Pitlo, R. & Dawson, F. H. (1989). The flow resistance of aquatic vegetation. In *Aquatic Weeds*, (eds A. H. Pieterse & K. J. Murphy). Oxford University Press.

Westlake, D. F. & Dawson, F. H. (1982). Thirty years of weed cutting on a chalk-stream. *Eur. Weed Res. Soc. 6th Int. Symp. Aquatic Weeds, Novi Sad, September 1982,* 132–40.

Westlake, D. F. & Dawson, F. H. (1986). The management of *Ranunculus calcareus* by pre-emptive cutting in southern England. *Eur. Weed Res. Soc.; Ass. of Appl. Biologists, 7th Int. Symp. Aquatic Weeds,* September 1986, 395–400.

Westlake, D. F. & Dawson, F. H. (1988). The effects of autumnal weed cuts in a lowland stream on water levels and flooding in the following spring. *Verh. int. Verein. theor. angew. Limnol.* 23, 1273–77.

FACTORS AFFECTING THE SUSCEPTIBILITY OF SALMONID FISH TO DISEASE

A. D. PICKERING

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

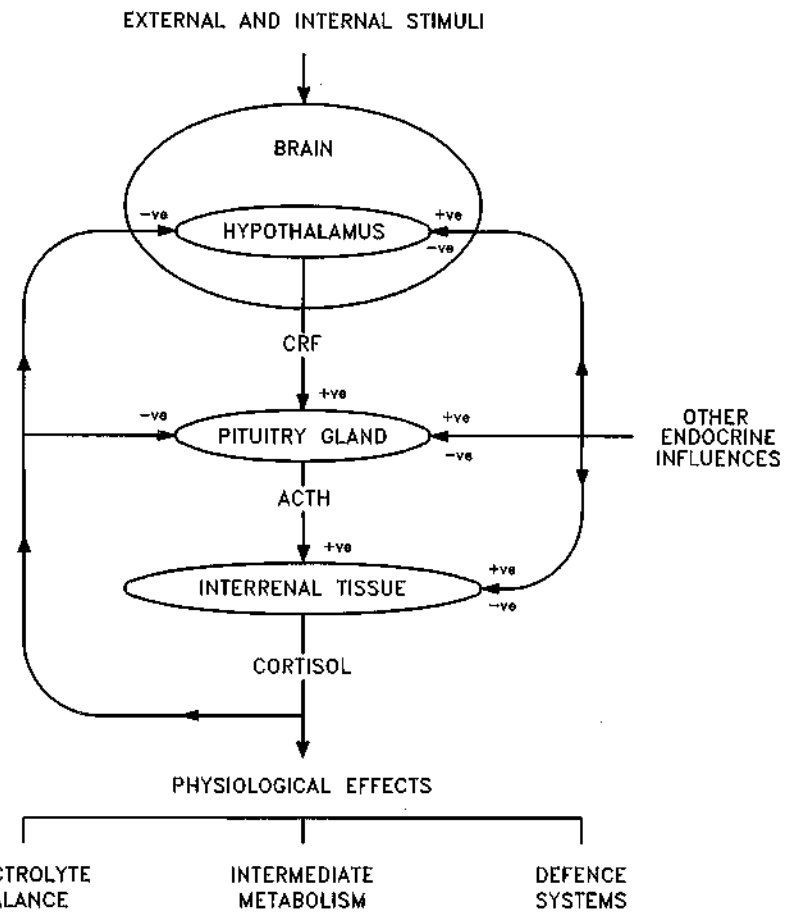

FIG. 2. A simplified, schematic diagram of the hypothalamic-pituitary-interrenal (HPI) axis in salmonid fish.

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

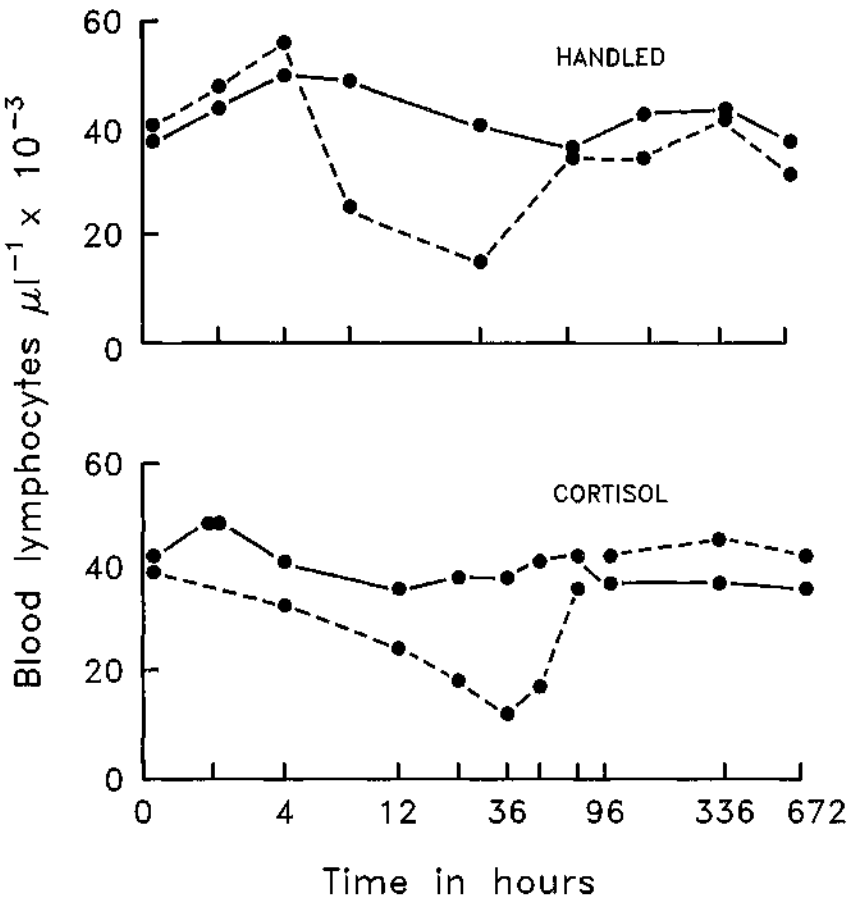

FIG. 3. Changes in the number of circulating lymphocytes in the blood of the brown trout following a single incidence of handling (upper graph) or the oral administration of a physiological dose of cortisol (lower graph). The broken line represents treated fish, the continuous line represents unstressed 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).

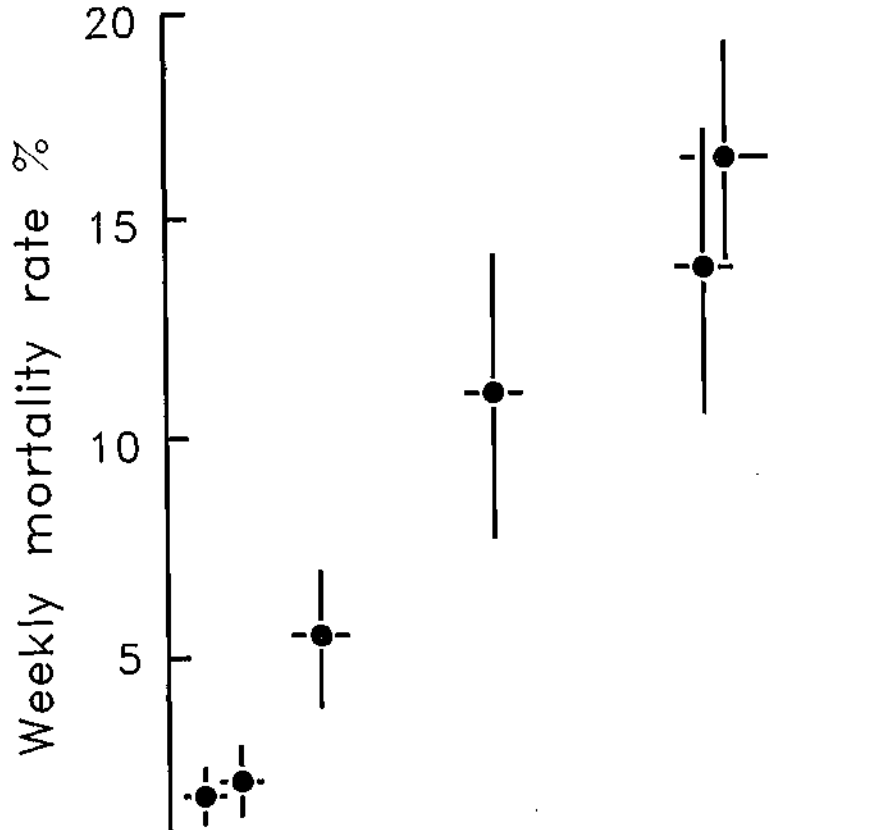

FIG. 5. Effect of chronic elevation of plasma cortisol levels by means of slow-release implants on the mortality rate of brown trout.

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 *Gyrodactylus* (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

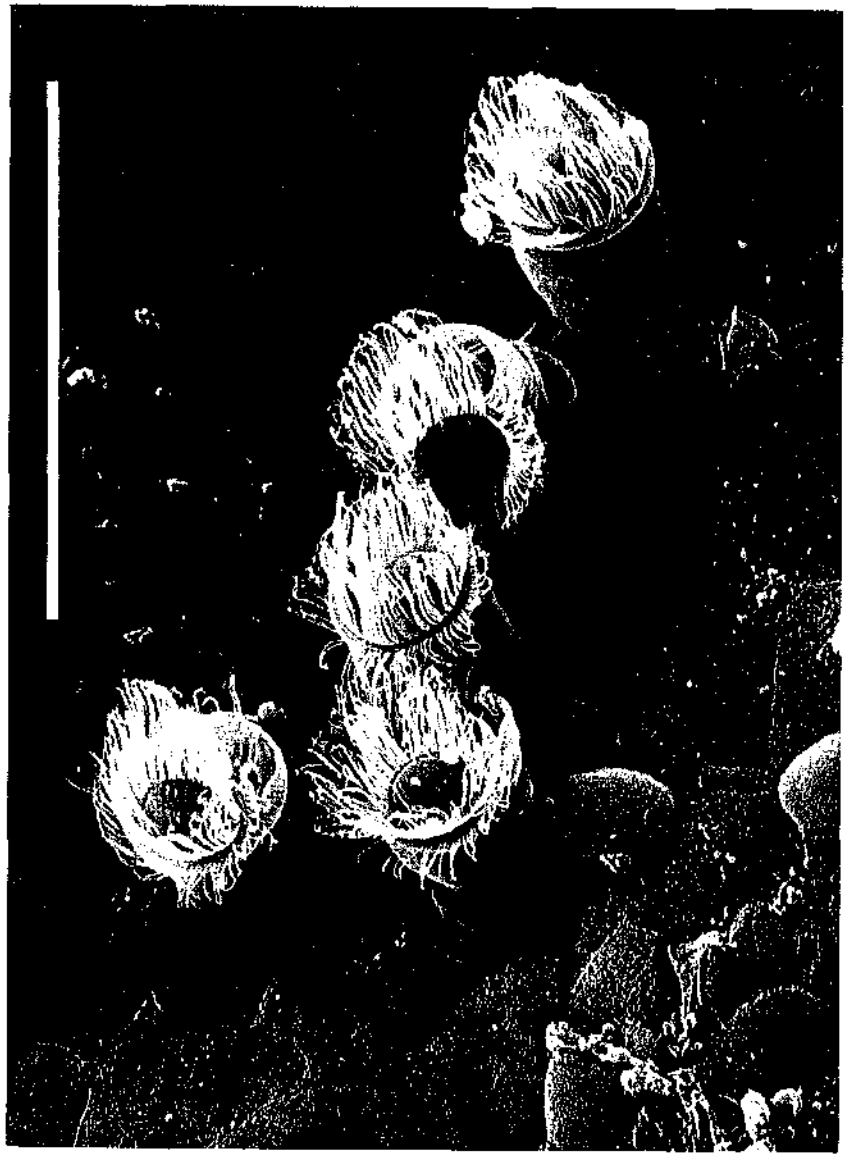

FIG. 6. A small colony of *Scyphidia* on the epidermis of the brown trout. These ciliated protozoans attach to the superficial epidermal cells of the fish and filter-feed on bacteria in the surrounding water (bar line 100 μm).

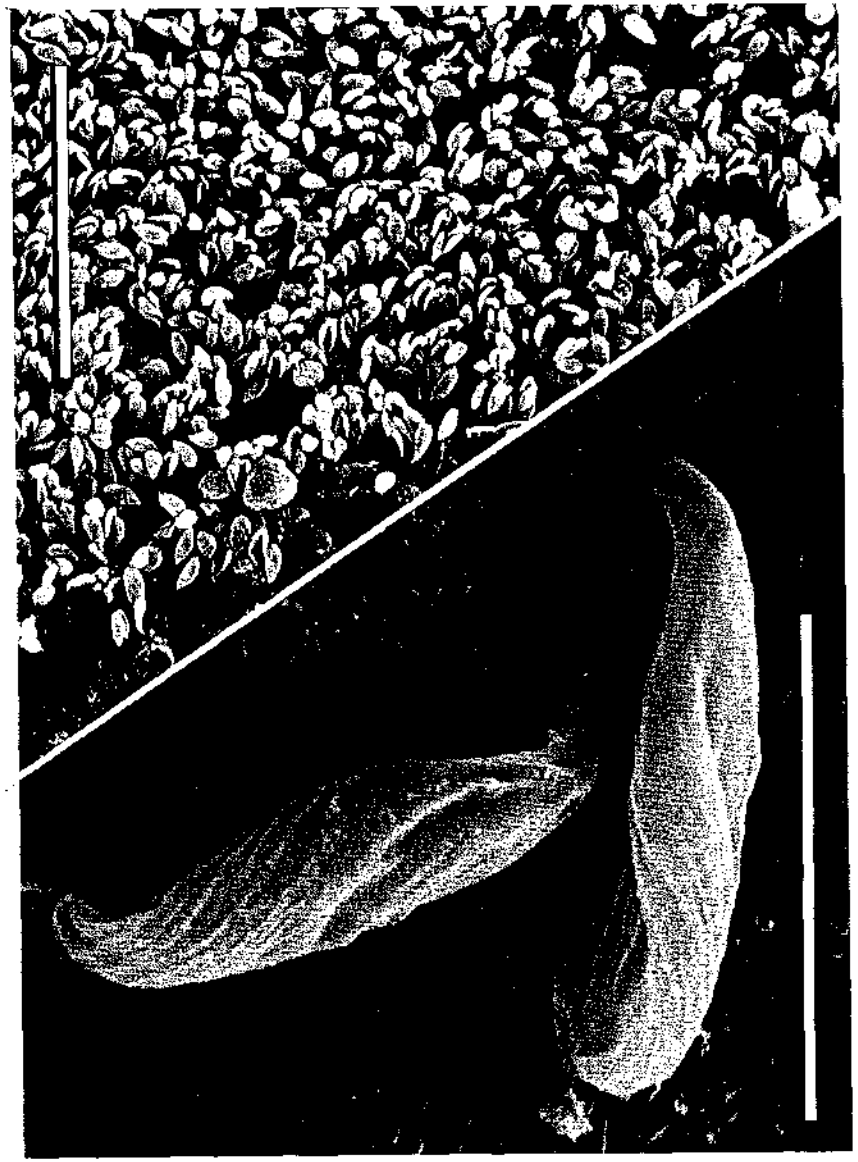

FIG. 7. Upper–severe infestation of the epidermis of the brown trout by the flagellate protozoan, *Ichthyobodo* (bar line 100 μm).
Lower–high power showing the attachment of two parasites to adjacent epidermal cells (bar line 10 μm).

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.

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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.

BIOLOGICAL SURVEILLANCE OF CHALK-STREAMS

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Introduction

Indices based on macroinvertebrate communities have been used for surveillance of the quality of river water for many years (e.g. Woodiwiss 1964; Chandler 1970). Two types of index are commonly used. Quantitative indices, which are a measure of faunal diversity, take account of the distribution of individuals among the represented taxa (e.g. Wilhm 1970) whereas qualitative indices, or biotic scores, weight taxa according to their known, or assumed, levels of tolerance to organic pollution (e.g. Chandler 1970). The latter have usually been developed in relation to particular rivers or areas, but have often been applied subsequently to a variety of situations for which they were not intended (Armitage et al. 1983). In 1981, the National Water Council published details of the 'Biological Monitoring Working Party Score' (BMWP or NWC Score) which was used in the 1980 survey of rivers in England and Wales. This index was designed to be widely applicable and acceptable in Britain. Its performance has been assessed by Chesters (1980) and by Armitage et al. (1983).

Chalk-streams

The chalk-streams of southern England are, in general, free from serious problems of pollution and are valuable as game-fisheries (Ladle & Casey 1979). However, they are being used to an increasing extent for a variety of other purposes, including domestic water supply, fish-farming, growing of watercress and the disposal of sewage and agricultural effluents.

Wessex Water have noted a decline in water quality in the headwaters of such streams (National Water Council 1981; Department of the Environment 1987) which is partly attributable to agricultural effluents. Casey & Clark (1970) reported a progressive increase in nitrate levels in the River Frome (Dorset) resulting from the increased use of inorganic, agricultural fertilizers.