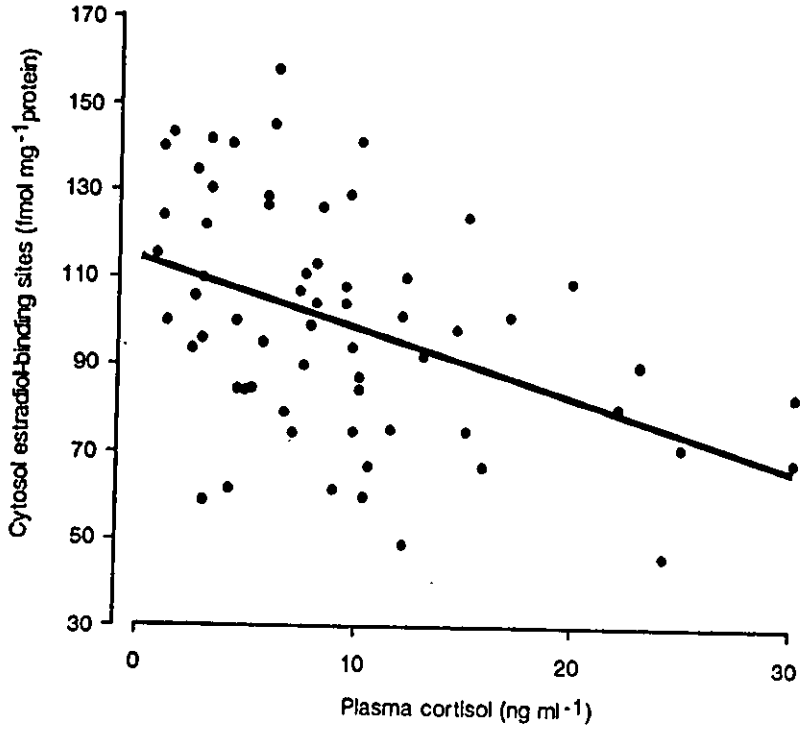

Cytosol estradiol-binding sites (fmol mg-1protein)
170
150
130
110
90
70
50
30
0
10
20
30
Plasma cortisol (ng ml-1)

Fig.6

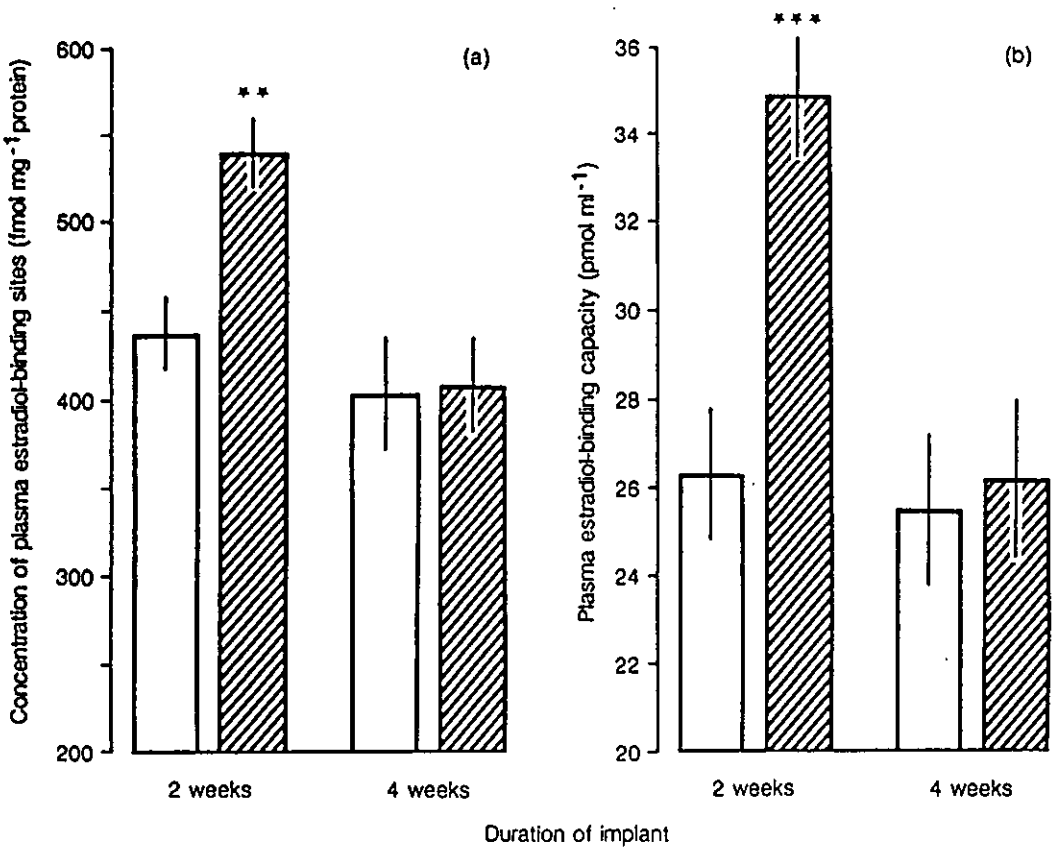

Concentration of plasma estradiol-binding sites (fmol mg-1protein)
600
500
400
300
200
**
(a)
Plasma estradiol-binding capacity (pmol ml-1)
36
34
32
30
28
26
24
22
20
***
(b)
2 weeks
4 weeks
2 weeks
4 weeks
Duration of implant

Fig.7

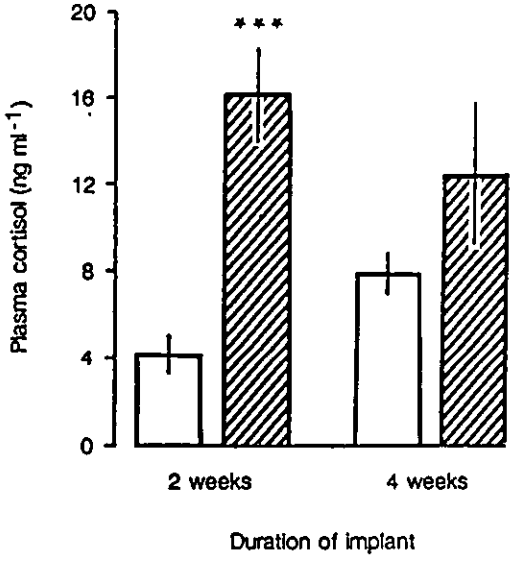

Plasma cortisol (ng ml^{-1})
20
16
12
8
4
0
***
2 weeks
4 weeks
Duration of implant

Fig.4

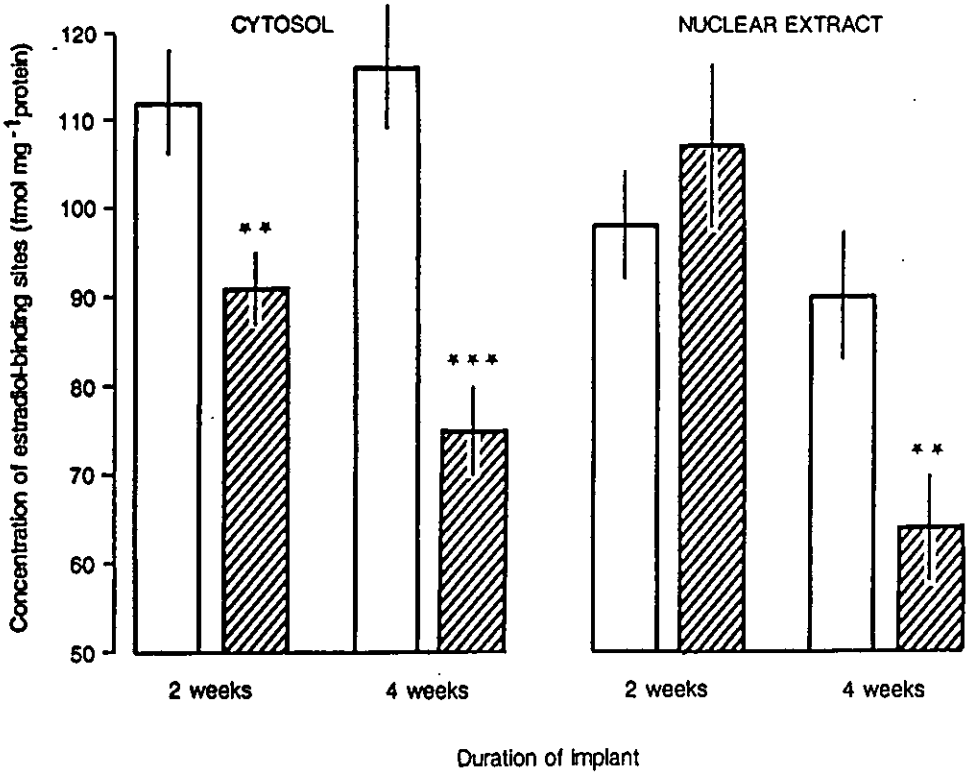

CYTOSOL
NUCLEAR EXTRACT
Concentration of estradiol-binding sites (fmol mg^{-1}protein)
120
110
100
90
80
70
60
50
**
***
**
2 weeks
4 weeks
2 weeks
4 weeks
Duration of implant

Fig.5

1990 In "Progress in Comparative Endocrinology" (ed. A.Epple)

STRESS AND THE SUPPRESSION OF SOMATIC GROWTH IN TELEOST FISH

A. D. Pickering

Institute of Freshwater Ecology, Windermere Laboratory,
Ambleside,
Cumbria LA22 OLP, U.K.

INTRODUCTION

One of the responses of fish to most forms of environmental stress is a reduction in growth rate, a response which may have important economic consequences for the aquaculture industry. Somatic growth in fish is influenced by many factors, some of which are hormonal. The aim of this paper is to identify the endocrine systems concerned with growth regulation and to examine, where possible, the effects of environmental stress on these systems.

The hormones known to influence growth in fish can be divided into two groups, the anabolic and catabolic hormones. Of the anabolic group, pituitary growth hormone is probably the one hormone most usually associated with growth promotion (see eg. Agellon et al., 1988) but gonadal steroids and thyroid hormones have also been shown to be effective growth promoters (McBride and Fagerlund, 1976; Higgs et al., 1977) and insulin is known to increase amino acid incorporation into the body musculature of teleost fish (see Donaldson et al., 1979). Of the catabolic hormones, the corticosteroids and catecholamines are probably the most important. This paper will concentrate on the effects of environmental stress on the corticosteroids, the gonadal steroids and pituitary growth hormone and changes in the plasma levels of these hormones will be interpreted in terms of the effects of stress on somatic growth.

THE HYPOTHALAMIC-PITUITARY-INTERRENAL (HPI) AXIS

Cortisol is the predominant corticosteroid in most of the teleost groups so far studied (Henderson and Garland, 1980) and the elevation of plasma cortisol in response to stress is probably the most well-documented hormonal response in the whole field of fish endocrinology. Plasma ACTH is elevated within 2 minutes of an acute stress such as handling and confinement (Sumpter et al., 1986) and the resultant cortisol elevation may persist for several hours. Usually, however, the plasma cortisol concentration returns to basal values within 24 hours (Pickering and Pottinger, 1989). When a fish is subjected to a chronic stress (ie. a continuous stress from which there is no escape) blood cortisol levels are again elevated but the elevation may persist for several days or even weeks. Thus, Pickering and Stewart (1984) showed that when brown trout, Salmo trutta, are subjected to overcrowding, blood cortisol levels were elevated for at least 25 days before acclimation ultimately occurred. In a more extreme example, cortisol levels in the brook trout, Salvelinus fontinalis, may be still elevated 6 months after exposure to low pH (Tam et al., 1987). Even in chronically-stressed fish whose plasma cortisol levels have returned to basal values (ie. acclimated fish), recent evidence suggests that the kinetics of cortisol secretion and clearance may be markedly altered (Redding et al., 1984a; Balm, 1986) and the number of cortisol receptors in the target tissues may also be significantly decreased in chronically-stressed fish (T.G. Pottinger, unpublished).

The catabolic action of cortisol is responsible for the mobilisation of energy reserves (gluconeogenesis, lipolysis; see Leach and Taylor, 1982; Sheridan, 1986), presumably of adaptive value to a stressed fish whose immediate concern is to avoid or overcome the immediate threat. However, the net effect of this catabolism is a reduction in the growth rate. Indeed, cortisol treatment causes a clear suppression of somatic growth in the rainbow trout, Salmo gairdneri (Barton et al., 1987) and in the channel catfish, Ictalurus punctatus (Davis et al., 1985). Thus, the elevation of blood cortisol levels in stressed fish is undoubtedly responsible for some of the observed growth suppression.

THE GONADAL STEROIDS

The androgens testosterone and 11-ketotestosterone act as potent growth promoters when administered to sexually immature fish (McBride and Fagerlund, 1976), although the situation with the oestrogens is not as clear. The synthetic oestrogen diethylstilboestrol is anabolic in the plaice, Pleuronectes platessa (Cowey and Sargent, 1972), but little is known about the potential anabolic effects of the natural hormone, oestradiol. During sexual maturation, both male and female salmonid fish have elevated plasma levels of testosterone and the observed acceleration of growth during the early phases of sexual development may be dependent upon such steroids.

The stress of handling, followed by 1 hour's confinement in small aquaria, was sufficient to suppress, temporarily, both testosterone and 11-ketotestosterone levels in the blood of sexually maturing brown trout (Pickering et al., 1987). The concentrations of both hormones returned to pre-stressed levels within 24 hours. Suppression of the plasma levels of the same two androgens in chronically confined trout was still evident 1 month after the onset of the stress (Pickering et al., 1987). Moreover, implantation of cortisol (at physiological doses) was sufficient to suppress the pituitary-gonadal axis of otherwise unstressed, sexually maturing, brown trout and rainbow trout of both sexes (Carragher et al., 1989). In maturing male fish, cortisol treatment caused a significant reduction in the pituitary GTH content and in the concentration of circulating testosterone. These changes were associated with a significant decrease in the size of the testes, relative to sham-implanted, control fish. In maturing female fish, cortisol treatment again caused a significant reduction in the pituitary GTH content together with a suppression of circulating testosterone and oestradiol. These changes were responsible for a signficant decrease in plasma vitellogenin levels and a decrease in ovarian size (Carragher et al., 1989). It seems probable, therefore, that the suppressive effects of stress on the gonadal steroids are mediated, in part at least, by the HPI axis. Whatever the precise mechanisms involved, environmental stress would seem to be capable of inhibiting growth in maturing fish via a reduction in the levels of circulating, anabolic, gonadal steroids.

PITUITARY GROWTH HORMONE

There is now an accumulated wealth of information on the growth-promoting effects of pituitary growth hormone in teleost fish. Natural and recombinant forms of mammalian, avian and teleost growth hormones are all markedly potent when administered to fish and act by increasing both food intake and food conversion efficiency (see Gill et al., 1985). Until recently, however, little was known about the levels of endogenous GH in fish but the development of specific radio immunoassays to measure teleost growth hormones is now beginning to open up this area of endocrinology (Cook et al., 1983; Bolton et al., 1986; Wagner and McKeown, 1986).

In a collaborative study between the IFE (A.D. Pickering and T.G. Pottinger), Brunel University (J.P. Sumpter and J.F. Carragher) and INRA (P.Y. Le Bail) we have examined the levels of plasma GH in rainbow trout under various environmental conditions using a salmonid GH RIA developed against chinook salmon, Oncorhynchus tshawytscha, GH and validated for use with other salmonid species (Le Bail et al., in preparation). In our experimental rainbow trout populations, the mean plasma levels of GH in unstressed fish lie in the range 0.5 - 3.5 ng ml^{-1}. Paradoxically, there appears to be an inverse relationship between GH levels and growth rate in these fish, with the fastest-growing strain of trout having the lowest plasma GH levels and the slowest growing strain having the highest GH levels.

When rainbow trout are subjected to an acute stress (24 hours confinement) there is a small, but statistically significant, drop in the mean plasma GH levels during the period of confinement (control fish 0.69 ± 0.10 ng ml^{-1} (n=84), confined fish 0.14 ± 0.01 ng ml^{-1} (n=60), mean ± SEM (n), $p<0.01$). It is not yet clear how long this suppression lasts and further work in this area is needed. In view of the low GH levels, a more sensitive assay would help to resolve this problem. Nevertheless, our evidence to date suggests that an acute stress could cause a temporary suppression of growth by reducing GH secretion from the pituitary gland.

In the case of a continuous, or chronic, stress blood GH levels may be elevated. Thus, we have observed an elevation of plasma GH levels from a mean of 0.51 ± 0.13 ng ml^{-1} (n=16) in control fish to 4.15 ± 1.68 ng ml^{-1} (n=16) under

conditions of crowding/water quality deterioration. Interestingly, the plasma GH levels were positively correlated with the cortisol levels, confirming the presence of a chronic stress response in these fish to the adverse environmental conditions. A similar elevation of plasma GH levels has been observed in other species of anadromous salmonid fish which have been prematurely transferred to sea water. This causes the problem of 'stunting', where the fish stops growing, turns dark, is more susceptible to disease (increase in mortality rate) and exhibits all the characteristics of chronically-stressed fish. Most importantly, plasma GH levels increase dramatically (Bolton et al., 1987; Bjornsson et al., 1988). Starvation also causes an elevation of GH levels in teleost fish (Wagner and McKeown, 1986) and we interpret the changes in chronically-stressed fish as a response to its overall metabolic state (ie. its state of starvation), rather than as a direct response to the environmental conditions. Thus, stress might suppress GH levels in the short-term but, over a longer period, other influences on the metabolic state of the fish may promote an increase in plasma GH levels. If the immunologically active GH measured in chronically-stressed fish represents biologically active hormone, it would seem that the block to growth must exist elsewhere in the system, perhaps at the level of insulin-like growth factors or the GH receptor. In this connection, it is interesting that Fryer and Bern (1979) has demonstrated a marked reduction in the number of GH receptors in stunted coho salmon, Oncorhynchus kisutch.

CONCLUSIONS

This paper has demonstrated 3 possible hormonal pathways involved in growth suppression of stressed, teleost fish:-

a) A state of catabolism induced by elevated cortisol levels.
b) Suppression the potentially anabolic steroids, testosterone and 11-ketotestosterone in sexually maturing fish.
c) Short-term suppression of plasma GH levels following acute stress.

It is likely that this represents only part of a complex series of hormonal events controlling growth processes in fish. Thus, the thyroid hormones (either alone or in combination with other hormones) are effective growth promoters (Higgs et al., 1977) and thyroidal physiology is undoubtedly influenced by environmental stress. In general, chronic stress reduces T_3 and T_4 levels (Osborn and Simpson, 1974; Simpson, 1975/76) whereas an acute stress has been shown to elevate T_4 levels (Brown et al., 1978). This latter effect is probably mediated by catecholamine release (Eales et al., 1986). Links between the HPI axis and the pituitary/thyroid axis have been postulated but the effects of cortisol on thyroidal activity are equivocal (Milne and Leatherland, 1980; Redding et al., 1984b; Leatherland, 1987). The recent finding by Young (1989) that growth hormone treatment increases the sensitivity of the coho salmon interrenal gland to ACTH and that cortisol stimulates the release of GH from the tilapia, Oreochromis mossambicus, pituitary gland in vitro (Nishioka et al., 1985) argues for links between the HPI axis and GH secretion. Virtually nothing is known about the effects of environmental stress on prolactin (PRL) secretion in fish whereas in mammals, PRL secretion is almost as characteristic a response as ACTH/ corticosteriod secretion to many forms of stress. As the techniques for the study of teleost endocrinology are developed and improved, it is envisaged that other hormonal pathways will be shown to be instrumental in the suppression of somatic growth in stressed fish.

ACKNOWLEDGEMENTS

The author is grateful to his collaborators Dr. T.G. Pottinger (IFE), Drs. J.P. Sumpter and J.F. Carragher (Brunel University) and Dr. P.Y. Le Bail (INRA) for access to unpublished data, to NERC and MAFF for financial support and to the organisers for the invitation to participate in the XIth International Symposium on Comparative Endocrinology.

REFERENCES

Agellon LB, Emery CJ, Jones JM, Davies SL, Dingle AD, Chen TT (1988). Promotion of rapid growth of rainbow trout (Salmo gairdneri) by a recombinant fish growth hormone. Can J Fish Aquat Sci 45 : 146-152.

Balm P (1986). "Osmoregulation in Teleosts by Cortisol and Prolactin". Nijmegen: Ph.D. Thesis, 176pp.

Barton BA, Schreck CB, Barton LD (1987). Effects of chronic cortisol administration and daily acute stress on growth,

physiological conditions, and stress responses in juvenile rainbow trout. Dis Aquat Org 2: 173-185.

Bjornsson BT, Ogasawara T, Hirano T, Bolton JP, Bern HA (1988). Elevated growth hormone levels in stunted Atlantic salmon, Salmo salar. Aquaculture 73: 275-281.

Bolton JP, Takahashi A, Kawauchi H, Kubota J, Hirano T (1986). Development and validation of a salmon growth hormone radioimmunoassay. Gen Comp Endocrinol 62: 230-238.

Bolton JP, Young G, Nishioka RS, Hirano T, Bern HA (1987). Plasma growth hormone levels in normal and stunted yearling coho salmon, Oncorhynchus kisutch. J Exp Zool 242: 379-382.

Brown S, Fedoruk K, Eales JG (1978). Physical injury due to injection or blood removal causes transitory elevations of plasma thyroxine in rainbow trout, Salmo gairdneri. Can J Zool 56: 1998-2003.

Carragher JF, Sumpter JP, Pottinger TG, Pickering AD (1989). The deleterious effects of cortisol implantation on reproductive function in two species of trout, Salmo trutta L. and Salmo gairdneri Richardson. Gen Comp Endocrinol (in press).

Cook AF, Wilson SW, Peter RE (1983). Development and validation of a carp growth hormone radioimmunoassay. Gen Comp Endocrinol 50: 335-347.

Cowey CB, Sargent JR (1972). Fish nutrition. Adv Mar Biol 10: 383-492.

Davis KB, Torrance P, Parker NC, Suttle MA (1985). Growth, body composition and hepatic tyrosine aminotransferase activity in cortisol-fed channel catfish, Ictalurus punctatus. J Fish Biol 29: 177-184.

Donaldson EM, Fagerlund UHM, Higgs DA, McBride JR (1979). Hormonal enhancement of growth. In Hoar WS, Randall DJ, Brett JR (eds): "Fish Physiology Vol. VIII," New York: Academic Press, pp 455-597.

Eales JG, Ransom M, Shostak S, Primean D (1986). Effects of catecholamines on plasma thyroid levels in Arctic charr, Salvelinus alpinus. Gen Comp Endocrinol 63: 393-399.

Fryer JN, Bern HA (1979). Growth hormone binding in tissues of normal and stunted juvenile coho salmon, Oncorhynchus kisutch. J Fish Biol 15: 527-583.

Gill JA, Sumpter JP, Donaldson EM, Dye HM, Souza L, Berg T, Wypych J, Langley K (1985). Recombinant chicken and bovine growth hormones accelerate growth in aquacultured juvenile Pacific salmon Oncorhynchus kisutch. Bio/Technol 3: 643-646.

Henderson IW, Garland HO (1980). The interrenal gland in pisces. Part 2. Physiology. In Chester Jones I, Henderson

IW (eds): "General, Comparative and Clinical Endocrinology of the Adrenal Cortex. Vol. 3," London : Academic Press, pp 473-523.

Higgs DA, Fagerlund UHM, McBride JR, Dye HM, Donaldson EM (1977). Influence of combinations of bovine growth hormone, 17α-methyltestosterone and L-thyroxine on growth of the yearling coho samon (Oncorhynchus kisutch). Can J Zool 55: 1048-1056.

Leach GJ, Taylor MH (1982). The effects of cortisol treatment on carbohydrate and protein metabolism in Fundulus heteroclitus. Gen Comp Endocrinol 48: 76-83.

Leatherland JF (1987). Thyroid response to ovine thyrotropin challenge in cortisol-and dexamethasone-treated rainbow trout, Salmo gairdneri. Comp Biochem Physiol 86A: 383-387.

McBride JR, Fagerlund UHM (1976). Sex steroids as growth promoters in the cultivation of juvenile coho salmon (Oncorhynchus kisutch). Proc World Maricult Soc 7: 145-161.

Milne RS, Leatherland JF (1980). Changes in plasma thyroid hormones following administration of exogenous pituitary hormones and steroid hormones to rainbow trout (Salmo gairdneri). Comp Biochem Physiol 66A: 679-686.

Nishioka RS, Grau EG, Bern HA (1985). In vitro release of growth hormone from the pituitary gland of tilapia, Oreochromis mossambicus. Gen Comp Endocrinol 60: 90-94.

Osborn RH, Simpson TH (1974). The effects of stress and food deprivation on the thyroidal status of plaice and rainbow trout. Cons Perm Int Explor Mer CM 1974 Gear and Behaviour Comm B9.

Pickering AD, Pottinger TG (1989). Stress responses and disease resistance in salmonid fish : Effects of chronic elevation of plasma cortisol. Fish Physiol Biochem (in press).

Pickering AD, Pottinger TG, Carragher J, Sumpter JP (1987). The effects of acute and chronic stress on the levels of reproductive hormones in the plasma of mature male brown trout, Salmo trutta L. Gen Comp Endocrinol 68: 249-259.

Pickering AD, Stewart A (1984). Acclimation of the interrenal tissue of the brown trout, Salmo trutta L., to chronic stress. J Fish Biol 24: 731-740.

Redding JM, Patino R, Schreck CB (1984a). Clearance of corticosteroids in yearling coho salmon, Oncorhynchus kisutch, in fresh water and seawater and after stress. Gen Comp Endocrinol 54: 433-443.

Redding JM, Schreck CB, Birks EK, Ewing RD (1984b). Cortisol and its effects on plasma thyroid hormone and electrolyte concentrations in freshwater and during seawater acclimation

in yearling coho salmon, Oncorhynchus kisutch. Gen Comp Endocrinol 56: 146-155.

Sheridan M (1986). Effects of thyroxin, cortisol, growth hormone and prolactin on lipid metabolism of coho salmon, Oncorhynchus kisutch, during smoltification. Gen Comp Endocrinol 64: 220-238.

Simpson T (1975/76). Endocrine aspects of salmonid culture. Proc Roy Soc Edinb (B) 75: 241-252.

Sumpter JP, Dye HM, Benfey TJ (1986). The effects of stress on plasma ACTH, α-MSH, and cortisol levels in salmonid fishes. Gen Comp Endocrinol 62: 377-385.

Tam WH, Birkett L, Makaran R, Payson PD, Whitney DK, Yu CK-C (1987). Modification of carbohydrate metabolism and liver vitellogenic function in brook trout (Salvelinus fontinalis) by exposure to low pH. Can J Fish Aquat Sci 44: 630-635.

Wagner GF, McKeown BA (1986). Development of a salmon growth hormone radioimmunoassay. Gen Comp Endocrinol 62: 452-458.

Young G (1989). Enhanced response of the interrenal of coho salmon to ACTH after growth hormone treatment in vivo and in vitro. Gen Comp Endocrinol (in press).

Husbandry and Stress in Fish

A.D. PICKERING Institute of Freshwater Ecology, The Ferry House, Far Sawrey, Ambleside, Cumbria, LA22 OLP, U.K.

INTRODUCTION

The definition of stress in biological systems has been the subject of considerable debate (see Pickering, 1981) yet there is still no concensus of opinion. One of the most severe definitions is that of Brett (1958) in which stress is defined as "a state produced by any environmental factor which extends the normal adaptive responses of an animal, or which disturbs the normal functioning to such an extent that the chances of survival are significantly reduced". From the aquaculture point of view, conditions which decrease the chances of survival (ie. increase the mortality rate) would certainly be described as stressful but there are other circumstances in which the mortality rate may not be affected yet other aspects of the performance capacity of the fish are impaired (Schreck, 1981). Environmental changes which suppress growth rate, for example, could also be described as stressful.

Despite such problems of definition, most fish farmers (and biologists) have an intuitive notion of those environmental conditions which are damaging to fish and the aim of this paper is to review what is known about the endocrinological changes that occur in stressed fish and then to consider the consequences of these changes in terms of the performance of the fish. Particular attention will be given to effects mediated via changes in the activity of the hypothalamic-pituitary- interrenal axis. The practicability of avoiding the worst effects of aquacultural stress is explored as is the potential for the selective breeding of fish with a reduced stress response

1990 Recent Advances in Aquaculture (eds J.Planas, S.Zanuy end N.Bromage)

(ie. accelerating the rate of domestication). Inevitably, this paper will draw heavily upon our own work on the stress responses of salmonid fish but an attempt will be made to include other species which are of importance to the aquaculture industry.

ENDOCRINOLOGY OF THE STRESS RESPONSE

The stress response in mammals has classically been described by the activation of two components of the neural/endocrine system, the adrenergic system and the pituitary-adrenal axis (Selye, 1956). Together, these effectively switch the animal's metabolism from an anabolic state (in which energy is being taken up and stored) to a catabolic state (in which energy reserves are broken down). This energy mobilization is presumed to be of adaptive value in the natural environment as the animal attempts to avoid or overcome the immediate threat.

Teleost fish show a similar response to most, if not all, forms of environmental stress. Thus, the concentrations of plasma catecholamines are rapidly elevated (Mazeaud and Mazeaud, 1981) as are corticosteroids in the blood (Donaldson, 1981). These two groups of hormones have multiple functions, many of which are of adaptive value to a stressed fish. For example, catecholamines are both hyperglycemic and lipolytic (Larsson, 1973; Sheridan, 1987), they promote branchial lamellar perfusion (Booth, 1979) and increase the oxygen affinity of the erythrocytes (Nikinmaa, 1982). Corticosteroids stimulate gluconeogenesis in fish (Janssens and Waterman, 1988; see also Suarez and Mommsen, 1988), they stimulate lipolysis (Sheridan, 1986) and promote ion-transport (Balm, 1986), an important property as the fish attempts to regain osmotic equilibrium with the environment during its recovery from stress.

In studies with salmonid fish, the activity of the hypothalamic-pituitary-interrenal (HPI) axis, in particular, has been used to assess the

degree of severity of the stress. Cortisol is the major corticosteroid in this group of fish. However, the cortisol response may be influenced not only by the nature of the stress, but also by other factors such as the age, sex and state of maturity of the fish (Sumpter et al., 1987), the environmental temperature (Sumpter et al., 1989), the species and strain of fish (Pickering and Pottinger, 1989) and even, under certain circumstances, by the chemical composition of the water (Pickering and Pottinger, 1987). Thus, considerable background information on the factors influencing HPI-axis responsiveness is required if changes in plasma corticosteroid levels are to be interpreted in terms of the severity of a stress response.

It has been the convention to describe environmental stresses as either acute or chronic, a distinction which is somewhat artificial and which may not adequately describe all situations. An acute stress is one of short duration (minutes or hours) in which the time-course of the response of the fish outlasts that of the stress. In the aquaculture industry, procedures such as netting, grading, handling, prophylactic treatment and hauling would all be described as acute stresses. Blood corticosteroid levels are rapidly elevated during the period of stress but return to basal levels within 24 hours or so (see e.g. Pickering et al., 1982). Repeated exposure of the fish to the same acute stress can result in acclimation to the stimulus. Thus, the cortisol response of brown trout, Salmo trutta, to the stress of cleaning the rearing tanks with the fish in situ was significantly reduced when the tanks were cleaned on a daily basis rather than a weekly or a monthly basis (A.D. Pickering, T.G. Pottinger and I.M.A. Armstrong, unpublished - Fig. 1). Similarly the cortisol response to a single prophylactic dose of the fungicide malachite green was completely abolished in fish treated on a daily basis (Pickering and Pottinger, 1984).

Chronic environmental stresses are continuous forms of stress from which, under aquaculture conditions, there may be no escape. Overcrowding,

poor water quality, social domination of one fish over another and exposure to novel environment are all examples of chronic stress associated with fish farming. Again the HPI axis is activated, but in such cases blood cortisol levels may be elevated for several days or even weeks (Pickering and Stewart, 1984). In many situations acclimation eventually occurs and blood cortisol levels return to basal values despite the continued stress (see Schreck, 1981). However, acclimation of the HPI axis, within a few days, to chronic stress is not an invariable consequence. For example, Tam et al. (1987) found that cortisol levels in male brook trout, Salvelinus fontinalis, were significantly elevated more than 6 months after chronic exposure to low pH and Redgate (1974) found that native carp, Cyprinus carpio, had not acclimated to confinement by 42 days post-capture. In our experience, submissive rainbow trout, Salmo gairdneri, showed no signs of acclimation to confinement/social interaction when kept with a dominant fish for periods of up to 6 weeks (A.D. Pickering and T.G. Pottinger, unpublished). Thus, plasma cortisol levels in chronically stressed fish may be elevated for prolonged periods. Even in those situations of chronic stress where plasma cortisol levels have returned to basal values, evidence from studies on the tilapia, Oreochromis mossambicus, indicates that this may be achieved by an increase in the clearance rate of the hormone rather than by a reduction in the secretion rate of cortisol by the interrenal tissue (Balm, 1986). Further work is needed on other species of fish with regard to this aspect of acclimation to chronic stress.

Whilst considerable attention has been given to changes in the adrenergic system and the HPI axis of fish subjected to both acute and chronic forms of stress, relatively little is known about the changes in other components of the fish's endocrine system. This dearth of information partly stems from the lack of suitable assays for many of the proteinaceous hormones although the situation is rapidly improving with the purification of a wide range of teleost hormones. Of the pituitary hormones (other than ACTH), it is

known that certain forms of stress stimulate the pars intermedia. Thus thermal shock, but not handling stress, stimulates the release of α-MSH and endorphin (Sumpter et al., 1985; Pickering et al., 1986). However, apart from a role of MSH in colour change, the function(s) of these hormones in fish (and hence the physiological significance of this aspect of the stress response) is unknown.

In mammals, environmental stress is also known to suppress the pituitary-gonadal axis and impair reproduction (Moberg, 1985) but little is known about the effects of stress on fish reproduction (see Billard et al., 1981). Recently, we have shown that both acute (handling) and chronic (confinement) stress can suppress androgen levels in maturing male brown trout (Pickering et al., 1987) and that some of these effects can be mimicked by cortisol implantation (Carragher et al., 1989). Cortisol implantation in otherwise unstressed fish also suppresses the pituitary-gonadal axis of sexually maturing female trout. These studies raise the possibility that the stress-induced suppression of the pituitary-gonadal axis is mediated by activation of the HPI-axis. The consequences of this phenomenon, in terms of reproductive success, remain to be elucidated (see below).

The development of specific radioimmunoassays for salmonid growth hormone (GH) now opens up another area of stress physiology/ endocrinology. Environmental stress is known to markedly suppress the growth rate of teleost fish yet, paradoxically, the available published evidence suggests that plasma GH levels are <u>increased</u> in response to acute stress (Cook and Peter, 1984). Moreover, when salmonid fish are prematurely transferred to sea water the fish become stunted, turn dark in colour and show poor survival, all characteristics of chronically-stressed fish. Under these conditions plasma GH levels are also invariably elevated (Bolton et al., 1987; Bjornsson et al., 1988). It has been proposed, therefore, that the problem of suppressed growth rates in such 'stunts' is related to target tissue sensitivity rather than the rate of pituitary GH secretion, a suggestion which is supported by the observation that

GH binding in membrane preparations from the liver and gills of coho salmon, <u>Oncorhynchus kisutch</u>, stunts is deficient compared with similar preparations from normal smolts (Fryer and Bern, 1979). Clearly, much more information is now needed on the mechanisms of action of GH in both normal and stressed fish and on the possible role of insulin-like growth factors.

The pituitary-thyroid axis of fish is sensitive to various forms of environmental stress although the evidence is fragmentory and somewhat contradictory. Osborn and Simpson (1974) found that transporting rainbow trout from a farm to the laboratory resulted in a rapid decrease of thyroxine (T_4) and triiodothyronine (T_3) levels in the blood and that those low levels were maintained for the next 30 days. Moreover, netting and confinement also had a prolonged, suppressive effect on T_4 levels in this species (Simpson, 1975/76). Similarly, Leatherland and Sonstegard (1978) reported that PCB contamination reduced T_4 and T_3 levels in coho salmon. By comparison, plasma T_4 levels in rainbow trout showed a transient <u>elevation</u> following the acute stress of saline injection or blood sampling (Brown et al., 1978). This effect is probably mediated by catecholamine release (Eales et al., 1986). Links between the HPI axis and the thyroid gland have also been postulated but the effects of cortisol treatment on thyroidal activity are even more equivocal than are the effects of stress. Cortisol has variously been shown to increase T_4 levels (Milne and Leatherland, 1980), have no effect on thyroid hormones (Leatherland, 1987), decrease T_3 but have no effect on T_4 (Redding et al., 1984) and decrease both T_4 and T_3 (Redding et al., 1986). Thus, the data indicate that the HPI axis does interact with the thyroid but much more work is needed to clarify the picture.

There is little or no information on the effects of stress on the other main components of the fish's endocrine system and this is clearly an area for future study. For example, in mammals prolactin (PRL) release is almost as characteristic a response to stress as is the release of ACTH. The recent

development and validation of teleost prolactin assays (Hirano et al., 1985; Prunet et al., 1985) should enable a further step to be taken towards our understanding of the hormonal changes associated with stress responses in fish.

CONSEQUENCES OF STRESS FOR FISH PRODUCTION

In its simplest terms, the life of a fish can be described by 3 essential features:- survival, growth and reproduction. The primary aim of aquaculture is to maximize these traits under artificial, usually intensive, rearing conditions.

a) Survival The most immediate effect of most forms of physical stress (netting, grading, transport etc.) is on the respiratory system. Adrenergic responses and the increased activity of the fish during such operations stimulate oxygen uptake. Smart (1981) demonstrated that the oxygen consumption of intensively-reared rainbow trout was still elevated by more than 50% one day after the fish had been graded. The problem of oxygen demand is exacerbated at high water temperatures when the oxygen requirements of the fish are high and the oxygen carrying capacity of the water is low. In extreme conditions (for example during transport at high stocking densities) respiratory stress is directly responsible for high mortality rates. Even when the oxygen requirements of the fish are met, toxic concentrations of the respiratory end products, ammonia and carbon dioxide, can build up. Ammonia toxicity is very pH dependent (see EIFAC, 1973) and problems can arise in recirculation systems with inefficient biological filters.

A second cause of mortality in stressed fish is osmoregulatory failure. The neural- and hormone-controlled respiratory adjustments used by stressed fish to meet their immediate energy requirements can also cause a temporary loss of salts from freshwater fish and an increase in salts in seawater fish. It seems likely that increased water permeability (Adedire and Oduleye, 1984), diuresis (Oduleye, 1975) and increased branchial ion fluxes (Lahlou et al., 1975) are all involved in this disturbance of osmotic and ionic regulation in stressed fish.

Perhaps the most far-reaching consequence of stress, with regard to survival, is the damaging effect on the defence systems of teleost fish. Currently, this is a large, active area of research, a full review of which is outside the scope of this paper and the reader is referred to Schreck (this volume) for a more detailed consideration of the effects of stress on disease resistance in fish. However, several relevant points will be emphasized here. Firstly, almost all forms of acute and chronic stress are capable of increasing the susceptibility of teleost fish to a wide range of common pathogens (viral, bacterial, fungal and protozoan), many of which would not normally cause disease problems (see Pickering, 1988). Thus, simulated transport stress for 21 h markedly increased the mortality rate of Atlantic salmon, Salmo salar, exposed to the bacterium Aeromonas hydrophila, a potential fish pathogen which seldom causes disease unless the fish are stressed (Johansson and Bergstrom, 1977). Secondly, the stress-induced increase in susceptibility to disease can be mimicked by the administration of physiological doses of cortisol to otherwise unstressed fish (Pickering and Duston, 1983). Thirdly, the concentrations of cortisol capable of producing this effect can be as low as 10 ng ml^{-1}, levels which, hitherto, had been thought to be representative of unstressed fish (see Pickering and Pottinger, 1985). This extreme sensitivity of teleost defence systems to corticosteroid hormones has been recently confirmed by Maule et al. (1987) and Tripp et al. (1987). Finally, in cortisol-treated fish the mortality rate due to disease is directly proportional to the plasma cortisol concentration (Pickering and Pottinger, 1989). Clearly, activation of the HPI-axis in stressed fish (with a resultant elevation of plasma cortisol levels) is a major factor in the predisposition to

disease. In fish, as in mammals, corticosteroids suppress many different components of the fish's defences, including the ability to develop a specific immune response (Maule et al., 1987). Immunization of fish against specific diseases is becoming a widely accepted technique for disease control but the effectiveness of what are often expensive vaccines will be markedly influenced by the degree of stress to which the fish are subjected before, during and after the vaccination procedure. During this period, therefore, it is in the fish farmer's interest to minimize, as far as is practicable, environmental stress.

b) Growth. One of the first behavioural responses of fish to any form of stress is a cessation of feeding activity, the duration of which varies according to species (Wedemeyer, 1976). This reduction in food intake, together with the catabolic effects of catecholamines and corticosteroids (see above), is undoubtedly responsible for much of the observed growth suppression in stressed fish. When administered exogenously, thyroid hormones (either alone or in combination with other hormones) act as growth promoters (Higgs et al. 1977, 1979) and, therefore, the decrease in thyroidal activity in chronically stressed fish may also contribute to the growth suppression. The role of growth hormone in this context is much more problematical. Recombinant forms of mammalian, avian and teleost growth hormones are all extremely effective stimulators of growth in fish (Agellon et al., 1988; Down et al., 1988) and act by increasing both food intake and food conversion efficiency (see Gill et al., 1985). However as noted above, the available evidence indicates that stressed fish have increased levels of circulating GH. If this represents biologically active hormone, it is most unlikely that administration of exogenous GH to chronically-stressed fish would restore their growth rate. Finally, it is known that some of the reproductive steroid hormones have anabolic effects in fish (Donaldson et al., 1979) and, therefore, the suppressive effects of stress on the pituitary-gonadal axis (see above) might also inhibit growth in maturing fish via this route.

c) Reproduction. It has been shown that the environmental stresses of acidification and sublethal pollution not only suppress reproductive endocrinology (Freeman et al., 1983) but also reduce the viability of the gametes (Macek, 1968; Lee and Gerking, 1980). It seems likely, therefore, that the suppressive effects of handling and confinement on the reproductive endocrinology of maturing fish will also result in reduce gamete quality/quantity although, at present, we have no direct evidence of this phenomenon. Chronic cortisol elevation certainly retards gonadal growth independently of somatic growth (Carragher et al., 1989) and in vitro studies indicate that some aspects of this suppression might be directly mediated by cortisol (Sumpter et al., 1987; Carragher and Sumpter, 1990 a, b). However, this whole aspect of stress physiology and its relationship with growth inhibition now requires rigorous investigation if the suppressive effects of aquaculture stress on broodstock endocrinology are to be interpreted in terms of the number and viability of both eggs and sperm and in terms of the survival and growth of the resultant offspring.

MINIMISING THE DAMAGING EFFECTS OF STRESS

It must be stated at the outset that, during normal aquaculture operations, it will be impossible to avoid many of the procedures known to induce stress responses in fish. Netting, grading and transport are integral components of the fish-farming routine and, at best, all the fish farmer can do is to minimize the effects of this type of stress. Some of the more chronic stresses such as overcrowding and poor water quality are avoidable, however, and must be avoided if the fish are to remain healthy, attain their full growth potential and, where appropriate, produce eggs and sperm of the highest quality. It is not the purpose of this paper to define optimal environmental conditions - indeed these vary with the species and size of fish and most farmers are familiar with the appropriate guidelines for stocking density,

water flow, feeding rate etc. It is the purpose of this paper to convince the reader of the damaging consequences of stress on survival, growth and reproduction should these guidelines be ignored.

In those circumstances where stresses (usually acute) cannot be avoided, there are still some tactics that the farmer can adopt to protect the fish:-

1. In general, the duration of the stress response is proportional to the duration of the stress. Thus, reducing the time-course of netting, grading or hauling will encourage a more rapid recovery of the fish. However, some of the secondary effects of a 30 secs handling stress may last for several days (Pickering et al., 1982) and we routinely use a recovery period of 2 weeks in our work with salmonid fish.

2. In both warmwater- (channel catfish, Ictalurus punctatus) and coldwater-species (chinook salmon, Oncorhynchus tshawytscha) stress-induced mortality increases with increasing water temperature (Strange, 1980; Barton and Schreck, 1987). It is safer, therefore, to undertake netting, grading and hauling at lower water temperatures.

3. The effects of multiple stresses may be additive or even synergistic. Barton et al. (1986) demonstrated that 3 repeated handling stresses 3 hours apart evoked cumulative stress responses in juvenile chinook salmon and that the cortisol response of acid-exposed rainbow trout to acute handling stress was more than twice that of unexposed fish (Barton et al., 1985). If repeated stresses are unavoidable, there is an advantage in allowing a sufficient recovery period between stresses. Other forms of multiple stresses, such as a sudden temperature change during or after hauling, should be avoided.

4. In freshwater fish, the use of dilute salt solutions during severe stresses (such as hauling) have been shown to be effective in limiting the loss of ions from the fish and significantly reducing the stress-associated mortality (Hattingh et al. 1975; Long et al., 1977; Kutty et al., 1980).

5. The withdrawal of food 2 or 3 days prior to any operation involving confinement of the fish not only prevents fouling of the water with faecal matter and regurgitated food but, more importantly, reduces the oxygen requirements of the fish (Brett and Groves, 1979) thereby ameliorating respiratory stress.

6. Anaesthesia can also be used, with effect, to promote survival during severe stress. Although anaesthesia itself may cause a considerable disturbance of the fish's physiology (Wedemeyer, 1970; Soivio et al., 1977), it can also suppress the cortisol response to an acute stress such as handling (A.D. Pickering and T.G. Pottinger, unpublished - Fig. 2) and reduce the mortality if the fish are subsequently exposed, without anaesthesia, to a second stress (Strange and Schreck, 1978). The beneficial effects of mild anaesthesia during the transport of tilapia, Oreochromis mossambicus, have been demonstrated by Ferriera et al. (1984). The anaesthetic causes a decrease in ammonia and carbon dioxide excretion (and, presumably, in oxygen uptake).

7. In the case of species (or strains) of fish new to cultivation, an understanding of the fish's natural habitat can provide insight into methods of stress control. Thus, the provision of floating overhead cover not only doubled the growth rate of underyearling Atlantic salmon (first generation fish from a wild stock) but also significantly increased the proportion of potential S1 smolts and minimized the haematological signs of chronic stress (Pickering et al., 1987). Moreover during the fish's first winter, overhead cover halved the mortality rate of potential S2 smolts (A.D. Pickering and T.G. Pottinger, unpublished). Overhead cover is an important component of the young salmons' natural environment, particularly during the winter months (Cunjak, 1988).

FUTURE DEVELOPMENTS

It has been shown that activation of the HPI axis, with a resultant elevation of blood corticosteroid levels, is at least partly responsible for a) the increase in susceptibility to disease, b) the reduction in growth rate and c) the suppression of reproductive endocrinology that occurs in stressed fish. Consequently, attention is now being given to the possibility of reducing the magnitude of this aspect of the stress response. The cortisol response of wild rainbow trout to aquacultural stresses is significantly greater than that of domesticated strains (Woodward and Strange, 1987). Moreover, marked differences in the magnitude of the stress response exist between different domesticated strains of the same species (Pickering and Pottinger, 1989). This indicates that a certain degree of empirical selection for low cortisol levels has already taken place during the history of rainbow trout domestication. With the exception of cyprinid rearing, aquaculture is a young industry and there must still be considerable scope for further selection within many species for the trait of a reduce cortisol response to common forms of aquaculture stress i.e. an acceleration of the rate of domestication. This approach has been extremely successful in the poultry industry where, for example, selection of turkey lines with reduced corticosterone responses to cold stress resulted not only in increased resistance to disease but also in increased growth and superior reproductive performance (Brown and Nestor, 1973).

In the aquaculture industry, some preliminary steps in this direction have already been taken. Refstie (1982, 1986) has shown that the magnitude of the cortisol response to stress is a heritable character in rainbow trout and Atlantic salmon and we have recently commenced a programme to select broodstock rainbow trout with a low cortisol response to the combined stress of handling and short-term confinement. The responses of 240 individually marked, all

female rainbow trout have been monitored on a two-monthly basis for more than a year. Despite considerable individual variation within the total population in the magnitude of the cortisol response with time, two groups (each of 25 fish) with markedly different and consistent cortisol responses have now been identified and isolated. Significant differences between the two groups in the 'stress' levels of plasma cortisol have been maintained for more than a year, indicating a considerable degree of stability in this trait and when last tested the 'high' responders had a mean peak cortisol level of 161.9 ± 10.2 ng ml^{-1} compared with 83.8 ± 4.9 ng ml^{-1} for the 'low' responders ($p < 0.001$). Current studies are now concentrated on the identification of similar groups of male fish for breeding purposes and future studies are planned to compare the stress physiology, survival, growth and reproduction of the resultant offspring. It is important to recognise that this is slow, demanding work but, by analogy with other forms of agriculture, the return, in terms of increased production, should be extremely rewarding.

Acknowledgements The author is grateful to Dr T.G. Pottinger (IFE) for his critical comments on the manuscript and to MAFF and NERC for financial support for the original research on which much of this article is based.

REFERENCES

Adedire, C.O. and Oduleye, S.O. (1984) Stress-induced water permeability changes in the tropical cichlid, Oreochromis niloticus (Trewavas). Journal of Fish Biology, 25, 463-471

Agellon, L.B., Emery, C.J., Jones, J.M., Davies, S.L., Dingle, A.D. and Chen, T.T. (1988) Promotion of rapid growth of rainbow trout (Salmo gairdneri) by a recombinant fish growth hormone. Canadian Journal of Fisheries and Aquatic Sciences, 45, 146-151

Balm, P. (1986) Osmoregulation in teleosts by cortisol and prolactin. Ph.D. Thesis, Nijmegen University, Holland.

Barton, B.A. and Schreck, C.B. (1987) Influence of acclimation temperature on interrenal and carbohydrate stress response in juvenile chinook salmon (Oncorhynchus tshawytscha). Aquaculture, 62, 299-310

Barton, B.A., Schreck, C.B. and Sigismondi, L.A. (1986) Multiple acute disturbances evoke cumulative physiological stress responses in juvenile chinook salmon. Transactions of the American Fisheries Society, 115, 245-251

Barton, B.A., Weiner, G.S. and Schreck, C.B. (1985) Effect of prior acid exposure on physiological responses of juvenile rainbow trout (Salmo gairdneri) to acute handling stress. Canadian Journal of Fisheries and Aquatic Sciences, 42, 710-717

Billard, R., Bry, C. and Gillet, C. (1981) Stress, environment and reproduction in teleost fish, in A.D. Pickering (ed.), Stress and Fish, Academic Press, London, pp. 185-208

Björnsson, B.Th., Ogasawara, T., Hirano, T., Bolton, J.P. and Bern, H.A. (1988) Elevated growth hormone levels in stunted Atlantic salmon, Salmo salar. Aquaculture 73, 275-281

Bolton, J.P., Young, G., Nishioka, R.S., Hirano, T. and Bern, H.A. (1987) Plasma growth hormone levels in normal and stunted yearling coho salmon, Oncorhynchus kisutch. Journal of Experimental Zoology, 242, 379-382

Booth, J.H. (1979) The effects of oxygen supply, epinephrine, and acetylcholine on the distribution of blood flow in trout gills. Journal of Experimental Biology, 83, 31-39

Brett, J.R. (1958) Implications and assessments of environmental stress, in P.A. Larkin (ed.), Investigations of Fish-power Problems, H.R. Macmillan Lectures in Fisheries, University of British Columbia, pp. 69-83

Brett, J.R. and Groves, T.D.D. (1979) Physiological energetics, in W.S. Hoar, D.J. Randall and J.R. Brett (eds), Fish Physiology, Vol. VIII, Academic Press, New York, pp. 280-352

Brown, K.I. and Nestor, K.E. (1973) Some physiological responses of turkeys selected for high and low adrenal responses to cold stress. Poultry Science, 52, 1948-1954

Brown S., Fedoruk, K. and Eales, J.G. (1978) Physical injury due to injection or blood removal causes transitory elevations of plasma thyroxine in rainbow trout, Salmo gairdneri. Canadian Journal of Zoology, 56, 1998-2003

Carragher, J.F. and Sumpter, J.P. (1990a) Corticosteroid physiology in fish. Proceedings of the XIth International Symposium on Comparative Endocrinology, (in press)

Carragher, J.F. and Sumpter, J.P. (1990b) The effect of cortisol on the secretion of sex steroids from cultured ovarian follicles of rainbow trout. General and Comparative Endocrinology, (in press)

Carragher, J.F., Sumpter, J.P., Pottinger, T.G. and Pickering, A.D. (1989) The deleterious effects of cortisol implantation on reproductive function in two species of trout, Salmo trutta L. and Salmo gairdneri Richardson. General and Comparative Endocrinology, (in press)

Cook, A.F. and Peter, R.E. (1984) The effects of somatostatin on serum growth hormone levels in the goldfish, Carassius auratus. General and Comparative Endocrinology, 54, 109-113

Cunjak, R.A. (1988) Behaviour and microhabitat of young Atlantic salmon (Salmo salar) during winter. Canadian Journal of Fisheries and Aquatic Sciences, 45, 2156-2160

Donaldson, E.M. (1981) The pituitary-interrenal axis as an indicator of stress in fish, in A.D. Pickering (ed.), Stress and Fish, Academic Press, London, pp. 11-47

Donaldson, E.M., Fagerlund, U.H.M., Higgs, D.A. and McBride, J.R. (1979) Hormonal enhancement of growth, in W.S. Hoar, D.J. Randall and J.R. Brett (eds), Fish Physiology, Vol. VIII, Academic Press, New York, pp. 455-597

Down, N.E., Donaldson, E.M., Dye, H.M., Langley, K. and Souza, L.M. (1988) Recombinant bovine somatotropin more than doubles the growth rate of coho salmon (Oncorhynchus kisutch) acclimated to seawater and ambient winter conditions. Aquaculture, 68, 141-155

Eales, J.G., Ransom, M., Shostak, S. and Primeau, D. (1986) Effects of catecholamines on plasma thyroid hormone levels in Arctic charr, Salvelinus alpinus. General and Comparative Endocrinology, 63, 393-399

EIFAC (1973) Water quality criteria for European freshwater fish. Report on ammonia and inland fisheries. Water Research, 7, 1011-1022

Ferreira, J.T., Schoonbee, H.J. and Smit, G.L. (1984) The use of benzocaine-hydrochloride as an aid in the transport of fish. Aquaculture, 42, 169-174

Freeman, H.C., Sangalang, G.B., Burns, G. and McMenemy, M. (1983) The blood sex hormone levels in sexually mature male Atlantic salmon (Salmo salar) in the Westfield River (pH 4.7) and the Medway River (pH 5.6), Nova Scotia. The Science of the Total Environment, 32, 87-91

Fryer, J.N. and Bern, H.A. (1979) Growth hormone binding to tissues of normal and stunted juvenile coho salmon, Oncorhynchus kisutch. Journal of Fish Biology, 15, 527-533

Gill, J.A., Sumpter, J.P., Donaldson, E.M., Dye, H.M., Souza, L., Berg, T., Wypych, J. and Langley, K. (1985) Recombinant chicken and bovine growth hormones accelerate growth in aquacultured juvenile Pacific salmon Oncorhynchus kisutch. Bio/Technology, 3, 643-646

Hattingh, J., Fourie, F. and van Vuren, J. (1975) The transport of freshwater fish. Journal of Fish Biology, 7, 447-449

Higgs, D.A., Fagerlund, U.H.M., McBride, J.R., Dye, H.M. and Donaldson, E.M. (1977) Influence of combinations of bovine growth hormone, 17α-methyltestosterone and L-thyroxine on growth of the yearling coho salmon (Oncorhynchus kisutch). Canadian Journal of Zoology, 55, 1048-1056

Higgs, D.A., Fagerlund, U.H.M., McBride, J.R. and Eales, J.G. (1979) Influence of orally administered L-thyroxine or 3', 5', 3' triiodo-L-thyronine on growth, food consumption and food conversion of underyearling coho salmon (Oncorhynchus kisutch). Canadian Journal of Zoology, 57, 1974-1979

Hirano, T., Prunet, P., Kawauchi, H., Takahashi, A., Ogasawara, T., Kubota, J., Nishioka, R.S., Bern, H.A., Takada, K. and Ishii, S. (1985) Development and validation of a salmon prolactin radioimmunoassay. General and Comparative Endocrinology, 59, 266-276

Janssens, P.A. and Waterman, J. (1988) Hormonal regulation of gluconeogenesis and glycogenolysis in carp (Cyprinus carpio) liver pieces cultured in vitro. Comparative Biochemistry and Physiology, 91A, 451-457

Johansson, N. and Bergström, E. (1977) Transportation in tanks of two-year old salmon (Salmo salar L.) as a predisposing factor for infection with Aeromonas hydrophila. Laxforskninginstitutet Meddelande, 3, 1-12

Kutty, M.N., Sukumaran, N. and Kasim, H.M. (1980) Influence of temperature and salinity on survival of the freshwater mullet, Rhinomugil corsula (Hamilton). Aquaculture, 20, 261-274

Lahlou, B., Crenesee, D., Bensahla-Talet, A. and Porte-Nibelle, J. (1975) Adaptation de la truite de'elevage à l'eau de mer. Journal de Physiologie, Paris, 70, 593-603

Leatherland, J.F. (1987) Thyroid response to ovine thyrotropin challenge in cortisol- and dexamethasome-treated rainbow trout, Salmo gairdneri. Comparative Biochemistry and Physiology, 86A, 383-387

Leatherland, J.F. and Sonstegard, R.A. (1978) Lowering of serum thyroxine and triiodothyronine levels in yearling coho salmon, Oncorhynchus kisutch, by dietary mirex and PCB's. Journal of the Fisheries Research Board of Canada, 35, 1285-1289

Larsson, A.L. (1973) Metabolic effects of epinephrine and norepinephrine in the eel, Anguilla anguilla L. General and Comparative Endocrinology, 20, 155-167

Lee, R.M. and Gerking, S.D. (1980) Survival and reproductive performance of the desert pupfish, Cyprinodon n. nevadensis (Eigenmann and Eigenmann), in acid waters. Journal of Fish Biology, 17, 507-515

Long, C.W., McComas, J.R. and Monk, B.H. (1977) Use of salt (NaCl) water to reduce mortality of chinook smolts, Oncorhynchus tshawytscha, during handling and hauling. United States Marine Fisheries Series, Marine Fisheries Review, 39, 6-9

Macek, K.J. (1968) Reproduction in brook trout (Salvelinus fontinalis) fed sublethal concentrations of DDT. Journal of the Fisheries Research Board of Canada, 25, 1787-1796

Maule, A.G., Schreck, C.B. and Kaattari, S.L. (1987) Changes in the immune system of coho salmon (Oncorhynchus kisutch) during the parr-to-smolt transformation and after implantation of cortisol. Canadian Journal of Fisheries and Aquatic Sciences, 44, 161-166

Mazeaud, M.M. and Mazeaud, F. (1981) Adrenergic responses to stress in fish, in A.D. Pickering (ed.), Stress and Fish, Academic Press, London, pp. 49-75

Milne, R.S. and Leatherland, J.F. (1980) Changes in plasma thyroid hormones following administration of exogenous pituitary hormones and steroid hormones in rainbow trout (Salmo gairdneri). Comparative Biochemistry and Physiology, 66A, 679-686

Moberg, G.P. (1985) Influence of stress on reproduction: Measure of well-being, in G.P. Moberg (ed.), Animal Stress, American Physiological Society, Bethesda, pp. 245-267

Nikinmaa, M. (1982) The effects of adrenaline on the oxygen transport properties of Salmo gairdneri blood. Comparative Biochemistry and Physiology, 71A, 353-356

Oduleye, S.O. (1975) The effects of calcium on water balance of the brown trout, Salmo trutta L. Journal of Experimental Biology, 64, 221-231

Osborn, R.H. and Simpson, T.H. (1974) The effect of stress and food deprivation on the thyroidal status of plaice and of rainbow trout. Conseil International pour l'Exploration de la Mer, Gear and Behaviour, Communication B9

Pickering, A.D. (1981) Introduction: The concept of biological stress, in A.D. Pickering (ed.), Stress and Fish, Academic Press, London, pp. 1-9

Pickering, A.D. (1988) Stress responses and disease resistance in farmed fish. Aqua Nor 87, Trondheim, Proceedings of Conference 3, 35-49

Pickering, A.D. and Duston, J. (1983) Administration of cortisol to brown trout, Salmo trutta L., and its effects on the susceptibility to Saprolegnia infection and furunculosis. Journal of Fish Biology, 23, 163-175

Pickering, A.D. and Pottinger, T.G. (1984) Acclimation of the brown trout, Salmo trutta L., to the stress of daily malachite green treatment. Aquaculture, 44, 145-152

Pickering, A.D. and Pottinger, T.G. (1985) Cortisol can increase the susceptibility of brown trout, Salmo trutta L., to disease without reducing the white blood cell count. Journal of Fish Biology, 27, 611-619

Pickering, A.D. and Pottinger, T.G. (1987) Poor water quality suppresses the cortisol response of salmonid fish to handling and confinement. Journal of Fish Biology, 30, 363-374

Pickering, A.D. and Pottinger, T.G. (1989) Stress responses and disease resistance in salmonid fish: Effects of chronic elevation of plasma cortisol. Fish Physiology and Biochemistry, 7, 253-258.

Pickering, A.D., Griffiths, R. and Pottinger, T.G. (1987) A comparison of the effects of overhead cover on the growth, survival and haematology of juvenile Atlantic salmon, Salmo salar L., brown trout, Salmo trutta L., and rainbow trout, Salmo gairdneri Richardson. Aquaculture, 66, 107-124.

Pickering, A.D., Pottinger, T.G., Carragher, J. and Sumpter, J.P. (1987) The effects of acute and chronic stress on the levels of reproductive hormones in the plasma of mature male brown trout, Salmo trutta L. General and Comparative Endocrinology, 68, 249-259

Pickering, A.D., Pottinger, T.G. and Christie, P. (1982) Recovery of the brown trout, Salmo trutta L., from acute handling stress: a time-course study. Journal of Fish Biology, 20, 229-244

Pickering, A.D., Pottinger, T.G. and Sumpter, J.P. (1986) Independence of the pituitary-interrenal axis and melanotroph activity in the brown trout, Salmo trutta L., under conditions of environmental stress. General and Comparative Endocrinology, 64, 206-211

Pickering, A.D. and Stewart, A. (1984) Acclimation of the interrenal tissue of the brown trout, Salmo trutta L., to chronic crowding stress. Journal of Fish Biology, 24, 731-740

Prunet, P., Boeuf, G. and Houdebine, L.M. (1985) Plasma and pituitary prolactin levels in rainbow trout during adaptation to different salinities. Journal of Experimental Zoology, 235, 187-196

Redding, J.M., DeLuze, A., Leloup-Hatey, J. and Leloup, J. (1986) Suppression of plasma thyroid concentrations by cortisol in the European eel Anguilla anguilla. Comparative Biochemistry and Physiology, 83A, 409-413

Redding, J.M., Schreck, C.B., Birks, E.K. and Ewing, R.D. (1984) Cortisol and its effects on plasma thyroid hormone and electrolyte concentrations in freshwater and during seawater acclimation in yearling coho salmon, Oncorhynchus kisutch. General and Comparative Endocrinology, 56, 146-155

Redgate, E.S. (1974) Neural control of pituitary adrenal activity in Cyprinus carpio. General and Comparative Endocrinology, 22, 35-41

Refstie, T. (1982) Preliminary results: Differences between rainbow trout families in resistance against vibriosis and stress, in W.B. van Muiswinkel (ed.), Developmental and Comparative Immunology, Suppl. 2, Pergamon Press, New York, pp. 205-209

Refstie, T. (1986) Genetic differences in stress response in Atlantic salmon and rainbow trout (Abstract). Aquaculture 57, 374

Schreck, C.B. (1981) Stress and compensation in teleostean fishes: Response to social and physical factors, in A.D. Pickering (ed.), Stress and Fish, Academic Press, London, pp. 295-321

Selye, H. (1956) The Stress of Life, McGraw-Hill, Toronto

Sheridan, M. (1986) Effects of thyroxin, cortisol, growth hormone and prolactin on lipid metabolism of coho salmon, Oncorhynchus kisutch, during smoltification. General and Comparative Endocrinology, 64, 220-238

Sheridan, M. (1987) Effects of epinephrine and norepinephrine on lipid mobilization from coho salmon liver incubated in vitro. Endocrinology 120, 2234-2239

Simpson, T. (1975/76) Endocrine aspects of salmonid culture. Proceedings of the Royal Society of Edinburgh B, 75, 241-252

Smart, G.R. (1981) Aspects of water quality producing stress in intensive fish culture, in A.D. Pickering (ed.), Stress and Fish, Academic Press, London, pp. 277-293

Soivio, A., Nyholm, K. and Huhti, M. (1977) Effects of anaesthesia with MS 222, neutralised MS222 and benzocaine on the blood constituents of rainbow trout, Salmo gairdneri. Journal of Fish Biology, 10, 91-101

Strange, R.J. (1980) Acclimation temperature influences cortisol and glucose concentrations in stressed channel catfish. Transactions of the American Fisheries Society, 109, 298-303

Strange, R.J. and Schreck, C.B. (1978) Anaesthetic and handling stress on survival and cortisol concentration in yearling chinook salmon (Oncorhynchus tshawytscha). Journal of the Fisheries Research Board of Canada, 35, 345-349

Suarez, R.K. and Mommsen, T.P. (1988) Gluconeogenesis in teleost fishes. Canadian Journal of Zoology, 65, 1869-1882

Sumpter, J.P., Carragher, J., Pottinger, T.G. and Pickering, A.D. (1987) The interaction of stress and reproduction in trout, in D.R. Idler, L.W. Crim and J.M. Walsh (eds), Reproductive Physiology of Fish, Memorial University of New Foundland, St. Johns, pp. 299-302

Sumpter, J.P., Pickering, A.D. and Pottinger, T.G. (1985) Stress-induced elevation of plasma α-MSH and endorphin in the brown trout, Salmo trutta L. General and Comparative Endocrinology, 59, 257-265

Tam, W.H., Birkett, L., Makaran, R., Payson, P.D., Whitney, D.K. and Yu, C.K.-C (1987) Modification of carbohydrate metabolism and liver vitellogenic function in brook trout (Salvelinus fontinalis) by exposure to low pH. Canadian Journal of Fisheries and Aquatic Sciences, 44, 630-635

Tripp, R.A., Maule, A.G., Schreck, C.B. and Kaattari, S.L. (1987) Cortisol mediated suppression of salmonid lymphocyte responses in vitro. Developmental and Comparative Immunology, 11, 565-567

Wedemeyer, G. (1970) Stress of anesthesia with MS 222 and benzocaine in rainbow trout (Salmo gairdneri) Journal of the Fisheries Research Board of Canada, 27, 909-914

Wedemeyer, G.A. (1976) Physiological response of juvenile coho salmon (Oncorhynchus kisutch) and rainbow trout (Salmo gairdneri) to handling and crowding stress in intensive fish culture. Journal of the Fisheries Research Board of Canada, 33, 2699-2702

Woodward, C.C. and Strange, R.J. (1987) Physiological stress responses in wild and hatchery-reared rainbow trout. Transactions of the American Fisheries Society, 116, 574-579

Legends to Figures

Figure 1: The cortisol response of brown trout to the stress of tank cleaning with the fish in situ. The cleaning procedure consisted of brushing the internal surfaces of the tank for exactly two minutes. Values are arithmetic means (± SEM, n = 16) and represent the pooled data from duplicate tanks. ●——● cleaned once per month, ●------● cleaned once per week, ●–·–·● cleaned on a daily basis (original data from A.D. Pickering, T.G. Pottinger and I.M.A. Armstrong).

Figure 2: The effect of three different anaesthetics on the cortisol response of rainbow trout to the stress of handling and subsequent confinement for a period of 1 hour. The shaded area denotes the duration of the anaesthesia, the unshaded area represents the recovery period. The typical cortisol response to the stress is completely inhibited in anaesthetized fish during the period of anaesthesia (20 min) but develops once the anaesthetic has been removed. The relatively slow cortisol elevation in those fish recovering from etomidate anaesthesia is caused by the specific effects of this drug on blocking steroid synthesis in the interrenal tissue. Each value is the arithmetic mean (± SEM) of 16 fish (original data from A.D. Pickering and T.G. Pottinger).

Fig. 2.

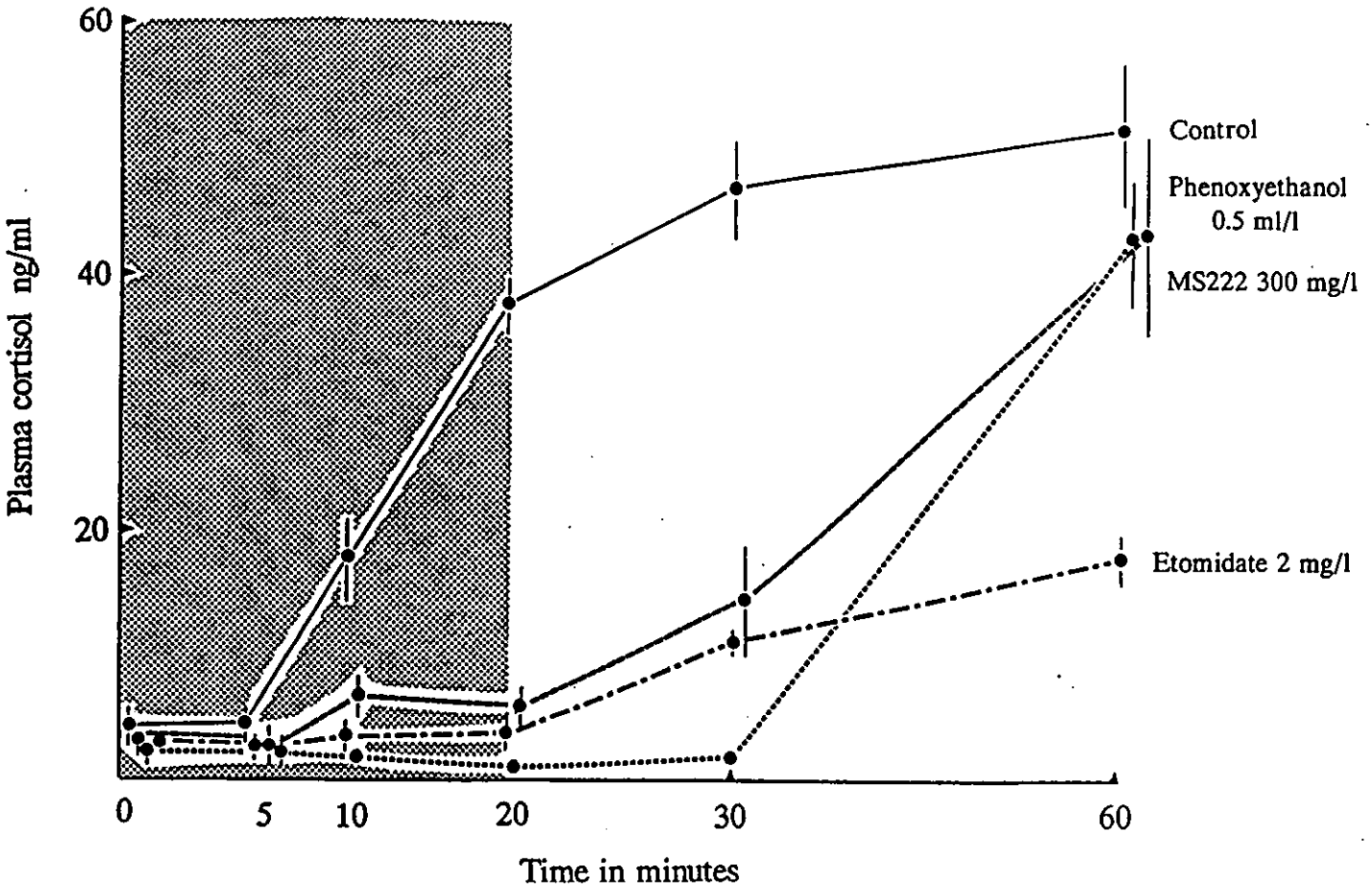
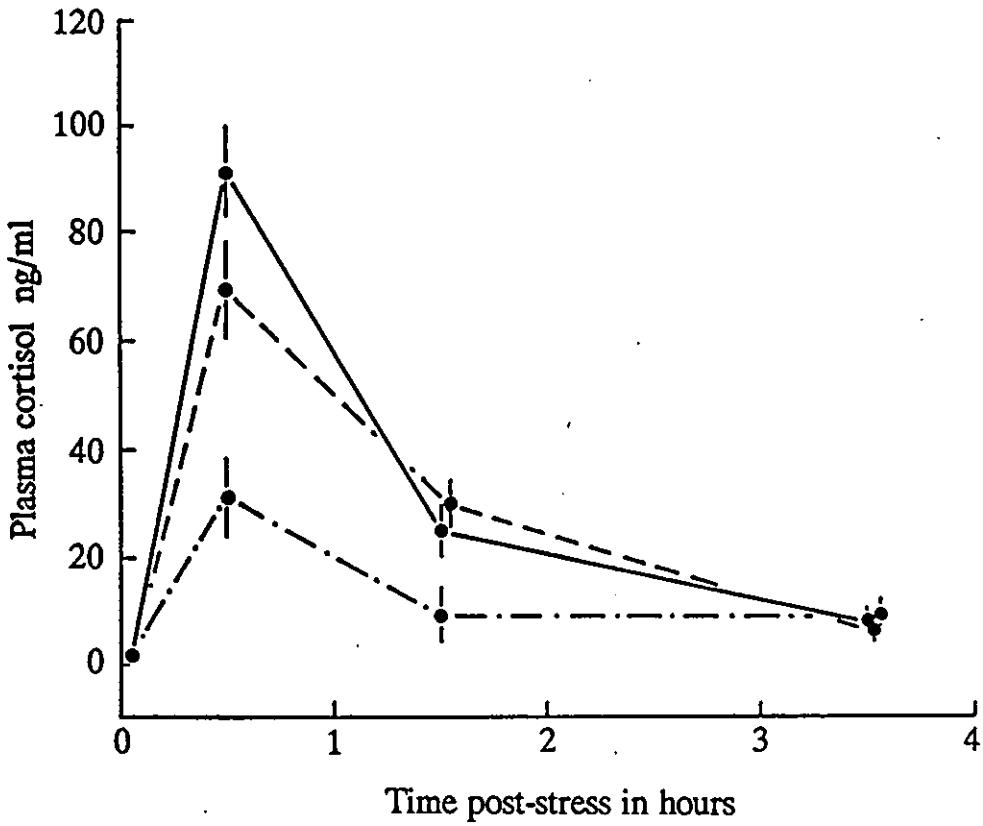

60
40
20
Plasma cortisol ng/ml
Control
Phenoxyethanol
0.5 ml/l
MS222 300 mg/l
Etomidate 2 mg/l
0
5
10
20
30
60
Time in minutes

Fig. 1


120
100
80
60
40
20
0
Plasma cortisol ng/ml
0
1
2
3
4
Time post-stress in hours

FISH HEALTH AND THE ENVIRONMENT

A.D. Pickering
Institute of Freshwater Ecology, Windermere Laboratory, Ambleside, Cumbria LA22 OLP

Introduction

Any deleterious change in the fish's environment is usually referred to as an environmental stress. However, the concept of stress when applied to biological systems, has been the subject of considerable debate and a universally acceptable definition has proved elusive (see Pickering, 1981). Despite this, most fish biologists and aquaculturists have an intuitive notion of what conditions are stressful to fish and in the aquaculture industry, stress is frequently diagnosed when the performance capacity of the fish is significantly reduced. The performance of a fish can be conveniently described in terms of its survival, growth and reproduction, and the aim of this paper is to identify some of the physiological and endocrinological changes that occur in stressed fish, changes which are largely responsible for a decrease in survival (by increasing disease susceptibility), a suppression of growth rate and, as is now becoming evident, an interference with sexual maturation and reproduction. Practical guidelines will be given to enable fish farmers to avoid or minimize the damaging effects of environmental stress.

The stress response

When a fish is subjected to an environmental stress, it initiates a series of hormonal and physiological responses which are designed to switch the fish from an anabolic state (one in which energy is being taken up and stored), to a catabolic state (one in which the body reserves are broken down). This gives the fish access to energy reserves not normally available to it, reserves which are used as it attempts to avoid or overcome the immediate threat. It is likely that many components of the fish's endocrine system are involved in this physiological change but to date, most research efforts have concentrated on the role of the hypothalamic-pituitary-interrenal axis in this response. In our own studies on brown trout and rainbow trout, we have examined the role of cortisol, the principle steroid hormone secreted by the interrenal tissue, in the stress response. Under aquaculture conditions cortisol is secreted in response to all forms of physical disturbance (see for example Pickering et al., 1982), overcrowding (Pickering & Stewart, 1984), prophylactic treatments (Pickering & Pottinger, 1984) and social interaction (Ejike & Schreck, 1980; Pickering and Pottinger, 1990). Under conditions of acute stress, i.e. the stress is of short duration and the time course of the stress response of the fish far outlasts that of the stress itself, cortisol levels may be elevated from basal values of less than 2 ng ml^{-1} to several 100 ng ml^{-1} within an hour or so but then return to basal values within 24 hours (Pickering & Pottinger, 1989). Under conditions of chronic (continuous) stress, plasma cortisol levels may be elevated for several days or weeks before acclimation occurs (Pickering & Stewart, 1984). Other periods of prolonged cortisol elevation occur during the later part of the spawning season (Pickering & Pottinger, 1987) and during smoltification (Young, 1986).

The catabolic properties of cortisol (Sheridan, 1986; Janssens & Waterman, 1988) are probably of adaptive significance in the natural environment when the most likely outcome of any stressful situation is flight from the immediate danger, but in aquaculture there is no escape and under certain conditions the fish's own stress response can result in a serious impairment of its state of health. In the following sections, it will be shown that elevated cortisol levels are largely responsible for increased mortality, reduced growth rate and suppressed reproductive activity.

Survival

The suppressive effects of corticosteroids, such as cortisol, on the defence systems of fish have been known for many years. However, much of the work has been undertaken using pharmacologically high doses of hormones (see for example Robertson et al. 1963; Roth, 1972; Chilmonczyk, 1982) or using synthetic steroids of unknown or unmeasured potency (Anderson et al. 1982; Houghton & Matthews, 1986). Various forms of stress have been shown to cause a significant suppression in the number of circulating white blood cells (lymphocytes in particular) and it has been shown that this effect can be mimicked by physiological doses of cortisol (Pickering, 1984). More recently, in vivo and in vitro studies by Maule et al. (1987) and Tripp et al. (1987) have shown that the immune system of the coho salmon is exquisitively sensitive to low levels of cortisol and may be markedly suppressed with doses lower than 10 ng ml^{-1}. It is to be expected, therefore, that cortisol treatment would increase the susceptibility of teleost fish to disease. This is certainly true for many species of fish and, for the brown trout at least, it has been shown that this effect is dose-dependent and is detectable at cortisol levels well within the physiological range for the species (Pickering & Pottinger, 1989). This demonstration fits in well with the observation that the small, but chronic, elevations of plasma cortisol levels during smoltification and sexual maturation are paralleled by increases in the susceptibility of the fish to common diseases (see for example Pickering & Christie, 1980).

Growth

Stressed fish are characterized by a marked suppression in their growth rate, even when they are provided with adequate food supplies. Clearly, one important aspect of this phenomenon is the behavioural one of refusal to feed. After an acute stress, this effect may last for several days, the exact period being dependent on the species (and strain?) of fish (Wedemeyer, 1976). In addition to this effect of stress on feeding behaviour, however, the catabolic effects of cortisol on the fish's own body reserves are also responsible for some of the observed growth suppression. Thus, Barton et al. (1987) and Davis et al. (1985) have shown that cortisol treatment significantly suppresses the growth rates of rainbow trout and channel catfish respectively. The magnitude of this effect appears to be species specific because Pickering et al. (1989) have shown that the sensitivity of brown trout to physiological doses of cortisol (in terms of both catabolic processes and disease resistance) is significantly greater than that of the rainbow trout.

It has already been stated that salmonid fish will acclimate to certain forms of environmental stress resulting in a reduction of cortisol levels to basal values despite the continued presence of the stress. However under these circumstances, somatic growth may still be suppressed (Pickering & Stewart, 1984) and it seems likely, therefore, that other factors are also responsible for stress-induced growth suppression. One potential candidate is, of course, pituitary growth hormone (GH) - a potent growth promoting agent in teleost fish (see review by Weatherley & Gill, 1987). The development of techniques for measuring GH in fish blood is relatively recent (Cook et al. 1983; Bolton et al. 1986; Wagner & McKeown, 1986; Le Bail et al. 1990) and the study of the physiological role of fish growth hormone is still in its infancy. Preliminary studies (Pickering et al. 1990) have shown that an acute stress, such as short-term confinement in small aquaria, can cause a significant suppression in the concentration of circulating GH in the rainbow trout. In contrast, the chronic stress of overcrowding combined with water quality deterioration promoted an increase in circulating GH levels. This latter observation may represent a response to a state of physiological

starvation caused by other stress-related factors. Clearly, much more work is now needed on the relationships between environmental stress and growth suppression in teleost fish under aquaculture conditions.

Reproduction

Until recently there was very little direct evidence linking environmental stress with the reproductive physiology of fish held under aquaculture conditions, although considerable circumstantial evidence suggested that various forms of stress were capable of suppressing reproductive activity in fish populations (see Pickering et al. 1987). The control and synchronization of sexual maturation is mediated by a set of endocrine glands which constitute the hypothalamic-pituitary-gonadal axis. Pickering et al. (1987) observed that prolonged (1 month) confinement stress not only caused a significant elevation of plasma cortisol levels but also reduced the circulating levels of the two androgens, testosterone and 11-ketotesterone, in sexually maturing male brown trout. Moreover, the same authors found that a period of 1 hour of confinement was sufficient to cause a prolonged suppression of these two androgens, with a recovery period of 24 hours. Paradoxically, plasma gonadotropin (GTH) levels were elevated. Following up this initial study, Carragher et al. (1989) found that implantation of physiological levels of cortisol to otherwise unstressed fish also interfered with the pituitary-gonadal axis of both sexes of brown trout and rainbow trout. In sexually maturing male fish, cortisol treatment significantly suppressed plasma testosterone levels and pituitary GTH content. In sexually maturing females, the effect was equally apparent with cortisol treatment causing a significant suppression of plasma oestradiol, testosterone and vitellogenin levels together with a 50% reduction in the pituitary GTH content. The net result in both sexes was a significant reduction in gonadal size. Even in sexually immature fish, more than 12 months away from spawning, cortisol treatment was shown to suppress circulating vitellogenin levels in the female. Thus, cortisol is undoubtedly capable of interfering with the hormonal processes leading to sexual maturation in salmonid fish.

It is not possible from these in vivo studies to elucidate the underlying mechanisms behind such reproductive suppression but Sumpter et al. (1987) have shown by means of in vitro studies that physiological levels of cortisol can suppress the basal secretion rate of oestradiol from cultured ovarian follicules from the rainbow trout. Similarly, Carragher & Sumpter (1990) demonstrated that cortisol can suppress pituitary GTH secretion in vitro. In a very recent study (Pottinger & Pickering, 1990), it has been shown that a further effect of cortisol is to suppress oestradiol binding-sites in the liver of female rainbow trout, sites which are important mediators of oestradiol-induced vitellogenesis during sexual maturation. Thus, cortisol may act directly at several different sites in the pituitary-gonadal axis to suppress reproductive processes. The level of cortisol needed to cause such changes are within the physiological range for the species and it seems likely, therefore, that aquacultural stress during the pre-spawning season will have damaging consequences for reproductive success. Further work is now needed to investigate the effects of stress on gamete quality and quantity.

Having shown that plasma cortisol elevation, as a result of aquacultural stress, is capable of reducing the probability of survival by increasing disease susceptibility, of suppressing somatic growth and of interfering with sexual maturation, I want to conclude this paper by examining some of the practical approaches a fish farmer can adopt to minimize such stress-related damage.

Practical approaches to minimizing stress

At the outset, it must be stated that, by the very nature of most fish farming operations, it will be impossible to avoid all instances of environmental stress. However in general terms, the duration of the stress response is proportional to the duration of the stress and, therefore, considerable benefit is obtained by minimizing, as far as possible, the length of time of any unavoidable period of stress. Moreover, the magnitude of the cortisol response to stress is temperature-dependent, with greater cortisol levels being developed at higher water temperatures. This is true for both coldwater species such as the trout (Sumpter et al., 1985) and for warmwater species such as the channel catfish (Strange, 1980). Thus, it is important to avoid stressing the fish at high water temperatures. Multiple stresses should be avoided at all times because the cortisol response to the various stressful components may be additive or even synergistic (Barton et al., 1985, 1986). Such effects might occur when, for example, fish are subjected to a sudden temperature shock following transport stress. One of the secondary problems associated with stress in freshwater fish is a loss of vital salts from the body fluids as a result of changes in tissue permeability and vascular flow. The use of dilute salt solutions for transporting freshwater fish has been shown to be effective in reducing stress-related mortality (Hattingh et al., 1975; Long et al., 1977; Kutty et al., 1980).

Withdrawal of food for 2-3 days is routinely recommended before any stressful procedure, not only because it prevents fouling of the water by faecal material and regurgitated food, but also because the oxygen requirements of the fish are reduced after food is withheld. Smart (1981) has shown that the oxygen consumption of rainbow trout is still markedly elevated 24 hours after the stress of grading. Mild anaesthesia has also been shown to be effective in reducing both the magnitude of the cortisol response to transport stress and in promoting post-stress survival (Strange & Schreck, 1978; Ferreira et al., 1984).

An understanding of the fish's natural habitat can also provide insight into methods of stress control. The natural habitat of young Atlantic salmon is characterised by immediate access to overhead cover and Pickering et al. (1987) have shown that the provision of floating annular covers to conventional tangential-flow rearing tanks significantly increased the growth rate of under-yearling Atlantic salmon during their first summer's growth. This had the effect of doubling the proportion of potential S1 smolts within the population. Moreover, overhead cover also reduced the mortality rate of the potential S2 smolts during their first winter (Pickering & Pottinger, unpublished). Our most recent approach to modifying the stress response under aquaculture conditions is to investigate the basis of the individual variation in the magnitude of the stress-induced cortisol response. Such an approach has been successfully adopted in the poultry industry (see for example Brown & Nestor, 1973). By careful examination of the cortisol response of individually identifiable rainbow trout, it has been possible to identify individual fish which give a consistently high or a consistently low cortisol response to a standard form of stress such as confinement for 1 hour. The "low responders" have been recently stripped and fertilized in an attempt to produce a strain of fish which respond with a reduced cortisol response to common aquacultural stresses (Refstie, (1982, 1986) has already shown that the magnitude of the cortisol response to a stress is a heritable character in rainbow trout and Atlantic salmon). In effect, what we are attempting to do is to accelerate the rate of domestication in these fish.

Summary

We have shown that stress-induced cortisol elevation plays a central role in increasing the susceptibility of fish to disease, in suppressing growth rates and in interfering with sexual development. There are several strategies that the fish farmer can adopt to minimize the damaging effects of stress but the long-term solution may be found in the development of stress-resistant strains of fish.

Acknowledgements

Specific acknowledgements have been made in the original research papers on which much of this review is based. In addition, the author is also grateful to Mrs M Thompson (IFE) for manuscript preparation and to NERC and MAFF for financial support.

References

Anderson, D.P., Roberson, B.S. & Dixon, O.W. (1982). Immunosuppression induced by a corticosteroid or an alkylating agent in rainbow trout (Salmo gairdneri) administered a Yersinia ruckeri bacterin. Dev. Comp. Immunol. Suppl. 2, 197-204.

Barton, B.A., Schreck, C.B. & Barton, L.D. (1987). Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout. Dis. Aquat. Org. 2, 173-185.

Barton, B.A., Schreck, C.B. & Sigismondi, L.A. (1986). Multiple acute disturbances evoke cumulative physiological stress responses in juvenile chinook salmon. Trans. Am. Fish. Soc. 115, 245-251.

Barton, B.A., Weiner, G.S. & Schreck, C.B. (1985). Effect of prior acid exposure on physiological responses of juvenile rainbow trout (Salmo gairdneri) to acute handling stress. Can. J. Fish. Aquat. Sci. 42, 710-717.

Bolton, J.P., Takahashi, A., Kawauchi, H., Kubota, J. & Hirano, T. (1986). Development and validation of a salmon growth hormone radioimmunoassay. Gen. Comp. Endocrinol. 62, 230-238.

Brown, K.I. & Nestor, K.E. (1973). Some physiological responses of turkeys selected for high and low adrenal responses to cold stress. Poult. Sci. 52, 1948-1954.

Carragher, J.F. & Sumpter, J.P. (1990). Corticosteroid physiology in fish. Proc. XI Int. Symp. Comp. Endocrinol., Malaga (in press).

Carragher, J.F., Sumpter, J.P., Pottinger, T.G. & Pickering, A.D. (1989). The deleterious effects of cortisol implantation on reproductive function in two species of trout, Salmo trutta L. and Salmo gairdneri Richardson. Gen. Comp. Endocrinol. 76, 310-321.

Chilmonczyk, S. (1982). Rainbow trout lymphoid organs : cellular effects of corticosteroids and anti-thymocyte serum. Dev. Comp. Immunol. 6, 271-280.

Cook, A.F., Wilson, S.W. & Peter, R.E. (1983). Development and validation of a carp growth hormone radioimmunoassay. Gen. Comp. Endocrinol. 50, 335-347.

Davis, K.B., Torrance, P., Parker, N.C. & Suttle, M.A. (1985). Growth, body composition and hepatic tyrosine aminotransferase activity in cortisol-fed channel catfish, Ictalurus punctatus. J. Fish Biol. 29, 177-184.

Ejike, C. & Schreck, C.B. (1980). Stress and social hierarchy rank in coho salmon. Trans. Am. Fish. Soc. 109, 423-426.

Ferreira, J.T., Schoonbee, H.J. & Smit, G.L. (1984). The use of benzocaine-hydrochloride as an aid in the transport of fish. Aquaculture 42, 169-174.

Hattingh, J., Fourie, F. & van Vuren, J. (1975). The transport of freshwater fish. J. Fish Biol. 7, 447-449.

Houghton, G. & Matthews, R.A. (1986). Immunosuppression of carp (Cyprinus carpio L.) to ichthyophthiriasis using the corticosteroid triamcinolone acetonide. Vet. Immunol. Immunopathol. 12, 413-419.

Janssens, P.A. & Waterman, J. (1988). Hormonal regulation of gluconeogenesis and glycogenolysis in carp (Cyprinus carpio) liver pieces cultured in vitro. Comp. Biochem. Physiol. 91A, 451-457.

Kutty, M.N., Sukumaran, N. & Kasim, H.M. (1980). Influence of temperature and salinity on survival of the freshwater mullet, Rhinomugil corsula (Hamilton). Aquaculture 20, 261-274.

Le Bail, P.Y., Sumpter, J.P., Carragher, J., Mourot, B., Niu, P.D. & Weil, C. (1990). Development and validation of a highly sensitive radioimmunoassay to chinook salmon (Oncorhynchus tshawytscha) growth hormone. (in preparation).

Long, C.W., McComas, J.R. & Monk, B.H. (1977). Use of salt (NaCl) water to reduce mortality of chinook smolts, Oncorhynchus tshawytscha, during handling and hauling. U.S. Mar. Fish. Ser., Mar. Fish. Rev. 39, 6-9.

Maule, A.G., Schreck, C.B. & Kaattari, S.L. (1987). Changes in the immune system of coho salmon (Oncorhynchus kisutch) during the parr-to-smolt transformation and after implantation of cortisol. Can. J. Fish. Aquat. Sci. 44, 161-166.

Pickering, A.D. (1981). Introduction: the concept of biological stress. In Stress and Fish (A.D. Pickering, ed.), pp. 1-9. London and New York : Academic Press.

Pickering, A.D. (1984). Cortisol-induced lymphocytopenia in brown trout, Salmo trutta L. Gen. Comp. Endocrinol. 53, 252-259.

Pickering, A.D. & Christie, P. (1980). Sexual differences in the incidence and severity of ectoparasitic infestation of the brown trout, Salmo trutta L. J. Fish Biol. 16, 669-683.

Pickering, A.D., Griffiths, R. & Pottinger, T.G. (1987a). A comparison of the effects of overhead cover on the growth, survival and haematology of juvenile Atlantic salmon, Salmo salar L., brown trout, Salmo trutta L., and rainbow trout, Salmo gairdneri Richardson. Aquaculture 66, 109-124.

Pickering, A.D. & Pottinger, T.G. (1984). Acclimation of the brown trout, Salmo trutta L., to the stress of daily exposure to malachite green. Aquaculture 44, 145-152.

Pickering, A.D. & Pottinger, T.G. (1987). Lymphocytopenia and interrenal activity during sexual maturation in the brown trout, Salmo trutta L. J. Fish Biol. 30, 41-50.

Pickering, A.D. & Pottinger, T.G. (1987). Lymphocytopenia and interrenal activity during sexual maturation in the brown trout, Salmo trutta L. J. Fish Biol. 30, 41-50.

Pickering, A.D. & Pottinger, T.G. (1989). Stress responses and disease resistance in salmonid fish : effects of chronic elevation of plasma cortisol. Fish Physiol. Biochem. 7, 253-258.

Pickering, A.D. & Pottinger, T.G. (1990). The influence of social interaction on the acclimation of rainbow trout, Oncorhynchus mykiss, to chronic confinement stress. (In preparation).

Pickering, A.D., Pottinger, T.G. & Carragher, J.F. (1989). Differences in the sensitivity of brown trout, Salmo trutta L., and rainbow trout, Salmo gairdneri Richardson, to physiological doses of cortisol. J. Fish Biol. 34, 757-768.

Pickering, A.D., Pottinger, T.G., Carragher, J. & Sumpter, J.P. (1987b). The effects of acute and chronic stress on the levels of reproductive hormones in the plasma of mature male brown trout, Salmo trutta L. Gen. Comp. Endocrinol. 68, 249-259.

Pickering, A.D., Pottinger, T.G. & Christie, P. (1982). Recovery of the brown trout, Salmo trutta L., from acute handling stress: a time-course study. J. Fish Biol. 20, 229-244.

Pickering, A.D., Pottinger, T.G., Sumpter, J.P., Carragher, J.F. & Le Bail, P.Y. (1990). Effects of acute and chronic stress on the levels of circulating growth hormone in the rainbow trout, Oncorhynchus mykiss. (in preparation).

Pickering, A.D. & Stewart, A. (1984). Acclimation of the interrenal tissue of the brown trout, Salmo trutta L., to chronic crowding stress. J.Fish Biol. 24, 731-740.

Pottinger, T.G. & Pickering, A.D. (1990). The effect of cortisol administration on hepatic and plasma estradiol-binding capacity in immature female rainbow trout (Oncorhynchus mykiss). Gen. Comp. Endocrinol. (in press).

Refstie, T. (1982). Preliminary results : differences between rainbow trout families in resistance against vibriosis and stress. In Developmental and Comparative Immunology, Suppl. 2. (W.B. van Muiswinkel, ed.), pp. 205-209. New York : Pergamon Press.

Refstie, T. (1986). Genetic differences in stress response in Atlantic salmon and rainbow trout (Abstract). Aquaculture 57, 374.

Robertson, O.H., Hane, S., Wexler, B.C. & Rinfret, A.P. (1963). The effect of hydrocortisone on immature rainbow trout (Salmo gairdneri). Gen. Comp. Endocrinol. 3, 422-436.

Roth, R.R. (1972). Some factors contributing to the development of fungus infection in freshwater fish. J. Wildl. Dis. 8, 24-28.

Sheridan, M.A. (1986). Effects of thyroxin, cortisol, growth hormone, and prolactin on lipid metabolism of coho salmon, Oncorhynchus kisutch, during smoltification. Gen. Comp. Endocrinol. 64, 220-238.

Smart, G.R. (1981). Aspects of water quality producing stress in intensive fish culture. In: Stress and Fish (A.D. Pickering, ed.), pp. 277-293. London and New York : Academic Press.

Strange, R.J. (1980). Acclimation temperature influences cortisol and glucose concentrations in stressed channel catfish. Trans. Am. Fish. Soc. 109, 298-303.

Strange, R.J. & Schreck, C.B. (1978). Anaesthetic and handling stress on survival and cortisol concentration in yearling chinook salmon (Oncorhynchus tshawytscha). J. Fish. Res. Bd Can. 35, 345-349.

Sumpter, J.P., Carragher, J., Pottinger, T.G. & Pickering, A.D. (1987). The interaction of stress and reproduction in trout. In Reproductive Physiology of Fish (D.R. Idler, L.W. Crim and J.M. Walsh, eds.), pp. 299-302. Memorial University of Newfoundland : St. Johns.

Sumpter, J.P., Pickering, A.D. & Pottinger, T.G. (1985). Stress-induced elevation of plasma α-MSH and endorphin in brown trout, Salmo trutta L. Gen. Comp. Endocrinol. 59, 257-265.

Tripp, R.A., Maule, A.G., Schreck, C.B. & Kaattari, S.L. (1987). Cortisol mediated suppression of salmonid lymphocyte responses in vitro. Dev. Comp. Immunol. 11, 565-576.

Wagner, G.F. & McKeown, B.A. (1986). Development of a salmon growth hormone radioimmunoassay. Gen. Comp. Endocrinol. 62, 452-458.

Weatherley, A.H. & Gill, H.S. (1987). The Biology of Fish Growth. 443 pp. New York : Academic Press.

Wedemeyer, G.A. (1976). Physiological response of juvenile coho salmon (Oncorhynchus kisutch) and rainbow trout (Salmo gairdneri) to handling and crowding stress in intensive fish culture. J. Fish. Res. Bd Can. 33, 2699-2702.

Young, G. (1986). Cortisol secretion in vitro by the interrenal of coho salmon (Oncorhynchus kisutch) during smoltification : relationship with plasma thyroxine and plasma cortisol. Gen. Comp. Endocrinol. 63, 191-200.


Natural
Environment
Research
Council