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Studies on precocious maturity in artificially reared 1+ Atlantic salmon parr *Salmo salar* L.

A thesis presented for the degree of Doctor of Philosophy to the University of Stirling

by

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This thesis is dedicated to the memory of my late father

ROBERT MURPHY, *and grandfather,* MAURICE HARTNETT.

The work presented in this thesis is the result of my own investigations and has neither been accepted nor is being submitted for any other degree.

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13/2/'80 ..... Date

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ABSTRACT

Studies on the testicular cycle of commercially reared precocious male Atlantic salmon have shown that spermatogenesis began in late spring/early summer and was completed by November/December. The spermatogenic cycle could be divided into three phases:

(i) Formation of spermatogonia which takes place during spring and summer.

(ii) Development of spermatids which occurs from August to December.

(iii) Spermiation which takes place from December until the next cycle begins.

Androgen secretion increased during the sexual cycle and was at a peak in November. Two types of basophils were identified in the meso-adenohypophysis and these resembled the gonadotrophic cells that have previously been described in salmonids. These precocious males had a greater interrenal activity than immature parr.

Both immature and mature 1+ parr in this study exhibited epidermal hyperplasia during the winter and the epidermis was considerably thicker amongst the precocious males. The skin thickness varied from area to area being thickest in the caudal peduncle and thinnest in the pectoral area.

There was a positive correlation between it and the body weight. There was a seasonal cycle in the superficial mucous cell concentration and the numbers of goblet cells increased in both sexes during autumn and winter. From October onwards, precocious males had significantly more mucous cells than immature females. The secretions of these cells were acid mucopolysaccharide with a large sialic acid component. The pectoral fin and tail were the regions most frequently infected with Saprolegnia and these had the greatest concentration of mucous cells.

When O+ parr were treated with exogenous steroids, it was found that androgens caused epidermal hyperplasia and that cortisol increased the mucous cell concentration in the fins and tail. Cortisol and oestrogen experimental groups had the greatest prevalence of Saprolegnia and the areas most frequently parasitised were the pectoral fin and tail. Comparison of mucohistochemical results from untreated 1+ parr and O+ parr fed steroids suggests that the endocrine status of the animal may influence the chemical composition of the mucus secretion.

Methallibure and cyproterone acetate failed to prevent gonad maturation when they were administered during the later stages of spermatogenesis. They did, however, lower plasma 11-ketotestosterone levels and caused a decrease in the gonadotrophic cell size. When these drugs were fed at the beginning of the sexual cycle from early spring to August

all the male parr in the methallibure-treated group were immature but 40% of the males in the cyproterone acetate group had developing gonads. None of the males in the methallibure experimental group showed any sign of gonad development 20 weeks after cessation of the treatment. The results of these experiments show that it is possible to prevent precocious sexual maturity in intensive aquaculture units using chemotherapeautic agents and that central inhibitors of gonadotrophic secretion are the drugs of choice.

ACKNOWLEDGEMENTS

I am very grateful to my supervisor, Prof. R.J. Roberts, for his advice and interest during this project. I thank Dr. D.J. Piggins for placing the facilities of the Farran Laboratory at my disposal, for his support and for the kindness he showed me while I was in Mayo.

This work could not have been undertaken without the financial assistance of the Atlantic Salmon Trust which is gratefully acknowledged. I particularly appreciated the readiness of Sir Hugh MacKenzie to allow me extra funds whenever they were needed.

My thanks also to Mr John Lawrie who helped to set up the experimental tanks, Miss Deirdre Cotter and the field assistants, Messrs M. Davitt, T. Lavelle, P. Mulchrone and A. Nixon who helped to make my stay in Newport so pleasant. The generosity of Major and Mrs. Blackwell is also acknowledged.

The help given to me by the staff of the Unit of Aquatic Pathobiology, University of Stirling, was greatly appreciated especially that of Dr. J.S. Buchanan, Mrs. S. Horne and Messrs. W. Dunn and I.H. MacRae. I take this opportunity to thank my colleague, Carmelo Agius,

for his companionship and ready wit which helped to make the histological work more agreeable.

My sincere thanks to Dr. T.A. Simpson for allowing me the use of the laboratory facilities at the Endocrinology Department, D.A.F.S. Marine Laboratory, Torry, Aberdeen. I am indebted to Dr. R.S. Wright for guidance with the 11-ketotestosterone assay and for his generous gift of 11-ketotestosterone radioactive label and antiserum. My thanks also to Miss Valerie Hunt for her help with the cortisol radioimmunoassay, the staff of the Analytical Chemistry Laboratory for use of the atomic absorption spectrophotometer and to Mr. R. Johnstone for some very productive discussions on my research topic.

I am obliged to Dr. Walpole (I.C.I.) and Dr. Clemens (Schering) for the gifts of methallibure and cyproterone acetate and to Miss Niamh Cavanagh, Biochemistry Department, Portiuncula Hospital, Ballinasloe, Co. Galway, for the use of the flame spectrophotometer.

I am deeply indebted to my wife and my mother for their constant encouragement during my studies.

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CHAPTER 1

GENERAL INTRODUCTION

1.1. Life cycle of Atlantic salmon Salmo salar L.

The life cycle of Atlantic salmon Salmo salar L. involves a freshwater and seawater stage. Atlantic salmon are anadromous - that is they return to freshwater to spawn. The young parr remain in the streams and rivers for 1 to 4 years before going to sea as smolts. Before they migrate to the sea, the parr undergo endocrinological, physiological and behavioural changes, known as smoltification, which make them able to adapt to the saltwater environment. Salmon smolts migrate to feeding grounds in the North Atlantic, off Greenland and possibly off Norway, for varying lengths of time. Early maturing Atlantic salmon return to freshwater to spawn after only one year at sea and are known as grilse, other adults remain at sea for 2 - 4 years before spawning and are called salmon.

The phenomenon of precocious sexual maturity in male parr has been known to occur amongst wild salmon for some time. They accompany spawning adults and participate in the fertilization of the eggs of the adult females (Jones, 1959). A varying number of young parr become ripe depending on the

river system (Pentelow, Southgate and Bassindale, 1933; Orton, Jones and King, 1938; Osterdahl, 1969; cited by Koch and Bergstrom, 1978).

1.2. Salmonid Fish Farming

Fish culture is an important and rapidly expanding enterprise all around the world. In developing countries fish are reared for food and in the more sophisticated nations fish culture is used to produce sport fishes for rivers and lakes as well as for food. The recent advance in technical expertise in rearing fish in seawater installations has given salmonid culture a new impetus because, until recently, artificial rearing of rainbow trout Salmo gairdneri Richardson and Atlantic salmon was confined to the freshwater stages of their life cycle. The decrease in commercial catches despite an increase in fishing effort, and the reduced numbers of wild adult salmon returning to spawn in their home rivers, has also added to the importance of salmon fish farms as means of preserving an endangered species.

Hjul (1976) quoting statistics from a conference on fish culture held under the auspices of the United Nations at Kyoto (Japan) showed that the world production of fin fish in 1975 was an estimated 3,980,492 tonnes, of which Norway produced 3,500 tonnes, U.K. 2,000 tonnes and Ireland 207 tonnes.

Norway produced 4,500 tonnes of salmonid fish in 1977, worth about £10m (Gjedrem and Edwards, 1978). Production in the U.K. in 1978 was about 2,500 tonnes of trout and 1,000 tonnes of other fish including salmon, turbot and carp (Anon., 1978). Projected figures for world aquaculture production have suggested an expansion to produce at least 20-25 million tonnes by the year 2000, which would be about 20-25% of the estimated world fish supply at that time (Edwards, 1976).

In England, the Ministry for Agriculture, Fisheries and Food has presented projections that by the mid 1980s fish farms will be making a significant contribution to the food supply of the U.K. by producing species that are in short supply from traditional sources (Anon.,1978). It is suggested that production will be 1-2 thousand tonnes of sea fish, up to 5,000 tonnes of salmon and 20,000 tonnes of trout, worth up to £30m at current market prices. Fish farming in Ireland is also expected to expand and Glenn (1977) suggests that within a decade, aquaculture could be contributing as much as 10-15% of Ireland's marine production.

To realize the full potential of salmonid farming, the fish are reared under intensive conditions. There are many disadvantages to this technique of fish husbandry; the high stocking densities lead to an increase in the prevalence of disease; the high level of nutrition leads to an increase in the number of male parr becoming sexually mature.

Unlike the situation in the wild, where it may have survival value, precocious sexual maturity lowers the efficiency of fish farms because such precocious fish have an increased susceptibility to bacterial and particularly fungal infections. Workers at the Salmon Research Trust of Ireland hatchery have shown that over 95% of all fish affected with the fungus Saprolegnia during the winter months were precocious males (Table 1.1), where the males constituted at least 50% of the overall population. Johnstone, Simpson and Youngston (1978) also suggest that gonad development lowers the food conversion efficiency, causes a deterioration of flesh quality and makes smolts less able to adapt to the change from freshwater to saltwater for on-growing in sea cages.

The profitability of salmonid culture depends on the rearing of good quality smolts. Smolts are a high value stage in the production process and thus any condition that increases the mortality of parr represents a large loss of income for the smolt production unit. In addition, good quality smolts have excellent growth rates and food conversion efficiency in sea installations which thus increase the profit margins of fish farming enterprises.

Precocious maturity also occurs in the post-smolt stage during the young fish's first winter in sea installations (Saunders and Henderson, 1965). Sexual development among fishes of this age group is a serious impediment to

economic grilse and salmon production, as these fish cease to feed, and their skin and muscle develop a reddish brown pigmentation which reduces their market value.

This thesis presents some studies of precocious 1+ male Atlantic salmon parr reared under normal commercial husbandry conditions at the hatchery of the Salmon Research Trust of Ireland, Newport, Co. Mayo. The aim of the project was to compare some of the endocrinological and physiological changes that occur in sexually mature parr with immature parr. Experiments in which chemosterilant compounds were used to prevent spermatogenesis in male parr are also described.

CHAPTER 2

ENDOCRINOLOGICAL AND PHYSIOLOGICAL STUDIES OF 1+ ATLANTIC SALMON PARR

2.1. Review of the Literature

2.1.1. Control of gametogenesis - general

Gametogenesis in male animals is known as spermatogenesis. It describes the process whereby the primary germ cell in the immature testis undergoes a series of mitotic and meiotic divisions to produce mature spermatozoa capable of fertilizing female ova. The process is regulated by hormones which are produced in minute quantities by ductless (endocrine) glands and are transferred to target organs where they co-ordinate interrelated physiological processes. Spermatogenesis in teleost fishes is a complex process involving the interaction of environmental factors with the endocrine glands of the hypothalamus, pituitary and the testis (Fig. 1).

Pickford and Atz (1957) and Dodd (1960) have reviewed the earlier work on sexual maturity in fishes. Since then a considerable amount of work in this field has been reported and this

account will contain a brief resume of the more important details as they are presently understood. In order to give a coherent account of the control of sex in Atlantic salmon it is necessary also to refer to studies in other salmonid and non-salmonid fishes.

2.1.2. Environment

Successful reproduction is essential for species survival and animals have adapted their breeding cycle in order to ensure success in the conditions of their particular environment. The majority of fish display a well marked seasonal cycle with the result that spawning and hatching occur at a time that is most propitious for the survival of the young. The endocrine system serves as a link between environment and reproductive organs. It is generally accepted that environmental stimuli are processed by the central nervous system (CNS) and transmitted to the gonads via the hypothalamus, pituitary and blood stream by means of hormones. De Vlaming (1972) stated that photoperiod and temperature are the two most important external stimuli in the initiation of gametogenesis. He described the work of Henderson (1963) who showed that the brook trout Salvelinus fontinalis Mitchill, a salmonid species, needed an initial long photoperiod (late winter, spring and early summer) followed by a short photoperiod before completing sexual maturation.

In the rainbow trout spawning was advanced by 6 and 12 weeks when the normal photoperiod cycle was compressed into 9 and 6 months respectively (Whitehead, Bromage, Forster and Matty, 1978). Changes in serum oestradiol 17β, calcium and phosphoproteins, associated with vitellogenesis of rainbow trout, appeared to be initiated by the decreasing phase of the light regimes which occurred after the longest day (Whitehead, Bromage and Forster, 1978).

2.1.3. Pineal gland

A pineal-gonadal relationship is well established in mammals (Reiter, 1973) and many workers have suggested that this may also be true in teleosts. De Vlaming (1975) has shown that in the cyprinid teleost Notemigonus crysoleucas Mitchill the pineal gland can either stimulate or inhibit gonadal development, depending on the temperature and photoperiod regime to which the fish are exposed. De Vlaming and Vodicnik (1978) using the technique of surgical pinealectomy showed that in the goldfish Carassius auratus L. the pineal inhibits gonadal development under long photoperiod conditions but stimulates gonadal development under short photoperiods.

The pineal may be a photo-receptor as several light and electron microscopy studies have shown that

the sensory cells present in the teleost pineal are similar to the ciliary type of photo-sensory cells present in the retina of the eye of fish (de Vlaming, 1974). Histological examinations have also shown that secretory type cells are present. There is a diurnal variation in stainable granulation of these cells, with degranulation occurring during the dark phase of the photoperiod. The activity of these cells also varies with the stage of gonadal development being inactive during the period of greatest gonadal activity (Cheze (1969) quoted by de Vlaming, 1974). It is not known how the pineal exerts its effect on gonadal activity; it could be either by neural or hormonal pathways. Either way, the effect is mediated through the hypothalamus and pituitary. De Vlaming and Vodicnik (1977) and Vodicnik, Kral, de Vlaming and Crim (1978) have shown that pinealectomy modifies gonadotrophin releasing factor activity in the hypothalamus and gonadotrophin secretion in the pituitary.

2.1.4. Hypothalamus

In all vertebrates the hypothalamus is connected with the pituitary (adenohypophysis) by means of neurohypophysis, which develops from a downgrowth of the brain. In mammals the hypothalamus is

responsible for the production and secretion of 9 hormones that control the release of trophic hormones from the pituitary. It is generally thought that amongst these releasing factors is a combined luteinising hormone and a follicle stimulating hormone releasing factor (LH-RH/FSH-RH) which controls the sexual maturation cycle (Schally, 1978). These releasing hormones are synthesised by nerve cell bodies with short axons. The neurosubstances flow down the axons into blood capillaries that enter the anterior lobe of the pituitary. This neurohaemal complex is known as the median eminence (Ham, 1974).

Two types of neurosecretory fibres penetrate the teleost adenohypophysis. Type A fibres originate in the nucleus preopticus (NPO) area of the hypothalamus and are positive to all the classical neurosecretory stains, aldehyde fuchsin, alcian blue, etc. They terminate in the distal part of the neurohypophysis on pituiticytes, around capillaries and against the basement membrane separating the neurohypophysis from the adenohypophysis. Ultra-structural studies have shown that these fibres contain vesicles 1200 - 1700A$^{\circ}$ in diameter (Ball and Baker, 1969; Sage and Bern, 1971; Holmes and Ball, 1974). Type B fibres are refractory to most neurosecretory stains. The majority of them originate in the nucleus lateralis tuberis (NLT) and they transverse the basement membrane separating the neurohypophysis from the adenohypophysis. In some species they come into more or less intimate contact with all the adenohypophyseal cells (Holmes and Ball, 1974;

Sage and Bern, 1971). The vesicles of type B fibres are about 700A° in diameter and may contain monamine transmitter substances (Ball and Baker, 1969; Sage and Bern, 1971, Holmes and Ball, 1974; de Vlaming, 1974).

Evidence of hypothalamic control of pituitary gonadotrophic function has been derived from experiments involving pituitary transplantation and stereotactic electrolytic lesions in various parts of the hypothalamus (Peter, 1973, Review). Peter (1970) showed that the gonadotrophic releasing hormone (GTH-RH) functions of the goldfish are controlled by the pars posterior and by the posterior region of the pars anterior of the NLT. Peter and Crim (1978) confirmed that lesions in the NLT prevented gonad development but they also found that lesions in the NPO prevented ovarian recrudescence and induced a degree of atresia in female goldfish. This would suggest some involvement of the NPO region in gonadotrophin secretion. Changes in the neurosecretory material (NSM) in the NPO have been correlated to phases of the reproductive cycle (Holmes and Ball, 1974; de Vlaming, 1974; Terlou, de Jong and van Oordt, 1978). In the rainbow trout a large number of nerve fibres from the NPO pass through the NLT and some of them come into contact with the NLT cells (Terlou and Ekengren, 1978) and this may indicate a functional relationship amongst these hypothalamic nuclei. The NPO has also been implicated in the spawning reflex of some species (Peter, 1973).

Extracts from carp Cyprinus carpio L. and trout hypothalamus have been shown to stimulate the release of gonadotrophin (GTH) from sheep pituitaries *in vitro*. Extracts of sheep hypothalamus similarly affect carp pituitaries (Breton, Kann, Burzawa-Gerard and Billard (1971) quoted by Dodd, 1975). When carp hypothalamus extracts and synthetic LH-RH/FSH-RH are injected into carp they induce a rise in plasma GTH within minutes (Breton and Weil (1973) quoted by Dodd, 1975). Intra-hypophyseal injections of synthetic LH-RH/FSH-RH stimulated ovarian maturation in female carp (Sokolowska, Popek and Bieniarz, 1978).

It is still not clear how this releasing hormone reaches the gonadotrophic cells as there is no median eminence in teleost fishes. Electron microscopy studies of Type B fibres have shown them to end very close to the cells of the adenohypophysis (Ball and Baker, 1969; Sage and Bern, 1971; Holmes and Ball, 1974; de Vlaming, 1974) and aminergic fibres have been seen to be in contact with cells in the meso-adenohypophysis of Tilapia sp. (Sage and Bern, 1971). Fridberg and Ekengren (1977) suggest that in the Atlantic salmon the brain mediates its information to the pituitary through neurovascular links. They suggest that the rostral neurohypophysis fulfils all criteria of being a median eminence although it lacks a well defined portal system. In their studies they found that nerve terminals were present on capillaries and on the perivascular spaces present in this area. These perivascular spaces form a complex network in the neurohypophysis and were continuous

with the basement membrane in the neurohypophyseal-adenohypophyseal interface and the intercellular channels of the pro and meso-adenohypophysis. Fridberg and Ekengren (1977) assumed that this network acted as a conveyor of neurocrine factors as they could find no innervation of the adenohypophyseal cells.

2.1.5. Pituitary

2.1.5 (a) General

The pituitary gland comprises two parts, the adenohypophysis and the neurohypophysis. Embryologically it is derived from ectoderm, the adenohypophysis from somatic ectoderm (buccal epithelium) and the neurohypophysis from neural ectoderm. The neurohypophysis arises from a downgrowth towards the oral cavity from that area of the neural tube that will later form the hypothalamus and retains a connection with this part of the brain by means of a stalk of nerve fibres. The adenohypophysis or glandular area of the pituitary develops from an extension of the oral fold that breaks away from the oral ectoderm. This island of epithelium becomes attached to the downgrowth of the brain to form the pituitary. In higher vertebrates the adenohypophysis secretes at least seven trophic hormones that are necessary for the control of physiological functions of the animal. Among these trophic hormones are the gonadotrophins, follicle stimulating hormone (FSH) and luteinising hormone (LH).

Pituitary control over gametogenesis in fishes was first established using the technique of surgical hypophysectomy followed by replacement therapy. The effects of pituitary ablation depend on the stage of gonadal development, season of the year, temperature and photoperiod. It is particularly effective during the prespawning period, the time of maximum gonadal activity in nature (Ahsan, 1966).

Hypophysectomy was found to arrest spermatogenesis and cause gonad regression. There is some confusion over the stage of spermatogenesis that is dependant on pituitary gonadotrophins. In the plaice Pleuronectes platessa L. (Barr, 1963), guppy Poecilia reticulata Peters (Pandey, 1969), lake chub Couesius plumbeus Agassiz (Ahsan, 1966), the mitotic division of spermatogonia was inhibited while in the Indian catfish Heteropneustes fossilis Bloch (Sundararaj and Nayyar, 1967) spermatogonial mitosis occurred but the transformation of secondary spermatogonia to primary spermatocytes was prevented. The later stages, spermatocytes and spermatids present at the time of surgery continued to develop and became mature spermatozoa. Spermiation was not impeded by pituitary removal. Fish operated on during the prespawning and spawning phase of their annual cycle appear to spermiate normally in the absence of the pituitary (Barr, 1963). Exceptions to this are the goldfish (Yamazaki and Donaldson, 1968) and the adult guppy

(Pandey, 1969) in which the later stages develop normally to form sperm after hypophysectomy, but after a while the spermatophores rupture and the sperm are phagocytosed by Sertoli cells.

Hypophysectomy is a very difficult operation and is often fatal as it deprives the animals of other trophic hormones that are essential for their well-being as well as depriving them of gonadotrophins. The drug methallibure has been used as an alternative to surgical hypophysectomy. It selectively inhibits pituitary gonadotrophin function (Paget, Walpole and Richardson, 1961). Methallibure caused the arrest of spermatogenesis and prevented the transformation of spermatogonia to spermatocytes in a number of teleost species (Hoar, Wiebe and Hui Wai, 1967; Wiebe, 1968, 1969; Pandey, 1970; Pandey and Leatherland, 1970; Martin and Bromage, 1970; Hyder, 1972; Hyder, Shah, Campbell and Dadzie, 1974). It did not prevent spermatogonial mitosis, nor did it prevent spermatozoa development, once secondary spermatogonia had been transformed into primary spermatocytes before treatment commenced.

Replacement therapy in both hypophysectomised and methallibure treated fish with mammalian gonadotrophins has shown that fish gonads are more receptive to LH. FSH did

not restore gametogenesis in hypophysectomised lake chub (Ahsan, 1966), injections of 50 µg/day had no effect on Indian catfish whereas 100 µg/day produced a slight increase in gonad weight but did not alter the immature histological appearance of the testis (Sundararaj and Nayyar, 1967). Exogenous LH overcame the effects of hypophysectomy in C. plumbeus (Ahsan, 1966) and in the viviparous seaperch Cymatogaster aggregata Gibbons treated with methallibure (Wiebe, 1969). Wiebe (1969) found that this was temperature dependent as he obtained a greater response in those chemically hypophysectomised fish kept in a warm temperature. Human chorionic gonadotrophin (HCG) like LH stimulated spermatogenesis in hypophysectomised H. fossilis (Sundararaj and Nayyar, 1967) and in methallibure treated Tilapia sp. (Hyder, 1972; Hyder et al., 1974).

Further evidence of pituitary control over the gonadal maturation cycle of fishes was produced by Hyder (1972) and Hyder et al. (1974) when they used a crude extract of Tilapia sp. pituitaries to stimulate spermatogenesis in male Tilapia sp. treated with methallibure. Partially purified salmon gonadotrophin also restored the spermatogenic cycle in hypophysectomised C. plumbeus (Ahsan, 1966). Funk and Donaldson (1972) have used partially purified chinook salmon Oncorhynchus tschawytscha Walbaum gonadotrophin (SG-G-100) to induce precocious sexual maturity in male pink salmon Oncorhynchus gorbuscha Walbaum. This same purified gonadotrophin has been used by Yamazaki and

Donaldson (1968) to produce spermiation in hypophysectomised goldfish. Bi-weekly injections of chinook salmon pituitary extracts induced spermatogenesis and the formation of mature sperm in immature 2 year old rainbow trout (Drance, Hollenberg, Smith and Wylie, 1976). Injections of the glycoprotein fraction of extracts from the American plaice Hippoglossoides platessoides Fabricus pituitaries produced oocyte maturation and ovulation in hypophysectomised winter flounder Pseudopleuronectes americanus Walbaum (Campbell and Idler, 1977).

2.1.5 (b) Piscine gonadotrophins

A generalised gonadotrophin (GTH) pattern has been described for the sexual development of male and female salmonid fishes. The plasma gonadotrophin levels were low or undetectable during the early phases of gametogenesis in both sexes but as vitellogenesis and spermatogenesis continued gonadotrophin concentrations rose and the highest values were seen in spawning animals (Crim, Meyer and Donaldson, 1973; Crim, Watts and Evans, 1975; Crim and Evans, 1978). Weil, Billard, Breton and Jalabert (1978) also showed that in the rainbow trout, pituitary response to synthetic LH-RH/FSH-RH varied during gametogenesis; there being no response until the spermatocyte stage. The appearance of pituitary sensitivity to LH-RH/FSH-RH corresponded to an increase in plasma gonadotrophin levels. Crim et al (1975) stated that it was surprising to find that the time of maximum gonad development was associated with

relatively low plasma GTH levels. They explained this phenomenon by citing Moore, Mohrman and Niswender (1972) who suggested that changes in the rate of gonad blood flow provide sufficient amounts of hormone for gametogenic activity.

Two gonadotrophins which stimulate all the stages of gametogenesis have been purified from the carp and chinook salmon (Donaldson, 1973, Review). They were both extracted using an initial ethanolic fractionation procedure. Gonadotrophins from chinook salmon (SG - G-100 preparation) (Donaldson, Yamazaki, Dye and Philleo, 1972) and from the chum salmon Oncorhynchus keta Walbaum (Yoneda and Yamazaki, 1976) were further purified using gel filtration and Sephadex G-100 and ion exchange chromatography on diethylamino-ethyl cellulose (DEAE cellulose). Biochemical and physiological tests on these preparations led to the belief that there was only one GTH in teleosts.

Idler, Bazar and Hwang (1975) produced evidence for a sex linked GTH in chum salmon when they used a chromatographic step involving agarose-coupled concanavalin A (Con A) which has been shown to selectively bind glycoprotein hormones (Dufau, Tsuruhara and Catt, 1972). They isolated two glycoproteins in the absorbed fraction (Con A.11) from a mixture of male and female pituitaries using a purification method involving aqueous buffer extraction, affinity chromatography on Con A coupled to Sepharose, gel filtration in Sephadex G-75 and finally chromatography on DEAE biogel A.

One of these gonadotrophins preferentially stimulated ovarian cyclic adenosine-3, 5 monophosphate (cAMP) production and the other stimulated testicular production of cAMP in immature rainbow trout. Differences were also found between the pituitary extracts from male and female chinook salmon (Breton, Prunet and Reinard, 1978). Breton et al (1978) found that most of the biochemical characteristics were similar but that the hormones differed in amino acid composition and in some biological activities. They stated that these differences were sex linked, would permit chromatographic separation, and could allow the affinity of each gonadotrophin for its specific receptor but they did not represent two distinct gonadotrophins.

Ng and Idler (1978) have provided the clearest demonstration yet of two distinct gonadotrophins. They isolated a non-glycoprotein fraction (Con A.I) which was not absorbed onto the Con A-Sepharose column from the pituitaries of chum salmon. This Con A.I. fraction exhibited the phenomenon of size heterogeneity and displayed vitellogenic activity but did not cause ovulation in hypophysectomised winter flounder (Ng and Idler, 1978). The glycoprotein fraction Con A.II. also had two forms with identical molecular weight and manifested discrete behaviour in isoelectric focussing. The gonadotrophic potency of Con A.II was manifested by its ability to stimulate uptake of ^{32}P into cockerel testes and it was also sex specific in stimulating *in vitro* production of cAMP in immature trout

gonad whereas Con A.1 fraction was inactive in these aspects (Idler et al, 1975; Ng and Idler, 1978).

Ng and Idler (1978) found that Con A.I does not interfere with the radioimmunoassay (RIA) for Con A.II. Pierce, Faith and Donaldson (1976) cited by Ng and Idler (1978) found that SG-G-100 gonadotrophin contained both Con A.I. and Con A.II fractions. Crim et al. (1973, 1975) and Crim and Evans (1978) used anti-SG-G-100 serum in their RIA of gonadotrophin in S. salar, S. fontinalis, in the brown trout Salmo trutta, L. and the sockeye salmon Oncorhynchus nerka Walbaum and found that the plasma levels of gonadotrophin were low or undetectable until the pre-ovulatory surge or spermatocyte stage. Ng and Idler (1978) speculated that the initial stages of vitellogenesis and spermatogenesis come under the control of a hormone distinct from Con A.II which is probably Con A.I and that their studies furnish a cogent argument for the existence of two gonadotrophins.

2.1.5 (c) Gonadotrophic cells

The glandular component of the pituitary (adenohypophysis) can be divided into 3 zones, pro-adenohypophysis (rostral pars distalis), meso-adenohypophysis (proximal pars distalis) and meta-adenohypophysis (pars intermedia). The distribution of cell types within the teleost adeno-hypophysis is extremely regular and gonadotrophic cells are most frequently located in the rostral ventral region of the meso-adenohypophysis (Ball and Baker, 1969; Sage and Bern, 1971; Holmes and Ball, 1974). They can spread

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into the pro-adenohypophysis at sexual maturity in some species - eel Anguilla anguilla L. and chinook salmon (Olivereau (1972) quoted by Holmes and Ball, 1974). Gonadotrophins are glycoproteins so the secretory granules in the cells are basophilic (cyanophilic) and are stained with periodic acid Schiff (PAS) reagent, aldehyde fuchsin, alcian blue and aniline blue.

Gonadotrophic cells have been identified by the changes that occur in the adenohypophysis during gonad maturation (Sokol, 1961; Robertson and Wexler, 1962; Rai, 1966 (a)(b); van Overbeeke and McBride, 1967). They exhibit hyperplasia, hypertrophy, vacuolisation and degranulation coinciding with the different stages of the gametogenetic cycle. Gonadotropes also show increased activity after gonadectomy thus proving that there is a feedback relationship between the pituitary and the gonads (McBride and van Overbeeke, 1969 a). Cytoimmunohistochemical techniques using fluorescent labelled antisera to pituitary hormones have also been used to identify the cells producing trophic hormones. Olivereau (1976) stated that immunofluorescent techniques were only valid when a specific antibody obtained from extracts of fish hypophyses is used. This may be only partly true, because in mammals interspecific immunological cross-reactions between a hypophyseal hormonal antiserum from one mammalian species and the corresponding hormone from another species of mammal can occur (Doerr-Schott, 1976). This also occurs in lower vertebrates; cross-reactions can occur between a mammalian antiserum and the corresponding

hormone from non-mammalian vertebrates (Doerr-Schott, 1976). McKeown and van Overbeeke (1971) found fluorescent antiovine LH bound to cells in the meso-adenohypophysis of the sockeye salmon, which were PAS + ve and basophilic with Masson's trichrome stain. These cells had previously been described as gonadotropes (van Overbeeke and McBride, 1967; McBride and van Overbeeke, 1969a).

There is some controversy over whether there is more than one gonadotrophic cell in the teleost pituitary. Only one gonadotrope has been seen in the rainbow trout (Robertson and Wexler, 1962); Tor (Barbus) tor (Ham) Rai, 1966 (a, b), Scarus croicensis Bloch, Pseudoscarus guacamaia Cuv., Perca fluviatilis L., Rutilis rutilus L. (Matty and Matty, 1959) and in the black molly Poecilia latipinna Le Sueur (Batten and Ball and Benjamin, 1975. A number of investigators using light and electron microscopy techniques have described two different gonadotrophic cell types (Knowles and Vollrath, 1966a; Cook and van Overbeeke, 1972; Olivereau, 1976). Knowles and Vollrath (1966a) described two gonadotropes in eels; the first had elongated electron dense vesicles about 1900A^{o} in diameter; and second had a few spherical, lightly staining vesicles about 1300A^{o} in diameter. The second type resembled mammalian FSH cells and became vacuolated as the eels migrated to the sea. Cook and van Overbeeke (1972) found that the gonadotrophic cells in sockeye salmon had

two types of inclusions; the first were large electron lucent globules 375A° and were PAS + ve. The second cell type was faintly PAS + ve and had a large amount of cytoplasm (that was of a conspicuous vesicular nature) consisting primarily of endoplasmic reticulum. Van Overbeeke and McBride (1967) also described a similar type of cell in sockeye salmon which was faintly PAS + ve and situated in the dorsal columns of the meso-adenohypophysis and they named it Type 7. They disputed the theory of two gonadotropes and said that this second cell was just another stage of the activity cycle of a single gonadotrope. Olivereau (1976) described two gonadotrophic cells in the meso-adenohypophysis of Atlantic salmon; the first was close to the follicular region of the pro-adenohypophysis, the second was situated dorso caudally. This second gonadotrophic cell was the only PAS + ve cell in the meso-adenohypophysis of salmon caught off Greenland and it gradually underwent vacuolisation as the gonad maturation cycle progressed. At spawning, they resembled the Type 7 cell of van Overbeeke and McBride (1967) and the vesicular cell of Cook and van Overbeeke (1972). Chestnut (1970) quoted by Cook and van Overbeeke (1972) and Olivereau (1976) described a cell in coho salmon Oncorhynchus kisutch Walbaum identical to the vesicular cell of sockeye salmon. This cell was present in great numbers in the months prior to spawning.

At present the biochemical and histological evidence for one or two gonadotrophins is contradictory. Haider and Blum (1977) put forward a hypothesis on the evolution of the pituitary gonadotrophin system. They believe that one should not only think in terms of the evolution of the hormone molecule, but one should also take into account the evolutionary changes at the level of the gonadotrophic cells (site of synthesis), and the hormone receptors at the target organs. They suggested that the pituitary gonadotrophin system in vertebrates was completed in several phases and postulated five phases based upon three levels of evolutionary change (a) gonadotrophin cell (b) hormone molecule (c) hormone receptor in the gonad, and that during phylogeny these three levels have undergone changes independent of each other (Table 2.1.1).

2.1.5. (d) Negative feedback mechanism

In higher vertebrates gonadotrophin secretion can be modified by a negative feedback of gonadal steroid on the pituitary and/or the hypothalamus. There is evidence which indicates that gonadotrophin release in teleosts is also influenced by gonadal steroids. McBride and van Overbeeke (1969a) showed that castration of mature sockeye salmon induced degranulation of the gonadotrophic cells, which could be interpreted as release of hormone. Billard, Richard and Breton (1977) found that castration of rainbow trout resulted in increased plasma levels of gonadotrophin. Bhatti and Javaid (1973) got a decrease

in the number of gonadotrophic cells in Colisa fasciata Bloch and Schn. treated with testosterone. Van den Hurk and van de Kant (1975) found that administration of exogenous methyltestosterone and 11-ketotestosterone to juvenile and adult black mollies produced degranulated PAS negative gonadotrophic cells. The nuclei of these cells were also significantly smaller than the nuclei of gonadotrophic cells in the control group. Ultra-structural studies on the pituitaries of black mollies treated with testosterone showed that the gonadotrophic cells were smaller, had fewer secretory vesicles and mitochondra than the untreated controls (Peute, de Bruyn, Seldenrijk and van Oordt, 1976). The above results are evidence of the existence of a feedback relationship between the pituitary and the gonads in teleost fishes.

2.1.6. Testes

In most teleosts the testes are paired elongated structures attached to the dorsal body wall and consist of an extensive system of branching seminiferous tubules embedded in a connective tissue stroma. The testes have both an exocrine and endocrine function.

The exocrine role involves the formation of mature spermatozoa by means of a cystic type germ cell production (Lofts, 1968). The germ cells proliferate in co-ordinated clusters enclosed in membranous cysts called follicles

or ampullae. At the height of the spermatogenetic cycle the gonads are filled with cysts at different stages of development. Within each follicle the germ cells are at the same stage and they mature at the same rate. Thus, a primary germ cell will divide mitotically a number of times to produce a cyst containing several spermatogonia. These cells undergo further divisions synchronously to produce secondary spermatogonia, thus increasing the cell numbers within each cyst. The spermatogonia differentiate at the same time into spermatocytes and after meiotic division become secondary spermatocytes and spermatids. Due to the increase in germ cells the cysts expand, eventually rupture, liberating the gametic contents into the tubulae lumina.

The endocrine function involves the synthesis and secretion of the male sex hormones (androgens) that are necessary for spermatogenesis and maintenance of the secondary sex characteristics. Experiments involving castration of fish and replacement therapy with testicular extracts have shown that the testes are the main source of male sex hormones (Lofts, 1968; de Vlaming, 1974). Two distinct arrangements of the endocrine cells occur in the teleost testes. The first is a typical vertebrate interstitial gland (Leydig cell) characteristically composed of modified connective tissue cells which occupy the interstices between the seminiferous tubules or lobules. This type of arrangement is present in teleosts such as the three spined stickleback Gasterosteus aculeatus L., Clupea sprattus L., Tilapia sp. and the chum salmon (Lofts, 1968). In the

second type of arrangement a different and less obvious distribution of Leydig cells occurs, the endocrine cells arise not in the interstices but in the lobule or tubule walls and are called lobule boundary cells. This perilobular location has been described in pike Esox lucius L., char Salvelinus willughbii Gunter (Lofts, 1968) and Atlantic salmon (O'Halloran and Idler, 1970).

Three methods have been used to localise the sites of steroidogenesis in the teleost testes. One is the application of cytochemical methods for the enzymes 3β, 3α hydroxy steroid dehydrogenase (3β HSD, 3α HSD) and glucose-6-phosphate dehydrogenase (G6PD) involved in the biosynthesis of steroids, the second is the use of electron microscopy, the third is the use of suitable fat stains such as sudan black to identify the cells containing lipids and cholesterol which are the precursors of steroids. Interstitial and lobule boundary cells are quite different in appearance but are cytologically very similar. They both undergo seasonal modification and accumulate cytoplasmic lipid droplets which give a positive reaction for unsaturated sterols (Lofts, 1968). Van den Hurk, Peute and Vermeij, (1978 a) and van den Hurk, Vermeij, Stegenga, Peute and van Oordt (1978 b) showed that the Leydig cells of rainbow trout had G6PD, 3α HSD activity and that this fluctuated with the gonad maturation cycle. The greatest enzyme activity was seen during three stages of the spermatogenic cycle; first, during the migration of the primordial germ cell, then during the accumulation of spermatogonia and

finally during the storage of mature spermatozoa in the seminiferous tubules. O'Halloran and Idler (1970) showed that the lobule boundary cells in mature Atlantic salmon were rich in lipids and exhibited 3β and 3α HSD activity. Ultra-structural studies have shown that the interstitial and lobule boundary cells have an abundance of a granular or smooth endoplasmic reticulum and tubular mitochondrial cristae which are characteristic of mammalian steroid producing cells (Lofts, 1968; van den Hurk et al., 1978 a, b).

Pituitary ablation experiments have shown that some of the stages of germ cell proliferation are under hypophyseal control. Gonadotrophic hormones secreted by the pituitary are necessary for the transformation of secondary spermatogonia to primary spermatocytes (see section 2.1.5 a). It is not known for certain how the gonadotrophins exert their effects. They could act directly on the germ cells, or as is thought to be more likely, indirectly, by stimulating the interstitial cells to produce androgens. Surgical or chemical hypophysectomy with methallibure, as well as causing a decrease in the gonadosomatic index, also produces atrophy, and a decrease in the enzyme activity in the interstitial cells (Wiebe 1968; 1969; Hyder, 1972; Hyder et al., 1974; van den Hurk and van de Kant, 1975; van den Hurk and Testerink, 1975). The interstitial cells exhibit a decrease in cell size, small pyknotic nuclei, weak sudanophilic reaction and a fall in 3β and 3α HSD enzyme activity. These degenerative changes are indicative of a depressed androgen secretion and are reversed by

replacement therapy with piscine pituitary extracts and mammalian gonadotrophins. LH reversed the effect of methallibure in the seaperch, stimulated spermatogenesis and caused an increase in the size, and in the HSD enzyme activity of the interstitial cells (Wiebe, 1968, 1969). Tilapia sp. pituitary extracts and HCG produced hypertrophy of the interstitial cells and restored spermatogenesis in methallibure treated Tilapia sp. (Hyder, 1972; Hyder et al 1974). Salmon gonadotrophin activated spermatogenesis as well as increasing the size and HSD activity of the interstitial cells of hypophysectomised goldfish (Yamazaki and Donaldson, 1969).

2.1.7. Androgens

The role of androgens in the sexual maturation of males is not clear and much of the evidence is contradictory. Administration of exogenous androgens accelerated spermatogenesis and produced secondary sex characteristics in intact fish (Eversole, 1941; Arai, 1967; Bhatti and Javaid, 1973). In hypophysectomised or methallibure treated fish, replacement therapy with testosterone propionate, methyltestosterone and 11-ketotestosterone restored full spermatogenesis (Sundararaj and Nayyar 1967; Yamazaki and Donaldson 1969; van den Hurk and van de Kant, 1975). Pandey (1969) showed that in hypophysectomised P. reticulata methyltestosterone stimulated division of spermatogonia and their differentiation into spermatocytes. On the other

hand, Wiebe (1969) and Hyder et al (1974) found that methyltestosterone or testosterone propionate had no effect on the regressed gonads of chemically hypophysectomised C. aggregata and Tilapia sp. Wiebe (1969) further reported that a combination of methyltestosterone and LH restored spermatogenesis in methallibure treated C. aggregata. Hyder et al. (1974) also found administration of testosterone propionate in conjunction with HCG produced a greater effect in chemically hypophysectomised Tilapia sp. than HCG on its own. It would appear from the results of Wiebe (1969) and Hyder et al. (1974) that the gonads may require stimulation by gonadotrophins before the germ cells will respond to androgens.

Testicular androgens are the principal stimuli for spermatogenesis in mammals and several different steps of the spermatogenic cycle are dependent on their availability at an adequate titre (Steinberger, 1971). In fish there is a direct correlation between plasma androgen concentrations and gonad development because as the gonadosomatic index increases so does the plasma steroid level (Schreck, Lackey and Hopwood, 1972; Campbell, Walsh and Idler, 1976).

Besides the normal androgen testosterone which is common to all vertebrates, salmonids and a few other teleosts secrete a second androgen called 11-ketotestosterone. This differs from testosterone in that it has a keto group at the 11 position of the steroid nucleus (Fig. 2). 11-ketotestosterone was first isolated and identified by

Idler, Schmidt and Ronald (1960) in postspawned sockeye salmon. Subsequent studies by Idler, Freeman and Truscott (1964) have demonstrated it in the plasma of spawned Atlantic salmon. Radioactive testosterone and 11-ketotestosterone were isolated in a free state from the plasma of sexually mature sockeye salmon 1.75 hours after an *in vivo* injection of 17α hydroxyprogesterone 4-C^{14} (Idler and Truscott, 1963). Idler, Horne and Sangalang (1971) have also shown that 11-ketotestosterone and testosterone were produced *in vivo* by the testes. They monitored the levels of androgens in testicular and peripheral plasma of Atlantic salmon and they found that 11-ketotestosterone was the major androgen in both testicular and peripheral plasma and that the concentration greatly increased as gonad maturation advanced. Campbell, et al. (1976) and Simpson and Wright (1977) also reported that plasma concentrations of 11-ketotestosterone increased during the gonad maturation cycle in the winter flounder and rainbow trout respectively.

11-ketotestosterone was shown to have androgenic activity in the chick comb weight bio-assay and its potency was 57.6% of testosterone propionate (Idler, Schmidt and Bieley, 1961b). Idler, Bitners and Schmidt (1961a) reported that 11-ketotestosterone influenced the skin thickness, colouration, secondary sex characteristics and spermatogenesis in male sockeye salmon. Administration of 11-ketotestosterone to gonadectomised sockeye salmon caused them to lose the red flesh colour of sexually immature salmon and develop

the secondary sex characteristics (hooked snout and premaxillary teeth) and the bright red skin colour typical of mature and spawned sockeye salmon (Fagerlund and Donaldson, 1969; van Overbeeke and McBride, 1971).

2.1.8. Summary

Environmental and hormonal factors are important in regulating the reproductive cycle of teleosts. The C.N.S. translates environmental stimuli into chemical messengers which activate and maintain the reproductive organs. Studies using pituitary ablation and replacement therapy techniques have shown that the hypophysis has a central role in controlling gonadal activity. Pituitary extracts and purified gonadotrophins have been shown to stimulate gametogenesis and gonadal sex steroid production. A functional relationship between the pituitary and hypothalamus has been established and certain areas in the NLT region of the hypothalamus control gonadotrophin secretion. There is still controversy over whether there are one or two gonadotrophic cell types and one or two gonadotrophins. Recent research in the purification of teleost gonadotrophins suggests the possibility that there may be two gonadotrophins in some species.

Androgens and oestrogens secreted by the gonads play a role in gametogenesis and are involved

in the development of secondary sexual characteristics. These steroids may also play a role in regulating hypothalamic and pituitary gonadotrophin activity through a negative feedback mechanism.

2.1.9. Sexual Maturity in Salmonids

2.1.9 (a) General

The majority of salmonid species are anadromous but a few species have become land-locked and spend all their lives in freshwater. Spawning in northern latitudes takes place during late autumn and early winter. Pacific salmon species (Oncorhynchus) all die after their first spawning but other salmonid species such as Atlantic salmon, rainbow trout and brown trout are capable of repeated spawning.

The changes that occur in salmonids during sexual maturation can be divided into two categories. The first category is associated with the reproductive function, gonad maturation, development of secondary sex characteristics and changes in behaviour. These changes have been discussed in the section on the control of sexual reproduction.

The second category concerns a complex of alterations, some degenerative, others hyperplastic, in a variety of organs and tissues including intestine, liver, spleen, kidney, the cardiovascular system and some endocrine glands.

2.1.9 (b) Corticosteroid Production

A feature of sexual maturity amongst salmonids is increased corticosteroid secretion (Hane and Robertson, 1959; Idler, Ronald and Schmidt, 1959; Heyl and Carpenter, 1972). Corticosteroids in fish are secreted by the interrenal gland, the homologue of the mammalian adrenal cortex (Nandi and Bern, 1960) which is situated in the head kidney. As in mammalia, the response of corticosterogenic tissue in teleosts to stress is mediated through the pituitary (Ball and Olivereau, 1966; Donaldson and McBride, 1967; Fagerlund, McBride and Donaldson, 1968; Fagerlund and McBride, 1969; Benjamin and Ireland, 1974). The corticotrophic cells have been identified as the epsilon cells which form a palisade-like layer in the pro-adenohypophysis of the pituitary (Ball and Olivereau, 1966; Fagerlund et al 1968; McKeown and van Overbeeke, 1969).

Corticosteroids are C_{21} compounds and they act by modifying RNA and enzyme synthesis in the tissues, with the result that they increase circulating glucose, fatty acids and amino acids. They also have anti-inflammatory and

immunosuppressive effects (Harper, 1973). A number of corticosteroids have been isolated from teleost fish, cortisol, cortisone, corticosterone from sockeye salmon (Idler et al 1959, Phillips, Holmes and Bondy, 1959). Cortisol, cortisone, corticosterone, 11 deoxycortisol, 11 deoxycorticosterone, and 11 dehydrocortisone have been identified in the plasma of the winter flounder (Campbell et al 1976).

Plasma levels of 17-hydroxycorticosteroids (17OHCS) increase during the spawning migration of Pacific salmon (Hane and Robertson, 1959; Idler et al 1959). A similar trend was seen in migratory and non-migratory steelhead trout Salmo gairdneri Richardson but the corticosteroid concentrations were not as high as in Pacific salmon (Robertson, Krupp, Thomas, Favour, Hane and Wexler, 1961 a). Atlantic salmon undergo cyclic changes in corticosteroid production during their spawning migration; from July to October 17OHCS levels are doubled but after spawning they fall to pre-spawning levels by April. The 17OHCS concentration in October (42 µg/100ml plasma) is considerably less than the levels found in Pacific salmon (60-100 µg/100ml plasma) of comparable sexual maturity (Heyl and Carpenter, 1972). Hane and Robertson (1959) cited Hatey (1954) and Fontaine and Hatey (1954) who showed that 17OHCS levels in spawning Atlantic salmon and Pacific salmon were alike (45-53 µg/100ml) but after spawning there was a drop in 17OHCS in both sexes of Atlantic salmon, whereas in the Pacific salmon

only the males showed this change and the females exhibited a rise. Robertson,Krupp, Favour, Hane and Thomas (1961 b) found that the longer the fish were in freshwater before spawning the higher the plasma corticosteroid concentration; spring run O. tschawytscha had a greater plasma 17OHCS concentration than autumn run salmon. Fagerlund (1967) on the other hand, found that cortisol concentration in adult sockeye salmon during the freshwater stage of their life cycle, was less than 5 µg/100ml when the fish were resting in captivity. This remained low even after captivity. He also reported that handling and exercise increased the plasma cortisol levels and that high plasma cortisol levels found in post spawning fish were more often than not associated with disease and approaching death. He implied that the methods of capture and the effort of migration were probably the reasons for high corticosteroid concentrations in wild Pacific salmon. Phillips et al (1959) also suggested that increased muscular activity during the spawning migration could be one of the reasons for increased corticosteroid secretion in Pacific salmon. Idler and Truscott (1963) found that cortisone and cortisol injected intra-arterially were cleared at a much slower rate from the plasma of sexually mature and spawned sockeye salmon than from the plasma of immature sockeye and spawned Atlantic salmon. They suggested that increased 17OHCS concentration in Pacific salmon was due not only to hypersecretion but to an impaired metabolism and clearance. Donaldson and Fagerlund (1970) and Fagerlund and Donaldson (1970) contrary to Idler and Truscotts'

findings, reported an increase in the metabolic clearance rate (MCR) and secretion of cortisol and cortisone in sexually mature male and female O. nerka and that this was reversed by gonadectomy. A companion study of gonadectomised salmon showed that the probable causative factors for these changes in cortisol dynamics during sexual maturation were oestrogens (Donaldson and Fagerlund, 1969) and androgens (Fagerlund and Donaldson, 1969) produced by the gonads.

Adrenal hyperplasia is concomitant with increased secretion of corticosteroids. Sockeye salmon develop extensive hyperplasia of the interrenal tissue during sexual maturation both in their natural environment and under laboratory conditions (McBride and van Overbeeke, 1969 b). This hyperplasia begins very early in the gonad maturation cycle of Pacific salmon and after spawning the interrenal cells undergo vacuolisation of the cytoplasm, pyknosis of the nuclei, karyorrhexis, karyolysis and eventually dissolution of the cell (Robertson and Wexler, 1959). In other post spawning salmonid species some degenerative changes take place in the hyperplastic interrenal but they never predominate (Robertson et al, 1961 a; Heyl and Carpenter, 1972; Olivereau, 1975).

The increased adrenal activity has been shown to be associated with gonad development by many investigators. Gonadectomy of sexually mature O. nerka resulted in a rapid involution of hyperplastic interrenal tissue and

if performed on fish in an early stage of gonad development it prevented interrenal hypertrophy (McBride and van Overbeeke, 1969 b). Robertson (1961) reported adrenal hyperplasia in castrated kokanee landlocked salmon Oncorhynchus nerka kennerly Suckley with varying amounts of regenerated gonad; hyperplasia did not occur in castrated fish with no regenerated gonad. Donaldson and Fagerlund (1970) found that in captive O. nerka the basal cortisol concentration showed a gradual increase during sexual maturation but decreased immediately after gonadectomy.

2.1.9 (c) Physiological and histological changes

Degenerative and hyperplastic changes take place in the tissues of all species of salmonids during sexual maturation but vary in the degree of severity from species to species. They are most drastic and indeed irreversible in the Pacific salmon species (Robertson and Wexler, 1960). The changes include a thickening of the skin, loss of gastric glands and villi, decrease in size of mucosal cells, thickening of the stratum compactum, atrophy of the circular muscle layer and hypertrophy of the longitudinal muscle in the stomach and intestine, lipolysis and degeneration of the liver cells, glomerular-capillary sclerosis and deterioration of the tubules in the kidney, diminution of lymphoid tissue and increase in the connective tissue stroma of the spleen, cytolysis of pancreatic acini, hypertrophy of the islets of Langerhans, atrophy and degeneration in some

of the follicles of the thyroid, degeneration of heart muscle and intimal proliferation of the coronary arteries (Robertson and Wexler, 1960, Robertson, Wexler and Miller, 1961). As well as an increase in 17OHCS concentrations, other changes occur in the blood constituents of spawning Pacific salmon. Glucose, protein and sodium values fall during the spawning migration; potassium either decreases or remains constant during migration but rises dramatically after spawning due to extensive tissue degeneration (Robertson et al, 1961b; Triplett and Calaprice, 1974).

Robertson and Wexler (1960) considered the stress of gonad development and prolonged starvation during the spawning migration to be the reasons for increased corticosteroid production and 100% mortality of Pacific salmon after spawning. Robertson, Hane, Wexler and Rinfret (1963) when they treated immature non-migratory rainbow trout with hydrocortisone, found that fish lost weight, developed Saprolegnia infection of the skin, and the histological changes of the tissues were similar to those occurring in spawning salmon.

McBride, Fagerlund, Smith and Tomlinson (1963) reported that force-feeding sexually mature sockeye salmon prolonged their life expectancy. Feeding, however, did not prevent the degenerative changes of an increase in the water and sodium and a decrease in the potassium contents of skeletal muscle that normally take place in

ripe sockeye salmon (Tomlinson, McBride and Geiger, 1967). Gonadectomy of Pacific salmon species prolonged the life span of the fish well beyond the time they would have spawned and died (Robertson, 1961, McBride et al, 1963). It prevented adrenal hyperplasia and the development of the deteriorative alterations in the skin, alimentary tract, pancreas, kidney, heart, etc. (McBride and van Overbeeke, 1971). Administration of oestrogens and androgens to gonadectomised sockeye salmon induced interrenal hyperplasia and brought about changes in various tissues similar to those that normally occur in sexually mature O. nerka (McBride and van Overbeeke, 1971; van Overbeeke and McBride, 1971). Cortisol, with the exception of the skin where it produced atrophy, evoked a similar but weaker response to those invoked by androgens and oestrogens in the stomach, liver, kidney and pancreas (McBride and van Overbeeke, 1971). These findings did not, however, answer the question of whether the alterations brought about by the androgens and oestrogens were mediated by the enhanced interrenal function or not.

The degenerative changes during spawning are not so severe in other salmonid species (Robertson et al 1961a)(Table 2.1.2). This could be due to the feeding behaviour of the other species during gonad development: non-migratory rainbow trout never stop feeding, migratory steelhead feed periodically, whereas Pacific salmon do not feed at all during gonad maturation. However, Atlantic salmon do not feed to any noticeable extent during their spawning

migration, and yet a number of them (especially females) survive to spawn again. The answer may lie in the degree of stimulation of the corticosterogenic tissue during sexual maturation where the species which can survive to spawn again usually have a less marked adrenocortical hyperplasia and only undergo a limited amount of degeneration in the interrenal gland after spawning (Robertson et al.,1961 a ; Heyl and Carpenter, 1974; Olivereau, 1975).

2.1.9 (d) Summary

Salmonid species undergo many hyperplastic, degenerative and behavioural changes during gonad maturation. These have been attributed to the increased secretion of the sex steroids by the gonads and have been prevented by gonadectomy. Only in the Pacific salmon species, which have a 100% mortality after spawning are these changes irreversible. This is most probably due to the greater stimulation of the interrenal tissue during gonad maturation and the extensive degenerative changes that take place in the adrenal gland after spawning.

2.1.10. Skin

As in other vertebrates the skin of teleost fishes forms an external protective covering of the body.

The integument of bony fishes can be divided into 3 areas, cuticle, epidermis and dermis (Roberts, Shearer, Elson and Munro, 1970; Whitear, 1970; Roberts, Young and Milne, 1971; Hawkes, 1974; Harris and Hunt, 1975; Bullock and Roberts, 1975; Bullock, Roberts and Gordon, 1976). It differs from the skin of higher vertebrates and is more akin to mammalian mucous membrane. There is no keratinization of the epidermal layer; all the cells are vital, and numerous apocrine mucous cells are present amongst the squamous epithelium.

The external layer is called the cuticle, is highly labile, and can easily be lost during normal histological procedures. It consists of protoplasm, some sloughed dead cells and mucopolysaccharides secreted by the surface epidermal cells, and is usually 1µm thick (Whitear, 1970). Whitear (1970) suggests that the cuticle may have a protective role against infectious agents, noxious chemicals and trauma.

The fundamental structural unit of the epidermis is the fibrous Malpighian or filament containing cell. Ultra-structural studies have shown that these cells contain bundles of filaments, many ribosomes and an endoplasmic reticulum but no Golgi apparatus (Roberts et al., 1970; Hawkes, 1974; Harris and Hunt, 1975). Unlike mammalian epidermis, there is no stratum corneum in fish and all the cells are living and capable of mitotic division at all

levels even in the external squamous layer (Henrickson, 1967; Bullock, Marks and Roberts, 1978). Most of the active enzyme sites are in the basal and upper layers. Bullock et al. (1978) suggest that the outer layers of the epidermis obtain their oxygen directly from the surrounding water. They also reported that although cells in all the epidermal layers were capable of proliferation, the greatest mitotic activity was found in the suprabasal layer of Malpighian cells.

Besides the Malpighian cell, the teleost epidermis contains other more specialised cells such as club cells, granule cells, and sensory structures such as taste receptor organs, canal organs, etc. (Bullock and Roberts, 1975). It also contains apocrine mucous cells which release their secretions onto the surface of the epithelium. This mucus is a characteristic of teleosts and there are many hypotheses as to its function. Pickering (1974) stated that "the continuous replacement of mucus prevents colonization by parasites, fungi and bacteria". Fletcher and Grant (1969) have shown immunoglobulins to be present in the mucus of plaice. Mucus has also been shown to regulate swimming speed by controlling the hydrodynamic resistance of the skin surface (Rosen and Cornford, 1971). Histochemical and biochemical studies on the mucus of salmonids and whiting have shown that the secretions are predominantly acid-mucopolysaccharide with varying amounts of sialic acid (Harris and Hunt, 1973; Harris, Watson and Hunt, 1973; Pickering and Macey, 1977; Bullock et al. 1976).

The dermis comprises two zones, the stratum spongiosum and the stratum compactum. The stratum spongiosum, the upper region of the dermis is composed of loosely organised collagen fibres containing scales, pigment cells, vascular and neural processes (Bullock et al, 1976; Hawkes, 1974). The stratum compactum contains a layer of horizontally condensed collagen bundles with vertical supporting bundles bearing blood vessels and nerves. A few fibroblasts may also be present (Hawkes, 1974; Bullock et al, 1976).

The skin is a labile tissue and is constantly changing. The skin dimensions, depend on the area of the body, sex of the fish and time of the year (particularly the stage of gonad development). The epidermis of the unscaled head regions of Atlantic salmon and brown trout Salmo trutta L. was thicker than that of the scaled areas (Harris and Hunt, 1975) and in the char Salvelinus alpinus L. the epithelium of the dorsal, pelvic and pectoral fins was significantly thinner than the rest of the body (Pickering, 1974). The skin was thicker in fish undergoing their spawning migration than in immature fish (Robertson and Wexler, 1960; Roberts et al, 1970). There was also a sexual dimorphism in skin in that Robertson and Wexler (1960) found that in spawning Pacific salmon the skin of males was 25% thicker than that of females. The epidermis of brown trout undergoes rhythmical changes during successive spawning cycles and for most of the year the male has a significantly thicker epidermis than the female (Pickering, 1977).

The concentration of mucous cells also depends on the area of the body, sex of the fish and stage of gonad maturation. In the brown trout and char the higher concentration of mucous cells occurs in the anterior regions and there are fewer mucous cells in the fins compared with the rest of the body (Pickering, 1974). During the spawning season there is a significant drop in the concentration of mucous cells in the epidermis of male brown trout and an increase in females (Pickering, 1977).

The changes in the skin during spawning seem to be due to the hormones produced by the gonads. McBride and van Overbeeke (1971) prevented an increase in the skin thickness of spawning sockeye salmon by gonadectomy. Idler et al (1961a) showed that 11-ketotestosterone induced an increase in skin thickness of sockeye salmon. McBride and van Overbeeke (1971) treated gonadectomised O. nerka with androgens and oestrogens and reported a marked increase in thickness of the epidermis and dermis, as well as an increase in the number of mucous cells. Administration of methyltestosterone to immature O. gorbuscha, O. keta, O. kisutch and O. tschawytscha brought about hyperplasia and hypertrophy of the epidermal cells, an increase in the thickness of the stratum spongiosum and a proliferation of the mucous cells

(Yamazaki, 1972; McBride and Fagerlund, 1973). Yamazaki (1972) suggested that sexual dimorphism of the skin may be caused by differences in the level of plasma androgen during sexual maturation.

2.1.11. Saprolegniasis

Teleost fish are very prone to fungal infections. The condition may be caused by a variety of aquatic fungi (Scott and O'Bier, 1962; Willoughby, 1970; Pickering and Willoughby, 1977). The fungal species most frequently isolated from salmonid fishes "has a low degree of homothallic sexuality" and belongs to the genus Saprolegnia. It has previously been known as Saprolegnia parasitica (Kanouse, 1932), Saprolegnia Type 1 (Willoughby, 1969) and Saprolegnia diclina Humphrey Type 1 (Willoughby, 1978).

Saprolegniaceae are ubiquitous in water systems, and some are present throughout the year while others show a degree of periodicity (Hughes, 1962). This periodicity can be correlated to changes in temperature, pH and organic content of the water (Alabi, 1971). Temperature plays an important role in determining the growth of Saprolegniaceae (Hughes, 1962; Roberts, 1963). Roberts (1963)

reported that the number of species found during the warm season was low while in autumn the number increased and reached a maximum in Spring. The presence of decomposing organic matter in the water also increased the number of species and an alkaline pH was more favourable for their growth (Roberts, 1963).

In salmonids Saprolegniasis is very common in sexually mature fishes (Robertson and Wexler, 1960; Roberts and Shepherd, 1974; White, 1975; Neish, 1977; Richards and Pickering, 1978). Salmonids spawn in late autumn and early winter at a time when the environmental conditions are optimal for Saprolegniaceae proliferation. Saprolegnia infection is generally considered to be the consequence of physical damage to the surface of the fish (Vishniac and Nigrelli, 1957; Egusa, 1963; Scott, 1964; Roberts and Shepherd, 1974; White, 1975) or secondary to bacterial or viral infections (Wolke, 1975). On the other hand, a number of workers have suggested that Saprolegnia sp. may also act as primary pathogens (Tiffney, 1939; Hoshina, Sano and Sunayama, 1960; Stuart and Fuller, 1968; Neish, 1977).

In the majority of adult salmonids there is no uniformity in the regions parasitised by Saprolegnia. Neish (1977) found that sockeye salmon were most frequently infected on the dorsal surface of the head, in front of the dorsal fin and in the regions of the fins. White (1975) and Richards

and Pickering (1978) found a definite sexual difference in the pattern of infection in adult brown trout, the heads and flanks of the male fish were more prone to Saprolegnia when compared to females and there was a greater susceptibility of the caudal and pectoral fins of the female when compared with the male. They suggested that the infection seemed to follow wounds induced by spawning activities; female redd digging with the caudal peduncle and the male territorial defence with the anterior end of his body. Richards and Pickering (1978) also reported that the prevalence of fungal infection prior to spawning was greater amongst the males than the females. They suggested that the reduction in the superficial mucous cell concentration in sexually mature male brown trout (Pickering, 1977) may increase the susceptibility of these fish to Saprolegnia.

Once Saprolegnia becomes established, it destroys the epidermis, penetrates the basement membrane and extends into the dermis. In a few cases it may continue growing into the hypodermis and musculature (Neish, 1977). There may or may not be an inflammatory reaction in the lesion and the surrounding tissue (Wolke, 1975; Neish, 1977; Pickering and Willoughby, 1977). Tiffney (1939) suggested three possible reasons for death due to Saprolegniasis

(a) the extensive destruction of tissue

(b) toxaemia

(c) the disruption of the skin may create osmotic stress due to dilution of the body fluids, etc.

Like the majority of fish pathogens, the Saprolegnia sp. are opportunistic facultative parasites. Wounding a fish frequently facilitates the establishment of Saprolegnia infection (Vishniac and Nigrelli, 1957; Egusa, 1963; Scott, 1964) but it does not assume that a fish will become infected even if it can be demonstrated that there are sufficient fungal spores present to initiate an infection, (Egusa, 1963; Neish, 1977). Neish (1977) suggested that a susceptible host must be further debilitated so that its defence mechanism is impaired. He proposes a hypothesis in terms of the normal physiological and endocrinological changes that take place in the spawning Pacific salmon. He stated that "increased corticosteroid production with the subsequent decreased immunocompetency and the depleted ascorbic acid reserves might provide the mechanism whereby normally non-pathogenic organisms including Saprolegnia sp. become pathogens". This theory is quite plausible as increased corticosteroid production lowers the resistance of fishes to pathogens (Wedemeyer, 1970) and lowered ascorbic acid levels decrease the ability of fish to repair wounds (Halver, 1972).

2.1.12. Precocious maturity (A Review).

Precocious maturity is common amongst male

Atlantic salmon parr (Orton et al, 1938) and also occurs in other salmonid species, rainbow trout (Johnstone et al, 1978), chinook salmon (Gebhards, 1960; Robertson, 1957) and steelhead trout (Schmidt and House, 1979). The prevalence of sexual maturity amongst male Atlantic salmon parr varies from river to river, 27.3% in the Tees estuary (Pentelow et al, 1933), 80% in the Welsh and Cheshire Dee (Orton et al, 1938) and 50% in Swedish rivers (Osterdahl (1969) cited by Koch and Bergstrom, 1978). Jones and King (1949) described the spawning behaviour of adult Atlantic salmon and they showed that ripe male parr accompany spawning adults and participate in fertilizing the females' eggs. They cited and repeated the work of Shaw (1839) and Alm (1943) and found that the percentage fertilization of ova by the ejaculate of precocious males was 99% which was comparable to adult sperm. The survival and growth of these alevins was similar to the progeny of adult males (Jones, 1959).

Jones (1940) and Jones and Orton (1940) described the paedogenetic cycle in male parr and said it was similar to the adult testicular cycle. Evidence that gonad development in salmon parr is under the control of the pituitary was produced by Olivereau (1976). She described PAS + ve gonadotrophic cells in the meso-adenohypophysis which became degranulated after spawning. Crim and Evans (1978) monitored the plasma gonadotrophin level in developing male parr and found that it increased as gonad maturation progressed. Precocious maturity has been induced in pink salmon parr by administration of the

salmon gonadotrophin SG-G 100 (Funk and Donaldson, 1972). Olivereau (1975) reported increased interrenal activity amongst precocious Atlantic salmon parr during December. Other physiological changes in ripe parr include increased plasma cholesterol and lipid levels (Koch and Bergstrom, 1978).

Precocious sexual maturity in Pacific salmon differs from adult sexual maturity in that the ripe male parr do not exhibit any secondary sex characteristics and do not die after spawning (Robertson, 1957; Gebhards, 1960; Funk and Donaldson, 1972). Funk and Donaldson (1972) noticed that the testes of the ripe pink salmon had few interstitial cells and that they exhibited low 3β HSD enzyme activity. They suggested that the biosynthesis of androgens in these fish was insufficient to induce the development of secondary sex characteristics.

Unlike adult males, precocious males continue to feed voraciously during gonad development (Jones, 1959). Many workers have failed to find erosion or spawning marks on the scales of ripe Salmo salar Juv. (Frost and Went, 1940; Jones, 1950). Robertson (1957) also found no spawning marks on the scales of precocious O. tschawytscha parr.

It is generally considered that the processes of sexual maturation are accompanied by a reduction in growth rate (Johnstone et al 1978). Robertson (1957) found that

in O. tschawytscha the immature control fish were 1.5 cm longer and 4 g heavier than the mature parr. On the other hand, Pentelow et al. (1933) found that precocious males occurred amongst all the size groupings of parr and that the prevalence of ripe parr was greatest amongst the larger size groups. They also reported that the spent male smolts were larger than the immature male smolts.

Unlike the ripe Pacific salmon parr, wild precocious male Atlantic salmon do exhibit a slight mortality after spawning (Jones and Orton, 1940). There is also an increased prevalence of bacterial and fungal diseases amongst ripe male parr in intensive aquaculture units (Table 1.1. Salmon Research Trust Annual Report, 1974; Johnstone et al, 1978). Fungal infections always manifest themselves in the pectoral and caudal regions of the fish and the causal agent is invariably Saprolegnia (Salmon Research Trust of Ireland Annual Reports, 1969, 1970, 1971, 1973).

Aims of this study

Various aspects of the physiology and endocrinology of farmed 1+ Atlantic salmon parr were studied, firstly to fill in some of the gaps in the knowledge to date on the development of precocious sexual maturity and secondly, to try and explain the increased susceptibility of ripe male parr to fungal infections. Endocrinological studies included histological examination of the pituitary and the gonads, monitoring the plasma androgen levels and correlating them with changes in the gonads during the sexual cycle.

Plasma cortisol levels were also measured, as well as histological studies on the interrenal tissue in order to confirm Olivereau's (1975) suggestion that increased corticosteroid secretion occurred in ripe male parr and to test Neish's (1977) hypothesis that increased adrenal gland activity is one of the precipitating factors in the disease process. Histological and histochemical studies of the skin and mucous cells were undertaken to see if any sexual dimorphism occurred in S. salar Juv.

2.2. Materials and Methods

2.2.1. Animals

The fish used in this study were 1+ Atlantic salmon parr (precocious males, immature females) of known parentage (Salmon Research Trust Annual reports, 1975, 1976, 1978).

The 1+ parr were kept under commercial rearing conditions in 10m circular tanks, with a water flow rate of 270 dm^3/min and a stocking density of 3.5 kg/m^2. The experimental fish used in this survey were randomly selected out of a population of 9,000 and were sampled during the months of October, November and December in 1975 and 1976. Monthly samples were also taken during 1978 from March to December. Skin, kidney, visceral organs and pituitary samples were collected from each fish for histological studies as well as plasma for steroid and electrolyte analysis.

2.2.2. Anaesthetic

After capture, the fish were quickly anaesthetised in a 150ppm solution of ethyl p-aminobenzoate (Benzocaine, Aldrich Chemical Co. Ltd., U.K., Laird and Oswald, 1975).

2.2.3. Plasma

Once anaethetised the fish were bled within three minutes by severing the caudal peduncle. The blood was collected in heparinised capillary tubes (Hawksley Ltd., U.K). The capillary tubes were then sealed in crista seal (Hawksley Ltd., U.K.) and centrifuged in a microhaematocrit centrifuge (Hawksley Ltd. U.K. for five minutes. The packed cell volume was recorded and the plasma was stored in plastic vials at -20°C until analysed.

2.2.4. Microscopy

2.2.4 (a) General reagents

Formaldehyde, picric acid, cupric acetate, mercuric chloride, sodium chloride, sodium dihydrogen phosphate (NaH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) were purchased from BDH chemicals Ltd., U.K., absolute alcohol from James Burrough Ltd., U.K., chloroform, xylene from May and Baker Ltd., U.K. The dyes described in the staining procedures listed below were purchased from either T. Gurr Ltd., BDH Chemicals Ltd. or Difco Ltd. Hyaluronidase and the dialysed iron solution were obtained from BDH Ltd. and neuraminidase from Koch-Light Laboratories Ltd.

2.2.4 (b) Fixation

Liver, spleen, stomach, pyloric caeca, large and small intestine and all the regions of the kidney were fixed in either 10% buffered formalin (Culling, 1974) or 10% formol saline (Culling, 1974).

Two pieces of skin about $1cm^2$ were taken from the dorsum, pectoral area, pectoral fin, caudal peduncle and tail (Fig. 3). One piece was fixed in 10% buffered formalin or 10% formol saline and used for histological and histochemical studies. The second piece which was used for quantitative study of the mucous cells was fixed in 5% formaldehyde in 0.6% NaCl. (Pickering, 1974).

The gonads were dissected from the abdominal cavity of the fish and weighed. They were then fixed in either 10% buffered formalin, 10% formol saline, Bouins fluid (Culling, 1974) or Bouins-Hollande sublimate (B.H.S., Kraicer, Herlant and Duclos, 1967).

The parr were decapitated and the roof of the cranium opened to expose the brain. As much as was possible of the skeletal structures of the head was dissected away to allow penetration of the fixative. The brain and pituitary (still in the sella turcica) were then fixed in 10% formol saline or Bouins fluid.

2.2.4. (c) Processing

All the tissues were processed in a Shandon Elliot Duplex processor (Shandon Elliot, U.K.) through ascending concentrations of alcohol, cleared in chloroform and impregnated with paraffin wax. 10 thin sections (5μm) were taken from each processed tissue using a rotary microtome (Leitz GFR), floated on to clean microscope slides (previously soaked in chromic acid for 12 hours and then rinsed in distilled water), dried in an oven (37^{o}C) and then stained.

Before processing, the brain samples were placed in a decalcifying fluid (R.D.C. Bethlehem Instr. Ltd.) for 5 - 10 minutes to soften the hard skeletal structures. After processing and impregnation with wax, the pituitary glands were sectioned either longitudinally or transversely at 5μm and mounted serially.

2.4. (d) Staining

For general examination of the skin, visceral organs, gonads, Mayers haematoxylin and eosin staining was used.

Mercury pigment was removed from tissues fixed in B.H.S. by soaking the sections in 0.5% iodine in 70% alcohol for 5 minutes and then in 5% sodium thiosulphate for 2 - 3 minutes before continuing with the staining procedure.

2.2.5. Mucous cell studies

2.2.5 (a) Quantitative Studies

Skin specimens specially fixed for quantitative studies of mucous cells were stained in 1% alcian blue and 1% acetic acid for 5 minutes (Pickering, 1974). 10 random cell counts using an 0.23mm quadrat were taken from each piece of skin and the result expressed as a mean figure/mm^2.

2.2.5 (b) Histochemical Studies

Mucohistochemical studies were carried out on skin sampled during the autumn and early winter of 1976 (and fixed in 10% formol saline). The skin samples were from 5 precocious males and 5 immature females sampled in October and 5 precocious males and 5 immature females sampled in December.

Previous experiments on the mucohistochemistry of salmonid skin by Harris et al (1973) and Pickering (1977) have shown that the mucous cells of brown trout and the char secrete mucoproteins. These mucoproteins have a large carbohydrate moiety and the aim of the following staining techniques was to identify the different types of mucopolysaccharide present in the mucus

of salmon parr. Histological methods used to demonstrate carbohydrates fall into two main groups (i) PAS reaction (ii) acid polysaccharide reaction. Table 2.2.1. gives the staining reaction of the main groups of carbohydrate found in the mucous cells of fish.

Periodic acid-Schiff (PAS) is very important in the identification of carbohydrates and involves the use of periodic acid as an oxidising agent. It breaks up C - C bonds where they are present as 1 - 2 glycol groups (CHOH - CHOH) converting them to dialdehydes (CHO - CHO) (Fig. 4). This configuration is present in carbohydrates and certain derivatives of 1 - 2 glycol groups, (amino - alkylamino and the oxidation product CHOH - CO) and these are also converted to aldehyde groups. These aldehydes can then be localised by combination with the colourless Schiff reagent, which is recoloured and the PAS + ve groupings show up red.

Acid mucopolysaccharides are a group of related heteropolysaccharides usually containing two types of alternating monosaccharide of which one has an acidic group, either a carboxyl or sulphuric group (Lehninger, 1975). Besides the PAS technique these acid mucopolysaccharides can be stained with (i) metachromatic dyes (ii) alcian blue (iii) colloidal iron (Hales dialysed iron). These techniques are not specific, so that in order to identify further the type of carbohydrate, blocking techniques, and enzyme controls must be used.

Methylation is used because it blocks subsequent staining of simple acid mucopolysaccharides by esterification of carboxyl groups (Fig. 5a) and also blocks complex sulphated mucosubstances by desulphation (Fig. 5b).

Among the constituents of mucus are glycoproteins. These may contain many different monosaccharides and monosaccharide derivatives attached covalently to the polypeptide chain. The linear or branched side chains of glycoproteins may contain many monosaccharide residues. The terminal monosaccharide unit is often N-acetylneuraminic acid, a sialic acid. Those compounds containing sialic acid give a similar staining reaction as the simple acid mucopolysaccharides and can be identified because they lose their affinity for stains (alcian blue) after treatment with sialidase (neuraminidase).

Other constituents of mucus include hyaluronic acid and chondroitin sulphate. These can be removed from mucous cells by incubating the sections in hyaluronidase. The major repeating carbohydrate unit of hyaluronic acid is a disaccharide composed of D-glucuronic acid and N-acetyl-D-glucosamine in β (1-3) linkage. Each disaccharide is attached to the next by β (1-4) linkage so hyaluronic acid contains alternating β (1-3) and β (1-4) linkage (Fig. 6). The enzyme hyaluronidase catalyses the hydrolysis of the β (1-4) linkage (Fig. 6)(Lehninger, 1975). Chondroitin is nearly identical in structure to

hyaluronic acid, the only difference being that it contains N-acetyl-D-galactosamine instead of N-acetyl-D-glucosamine residues and is affected by hyaluronidase in the same way as hyaluronic acid.

Techniques using alcian blue in increasing ionic strength of magnesium chloride, alcian blue at different pH values or in combination with other dyes such as aldehyde fuchsin or safranin can allow distinguishing of sulphate mucopolysaccharides from carboxyl-mucopolysaccharides.

The staining techniques used in this study are given in Table 2.2.2. A modified periodic acid-Schiffs method was used. The sulphurous acid rinse step was excluded and the Schiffs reagent was prepared by the technique of Drury and Wallington (1967). Control staining procedures were employed with the active and mild methylation, neuraminidase and hyaluronidase techniques. Intensity of staining was visually estimated and staining activity was represented as +++ very dense, ++ dense, + moderate, ± weak, ± very weak, - negative.

2.2.6. Pituitary cytology

A variety of staining techniques were used but Mayer's haematoxylin and eosin was used for the

purpose of orientation and general cytological surveying. For more specialised work in distinguishing chromophobic, acidophilic and basophilic cells, the following techniques were used: Slidder's (Disbrey and Rack, 1970), Masson's trichome (Culling, 1974), one stage Martius scarlet blue (M.S.B., Drury and Wallington, 1967), aldehyde-fuchsin with and without oxidation in $KMNO_4$-H_2SO_4 (AF, Elftman, 1959a), periodic acid-Schiff and orange G (PAS - OG, Disbrey and Rack, 1970), alcian blue + periodic acid-Schiff + orange G (AB-PAS-OG, Disbrey and Rack, 1970), aldehyde fuchsin + periodic acid Schiff + orange G (AF-PAS-OG, Elftman, 1959b), Barrett's stain (Disbrey and Rack, 1970), Bargmann's chrome haematoxylin (Bargmann's) for distinguishing neurosecretory material (Pearse, 1968).

It was found that better staining results were achieved when the sections were allowed to stand in a formol mercuric chloride solution (Drury and Wallington, 1967) for 24 - 72 hours. The mercury pigment was removed by placing the sections in Lugol's iodine (Culling, 1974) for 10 minutes, rinsing in water and then in 2.5% sodium thiosulphate for 2 - 3 minutes before continuing with the staining procedure.

2.2.7. Measurements

All measurements were made using a calibrated

eyepiece graticule in a Gillet and Sibert binocular microscope.

2.2.7 (a) Skin Measurements

The thickness of the epidermis and hypodermis was measured and the number of cell layers in the epidermis was counted. The mean values of these parameters for each skin sample were determined from 10 measurements. Measurements were made in the dorsum, pectoral, pectoral fin, caudal peduncle and tail regions of fish sampled in autumn and winter of 1975 and 1976 (Fig. 3).

2.2.7 (b) Interrenal

Nuclear diameter of the interrenal cells was measured in parr sampled during the autumn and early winter of 1975 and 1976. As the nuclei were not perfect circles, the nuclear diameter was determined as the mean of two measurements taken at right angles to each other. The mean value for each fish was determined from measurements of 50 randomly selected cells.

2.2.7 (c) Pituitary

The nuclear diameter of the basophilic cells in the pro and meso-adenohypophysis was measured in the same way as in the interrenal gland. 50 cells were randomly selected in the meso-adenohypophysis. 50 basophilic cells were also selected from the palisade layer and from the islands between the follicles of the pro-adenohypophysis. The mean value for each of these areas was calculated.

The majority of basophilic cells in both the pro and meso-adenohypophysis were very irregular in shape. The cellular diameter was determined as the mean of two measurements taken at right angles to each other and the formula πr^2 was used to give a rough approximation of the cell area.

2.2.7 (d) Testes

In every gonad examined, 100 cysts were counted and each cyst was classified into one of the five spermatogenic stages - primary spermatogonia, secondary spermatogonia, primary spermatocyte, secondary spermatocyte and spermatids or spermatozoa.

2.2.7 (e) Ovary

Measurements of the diameter of oocytes and their nuclei were made in the ovaries of 1+ female parr. Three stages of oocyte development, early and late perinucleolus and early yolk vesicle stages were present. In each fish examined the mean oocyte diameter and nuclear diameter for each stage was determined from measurements of 35 randomly selected cells.

2.2.8. Electron Microscopy

Small pieces of skin (1-2mm^2) were taken from

the pectoral and caudal peduncle areas of 3 precocious males and 3 immature females in October, 1977. They were fixed in 0.75% glutaraldehyde, 2% formalin and 1% acrolein in 0.1 M sodium cacodylate buffer with 0.2% $CaCl_2H_2O$, 0.2 M s-collidine, 5.5% sucrose and 2% dimethyl sulfoxide. After a buffer wash (0.1 M sodium cacodylate, 0.2% $CaCl_2H_2O$ and 5.5% sucrose) the specimens were post-fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer. After dehydration in ethanol the tissues were embedded in resin made up as follows:

A)	EPON 812	26g
	DDSA	41g
B)	EPON 812	18g
	MNA	15g

Solutions A and B were mixed in a ratio of 7:3 and an accelerator DMP-30 was added to a concentration of 1.5 - 2.0%. Ultra thin sections (500 - 600A^{O}) were cut on an ultra microtome (LKB ultrotome III) and mounted on carbon coated grids and stained with lead citrate. The sections were then examined in Jeol Jem-100C microscope.

2.2.9. Radioimmunoassay (R.I.A.)

2.2.9 (a) General reagents and materials

All the solvents were "Analar" grade and purchased from B.D.H. Chemicals Ltd., U.K. and were

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redistilled before use. NaCl, NaH_2PO_4, Na_2HPO_4, NaN_3, trichloroacetic acid (TCA),gelatine, were also "Analar" grade, and were obtained from B.D.H. Chemicals Ltd. Bovine serum albumin was purchased from Sigma Ltd. Activated charcoal was also purchased from Sigma Ltd. and either sieved through a fine mesh (53 µm) or repeatedly suspended in distilled water and the fines removed by decantation, filtration, and then dried in vacuo over phosphorus pentoxide before use. Dextran T70 was bought from Pharmacia Fine Chemicals Ltd., Instagel, 2,5-diphenyloxazole (PPO), and 1,4-di 2-(phenyloxazole) denzene (POPOP) from Packard Ltd. Precoated aluminium, plastic and glass silica gel plates (F_{254})(20 x 20cm) were obtained from Merck Ltd. (G.F.R.) and were used after prewashing in ascending ethanol. The radioactive labels were purchased from the Radiochemical Centre, Amersham.

2.2.9 (b) 11-ketotestosterone (11-oxotestosterone, 17 β-Hydroxyandrost-4-ene 3 11-dione) assay

The radioactive label and antiserum were prepared and kindly supplied by Dr. R. Wright at the D.A.F.S., Marine Laboratory, Aberdeen. (1,2-^{3}H) 11-ketotestosterone was chemically prepared from (1,2-^{3}H) cortisone (specific activity 37 Ci/m.Mol) (Simpson and Wright, 1977) and the anti-11-ketotestosterone-3-(O-carboxymethyl) oxine - BSA serum was produced in rabbits (Simpson and Wright, 1977).

Plasma from parr sampled in October and December, 1976, was assayed according to the technique described by Simpson and Wright (1977). This assay was modified when the 1978 plasma samples were analysed. The modifications were as follows:

All the solutions were prepared in 0.1% gelatine, 0.14 M Nacl, 0.01% NaN_3, 0.01 M NaH_2PO_4 - NaOH, pH7.0 (P.B.S.).

The (1,2-^{3}H) ketotestosterone label was first purified by thin layer chromatography (glass coated silica gel plates F_{254}) in dichloromethane: ethanol (47.3) solution before use.

Label (^{2},500 dpm in 0.1M P.B.S.) was added to all plasma samples in order to estimate procedural losses during the extraction and separation stages.

A blank and 4 plasma samples were spotted on to each glass chromatography plate and 2 - 3 μg of authentic 11-ketotestosterone was spotted on to the centre and end lanes for reference. The plates were developed as described by Simpson and Wright (1977). After examination under ultra-violet light (Hanovia Lamps U.K. 2537A^o), areas that were isopolar with reference 11-ketotestosterone were eluted with 4 or 5 mls of dichloromethane:ethanol (9:1) solution. The elutes were then dried under air and the steroid fractions dissolved in 1 ml of buffer: 0.4 ml of this was counted for estimation of manipulative losses and 2 x 0.2 ml were transferred to clean glass tubes for assay.

To each of these 0.2 ml was added 0.1 ml of antiserum (1:4,000 dilution) and 0.1 ml of label containing 22,000 dpm. After overnight incubation at 4°C, 0.6 ml of 0.25% dextran coated charcoal solution was added and allowed to stand for 15 minutes at 4°C. After centrifugation at 2500rpm (MSE Mistral 6L) for 10 minutes at 4°C the supernatant was poured into counting vials and 10 ml of "scintillating cocktail" added. The cocktail was made up of 0.2g POPOP and 4g PPO/l of toluene. After 12 hours equilibration the vials were counted in a Packard tricarb Liquid Scintillation Spectrophotometer (Model 3330) for 10 minutes.

Fig. 7 shows the effect of different charcoal concentrations and two incubation regimes on the non-specific binding (NSB) and on the binding of labelled 11-ketotestosterone to its antiserum (BØ). 0.25% charcoal concentration falls towards the edge of the stable region and changing the incubation time to 15 minutes does not markedly affect the assay.

To test the accuracy of the assay, 50 µl of canine plasma spiked with 500pg, 1ng, 2ng and 4ng of steroid were assayed and also to act as a check on interassay precision 50 µl of canine plasma spiked with 1ng of 11-ketotestosterone was included in each batch of samples assayed. The coefficient of variation for 8 assays was 17.6% and the mean recovery for each of the canine samples spiked with exogenous 11-ketotestosterone are shown in Table 2.2.3.

2.2.9 (c) Cortisol assay

Rabbit anti-cortisol-21-thyroglobulin serum was obtained from Miles Yeda Ltd., Israel. (1,2-^{3}H) cortisol (specific activity 49 Ci/m mol) was the radioactive label.

All the solutions in the assay were prepared in 0.6% bovine serum albumin (BSA), 0.03 M NaH_2PO_4, 0.06 M Na_2HPO_4 - NaOH, pH 7.4.

The freeze dried antiserum was reconstituted with 7.5 ml of buffer and this dilution gave about 45% binding of labelled cortisol. Standards were also made up in buffer to give 500pg, 250pg, 100pg, 50pg and 10pg/assay.

Plasma from precocious males and immature female parr sampled in October and December, 1976, was assayed.

Standards and plasma were assayed in duplicate. 0.5 ml of label (18,000 dpm) was added to 0.1 ml standard, 0.25 ml of label (18,000 dpm) to 0.05 ml plasma, to give equal proportions of label and plasma. These were mixed and allowed to equilibrate overnight at 4^{o}C. 0.4 ml of 2.5% aqueous TCA was added to the standards and 0.2 ml to the plasma samples, mixed well and centrifuged at 2000 rpm for 15 minutes (MSE GF8 centrifuge with multiple heads). 0.2 ml of the TCA supernatant was added to 3.8 ml of buffer and mixed. 0.6 ml was transferred to clean tubes for assay and a further 0.6 ml transferred to counting vials and 10 ml of scintillating cocktail (Instagel, Packard Ltd.) added. They were then counted for 40,000 counts or 100

minutes. These counts gave the total radioactivity in each assay tube and they were also used to estimate the procedural losses during the deproteinisation stage.

0.1 ml of antiserum was added to the 0.6 ml in the assay tubes and this was allowed to equilibrate overnight at $4^{o}C$. 0.6 ml of dextran coated charcoal solution, made up of equal volumes of 1.25% charcoal and 0.125% dextran was then added and the charcoal was kept suspended by slowly rotating the tubes in a rack for 1 hour. The charcoal was then separated by centrifugation, 2000 rpm for 10 minutes and 0.5 ml of the supernatant removed from each tube for counting.

The amount of cortisol in the label was taken into account in the construction of the standard curve, the % of label bound was plotted against the amount of cortisol (standard + label). The amount of cortisol in the unknown plasma was then interpolated from this and corrected according to the amount of cortisol in the label.

To overcome any intraassay variation, plasma samples from all the experimental groups were assayed in each run. Fig. 8 shows the intraassay variation ($\bar{x} \pm$ S.D.) of 14 consecutive standard curves for the cortisol radioimmunoassay. Pooled fish plasma in which the cortisol level was known was assayed with each batch of samples to act as a control on the precision of the assay. Intraassay precision was reasonably satisfactory: the coefficient of variation for 14 determinations of pooled fish plasma was 16.4%.

2.2.10. Electrolyte Analyses

1975:

Electrolyte analyses were carried out on plasma from 12 precocious males and 8 immature parr sampled in December. Sodium and potassium values were determined on whole plasma using a digital flame spectrophotometer 1L343 (Instrumentation Laboratories U.S.A.).

1976:

Sodium (Na^+) potassium (K^+) calcium (Ca^{++}) and magnesium (Mg^{++}) values were determined in plasma from 7 precocious males, 8 immature parr sampled in October and 15 precocious males and 7 immature parr sampled in December, using an atomic absorption spectrophotometer 1L251 (Instrumentation Laboratories, U.S.A.).

Na^+ and K^+ were determined by the flame emission method and Ca^{++} and Mg^{++} by the atomic absorption method.

Standards were made up from magnesium chloride, calcium chloride, potassium chloride and sodium chloride, all "Analar" grade and purchased from B.D.H. Chemicals Ltd., U.K.

Standards and plasma dilutions were made up with double glass distilled water. Plasma samples for K^+, Ca^{++}, Mg^{++}, determination were diluted 1:100 and for Na^+ determinations 1:1000.

2.2.11. Saprolegniasis Studies

From October to April, the hatchery stocks at the Salmon Research Trust are treated daily with 0.5ppm malachite green as a precaution against outbreak of fungal infection (Murphy, 1975). In order to determine the prevalence of Saprolegnia 2,000 1+ parr were selected at random from the hatchery population and transferred to the smolt release pond (see Salmon Research Trust Annual Report, 1972). Here they were kept at a stocking density of 1.3 kg/m^2 with a water flow rate of 705.25 dm^3/min. They did not receive any prophylactic medication with malachite green.

When fish infected with fungus were observed they were netted out of the pond, the areas of the body parasitised were recorded and the sex determined. Small pieces of the infected sites were fixed in either 10% formol saline or 10% buffered formalin, processed and sectioned in the usual way (as described in Section 2.2.4).

and stained with Mayer's haematoxylin and eosin (H. and E.).

Saprolegnia was identified from each infected fish by comparing the chlamydospores and zoosporangia with the diagrams of Coker (1923) Kanouse (1932) and Scott (1964). A few of the isolates were grown on corn meal agar (Difco Ltd.) at room temperature and attempts to induce sexual formation on sterile hemp seeds failed. Identification of the fungal agents was not taken further than the genus level. A χ^2 analysis was performed on the number of infected and non-infected fish and on the patterns of infection amongst the precocious males and immature parr using a 2 x 2 contingency table (Moroney, 1951).

2.2.12. Gonadosomatic Index (GSI)

GSI expresses the gonad weight as a percentage of the body weight and was calculated thus:

$$GSI = \frac{\text{Gonad Weight}}{\text{Body Weight}} \times 100$$

2.2.13. Condition Factor ('K')

The condition factor was calculated thus:

$$'K' = \frac{\text{Body Weight - Gonad Weight}}{(\text{Length})^3} \times 100$$

2.2.14. Statistics

The mean and standard error of the mean was calculated for all the data. Where applicable the results from precocious males and immature parr were compared using the Student t-test.

2.3. Results

2.3.1 General

Precocious maturity was most prevalent amongst the 1+ age group where 100% of the males became mature (Table 2.3.1). A number of underyearling parr are thought to have become sexually mature as some of the 0+ male parr sampled in Spring, 1978, had the appearance of having previously spawned (Table 2.3.2).

Precocious males were indistinguishable from immature parr on external appearances. They did not develop secondary sex characteristics, the kype and the red colouration of the musculature which is characteristic of adult males did not occur. Ripe male parr continued to feed voraciously and the degenerative changes of the intestines, pancreas, liver and kidney, that are associated with non-feeding spawning adults did not occur in these parr (Plate 1). There was no increase in the amount of brown pigment (melanin macrophage centres) in the spleen of precocious males, and no degenerative changes took place in the cardiovascular system (Plate 2).

From Fig. 9 it can be seen that the 1+ parr gained weight at a steady rate throughout the year and

that in April, May and June potential precocious males were slightly heavier than immature females. Precocious males reached their maximum weight by October and maintained it for the duration of the spawning season, whereas immature parr continued growing and were significantly heavier in December ($p \leq 0.05$) and January ($p \leq 0.002$). Fig. 10 shows that in spring and early summer the potential 1+ precocious males were slightly longer than the rest of the population and this was significant in June ($p \leq 0.01$) but by September they were significantly shorter ($p \leq 0.025$) than immature female parr. Although precocious males continued feeding, they stopped growing by October/November but they maintained their length and weight measurements (Figs. 9 and 10) during the latter half of the gonad developmental cycle. Immature parr continued growing in length throughout the whole year and were significantly larger than precocious males in October ($p \leq 0.01$), December ($p \leq 0.025$). and January($p \leq 0.05$).

Fig. 11 shows that the condition factor of both precocious males and immature parr followed a similar pattern. There was a peak in mid-summer and by early autumn the 'K' value fell in both groups. The 'K' value amongst precocious males during this period was slightly lower than in immature parr. During December and January it began to rise again in immature parr, whereas in precocious males it fluctuated slightly and was significantly lower than immature parr in January ($p \leq 0.01$)(Fig. 11).

2.3.2. Gonads

2.3.2 (a) Developing males

The majority of yearling parr examined during March, April and May had immature testes composed entirely of closely packed cysts of primary spermatogonia (germ cells). The cells had large nuclei with masses of chromatin and prominent nucleoli (Plate 3). A small number of the male parr showed evidence of having previously undergone a sexual cycle as 0+ parr (Table 2.3.2). These spent gonads had numerous primary spermatogonia either singly or in groups situated along the lobule boundary walls and clumps of spermatozoa remained in the lumen of a few lobules (Plate 4).

The onset of spermatogenesis in immature parr occurred sometime during May or June. The testes increased in size slowly, but the number of primary spermatogonia increased dramatically. They then divided mitotically to form secondary spermatogonia. The lobules did not all develop at the same rate and some were more advanced than others. Table 2.3.3. shows that by August spermatocytes were present in some of the lobules and a few lobules also contained spermatids (Plate 5). Spermatids and spermatozoa were the major cell types in developing testes from October onwards. At this

time, sperm could be extruded from the parr by gentle pressure on the abdomen. By December all the lobules were at the spermatozoa stage (Plate 6). As the testes matured the lobule boundary wall became stretched due to the increasing number of germ cells. In the fully mature testes the lobule boundary cells became sparse and primary spermatogonia, either singly or in groups of 2 - 4 lined the lobule wall. The testes, at this stage, had a milky white colour and occupied all the available space in the abdominal cavity.

Fig. 12 shows the gonad weight (GSI) in the developing parr did not markedly increase until the appearance of the spermatocyte stage during July and August. It reached a peak in October when the major cell types were spermatocytes and spermatozoa. The decrease in GSI during November, December and January was partially due to loss of milt during preparation but may also be due to re-absorption of the sperm cells as sperm release seldom occurs in fish kept in captivity.

2.3.2 (b) Immature females

Three stages of oocyte development were present in the ovaries of 1+ parr examined during October and December, 1976. These were (i) early perinucleolus stage (ii) late perinucleolus stage (iii) early yolk vesicle stage (Plate 7). Table 2.3.4 gives the dimensions of each cell type.

(i) Early perinucleolus stage

These cells were irregular in shape and had a large nucleus relative to the size of the cell. The nucleus

had numerous basophilic nucleoli along the nuclear membrane. The cytoplasm of these cells had a marked affinity for haematoxylin and a single layer of follicle cells bordered the cytoplasm.

(ii) Late perinucleolus state

This was the most common cell type present. The cells were more regular and larger than the early perinucleolus stage cells. Their nuclei were more eosinophilic but the nucleoli remained basophilic and arranged around the periphery of the nucleus. The cytoplasm lost its affinity for haematoxylin and a non-cellular membrane (zona radiata) formed between the follicle layer and the cytoplasm.

(iii) Early yolk vesicle stage

A small number of late perinucleolus stage cells developed small vesicles in their peripheral cytoplasm just under the zona radiata. These cells were slightly larger than the late peri-nucleolus stage. There was a small increase in their numbers from October to December.

2.3.3. Plasma Androgen Levels

2.3.3 (a) Developing males

Fig. 12 shows that there was a parallel rise in plasma 11-ketotestosterone with the increasing GSI of developing males. It was a steady increase

throughout the gonad development cycle. Peak plasma androgen levels occurred in December, a month after the peak in GSI. Once the maturation period was over and spermiation had begun, the 11-ketotestosterone levels decreased very quickly.

2.3.3 (b) Immature Females

Plasma 11-ketotestosterone values were low in immature females and showed no change when measured at two different times of the year (Table 2.3.5).

2.3.4. Adrenocortical (interrenal) cell activity

Adrenal cortical cells in Atlantic salmon were situated in the 'T' shaped head end of the kidney just dorsal to the heart. This area of the kidney consisted mostly of haematopoietic tissue and spotted through it were perivascular cuffs of adrenal cortical cells known as interrenal cells. The interrenal of salmon parr consisted of groups of small cells with almost spherical nuclei containing a considerable amount of chromatin material and finely granulated cytoplasm (Plate 8). The phenomenon of size heterogeneity did not seem to occur in the interrenal cells of these farmed parr.

Fig. 13 shows that the interrenal activity increased during autumn and early winter as manifested by the

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changes in the nuclear diameter of the adrenocortical cells. There appeared to be greater activity amongst the precocious males as the nuclear diameter in these fish was significantly larger than in immature parr. There were no signs of degenerative changes in the interrenal of the ripe males.

2.3.5. Cortisol

Table 2.3.6 shows that there was a significant increase ($p \leq 0.001$) in plasma levels of cortisol in both precocious males and immature parr during the winter period. Sexual dimorphism occurred as cortisol production was greater amongst developing males, although the October values were not statistically different from immature parr, but in December the difference between the two groups was highly significant ($p \leq 0.01$).

2.3.6. Electrolyte Analysis

Tables 2.3.7 and 2.3.8 show that plasma Na^+, Ca^{++}, Mg^{++} levels varied slightly during the experimental period but the differences were not statistically significant. Plasma K^+ levels in precocious males were lower than in immature parr at all sample times. This difference was statistically significant in December, 1975, October, 1976 ($p \leq 0.01$) and in December, 1976, ($p \leq 0.05$).

There was a slight amount of haemodilution during the early winter and the December PCV values in both groups of parr were a little higher than the October values but not significantly so. Precocious males also had a higher PCV in October and December than immature parr, but this difference was not statistically significant.

The difference in Na^+ and K^+ values between the two tables 2.3.7 and 2.3.8 is probably due to the fact that they were estimated by two different techniques.

2.3.7. Pituitary

2.3.7 (a) General

A wide variety of terms are used to describe the various regions of the teleost pituitary. The terminology proposed by Pickford and Atz (1957) in their review on the general structure and cellular composition of the fish pituitary has been employed in this description.

The pituitary of 1+ Atlantic salmon parr can be divided into two distinct regions - the adenohypophysis and neurohypophysis. The adenohypophysis can be further subdivided into the pro-adenohypophysis, meso-adenohypophysis and meta-adenohypophysis, which correspond to the rostral pars distalis (anterior lobe), proximal pars distalis

(dorsal lobe) and pars intermedia (ventral lobe) of mammals.

Fig. 14 is a diagrammatic representation of the pituitary of salmon parr. It shows that the pro-adenohypophysis is the smallest region and occupies the anterior part of the gland and extends ventrally and laterally. It is composed of cells arranged in compact follicles with ovoid or spherical lumina. A distinct layer of cells, 3 - 4 thick, separates the pro-adenohypophysis from the neurohypophysis and part of the meso-adenohypophysis. These cells are elongated with their long axis perpendicular to the adeno-neurohypophyseal interface; their nuclei are irregularly shaped and the cytoplasm has fine granules.

The meso-adenohypophysis comprises about a quarter of the pituitary and is situated in the central portion of the gland. It is bounded centrally and ventro-posteriorly by the meta-adenohypophysis, dorsally by the neurohypophysis and anteriorly by the pro-adenohypophysis (Fig. 14). It is composed of diversely staining cells, many of which are arranged in columns which project into the neurohypophysis like fingers (Fig. 14).

The meta-adenohypophysis occupies the ventral half of the pituitary. It is composed of strands of columnar cells separated from one another by the neurohypophysis and connective tissue (Fig. 14). The cells are either cuboidal or columnar with large irregularly shaped nuclei.

The neurohypophysis is an extension of the brain and is attached to the hypothalamus by means of the hypophyseal stalk. It is highly branched and the neural elements extend in connective tissue trabeculae throughout the various lobes of the adenohypophysis. Its largest portion is found in the ventro-posterior part of the pituitary where it ramifies between the strands of the meta-adenohypophysis (Fig. 14). It also covers all the dorsal aspects of the meso-adenohypophysis and its interdigitations in this area are not as pronounced as in the meta-adenohypophysis (Fig. 14).

Four different cell types can be distinguished in the adenohypophysis of salmon parr on the basis of their tinctorial properties.

Cell type 1: These are small undifferentiated cells with spherical nuclei and little or no cytoplasm. They are present in the dorsal columns of the meso-adenohypophysis.

Cell type 2: The cytoplasm in these cells has a strong affinity for acidic dyes such as orange G, eosin, acid fuchsin and ponceaux de xylidine. They are present in great numbers in the pro and meso-adenohypophysis.

Cell type 3: These cells have a basophilic granular cytoplasm that is PAS+ve and which is also stained with aldehyde fuchsin, light green and aniline blue. They are found in the pro and meso-adenohypohysis.

Cell type 4: The layer of columnar cells separating the pro from the meso-adenohypophysis and the strands of cuboidal and columnar cells in the meta-adenohypophysis are composed almost entirely of this type. They have large elongated

nuclei with varying amounts of chromophobic cytoplasm that was refractory to all the dyes used in this survey.

All four cell types were present in both precocious males and immature parr, the only difference being in the relative proportion of each type. Table 2.3.9 summarises the tinctorial properties of each cell type. The use of aldehyde fuchsin with and without oxidation in $KMNO_4$ and in combination with PAS and orange G did not differentiate between the thyrotrophic and gonadotrophic basophils. When Barrett's procedure was used it was impossible to distinguish basophils from chromophobes as they were both stained grey. The methods of Slidders, Masson, one-step MSB and AB + PAS + OG were found to be the most useful as they differentiated clearly the major cell types.

2.3.7 (b) Precocious males

Pro-adenohypophysis

The pro-adenohypophysis of precocious males was composed of acidophilic cells arranged in compact follicles. The nuclei of these cells were spherical and occupied the polar position of the cell. The cytoplasm was more deeply stained around these nuclei. Colloid material was present in some of the lumina and exhibited variable staining properties either with orange G, PAS, alcian blue and light green (Plate 9)

PAS + ve basophilic cells were present amongst the chromophobic layer separating the pro-adenohypophysis

from the neurohypophysis. They were also scattered in the connective tissue between the follicles and a few were in the body of the follicles pushing the adjacent acidophils aside (Plate 9). Table 2.3.10 shows that in the period between October and December, the basophils in the palisade layer and amongst the acidophilic follicles increased in size. A large number of basophils were also present in the ventral border of the pro-adenohypophysis with the meso-adenohypophysis. These PAS + ve cells were quite large with irregular eccentric nuclei and with varying amounts of coarse and fine granular cytoplasm. When the staining procedure AB + PAS + OG was used, the large coarse granules were stained dark blue and the fine cytoplasmic granules exhibited a PAS + ve reaction.

Meso-adenohypophysis

All four cell types were present in this zone. The majority cell type was the PAS + ve basophils. These cells were large, irregular in shape and had eccentrically placed nuclei. Their cytoplasm contained both large and fine granules distributed evenly throughout the cell, which exhibited a similar staining reaction as the basophilic cells in the ventral border of the pro-adenohypophysis.

These basophils were most abundant on the lateral edges of transverse sections, in the ventral edge of sagittal sections near the ventral extension of the pro-adenohypophysis and along the connective tissue border separating the meso-adenohypophysis from the meta-adenohypophysis (Fig. 14, Plate 10 a). Scattered amongst the densely staining basophils were a few cells

that were either negative or faintly PAS + ve. The nuclei and cytoplasm of these cells were normal and showed no signs of degranulation or vacuolisation.

Individual basophils were also present, scattered amongst the undifferentiated cells at the tip of the fingerlike processes. These were nearly always situated on the connective tissue border with the neurohypophysis (Plate 11 a and 11 b). In a few specimens, some of these cells were not as densely stained as the basophils on the ventral and lateral edges.

The relative number of basophils did not change during the later stages of spermatogenesis and as Table 2.3.10 shows they were significantly larger and more densely stained in December. The rest of the zone was composed of densely stained acidophils and undifferentiated cells. The acidophils were variable in shape but the majority were elongated with the nuclei just off centre. They were most common in the body of the meso-adenohypophysis and extended into the fingerlike processes (Plates 10 and 11). The undifferentiated cells were mostly present at the tips of the fingerlike processes but a few individual cells were also scattered amongst the acidophils.

(iii) Meta-adenohypophysis

The cytoplasm of the cuboidal and columnar cells was chromophobic and had no affinity for any of the dyes used. PAS + ve granules were present in the cytoplasm

of a few cells (Plate 12). This zone did not change during late spermatogenesis and its appearance in December was similar to that in October.

(iv) Neurohypophysis

This area contained a great mass of neurosecretory material (NSM). This was most abundant in the posterior part of the neurohypophysis in the region of the meta-adenohypophysis. The NSM stained strongly with alcian blue, aniline blue, Bargmann's technique and faintly with aldehyde fuchsin before oxidation (Plate 13). The nerve fibres around the infundibulum and meso-adenohypophysis showed a positive PAS reaction. There was a great deal of PAS + ve NSM in this region, as well as an odd PAS + ve cell (Plate 14).

2.3.7. (c) Immature Parr

(i) Pro-adenohypophysis

The only difference in this zone was that the basophilic cells were significantly smaller ($p \leq 0.05$) than those in the precocious males (Table 2.3.10). They were present only amongst the palisade layer of cells separating the pro-adenohypophysis from the neurohypophysis and they were less abundant than in precocious males. Their response to dyes was similar to precocious males. There were no changes amongst the acidophilic follicles and chromophobic border cells and they exhibited staining patterns similar to those of

precocious males.

(ii) Meso-adenohypophysis

The numbers and staining pattern of acidophilic and undifferentiated cells were unchanged. The acidophilic cells were the most common cell type in the meso-adenohypophysis of immature parr. There was an increase in the number of chromophobe cells; these were present mostly in the lateral edges (transverse section) and at the ventro-median border (sagittal section) (Plate 15). These were areas which in precocious males were packed with PAS + ve, basophilic cells. A few basophils were present amongst the lightly staining chromophobes. They were more numerous in the fish sampled in October, but they never approached the quantity present in ripe male parr. They were also much smaller than the basophils in sexually mature males ($p \leq 0.001$) (Table 2.3.10). In the majority of these basophils the nuclei covered most of the cell area, and there was only a small strip of very faint PAS + ve and lightly basophilic cytoplasm present. Just as in precocious males, individual PAS + ve basophils were also scattered along the border of the fingerlike processes with the neurohypophysis in immature parr. The basophils in the meso-adenohypophysis of immature parr did not have the same affinity for dyes as those in precocious males and were not as densely stained.

(iii) Meta-adenohypophysis, Neurohypophysis

There was no change in the meta-adenohypophysis. Many of the chromophobic cells had a few PAS + ve granules in their cytoplasm. The amount and staining reaction of NSM in the neurohypophysis was similar to precocious males, the only difference being that no PAS + ve cells were present in the neurohypophysis surrounding the meso-adenohypophysis.

2.3.8. Skin

2.3.8 (a) General

The structure of salmon parr skin was similar to most teleost fishes. Plate 16 illustrates that the morphology of the Malpighian cells varied throughout the epidermal layers. In the basal layers these cells were either columnar with an elongated nucleus positioned at right angles to the basement membrane or cuboidal with a large spherical nucleus. In the mid-epidermal region, the cells were either rounded or slightly angular with dense nuclei. On the outer layers the Malpighian cells were flattened and the nuclei were in a horizontal position. Also in the outer layers some of the nuclei had lost their affinity for the nuclear stain and were large opaque masses.

2.3.8 (b) Changes in the skin

The skin measurements in 1+ parr varied with the position on the body surface, time of the year and with sexual maturity.

(i) Dermis

The dermis varied in thickness depending on the site from which it was obtained. It was thinnest in the pectoral region between the pectoral fins, and thickest in the caudal peduncle. Tables 2.3.11 and 2.3.12 show that the changes in the size of the dermal layer during the period of sampling, October to December, were small. In general, there was no difference in the thickness of the dermal layer between the sexes. Exceptions to this were in October, 1976, when the dermis in the caudal peduncle and pectoral regions of immature parr was statistically thicker ($p \leq 0.02$) than in precocious males and in December, 1975, and 1976, when it was significantly larger in ripe males than in immature female parr ($p \leq 0.05$ and 0.02).

(ii) Epidermis

In all areas examined there was an increase in the thickness of the epidermis in both sexes during late autumn and early winter (Tables 2.3.11, 2.3.12, 2.3.13 and Fig. 15). Sexual dimorphism also occurred and the epidermal layer in precocious males was thicker than in immature females (Tables 2.3.11, 2.3.12, 2.3.13 and Fig. 16). This difference was statistically significant in the majority of the regions examined, but it was not so marked in 4 cases (dorsal and pectoral areas sampled in October, 1976, pectoral fin and tail

sampled in December, 1976, (Tables 2.3.12 and 2.3.13). The enlarged epithelial layer in sexually mature parr was due to an increase in the number of Malpighian cell layers (Tables 2.3.11, 2.3.12 and 2.3.13). This could suggest increased mitotic activity but no evidence of this was seen. Often small gaps occurred in the outer epithelial layer where dead cells had sloughed off and were not filled by cells from the lower layer (Plate 16). These breaks in the continuity of the outer epidermal border were present in both ripe and immature parr but were more numerous amongst the precocious males especially in the pectoral and caudal peduncle areas.

Correlation coefficients ('r') were estimated to see if a relationship existed between the skin measurements in the different regions of the body, the body weight and the condition factor ('K'). There was a slight positive correlation between the epidermal thickness and the body weight in most regions examined and in 3 areas this was statistically significant (Figs. 17 and 18). When the total skin (epidermis and dermis) measurement was considered it was found that there was a direct and highly significant correlation between it and the body weight in both sexes (Fig. 19 and 20). In order to see if the increased gonad weight amongst the precocious males was obscuring any relationship with the skin measurements, adjustments were made by substracting the gonad weight from the body weight, but this did not alter the 'r' value significantly (Tables 2.3.14 and 2.3.15).

Amongst immature female parr there was a negative correlation between the total skin measurement and 'K' (Fig. 24) in October. This interesting trend was also observed when the epidermal thickness was plotted against 'K' (Fig. 22). The immature parr with the highest condition factor had the thinnest epidermis in the pectoral and caudal peduncle, pectoral fin and tail areas. By December this negative correlation had become a positive relationship. Precocious males did not exhibit this trend as there was a positive but not significant relationship between the epidermal thickness, total skin thickness and the 'K' factor during October and December (Figs. 21 and 23).

From the above results it would appear that the thickness of the salmon parr skin undergoes a seasonal cycle. These changes are most obvious in the epidermis and are influenced by the size of the fish and especially the stage of sexual maturity.

2.3.8 (c) Electron Microscopy

There were no ultra-structural differences in the epidermis of mature and immature parr. The major cell type in the epidermis was the Malpighian filament cell which had a large highly indented nucleus (Plate 17). Prominent nucleoli were present in some of the nuclei in the mid-epidermal and outer layers. Plate 17 shows that the nuclear chromatin was organised in a series of elongated clumps separated from each other by a small clear space (pore complex) along the nuclear membrane.

The cytoplasmic inclusions of these cells included some mitochondria in the perinuclear region and numerous tonofilaments in the peripheral zone. Cells near the outer edge of the epidermis also had a large number of vesicles (Plate 18). A highly convoluted cell membrane separated adjacent filament containing cells and consisted of an extreme network of microvilli interdigitations and desmosomal complexes (Plate 19). Numerous large and small intercellular spaces occurred between the cells and highlighted the microvilli connections (Plates 17 and 19). These intercellular spaces extended from the mid-epidermal layer to the outer layers of cells and often contained filamentous material (Plate 19).

The majority of epithelial cells in the outer layer were electron lucent; they had lost all of their nuclear and cytoplasmic elements and were undergoing degenerative changes. In the precocious males examined, some of these cells had large round electron dense masses along the nuclear membrane of clear ovoid nuclei (Plate 18). These could have been aggregations of chromatin material. Many of the cells in this area were undergoing desquamation. Where one of the cells had sloughed, the microvilli of the underlying cell were stretched and gave the epithelium a spiked appearance (Plate 18). Sometimes one or two of the cells remained in contact with the underlying epithelium through one or two microvilli attachments and formed the protoplasmic constituents

of the outer cuticle layer. Electron lucent cells also occurred at irregular intervals throughout the penultimate epidermal layer.

Mucous cells were also present and were similar to those described by Roberts et al (1970) and by Harris and Hunt (1975). They had numerous pockets of secretory material and their nuclei were displaced to the back edge of the cells.

2.3.8 (d) Superficial mucous (apocrine, goblet) cell concentration

Mucous cells that are on the outer surface of the epidermis release their secretions to the exterior through a small pore (Pickering and Macey, 1977). Alcian blue (pH 2.5) stains those superficial cells that are open to the environment by the apical pore (Pickering, 1977). Fig. 25 shows that the concentrations of superficial mucous cells in 1+ salmon parr underwent marked fluctuations from February to December. This would suggest a seasonal cycle. These changes occurred in both mature and immature parr and were the same in all the areas of the body examined. The number of goblet cells increased during the first two months of the survey. The peak in May may have been artificial as the parr were subjected to the stress of grading a few days prior to sampling.

During the summer months the concentration of mucous cells decreased with the exception of July, when there was a sharp rise (Fig. 25). This was probably due to an epidemic of the

bacterial disease furunculosis (Aeromonas salmonicida) amongst the hatchery population in late June and early July. There was no significant difference (5% level) between the number of mucous cells in precocious and immature parr during spring and summer.

From September to early winter the number of mucous cells increased again. Sexual dimorphism was apparent at this time and Figs. 25 and 26 and Table 2.3.16 illustrate that from autumn onwards precocious males had a significantly greater number of goblet cells in most regions than immature parr in both 1976 and 1978.

The distribution of mucous cells varied from area to area and at all times the greatest concentration was in the head, tail, and pectoral fin regions. The gradient of mucous cells observed by Pickering (1974) in brown trout, where the anterior of the fish had a higher concentration of goblet cells than the posterior zones, was not present in salmon parr, as the tail, dorsal and pectoral fins had the greatest number.

2.3.8 (e) Mucoid Histochemistry

The results of the histochemical analysis are presented in Tables 2.3.17, 2.3.18, 2.3.19, 2.3.20. Mucous cells stained densely with colloidal iron and alcian blue (pH 2.5) but not with alcian blue (pH 1.0) which suggests the presence of acidic mucins with carboxyl groups. The results of the combined alcian blue/PAS procedures were more complicated.

With alcian blue (pH 2.5)/PAS some of the cells stained blue (periodate unreactive alcianophilic), others blue/purple (periodate reactive alcianophilic) and a small number red (neutral polysaccharides)(PAS + ve)(Plates 20 a and 20 b). The proportion of these cells depended on the area of the body surface and varied with the state of sexual maturity and time of the year (Tables 2.3.17, 2.3.18, 2.3.19 and 2.3.20).

During October, when the alcian blue (pH 2.5)/PAS procedure was used, precocious males had more periodate unreactive alcianophilic and less periodate reactive alcianophilic cells in the dorsal, pectoral and caudal peduncle areas than immature parr. This was reversed in December, when there was a decrease in the number of blue cells in the ripe males and an increase in immature parr. Precocious males and immature parr had about the same proportion of blue to blue/purple cells in the pectoral fin and tail regions during December. Red (PAS + ve) cells containing neutral polysaccharides were present in both sexes but their distribution varied. In most of the areas examined in October, with the exception of the caudal peduncle, precocious males had more PAS + ve cells than immature parr. Immature parr, at this time, also had a large amount of PAS + ve cells in the pectoral fin. No comparison could be made with the corresponding area in precocious males as both the pectoral fin and tail specimens were lost during histological processing. During December no PAS + ve cells

were present in the dorsal and caudal peduncle regions of ripe males and in the dorsal and tail areas of immature parr. In the other areas at this time, precocious males had more neutral polysaccharide cells than immature parr in the tail and pectoral fin regions and immature female parr had more PAS + ve cells in the pectoral and caudal peduncle than precocious males.

The staining pattern of mucous cells with colloidal iron/ PAS technique should resemble that of AB/PAS. The number of blue cells was low except for the dorsal and caudal peduncle regions of the precocious males. This was because colloidal iron was not as specific as alcian blue for acidic mucopolysaccharides. The majority of cells were strongly stained blue/purple and the purple tinge was due to the periodate reactivity of the acidic mucins (Plates 21 a and 21 b). There appeared to be no difference between the precocious males and immature parr in the proportion of blue to blue/purple cells present except in the dorsum where precocious males had more blue cells than immature parr. The amount of cells having neutral polysaccharides was variable and only in the pectoral fins of both experimental groups did they occur in great numbers. When the alcian blue (pH 1.0)/ PAS combination was used, all the cells were stained either red or purple (Plates 22 a and 22 b). These red/purple cells contained a mixture of acid and neutral mucin compounds.

Alcianophilia was unaffected by mild methylation but was nearly completely inhibited with active methylation (Plate 23). Alcianophilia was weak when alcian blue was made up in 0.1M $MgCl_2$ and it decreased still further with increasing ionic strengths of Mg Cl_2 and was either very weak or negative when 0.8M $MgCl_2$ was used. This suggests that the acidic mucosubstances were very weakly sulphated.

Alcian blue, in combination with safranin, produced a dense blue colour as it blocks the reaction of safranin with sulphated mucins and if any strong acidic mucopolysaccharides had been present, they would have been stained red (Plate 24). Aldehyde fuchsin, which reacts specifically with sulphate mucopolysaccharides in unoxidised formalin fixed tissue, gave a negative result. When it was used in combination with alcian blue only, the non-sulphated acid mucosubstances were stained blue, indicating that sulphated mucins were not present in large amounts.

Hyaluronidase reduced alcianophilia slightly, suggesting that a little hyaluronic acid or chondroitin sulphate was present in the acidic mucins. Alcianophilia was almost completely inhibited after treatment with neuraminidase indicating that the majority of the acid mucopolysaccharides had a terminal sialic acid.

The above results show that the major carbohydrate component in mucous cell secretions of 1+ parr is an acid mucopolysaccharide containing sialic acid. Some neutral

polysaccharides are also present. The amount of these mucins varied from area to area and it would appear that the state of sexual maturity and the time of year may also have an effect on their distribution.

2.3.9. Saprolegniasis

Table 2.3.21 shows that the prevalence of fungal infections amongst 1+ parr was 4.45%. 58.4% of the infected fish were precocious males. Assuming from Table 2.3.1 that precocious males comprised 50% of the 1+ parr population, the prevalence of Saprolegnia amongst ripe males was not significantly different from immature parr.

Table 2.3.22 shows the number of fish parasitised in the different areas of the body. Many of the parr were infected in more than one area. The areas most frequently infected were the tail, caudal peduncle, pectoral fin and the pectoral areas between the fins. The caudal peduncle ($p \leq 0.01$) and tail ($p \leq 0.001$) of precocious males appeared to be more prone to Saprolegnia when compared to the immature parr.

In all the infected fish the fungus destroyed the epidermis and in many fish only the stratum compactum remained of the dermis (Plate 25) and in addition degenerative changes took place in the underlying muscle. In a few of the more severely infected fish, the fungus had broken up both the epidermal and dermal layers and the hyphae were present amongst the subcutaneous muscle layer. In only a few cases was an inflammatory response of leucocytic infiltration observed.

2.4. Discussion

2.4.1. General

Simpson and Thorpe (1976) state that sexual maturation in salmon, as in higher vertebrates is influenced more by age than by nutrition. The males in salmonid species appear to mature earlier than females; in the brown trout and rainbow trout the ovary seldom develops before 3 years, while the testis normally matures in the second year (Gardner,1976, Johnstone et al 1978). Gardner (1976) cites a number of workers who have reported a predominance of males amongst the early maturing wild salmon. This is not always the case, as workers at the Salmon Research Trust of Ireland have found that the sex ratio in returning grilse varied between 60:40 to 50:50, females to males (Piggins, pers. comm.). Simpson and Thorpe (1976) also stated that there is a preponderance of males amongst artificially reared grilse but this has not been observed amongst the early maturing fish at the sea installations of the Salmon Research Trust of Ireland (Piggins, pers. comm.). It is generally accepted that the offspring of grilse are more likely to mature early, and Thorpe (1975) mentions that there is a possible genetic link between precocity and strain of fish.

The relationship between food, age and sexual maturation is not a simple one although accelerated growth rate of salmon parr induces early smoltification (Piggins (1969, 1974) cited by Gardner, 1976). Simpson and Thorpe (1976) stated that fish destined to be early maturing grilse are recruited from the larger members of the sea-caged stock. The results of this study show that in spring the 1+ male parr were slightly heavier and longer than the immature females so there may be a possible link between growth rate and sexual maturity. Feeding a balanced artificial pellet diet to increase the growth rate is now common practice in fish farms. These diets have been shown to have an ageing effect on salmon parr (Bergstrom and Koch, 1969), and in hatchery populations the majority of male salmon parr become sexually mature within 2 years (Table 2.3.1)(Carlin (1969) cited by Koch and Bergstrom, 1978).

In all hatchery populations there are two groups, the larger (upper mode) fish which invariably smoltify after one year, while the smaller (lower mode) fish continue developing in freshwater for one or more further years (Thorpe, 1977). New husbandry regimes have been developed using heated water (10°C) to incubate eyed ova and alevins, so as to advance first feeding (Lawrie, 1975). This increases the growth rate and the proportion of fish in the upper mode group. It also had the effect of inducing sexual maturation in a small proportion of 0+ parr (Table 2.3.2). Murza (1976) found that heated water regimes increased the number of precocious males in underyearling Neva salmon. MacKinnon and Donaldson (1976) and Schmidt

and House (1979) also found that heated water induced precocious sexual development in male pink salmon and steelhead trout respectively. MacKinnon and Donaldson (1976) suggested that precocious development may be size dependent and that the fish that grow the fastest reach the critical size for initiation of gonad development. They further stated that if this occurs at a time when the environmental conditions are conducive to promote sexual maturity, precocious development proceeds. This may be true as the potential precocious males in this study were slightly longer, heavier and also had a greater 'K' value than the immature parr during spring (Figs. 9, 10 and 11).

The results of this study show that sexual maturation retards the growth of salmon parr (Figs. 9, 10 and 11). Thorpe (1977) reported that precocious males occurred in both modes of the population, but that in the upper mode the immature fish were longer than the mature ones. In precocious males the food conversion ratio falls from September onwards, most of the energy is used for development of the gonad, and at spawning the testicular weight represents 16% of the total body weight. It would clearly be an advantage to fish farms if paedogenesis in male parr could be prevented and the food used more efficiently to increase growth rate. At present, because of economic necessity, aquaculture regimes are conducive to the development of precocity. The fish stocks may be bred from early maturing grilse, and the husbandry and nutritional

practices, besides increasing the growth rate, also accelerate the ageing process, thus inducing sexual maturity in a large number of young parr.

Very little information has been documented about the changes that take place in the internal organs of precocious salmonids. MacKenzie, House, MacWilliam and Johnston (1978) reported that precocious male steelhead trout had coronary myointimal hyperplasia. In contrast to this, the paedogenetic male S. salar in this survey did not exhibit any degenerative changes in the cardio-vascular system, despite the fact that Koch and Bergstrom (1978) have reported elevated cholesterol levels in precocious Atlantic salmon parr. The visceral organs of precocious males did not undergo any degenerative changes as one would expect since they continue feeding voraciously during testicular development. Precocious males survive sexual maturation and spawning and smoltify like normal parr (Jones and Orton, 1940). They smoltify slightly later than immature parr (Piggins, pers. comm.) probably because of the cessation in their growth rate during winter and the endocrinological changes of sexual maturity.

2.4.2. Interrenal, plasma cortisol and electrolyte levels.

Many workers have used the nuclear diameter of adrenal cortical tissue as an indication of its activity

(Davis and Fenderson, 1971; Olivereau, 1975; Bromage and Fuchs, 1976). Mudge, Dively, Neff and Anthony (1977) have reported that there is a reduction in cell size and nuclear diameter of inactive interrenal cells. Fuller (1974) cited by Bromage and Fuchs (1976) has shown that increased interrenal cell nuclear diameter closely parallels increases in cortisol secretion.

One of the hazards of sampling fish is that they are very susceptible to handling stress (Wedemeyer, 1972) and the common anaesthetic agents, (tricanemethanesulphonate (M.S.222) and benzocaine) can also act as stressors (Houston, Madden, Woods and Miles, 1971 a, b; Soivio, Hyholm and Huhti, 1977). The effects of stress can be divided into primary endocrine effects which result in immediate increased secretion of adrenaline, nor-adrenaline and cortisol and secondary effects such as hyperglycaemia, changes in plasmatic free fatty acids and electrolyte balances. The secondary effects occur later as a result of the primary endocrine effects (Mazeaud, Mazeaud and Donaldson, 1977). Strange and Schreck (1978) said that brief anaesthetisation with mild handling does not affect cortisol concentration. The recommended time for anaesthetisation is not more than 3 minutes (Reinitz and Rix, 1977; Wedemeyer and Yasutake, 1977), and anaesthetic doses that immobilise the fish quickly have been found to be less stressful than doses that merely depress the fish (Strange and Schreck, 1978).

The fish in this survey were netted as quickly as possible, anaesthetised in 150ppm of Benzocaine and were bled within 3 minutes of capture to avoid the primary endocrine effects of stress. Thus the increased cortisol concentration observed in the 1+ parr in this study during early winter can be taken as a normal physiological occurrence. It occurred simultaneously with enlarged adrenal nuclear diameter. Enlarged interrenal cells are a sign of chronic corticosteroid secretion. Any sudden stressful stimuli such as handling and anaesthetisation would manifest itself in elevated cortisol levels but the interrenal tissue would remain unchanged.

Both groups of 1+ parr, when examined in December, had significantly greater plasma cortisol concentration and larger interrenal cell nuclear diameter than the 1+ parr sampled in October. This would suggest a seasonal variation in interrenal activity. Campbell et al (1976) said that the seasonal cycle of corticosteroid production in winter flounder was associated with feeding activity. Davis and Fenderson (1971) found that Atlantic salmon parr examined in May had significantly larger interrenal cell nuclear diameter than those examined in August. They also mentioned that the social interactions and general movement of salmon parr are markedly higher in winter and spring than in summer and fall. This could probably explain the increased nuclear diameter of the interrenal cells and cortisol secretion during early winter seen in the 1+

parr in this survey. The seasonal elevation in corticosteroid production may also be due to environmental stimuli such as falling water temperature and photoperiod.

Precocious males had greater interrenal activity than immature parr when examined during October and December and this could have been due to sexual maturity. Olivereau (1975) also found that wild precocious males, when sampled in December, had larger interrenal cells than immature parr. The period of reproduction in teleosts is frequently associated with hyperactivity of the interrenal gland. All spawning adult salmonids exhibit, to varying degrees,increased interrenal secretion (Robertson and Wexler, 1959; Robertson et al, 1961 a; Donaldson and Fagerlund, 1970; Heyl and Carpenter, 1972). Gonadectomy resulted in a rapid involution of the hypertrophied interrenal gland in O. nerka (McBride and van Overbeeke 1969 b). Administration of gonadotrophins to castrated sockeye salmon did not increase the plasma concentration of cortisol or cortisone, nor did it stimulate the activity of the interrenal (Donaldson and McBride, 1974). Androgens and oestrogens, on the other hand, restored the interrenal hypertrophy in these gonadectomised sockeye salmon (van Overbeeke and McBride, 1971). Since these steroids did not affect the pituitary-interrenal axis (van Overbeeke and McBride, 1971) it was thought that they exerted their effects by acting directly on the interrenal.

One of the consequences of increased corticosteroid production is a change in electrolyte balance (Mazeaud et al, 1977). There is an increased Na^+ retention and K^+ excretion by the kidney (Wedemeyer, 1970). Houston et al. (1971 a, b) found that anaesthetic and surgical stress caused a slight increase in plasma Na^+ and Ca^{++}, no change in Mg^{++} and a decrease in K^+ levels of brook trout. Mazeaud et al. (1977) mentions that stressing freshwater fish results in haemoconcentration and thus an increased PCV.

The 1+ parr in this study exhibited a slight increase in PCV and a decrease in plasma K^+ levels during the experimental period October to December. Na^+, Ca^{++} and Mg^{++} values fluctuated but were not significantly different from each other. These changes in the plasma electrolyte balance and the red cell concentration are probably a part of the secondary effect of the seasonal increased corticosteroid production. Precocious males had a slightly greater interrenal activity and this was reflected in them having a greater PCV and lower K^+ values than immature parr.

4.3. Gonads

2.4.3 (a) Testis

The above description of the spermatogenic cycle in precocious males of S. salar Juv. corresponds to "observations" of the same species by Jones (1940) and Crim and Evans (1978). These workers mentioned that the spermatogenesis was complete by November,

whereas in this study, it was finished in December. Crim and Evans (1978) also reported that the peak GSI in wild precocious males occurred in September, but in the artificially reared parr of this study, the peak GSI was noticed in October. The GSI in farmed fish (15%) was much higher than that of the wild precocious males(10%) in the study of Crim and Evans (1978).

On the basis of these histological studies the spermatogenic cycle in paedogenetic males can be divided into 3 periods. These periods are not clearly distinguishable from each other and overlap to a certain extent. The first period is from May/June to September and is characterised by the formation of cysts with secondary spermatogonia. The second period is a period of maturation and spermiogenesis and starts in September and continues until early December. The third period is a phase of spermiation and lasts from December until the sperm cells are reabsorbed and the numbers of primary spermatogonia begin to increase again, usually in June. In cultured salmon the spermatozoa are not released spontaneously and so the sperm cells are progressively reabsorbed.

2.4.3 (b) Ovary

At present there is no comparable work available to compare with the histological studies on the gonads of immature female parr described above. The immature parr were still in the early stages of oogenesis. Leizerovich and Murza (1976) have shown that the techniques of intensive aquaculture (heated water, etc.) can accelerate ovarian development, so possibly the female parr in this study were more advanced than the wild female parr described by Olivereau (1975) and Crim and Evans (1978).

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2.4.4. Plasma androgen levels

Androgens have been shown to be necessary for spermatogenesis (Eversole, 1941; Arai, 1967; Sundararaj and Nayyar, 1967; Yamazaki and Donaldson, 1969; Bhatti and Javaid, 1973; van den Hurk and van de Kant, 1975). 11-ketotestosterone has been found to be the major androgen in salmonids and its concentration increases in the peripheral plasma of adult Pacific and Atlantic salmon and rainbow trout during gonad development (Schmidt and Idler, 1962; Idler et al 1971; Simpson and Wright, 1977). The observation reported here that plasma 11-ketotestosterone levels increased during gonad development of male paedogenetic Atlantic salmon agrees with these authors. 11-ketotestosterone levels reached a peak at the end of the maturation period in December and then declined sharply during the period of spermiation (Fig. 12). Schmidt and Idler (1962) also found that plasma androgens decreased markedly in post-spawned Pacific salmon. Thus the pattern of androgen secretion in precocious males is similar to that of sexually mature adult males.

Crim and Evans (1978) reported that pituitary gonadotrophin values in precocious males were low from May to August (the period of rapid gonad development) but increased from September to

January (synthesis and storage) and then decreased during the period of spermiation. This coincides with the cycle of 11-ketotestosterone secretion reported here. Plasma androgen gradually increased during the period of gonadotrophin synthesis and storage in the pituitary. Pituitary gonadotrophin levels decreased with a resultant rise in plasma gonadotrophin (Crim and Evans, 1978) during spermiation in January. This occurred at a time when the plasma 11-ketotestosterone levels were found, in this study, to be falling. These findings, in conjunction with the results of Crim and Evans (1978), provide proof of a negative feedback relationship between the gonad and pituitary in precocious males.

The maximum value for plasma 11-ketotestosterone (1.2 µg/100 mls) recorded in these experiments at the end of spermatogenesis is much lower than the reported values for mature adults. Schmidt and Idler (1962) reported that spawning adult Pacific salmon had values of 17 µg/100 ml and Idler et al (1971) mentioned levels of 10-12 µg/100 ml in adult Atlantic salmon. Simpson and Wright (1977) found that the levels in mature rainbow trout could range from 2 µg-9 µg/100 ml. The relatively low plasma 11-ketotestosterone level in precocious males even at the height of sexual maturity could be the reason for them not developing secondary sexual characteristics. The tissue (skin, muscle, etc.) may require a certain level of circulating androgens before the development of the

secondary sexual characteristics is initiated. Funk and Donaldson (1972) have stated that the rate of synthesis of androgens in precocious male pink salmon was insufficient to induce the development of secondary sex characteristics. The presence of male androgens in female salmonids is normal (Schmidt and Idler, 1962; Schreck et al, 1972). Simpson and Wright (1977) reported levels of 11-ketotestosterone, 0.03 - 0.04 µg/100 ml in the plasma of immature female (GSI 0.1-0.2%) rainbow trout. The levels reported here (Table 2.3.5) are slightly higher than Simpson and Wright's but this could be due to the ovaries being more advanced.

2.4.5. Pituitary

The pituitary of teleost fishes has been subjected to many studies and the functions of the different cell types have been identified using a number of techniques, including culturing, transplantation, biochemical isolation, immunohistochemical, ultra-structural and surgical or chemical ablation of the target organs. Holmes and Ball (1974) summarised all the experimental data in their review and mentioned that in the pro-adenohypophysis the acidophilic follicle cells secrete prolactin, the palisade layer of chromophobes that separate the prolactin cells from the neurohypophysis are

adrenocorticotrophic cells. The prominent acidophils of the meso-adenohypophysis are somatotropes. They also stated that since gonadotropes and thyrotropes secrete a glycoprotein substance they have the same staining properties and their distribution varies from species to species.

The observations on the pituitaries of 1+ parr of Atlantic salmon reported here show that their structural morphology is the same as that of rainbow trout (Robertson and Wexler, 1962) and that of sockeye salmon (van Overbeeke and McBride, 1967). The principal change in the pituitaries of all sexually mature fishes takes place in the pro and meso-adenohypophysis and consists of an increase in the number and size of basophils (Robertson and Wexler, 1962; Rai, 1966 a, b; van Overbeeke and McBride, 1967; Ball and Baker, 1969; Sage and Bern, 1971; Schreibman, Leatherland and McKeown, 1973; Holmes and Ball, 1974; Olivereau, 1976). In this study, both immature and mature parr had PAS + ve cells in the pro and meso-adenohypophysis. The basophilic cells were more abundant and larger in precocious males than in the immature females (Table 2.3.10). Sexually mature sockeye salmon had a greater number of basophils in both the pro and meso-adenohypophyseal regions of the pituitary than immature fish (van Overbeeke and McBride, 1967). McBride and van Overbeeke (1969a) stated that all these basophils were gonadotropes as they were degranulated after castration. They also stated that the number of

thyrotrophic cells were so small that they could not be identified and they cited other workers who showed that thyroid activity in Atlantic and Pacific salmon is reduced during sexual maturity. McKeown and van Overbeeke (1969), on the other hand, found that only the PAS + ve cells in the rostro-ventral area of the meso-adenohypophysis reacted with flourescent anti-ovine LH serum. In migratory and non-migratory spawning rainbow trout, basophils are only found in the meso-adenohypophysis (Robertson and Wexler, 1962).

Olivereau (1976) stated that in Atlantic salmon the basophils present in the meso-adenohypophysis are gonadotropes, and those in the pro-adenohypophysis are thyrotropes. A small number of basophilic gonadotrophic cells can spread into the pro-adenohypophysis at sexual maturity in various teleosts including the eel, salmon and trout (Olivereau(1972) cited by Holmes and Ball, 1974). This may account for the large accumulation of glycoprotein + ve cells on both sides of the border of the meso-adenohypophysis with the pro-adenohypophysis in the paedogenetic males of this study. Ekengren, Peute and Fridberg (1978) have reported that basophilic cells in the pro-adenohypophysis near the border with the meso-adenohypophysis reacted with rabbit anticarp gonadotrophin serum in the double antibody flourescent technique.

Olivereau (1976) described two types of gonadotrophic cells in both sexually mature male parr and adult Atlantic salmon. The first group of cells, called the "g" cells, were present in the ventro-lateral border of the meso-adenohypophysis and their development paralleled that of the gonad. The second cell type "G" cells were situated dorso-caudally in the meso-adenohypophysis and were poor in mucopolysaccharides, weakly PAS + ve and often vacuolised. These "G" cells were first seen in the adult salmon off Greenland before the fish had begun their spawning migration. The "G" cells, at this time, had an active appearance and they persisted throughout all the stages of the sexual cycle. Van Overbeeke and McBride (1967) also described two similar types of basophilic cells in the mature sockeye salmon but Robertson and Wexler (1962) found only one glycoprotein cell type in the spawning rainbow trout.

Many investigators have identified two different gonadotrophic cell types (Schreibman et al 1973; de Vlaming, 1974). Knowles and Vollrath (1966a) reported two gonadotropes in the eel, one containing vesicles and becoming vacuolated at the beginning of the spawning migration. Ultra-structural studies have also revealed two gonadotropes in O. nerka, one with globular inclusions and the other with vesicles (Cook and van Overbeeke, 1972). Olivereau (1976) cited Chestnut (1970) who described two types of gonadotrophic cells in O. kisutch. The first cell type was present before there was a marked increase in the GSI and as gonad development progressed it degranulated and

became vacuolised. The second gonadotrope appeared during the maturation cycle and was the most common cell type during spawning.

Donaldson et al (1972) and Yoneda and Yamazaki (1976) have partially purified a gonadotrophin from Pacific salmon. Biological and biochemical tests on this gonadotrophin preparation have shown it to contain one gonadotrophin which was capable of stimulating all the stages of gametogenesis. Recently, Ng and Idler (1978) have put forward evidence for two gonadotrophins. They isolated a non-glycoprotein fraction that induced vitellogenic activity only and a glycoprotein that caused both vitellogenesis and ovulation.

Thus, it is very probable that the vesicle cells described by Knowles and Vollrath (1966a) and Cook and van Overbeeke (1972) the degranulated basophil of Chestnut (cited by Olivereau, 1976), the type 7 cell of van Overbeeke and McBride (1967) and the "G" cell in Atlantic salmon (Olivereau, 1976) secrete a gonadotrophin (not LH) that initiates gametogenesis and that later in the cycle the globular "g" cells (Olivereau, 1976) secrete an LH like hormone that stimulates further maturation and spermiation. To resolve this question, pituitary extracts should be prepared from fish in the early stages of gametogenesis and tested on immature animals.

In the present study, basophils were situated in two areas of the meso-adenohypophysis of precocious males. The majority of the PAS + ve cells were in the ventral and lateral border of the meso-adenohypophysis, wedged in between the pro and meta-adenohypophysis. These cells were irregular in shape, densely stained and increased in size during the second phase of spermatogenesis (Table 2.3.10) and thus resembled the "g" cells of Olivereau (1976). The increase in cytoplasmic area and greater affinity for stains, represented synthesis and storage of the cell contents. This agreed with the results of Crim and Evans (1978) who reported an increase in pituitary gonadotrophin content during the gonad development cycle in wild precocious males.

Glycoprotein + ve cells were also present amongst the somatotropes and undifferentiated cells in the fingerlike processes of the meso-adenohypophysis. These cells were a little larger than the "g" cells. Their affinity for basophilic dyes varied from moderately weak to dense. In many ways, they resembled the "G" cells of Olivereau (1976) but they never exhibited vacuolisation. This discrepancy could be due to the type of fixative used; buffered formol saline was used in this study whereas Olivereau (1976) used Bouins-Hollande sublimate.

This report does not add any further information to the unresolved question of whether one or two gonadotrophic cells control sexual maturity in fishes. The cells in the ventro-lateral border ("g" cells) of the meso-adenohypophysis were definitely one kind of gonadotrophic cell. The question of whether or not the larger PAS + ve cells found amongst the somatotropes represented early gonadotropes could not be answered, as this study was started in October when testicular development was well advanced. These "G" type cells were also present in small numbers in the immature female parr so that they could be an early gonadotrope.

The smaller basophilic cells were also present in immature parr along the ventro-lateral area of the meso-adenohypophysis. They were never as numerous as in precocious males and were also much smaller (Table 2.3.10). They exhibited the phenomenon of being weakly PAS + ve but had a moderate affinity for the other basophilic stains. This finding disagrees with the results of Olivereau (1976) who reported that there were no glycoprotein + ve cells in the meso-adenohypophysis of immature female wild Atlantic salmon parr. Robertson and Wexler (1962) reported an occasional basophil in the meso-adenohypophysis of juvenile rainbow trout but they did not say whether these were gonadotropes or thyrotropes. Olivereau (1976) cited the study of McKeown and Leatherland (1973) on immature sockeye salmon, in which they described a basophil that resembled the gonadotrope of the adult; she also stated that the existence of such glycoprotein + ve cells in immature parr was surprising. The observation reported

here of a small number of gonadotropes being present in the pituitary of immature female parr is not considered surprising. Leizerovich and Murza (1976) have reported that intensive fish farming techniques advanced gonad development in female salmon albeit not as rapidly as in males. Although there is little supporting evidence, a certain level of gonadotrophic cell activity may be necessary for the early development of oocytes. Rai (1966 b) stated that the cyanophils in the meso-adenohypophysis of female Tor tor have a low level of secretory activity in the early stages of oogenesis. Crim and Evans (1978) detected gonadotrophin in the pituitary and plasma of wild immature female S. salar parr with GSIs similar to the GSIs reported for the immature parr of this study.

The basophilic cells in the palisade layer and in the islands between the follicles of the pro-adenohypophysis of 1+ parr increased in size during the experimental period. They were more abundant and larger in precocious males than in immature females at all sampling times (Table 2.3.10). Ekengren, et al (1978) using rabbit anti-carp gonadotrophin in the double immunofluorescence technique, reported that gonadotrophic cells were distributed all over the pro-adenohypophysis of Atlantic salmon. Olivereau (1976) mentioned that this anti-carp gonadotrophin serum was not specific and in some species it bound thyrotrophic cells. The cells described in this study were most probably thyrotrophic cells as Olivereau (1976) mentioned that the thyrotropes are situated

in the pro-adenohypophysis close to the corticotropes and Holmes and Ball (1974) stated that they may also lie between the prolactin follicles. The increase in the cytoplasmic area of these cells during early winter probably represented storage of thyroid stimulating hormone due to an enhanced thyroxine secretion. Thyroid activity in salmon parr is normally low but it increases when the fish are smoltifying (Chester-Jones, Ball, Henderson, Sandor, Baker, 1974). Osborn, Simpson and Youngson (1978) stated that plasma thyroxine increased in farmed one to two year old rainbow trout during autumn and reached a peak in November. This could explain the hypertrophy of the thyrotrophic cells of the parr in this report, but since no examination was made of the thyroid gland, it is only conjecture.

The presence of larger basophils in the pro-adenohypophysis of ripe males is more difficult to explain, but may be due to sexual maturity. Studies on the interaction of the gonads, thyroid and pituitary have produced conflicting results. In some species gonadotrophins, androgens and oestrogens inhibit thyrotrophic cells while in others they stimulate them. In adult Atlantic and Pacific salmon there is a marked reduction in the thyroid gland's function during the latter part of sexual maturation (Barannikova, 1961; van Overbeeke and McBride, 1971). Holmes and Ball (1974) cited Singh (1969) who showed that androgens and oestrogens stimulated the thyroid directly

in hypophysectomised catfish Mystus vittatus Bloch. Bentley (1976) said that in many teleost fishes the thyroid displays an increased activity during the spawning season. Zanuy and Carrillo (1977) found that there was an increase in the thyrotrophic cells of Spicara chryselis C.V. during the pre-spawning period. White and Henderson (1977) have demonstrated that the thyroid hormone levels in the brook trout rise during gonad development.

The physiology of paedogenetic males differs from adult Pacific and Atlantic salmon in that they continue feeding while the gonad is developing. Ripe males also differ markedly from immature parr because of the presence of mature gonads. These differences may be reflected in a higher metabolic rate and increased thyroid secretion in precocious males.

There was no difference in the amount of type A or type B NSM in the neurohypophysis of both groups of 1+ parr examined in this study. Holmes and Ball (1974) said that many workers have observed fluctuations in the amount of AF + ve and AB + ve type A NSM that could be related to the reproductive cycle. Type B fibres which are PAS + ve originate in the NLT and Peter (1973) mentioned that part of the NLT is involved in the control of gonadotrophin and also thyrotrophin secretion. Type B fibres are most frequently found in the anterior region of the

neurohypophysis (Holmes and Ball, 1974; Batten and Ball, 1977), and have been shown to synapse with glial cells (pituiticytes) and all the adenohypophyseal cell types (Knowles and Vollrath, 1966 b; Holmes and Ball, 1974; Batten and Ball, 1977). Barannikova (1961) reported that in spawning salmon there is an increase in PAS + ve material in the neurohypophysis. In this study no difference was observed in the amount or staining intensity of PAS + ve NSM in the infundibular region of the pituitaries of both groups of parr. The probable explanation for this phenomenon was that active thyrotropes and a small number of gonadotropes were present in immature parr as well as in precocious males.

2.4.6 Skin

The skin of Atlantic salmon parr is essentially similar to that of other salmonids (Roberts et al 1970; Pickering and Macey, 1977). These investigators only examined one area of the body, whereas in this study, five regions were examined. There was an increase in the epidermal thickness of mature and immature 1+ parr during late autumn and early winter. These changes occurred in all the zones examined and suggest a seasonal fluctuation

in the dimensions of the integument of salmon parr. Pickering (1977) showed that changes occurred in the epidermal thickness of hatchery-reared sexually mature adult brown trout. He stated that "the changes were related to the degree of sexual maturity of the fish rather than the time of year because immature hatchery-reared brown trout of similar size showed no change". This report is the first evidence that there may be a seasonal cycle in the epidermal thickness of some immature salmonid species. No comparison could be made between the skin of immature male and female parr as all the males examined were sexually mature.

The observation that precocious males had a thicker epidermis than immature parr agrees with the results of Robertson and Wexler, 1960; Roberts et al, 1970, and McBride and van Overbeeke, 1971, who have shown that sexual maturity increased the skin thickness in teleosts. During the experimental period, October to December, there was an increase in the epidermis of the ripe males. This confirms Pickering's (1977) observation that in sexually mature fishes, skin thickness is related to the stage of gonad development. The reason for the changes in the skin is probably the enhanced secretion of sex steroids during gametogenesis. McBride and van Overbeeke (1971) induced hypertrophy of the dermal and epidermal layer of gonadectomised sockeye salmon with exogenous androgens and oestrogens. Yamazaki (1972) also increased the epidermal thickness in O. gorbuscha and O. keta by oral administration of methyltestosterone.

The increase in thickness of the epidermis in precocious males was due to hyperplasia of the Malpighian cells. McBride and van Overbeeke (1971) also found the same in O. nerka; in their androgen treated fish the epithelium was 10-12 cells thick, whereas in the control gonadectomised salmon the epidermal layer was only 3-6 cells thick. Exogenous androgens also produced hyperplastic changes in the epidermis of 0+ salmon parr (see Chapter 3).

The thickness of the skin (dermis and epidermis) was found, in general, to be influenced by the weight of the fish (Figs. 19 and 20). Pickering (1977) also recorded similar findings in the brown trout during the interspawning period, June-August, when the sexual differences in the skin structure were minimal. At other times, he found that the variation due to the seasonal cycle and sex differences obscured any visible relationship between size and skin thickness. The statistically negative correlation between the epidermal thickness and the condition factor in immature parr in autumn is unusual. One explanation for its occurrence is that, at this time, growth in length is faster than the gain in weight (hence the fall in "K") and that this may stretch the epidermis. In December, the epidermis increased in thickness due to an increase in the number of cell layers and the correlation coefficient between total skin thickness and "K" was slightly positive.

There was very little difference in the epidermal thickness of the different areas of the body. Both the tail and pectoral fin had slightly thicker epidermis than the rest of the body. There appears to be some variation amongst amongst salmonids, as Pickering (1974) found that the dorsal, pelvic and pectoral fins of the char had a significantly thinner epidermis than the rest of the body but in the brown trout, the epidermis of the tail, ventral and pectoral fins was slightly thicker than the other areas examined. In this study, a definite pattern emerged when the total skin thickness (epidermis and dermis) was considered; the caudal peduncle was the thickest area and the pectoral or belly area had the thinnest skin.

The present investigation clearly shows that a seasonal cycle in the concentration of superficial mucous cells exists. The number of mucous cells were high during the winter and early spring and fell during summer. It was only in the latter part of the spermatogenic cycle, October to December, that the mucous cell concentration in precocious males was significantly greater than immature parr. This was contrary to the results of Pickering (1977) who found that in sexually mature brown trout the females had an increased mucous cell concentration and the males had few or no mucous cells.

In many fish the activity and number of mucous cells were reduced by hypophysectomy but were restored by replacement therapy with prolactin (Ball, 1969, Review). Lemoine and Olivereau (1973) & Wendelaar and Sjoerd (1978) found that there was a positive correlation between the number of mucous cells and prolactin cell activity in the stickleback. In some lower vertebrates there is a seasonal cycle in prolactin secretion. Sotowska-Brochocka (1977) found that in Vipera berus L. prolactin accumulates in the pituitary during spring and summer and is rapidly released in autumn. Plasma and pituitary prolactin levels have not been monitored in salmonids so it is not known if there is a seasonal cycle in these fish. If a seasonal cycle in prolactin cell activity,with a peak in autumn,did exist in salmon parr, it could explain the increase in mucous cell concentration reported here.

In mammals,thyrotrophin releasing hormone induces prolactin secretion (Schwinn, von jur Muehlem, Koebherling, Halves, Wenzel and Meinhold, 1975; Paneral, Salerno, Manneschi, Cocchi and Muller, 1977; Spitz, Almaliach, Polishuk and Rabinowitz (1977). TSH and thyroxine restores mucus secretion in hypophysectomised P. latipinna (Ball, 1969). It is possible that the seasonal increase in thyroid activity which has been shown to occur in rainbow trout (Osborn et al 1978) and which appears to occur also in salmon parr (Table 2.3.10) may enhance prolactin production and cause the elevated mucous cell concentration during winter.

From the results it appears that stress, such as grading or bacterial infections, also increases mucous cell numbers. Pickering and Macey (1977) have shown that a single incidence of handling can promote an increase in concentration of the apocrine cells in the epidermis of char. Increased corticosteroid production is one of the consequences of stress (Wedemeyer, 1970) Feeding exogenous cortisol to O+ salmon parr produced a significant increase in the superficial mucous cell concentration of the belly, dorsal fin, pectoral fin and tail areas (see Chapter 3). There was a seasonal rise in the interrenal activity of the farmed parr in this study, so it is possible that the elevated plasma cortisol levels during early winter, in conjunction with prolactin and the seasonal rise in thyroid activity increased the mucous cell numbers. The greater number of goblet cells amongst the paedogenetic males may also be explained by the effect of the slightly higher level of cortisol and greater thyroid activity in these fish. McBride and van Overbeeke (1971) and Yamazaki (1972) found that androgens, besides increasing the skin thickness, also increased the number of mucous cells. This may not be true for salmon parr as 17α methyltestosterone and testosterone did not markedly alter the mucous cell concentration of O+ parr (see Chapter 3).

The distribution of mucous cells in the salmon parr of this study differed from both the brown trout and the char. Pickering (1974) recorded that the greatest concentration of apocrine cells in the trout and char occurred on the anterior regions and that the fins had significantly fewer

cells than the rest of the body. In this study, the head, tail and pectoral fins were the areas that had the greatest concentration of goblet cells.

The mucoid histochemistry of a few teleost species has been described (Asakawa, 1970; Harris et al, 1973; Bullock et al 1976). These studies have indicated major diversity between groups with regards to the carbohydrate moiety. Bullock et al (1976) found in whiting that the epithelial mucins were mainly acid mucopolysaccharides with a small sialic acid component and a greater proportion of sulphated mucins. Asakawa (1970) found that the predominent carbohydrate component of mucus in the eel, Anguilla japonica Temminck et Schlegel contained sialic acid. The results of the single and combined staining procedures used in this study suggested that the major carbohydrate component of mucus in 1+ Salmo salar Juv. was mucopolysaccharide containing sialic acid. A few neutral polysaccharides were also present as well as some polysaccharides containing hyaluronic acid.

The results recorded here suggest that the composition of mucus is not constant, that the proportions of the different components depends on the area of the body examined, sex of the fish and the time of the year. Asakawa (1970) and Harris et al (1973) did not mention in their reports the time of the year the fish were

sampled, the stage of sexual maturity of the fish, nor did they describe any differences in the histochemistry of the various regions of the body examined. No clear pattern emerged from this study. The major changes were the different proportion of non-periodate alcianophilic and periodate alcianophilic cells and the amount of neutral polysaccharides (especially in the pectoral fin of both sexes) during the experimental period. One can only speculate as to the reason for the changes in the chemical composition of mucus during early winter. Many hormones have been shown to have an effect on mucus production; prolactin (Sage and Bern, 1971); thyroxine (Graham, 1970) and androgens (McBride and van Overbeeke, 1971; Yamazaki, 1972). Environmental factors have also been shown to have an effect on mucus secretion. Excessive acid or alkaline conditions can cause excessive mucus production in brook trout (Daye and Garside, 1976), and Varanasi, Robisch and Malins (1975) have shown that low concentrations of lead and mercury in water can produce alterations in the epidermal mucus of rainbow trout.

A number of ultra-structural studies on the skin of the head, dorsal and lateral regions of adult Atlantic salmon, brown trout and coho salmon parr have been reported in the literature (Roberts et al, 1970; Hawkes, 1974; Harris and Hunt, 1975). It was thought necessary to investigate

the structure of the epidermis of the caudal peduncle and pectoral zones of salmon parr to try and explain the increased susceptibility of these areas to fungal infections. It was found that the ultra-structure of these areas was similar to that described for other salmonids (Roberts et al, 1970; Hawkes, 1974; Harris and Hunt, 1975), the only difference being an increase in the amount of peripheral condensed chromatin in the nuclei of the mid and upper epidermal layers of the parr in this study. The only factor that might increase the susceptibility of these parr to fungal infections was the presence of numerous intercellular spaces which extended from the mid-epidermis to the superficial layers. These intercellular spaces were present in the fish examined by Roberts et al (1970) and by Harris and Hunt (1975) but the authors did not discuss their significance. Fungal hyphae may be able to penetrate these spaces and become well established in the epidermis whilst the sloughing cells of the outer cuticle layer would be an excellent medium for Saprolegnia growth.

2.4.7. Saprolegniasis

The low level of fungal infection amongst the experimental fish in this study compared to the

prevalence of Saprolegnia in young parr at the Salmon Research Trust in previous years may be ascribed to this group having being reared in much better husbandry conditions. They were held at a stocking density of 1.3 kg /m^2 which is much lower than normal hatchery conditions. A surprising outcome of this experiment was that both immature and mature parr were more or less equally parasitised with the fungus. All the literature to date has reported that sexually mature salmonids are more susceptible to Saprolegnia than immature fish (Roberts and Shepherd, 1974; Neish, 1977; Richards and Pickering, 1978).

A definite pattern of infection existed and precocious males were more often parasitised in the caudal peduncle, tail, pectoral and pectoral fin regions than immature parr (Table 2.3.22). These were the areas that were most frequently seen by the workers of the Salmon Research Trust to be infected in previous years. Richards and Pickering (1978) said that the "fins of hatchery-reared trout were particularly prone to Saprolegnia infection". It is generally believed that the behaviour of hatchery fish, such as tail biting, rubbing the belly and pectoral areas along the bottom of the ponds, causes damage to the skin and makes them more susceptible to fungal infections in these areas.

Richards and Pickering (1978) also mentioned that one of the reasons for the high level of Saprolegnia infections on the fins of brown trout was the low concentration of mucous cells in these areas. It has been suggested that mucus, because it is continually being replaced, is protective against infection (Willoughby, 1971). Antibodies have been

shown to be present in the mucus of plaice (Fletcher and Grant, 1969). The observation noted here of increased mucous cell concentration in the areas most frequently parasitised by fungus (especially the tail and pectoral fin during early winter) suggests that mucus may not have a pronounced protective action.

Willoughby (1971) stated that "encysted aquatic fungi would seem to find in fish mucus all the elements of an ideal growth medium even including an amenable initial pH and trigger amino acids to facilitate early growth and make possible the later production of adaptive enzymes for attacking the larger proteinaceous molecules". Willoughby and Pickering (1977) have reported that mucus enhances germination and growth of secondary Saprolegnia zoospores. They also found that fungal spores readily adhered to the surface of trout and char and a number of them remained on skin even after 24 hours. Thus the increased mucus production reported here in salmon parr during early winter may aid absorption of many Saprolegnia zoospores that are present in the water at this time. The changes in its chemical composition, particularly the increase in neutral polysaccharides, could provide an optimal environment for the growth of the zoospores. The many microscopic lesions on the superficial epithelium due to sloughing dead cells may help the fungal spores encyst and prevent them being washed off, as mucus is continually being replaced. Peduzzi, Nolard-Tintigner and Bizzorero (1976) have isolated a proteolytic enzyme in Saprolegnia that may help hyphae penetration of the host's tissue once the zoospore has germinated

This would suggest that Saprolegnia may have the ability to be a primary infectious agent as well as being an opportunist secondary invader.

Neish (1977) mentioned that wounding a fish does not necessarily ensure fungal infection and that the host must be further debilitated, so that its normal defences and reparative capacity are impaired. In the normal hatchery life of fish, stressors such as injury, overcrowding, handling, etc. can cause an increase in corticosteroid production (Wedemeyer, 1970). A consequence of increased corticosteroid secretion is a depletion of ascorbic acid reserves so that the fish are not able to repair tissue damage. Corticosteroids are also anti-inflammatory and immuno-suppressive agents and thus lower the ability of fish to cope with infections. Administration of corticosteroids has aided in the induction of fungal infections in fishes (Robertson et al 1963; Roth, 1972) (see also Chapter 3). The present investigation has shown that there is an increase in plasma cortisol levels during early winter in 1+ salmon parr. This enhanced interrenal activity allied with the microscopic lesions on the skin and the increase in mucus production may contribute to the vulnerability of young parr to fungal infections.

CHAPTER 3

THE EFFECTS OF EXOGENOUS STEROIDS ON THE SKIN OF IMMATURE 0+ PARR.

3.1. Introduction

The results of the previous section show that there were seasonal changes in the skin of artificially reared 1+ Atlantic salmon parr. There was a seasonal fluctuation in the mucous cell concentration with an enhanced mucus production in both sexes during winter. Precocious males at this time had a significantly greater concentration of apocrine cells than immature parr. Hyperplastic changes also occurred in the epidermis of 1+ parr during the period of October to December. These changes were more pronounced amongst the sexually mature male parr.

Both groups of 1+ parr exhibited increased corticosteroid secretion during late autumn and early winter, and the paedogenetic males had also an enhanced androgen production. The following experiment was designed to test the hypothesis that the skin changes could be due to the endocrine status of the fish during the experimental period. Yamazaki (1972) and McBride and Fagerlund (1973)

have reported that administration of androgens as a supplement in the diet caused a hypertrophy of the skin. Corticosteroid implants have produced atrophy of the epidermis in rainbow trout (Robertson et al, 1963). No comparable studies have been described for Atlantic salmon.

3.2. Materials and Methods

3.2.1. Animals

All the fish in this experiment were underyearling (O+) Atlantic salmon parr of known parentage (Salmon Research Trust Annual Report, 1976). 299 fish were selected at random from the O+ hatchery population. Eight fish were sacrificed and used as initial controls. The remaining parr were divided into six groups of which one group acted as controls and the rest were treated with exogenous steroids in their diet. The fish were kept in 0.91m^2 fibre glass tanks, held at a stocking density of 0.93 kg/m^2 and a water flow rate of 16 dm^3/min.

3.2.2. Steroids

The steroids used in this experiment were purchased from Sigma Chemicals Ltd. (U.K.) and were 17β oestradiol, testosterone, 17α methyltestosterone, cortisol and cortisone. They were administered at the rate of 100ppm in the diet.

3.2.3. Diet

The basic diet was B.P. Nutrition fish pellets No. 2 (B.P. Nutrition). The steroid was incorporated into the pellets in the following manner. The appropriate amount of steroid was

dissolved in about 20 ml of ethanol and then dispersed over 100g of pellets. The ethanol solution was thoroughly mixed with the pellets and then allowed to evaporate at room temperature. The control diet was prepared in the same way except for the exclusion of the steroids.

3.2.4. Experimental regime

The experiment lasted for 12 weeks and was carried out during the period from mid-September to mid-December, 1976. Fresh pellets were prepared every week and the fish were hand-fed three/four times a day, Monday to Friday and once a day on Saturday and Sunday. Undigested food, faeces, dead and moribund fishes were removed from the ponds daily. The number of fungussed fish and the area of the body parasitised were recorded.

3.2.5. Histology

The methods used to collect specimens for histological, histochemical and quantitative mucous cell studies have been described in the previous chapter. Fixation, processing, staining and calibration procedures on the skin and interrenal were the same as reported in Section 2.2.

3.2.6. Statistics

An analysis of variance was performed on the data. When the overall analysis of variance was significant a further 'f' test was performed between the different levels (using the residual mean squares and the residual degrees of freedom) to compare the different treatment groups with the controls (Campbell, 1967). χ^2 analyses using 2 x 2 contingency tables were performed on the numbers of fungal infected and non-infected fish from the different treatment groups A further χ^2 analysis was made between the patterns of infection obtained from the different experimental groups.

3.3. Results

3.3.1. Epidermis and dermis

Administration of exogenous steroids did not affect the general structure of the skin. Fig. 27 and Tables 3.3.1, 3.3.2, 3.3.3, 3.3.4 and 3.3.5 illustrate that the androgens, methyltestosterone and testosterone induced hyperplastic changes in the epidermis and dermis (Plate 26). Fig. 27 and Tables 3.3.1, 3.3.2, 3.3.3, 3.3.4, 3.3.5 show that this increase in the skin measurements was significantly greater than the control group, in most areas of the body examined. The exceptions were the caudal peduncle and tail, where because of the small number of control animals, a statistical analysis was not valid but the trend was similar to the other regions. The other steroids did not markedly affect the skin measurements except that the cortisol and to a lesser extent the β oestradiol experimental groups had a thinner dermal layer than the control group.

The results in Table 3.3.6 show that there was a significant increase in the epidermal and dermal thickness of the untreated fish during the experimental period.

3.3.2. Mucous cell concentration

The effect of exogenous steroids on the superficial mucous cell concentration was varied. No clear pattern emerged from the quantitative studies in the head, dorsal and caudal peduncle areas. In most of the areas examined, the steroids did not alter the number of goblet cells, but Fig. 28 shows that oestradiol ($p \leq 0.001$) and cortisone ($p \leq 0.01$) treated groups had significantly fewer cells in the head region and the cortisone ($p \leq 0.05$) and methyltestosterone ($p \leq 0.01$) groups had less apocrine cells in the dorsum than the control group.

In the pectoral area both the oestradiol ($p \leq 0.05$) and cortisol ($p \leq 0.05$) groups had significantly more mucous cells than the control group. Cortisol induced a highly significant increase in the number of apocrine cells in the dorsal fin ($p \leq 0.01$), pectoral fin ($p \leq 0.01$) and tail ($p \leq 0.001$). The other steroids did not markedly alter the mucous cell concentration in the fin areas, except for the pectoral fin of the testosterone experimental group which had a significantly greater concentration of goblet cells ($p \leq 0.001$) than the controls.

3.3.3. Mucoid histochemistry

The results of the muco-histochemical staining precedures are presented in Tables 3.3.7, 3.3.8, 3.3.9, 3.3.10, 3.3.11, 3.3.12 and 3.3.13. The mucous cells reacted intensely with alcian blue (pH 2.5) and colloidal iron and were not stained with alcian blue (pH 1.0). Mild methylation had no effect on alcianophilia but active methylation inhibited staining with alcian blue. All the mucous cells were refractory to aldehyde fuchsin and when this stain was used in combination with alcian blue, they were stained blue which indicated that the mucus secretion was non-sulphated. This was supported by the weak staining reaction of the apocrine cells with alcian blue in increasing strengths of $MgCl_2$ solutions. Hyaluronidase only slightly decreased alcianophilia in a few areas which suggested that hyaluronic acid did not form a major component of O+ parr mucus. Neuraminidase treatment blocked alcianophilia but did not prevent periodate reactivity. The proportions of periodate reactive alcianophilic, periodate unreactive alcianophilic and periodate reactive cells after the combined alcian blue/PAS and colloidal iron/PAS techniques varied with the experimental group and with the area of the body examined.

When the AB (pH 2.5)/PAS technique was used, all the areas of the cortisol (with the exception of

the pectoral fin) and oestrogen experimental groups had fewer periodate unreactive alcianophilic (blue) cells than the control group. The pectoral fin and tail of the methyltestosterone and testosterone experimental groups, the tail of the cortisone group and the caudal peduncle of the testosterone and cortisone groups also had fewer blue cells than the control group. The other areas examined had the same number of periodate unreactive alcianophilic cells as the control group. The pectoral area of the cortisone and testosterone experimental groups, the caudal peduncle of the oestrogen and testosterone groups and the pectoral fin in the cortisol group had similar amounts of periodate reactive alcianophilic (blue/purple) cells as the control group. The caudal peduncle of the methyltestosterone group had fewer blue/purple cells, whereas all the other areas exhibited an increase in the number of blue/purple cells over the control group. All the exogenous steroids increased the number of neutral polysaccharide mucous cells in the dorsum and caudal peduncle and decreased them in the pectoral fin areas.

The results of the colloidal iron/PAS staining procedure show that there was a decrease in the amount of cells containing neutral polysaccharides and an increase in the blue/purple cells during the experimental period. The staining pattern of the steroid treated experimental fish was more or less similar to the control group.

The majority of cells in all experimental groups stained by AB (pH 1.0)/PAS contained non-sulphated acidic mucins and stained red or red with a purple tinge.

3.3.4. Interrenal

Administration of exogenous steroids had an effect on interrenal activity as shown by Table 3.3.14. Testosterone ($p \leq 0.01$), methyltestosterone ($p \leq 0.05$) and cortisone ($p \leq 0.001$) induced a significant increase in the nuclear diameter of the interrenal cells. Treatment with cortisol and oestrogen produced a decrease and an increase in the size of the interrenal nuclei respectively but these changes were not statistically (5% level) different from the control group.

3.3.5. Saprolegniasis

Table 3.3.15 lists the mortalities due to Saprolegnia amongst the 0+ parr during the experimental period. Oestrogen and cortisol were the only treatment groups that were infected with Saprolegnia and had 57.1% and 37.1% mortalities respectively. All the infected fish were parasitised in either the

caudal peduncle, tail regions or the pectoral & pectoral fin areas. Table 3.3.16 illustrates that a definite pattern of infection existed and that the oestrogen treated fish were more frequently parasitised in the pectoral and pectoral fin region ($p \leq 0.025$) than were the cortisol group.

3.4. Discussion

3.4.1 Skin

The hyperplastic changes in the epidermis and dermis of the androgen treated parr reported here agree with the studies of Idler et al (1961 b), McBride and van Overbeeke (1971), Yamazaki (1972), and McBride and Fagerlund (1973). Although 17 α methyltestosterone and testosterone are not the major androgens in sexually mature salmonids (Idler et al, 1961 a; 1964), the results of this study provide evidence that the increase in skin thickness of precocious males (Section 2.3.8) may be due to gonadal hormones.

None of the other steroids markedly affected the integument. This is in contrast to the results of McBride and van Overbeeke (1971) who found that oestradiol increased the skin thickness of gonadectomised adult female O. nerka. It appears from this study that oestrogens do not affect the skin of Atlantic salmon parr. Yamazaki (1972) suggested that gonadal androgens were the main cause for the changes in the skin of spawning adult salmonids and that the reason for the sexual dimorphism was the difference between the sexes in the plasma levels of androgens.

There was also an increase in the epidermal and dermal thickness in the untreated control fishes during the experimental period. This could have been due to seasonal fluctuation in the skin dimensions as an increase in the skin thickness was noted amongst the immature 1+ female parr in the previous section (see Section 2.3.8).

3.4.2 Mucous cell studies

The distribution of mucous cells in 0+ parr was similar to that described for 1+ parr and the greatest concentration was in the dorsal fin, pectoral fin and tail regions (see Section 2.3.8). No clear pattern emerged as to the effect of exogenous steroids on the mucous cell concentration in the dorsum, pectoral and caudal peduncle areas. Pickering and Macey (1977) found that the stress of handling increased the number of superficial apocrine cells. The results reported here would support this as cortisol significantly increased the number of goblet cells in the dorsal fin, pectoral fin and tail regions (see Fig. 28).

There are conflicting reports concerning the effects of sexual maturity and the administration of androgens and oestrogens on the superficial mucous cell concentration in salmonids. McBride and van Overbeeke (1971) and Yamazaki (1972)

have described a large increase in the numbers and size of goblet cells in the skin of oestrogen and androgen treated Pacific salmon. Pickering (1977), on the other hand, found that there was a significant fall in the concentration of mucous cells in normal sexually mature adult brown trout. In this experiment the number of apocrine cells in the methyltestosterone, testosterone and oestrogen groups did not differ significantly from the control group.

There was a significant increase in mucus secretion amongst the control fishes during the experimental period. This could have been due to either a seasonal fluctuation in mucous cell numbers or to the initial handling stress. The increase in the superficial goblet cell concentration after a single handling only lasts for one month (Pickering and Macey, 1977), so the increase reported here after 14 weeks was probably due to a seasonal cycle, similar to that described in 1+ parr (see Section 2.3.8). The mucus secretion in O+ parr was histochemically similar to that of 1+ parr. It contained mostly non-sulphated acidic mucins with a sialic acid component and a little hyaluronic acid and some neutral polysaccharides. In general, the mucohistochemistry of the different experimental groups, with a few exceptions, was similar to the control group. Administration of the exogenous steroids did not affect the overall composition of mucus but in some treatment groups the proportion of the various components differed

especially after the AB/PAS procedures. Cortisol caused a decrease in the number of periodate unreactive alcianophilic and an increase in the periodate reactive alcianophilic cells.

Comparison of the staining reaction of the mucous cells of 0+ parr, fed different steroids, with the comparable results from 1+ parr, showed that there may be a relationship between the hormonal status of the animal and the chemical composition of the mucus. The staining reaction of precocious males in October (Table 2.3.18) after the AB (pH 2.5)/PAS combination resembled that of the androgen treated groups (Tables 3.3.10 and 3.3.11). In December the mucohistochemistry (AB (pH 2.5)/PAS) of ripe males (Table 2.3.20) was similar to the cortisol experimental group (Table 3.3.13). No obvious relationship existed between the results of immature 1+ parr (AB (pH 2.5)/PAS) and the different steroids and most of the areas examined had the same pattern (Table 2.3.19) as the 0+ control group at 0 and 14 weeks. The only exceptions to the generalisation that the histochemistry of 1+ immature parr resembled the 0+ control group were the tail and to a lesser extent the pectoral fin of 1+ parr examined in December (Table 2.3.19). These were the only two areas that had a reaction similar to the cortisol experimental group (Table 3.3.13).

The staining reaction of the mucous cells in the control and steroid treated 0+ parr resembled that of the precocious male and immature 1+ parr after the colloidal iron/PAS procedure. The greater proportion of PAS + ve neutral polysaccharides in the pectoral fin area of the precocious males may be due to enhanced androgen secretion as both testosterone and methyltestosterone increased the number of red cells in the pectoral fins of 0+ parr.

The results of this study show that androgens, oestrogens and corticosteroids have an effect on production and histochemistry of mucus in salmon parr. Cortisol was the most active physiologically as it had an affect on the quantity as well as the chemical composition of mucous cells.

3.4.3 Interrenal

With the exception of cortisol all the steroids used in this study increased the nuclear diameter of the interrenal cells which probably resulted in enhanced corticosteroid production. This could be the normal physiological effect of androgens and oestrogens on the adrenal, or it

could be due to the pharmacological effects of the relatively high levels of steroids used in this study. There is evidence to suggest that gonadal hormones do have an effect on cortisol secretion. Donaldson and Fagerlund (1970) found that in O. nerka the basal cortisol concentration increased during sexual maturation but decreased immediately after gonadectomy. Van Overbeeke and McBride (1971) mentioned that both androgens and oestrogens caused interrenal hypertrophy in gonadectomised Pacific salmon. The observation reported here suggests that these steroids react in a similar manner in Atlantic salmon parr and in the previous section (Table 2.3.6) it was shown that precocious males had significantly higher plasma cortisol levels than immature parr.

Cortisol induced a degree of atrophy in the adrenocortical tissue as would be expected but there was no evidence of the degeneration and pyknosis that occurred in rainbow trout and Pacific salmon (Robertson et al.,1963; van Overbeeke and McBride, 1971).

3.4.4. Saprolegniasis

Previous workers have shown that administration of exogenous corticosteroids to teleost fishes

increased their susceptibility to fungal infections (Robertson et al., 1963; Roth, 1972). Neish (1977) suggested that there is a positive correlation between the incidence of Saprolegniasis and increased plasma corticosteroids. The results of this study confirm this suggestion (Table 3.3.16). The high mortalities due to Saprolegnia in the oestradiol-treated group have not been reported before (Table 3.3.15). One possible explanation for this increased prevalence of fungal infection is the increased adrenal activity. This may not be plausible as all the other experimental groups were not infected and they also exhibited an increased interrenal nuclear diameter. Johnstone et al. (1978) have reported that oestradiol-treated rainbow trout had a slower growth rate and were more susceptible to adverse environmental conditions than the controls.

In both affected groups the areas most frequently parasitised with fungus had the greatest concentration of mucous cells (Table 3.3.16 and Fig. 28). This contradicts the general consensus that mucus protects the fish from infection. Richards and Pickering (1978) suggested that the reason why male brown trout are more often infected with Saprolegnia than females is that there is a reduction in the superficial epidermal goblet cell concentration in the males.

Cortisol caused changes in the histochemical staining reaction of the mucous cell secretion (Table 3.3.13). Oestradiol treated fish also exhibited a similar muco-histochemical reaction in the dorsum, caudal peduncle and pectoral areas and it is reasonable to assume that these changes were consistent throughout the whole body (Table 3.3.9). These differences in the staining pattern could be due to changes in the chemical composition of mucus and it is possible that these changes could enhance fungal growth. Willoughby (1971) has reported that fish mucus contains all the elements of an ideal growth medium for Saprolegnia.

CHAPTER 4

CONTROL OF PRECOCIOUS SEXUAL MATURITY IN ATLANTIC SALMON PARR USING CHEMOSTERILANT AGENTS

4.1. Introduction

The control and manipulation of sex has become a very important part of fisheries biology. The benefits of controlling reproduction in fishes are obvious. Sex manipulation can help in the formation of sterile fishes to prevent indiscriminate breeding which would cause stunting due to over-population, or decimation of other species. Prevention of precocious maturity in intensively reared salmonid and Tilapia sp. allows for the channelling of the energy directed towards gonad development into protein formation and increased growth rate. Precocious sexual maturity has been induced in salmon in an attempt to produce a wild salmon run in rivers of the Canadian Pacific coast in the years when they do not occur naturally (Donaldson, 1973).

Schreck (1974) said that 'the sex and reproduction of fishes can potentially be artificially manipulated by affecting any link in the sequences of events controlling the reproductive process'. Manipulation of the environment has been shown to be very effective in regulating the reproductive cycles of fishes (de Vlaming, 1972), but it is not feasible in the

majority of fish farm units. Most of the research effort has been directed towards the production of unisex populations. One of the ways in which this is achieved is by gynogenesis whereby sperm is attenuated by irradiation so that it is able to penetrate the egg but the genetic material cannot enter the karyocyte. They are then given either a hot or cold shock to produce viable diploid eggs. Normal fertilised eggs have also been treated with radiation to alter the sex ratio. Both these techniques produce a greater proportion of females but have the disadvantage of high mortalities.

Oral administration of androgens or oestrogens have been used during the period of gonad differentiation to successfully produce unisex populations. Genetic males can be induced to become functional females by treatment with oestrogenic compounds and genetic females can be turned into functional males by administration of androgenic compounds. This technique has the disadvantage of producing a proportion of fish that are hermaphrodite (Jalabert, Billard and Chevassus, 1975; Johnstone et al, 1978). These sex reversed individuals can develop into functional fertile adults and it has been suggested that they could sire unisexual broods i.e. homogametic females which have been changed into functional homogametic males can be bred with normal homogametic females to produce an all female population. Similarly, oestrogens can be employed in species where the male is homogametic to produce an all male population.

Laird, Ellis, Wilson and Holliday (1978) have developed a technique which utilizes an auto-immune rejection of the testes

to prevent precocious sexual maturity. Young fish (first feeders) are vaccinated with a suspension of male gonad and the antibodies that are raised are sex and organ specific and thus prevent testicular development.

Chemosterilisation has been used with success to manipulate reproduction in domesticated animals and theoretically these chemicals could be used in hatchery-reared fish. The advantage of these agents is that they would be used on older fish and thus avoid the hazard of feeding drugs during the highly vulnerable first feeding stage of the fishes' life cycle. The effects of the majority of these drugs are reversible once treatment is terminated and so there is no need for special rearing regimes for brood stocks.

Methallibure (Fig. 29b) which selectively inhibits gonadotrophic cell function (Paget et al., 1961) has been used extensively in mammals and fishes as a chemosterilant. Amongst fishes, the majority of workers have concentrated on the effects of methallibure on the reproductive physiology. Methallibure has been shown to block spermatogenesis in C. auratus, G. aculeatus, C. aggregata (Hoar et al., 1967), C. aggregata (Wiebe, 1968), P. reticulata (Martin and Bromage, 1970; Pandey and Leatherland, 1970), Tilapia sp. (Hyder, 1972) and P. latipinna (van den Hurk and Testerink, 1975). It has also been shown by some of the above mentioned authors to prevent the formation of secondary sex characteristics, to lower steroidogenic enzyme activity and to cause degenerative changes in the Leydig cells. The report of Dadzie (1975) is the only one where methallibure was

administered to fish reared in normal commercial hatchery conditions. He showed that it prevented sexual maturity in Tilapia sp. and that the treated fish had a better growth rate than the controls.

Anti-androgen compounds have been used successfully to prevent reproduction in laboratory animals. They have also been used clinically in man, to prevent hypersexuality, idiopathic precocious puberty in children, hirsutism in adult females and on sexual deviants (Neumann and Steinbeck, 1975). The mechanism of action of these compounds is that they compete with androgens at the androgen receptor sites wherever these receptors are located in the body. The most potent anti-androgenic compound is the steroid cyproterone acetate (Fig. 29a). It competes with dihydroxytestosterone (DHT), the major metabolite of testosterone, for the cytoplasmic and nuclear binding proteins, thereby lowering DNA activity in the target sites (Kenjiand Kano, 1972; Geller and McCoy, 1974; Neumann and Steinbeck, 1975). Cyproterone acetate does not affect the 5α reduction metabolism of testosterone to DHT (Geller and McCoy, 1974).

There are conflicting reports regarding its affects on plasma testosterone levels. Geller, van Damne, Garabieta, Loh, Rettura and Seifter (1969) and Rajalakshmi and Prasad (1976) reported that cyproterone acetate did not reduce circulating levels of plasma testosterone. Other workers, Stern and Eisenfield (1971); Brotherton (1974); Brotherton and Barnard (1974); Neumann and Steinbeck (1975); Murray, Bancroft, Anderson, Tennet and Carr (1975); Koch, Lorenz, Danehl, Ericsson, Hasan, von Keyserlingk, Luebe, Mehring and Roemmler (1976) have shown

that plasma testosterone levels were significantly lower in men treated with cyproterone acetate than in the controls. Cyproterone acetate has anti-spermatogenic effects, in addition to its antiandrogenic action (Neumann and Steinbeck, 1975). Other workers have reported that it can inhibit spermatogenesis and cause the formation of large numbers of abnormal sperm (Brotherton and Barnard, 1974; Koch et al 1976). Cyproterone acetate has the same effect as castration and produces degenerative changes in the accessory sexual glands of laboratory animals (Neumann, van Bersworth-Wallrabe; Elger, Steinbeck and Hahn, 1969; Neumann and Steinbeck, 1975; Djoseland, 1976; Rajalakshmi and Prasad, 1976; Jones, 1977). It is also a potent gestagen and so does not markedly affect gonadotrophin release (Brotherton, 1974; Neumann and Steinbeck, 1975).

There have been only a few studies on the effects of cyproterone acetate on lower vertebrates and some of the evidence is conflicting. Della Corte, Angelini, Galgano and Marinucci (1973) showed that in the newt Triturus cristatus connifer Laur. cyproterone acetate treatment produced a loss of secondary sex characteristics and that gonadotrophic cells in the pars distalis had the appearance of a castrated animal. Chieffi, Iela and Rastogi (1974) found that cyproterone acetate had a masculinisation effect on the embryonic gonads of the frog Rana esculenta L. Rastogi and Chieffi (1975) reported that in the adult frog, it had a central and a peripheral antiandrogenic effect,

it did not lower plasma testosterone levels and it appeared to compete with the androgens for the receptor molecules at the target cells just as in mammalian species. Schreck (1973) showed that cyproterone acetate decreased plasma levels and blocked the uptake in the testes of ^{3}H testosterone in rainbow trout. Rouse, Coppenger and Barnes (1977) reported that cyproterone acetate caused a reduction in the aggressive courtship behaviour, regression of the nuptial colouration and inhibition of the later stages (spermatids and spermatozoa) of spermatogenesis in the stickleback. On the other hand, Rastogi and Chieffi (1975) found that cyproterone acetate did not prevent the masculinisation effects of exogenous testosterone propionate and 11-ketotestosterone in the swordfish Xiphophorus helleri Heckel.

This report describes the effects of methallibure and cyproterone acetate on the reproductive cycle in precocious male salmon parr. The aim of the experiment was to compare the efficiency of these chemical agents in preventing sexual maturity in 1+ parr and to see whether either could be used in future experiments designed to assess their potential in a commercial fish farming situation. Studies on the effects of these drugs on the pituitary, skin, plasma androgen and corticosteroids of both immature and mature parr are also reported.

4.2. Materials and Methods

4.2.1. Animals

4.2.1 (a) Experiment A

154 1+ salmon parr (sex unknown) of known parentage (Salmon Research Trust Annual Report 1975) were selected at random from the hatchery population during early October, 1976. 24 fish were killed immediately (12 immature females and 12 developing males) and used as initial controls. The remaining 135 were separated into three groups of 45. During the first two weeks of the experiment, a number of parr escaped with the result that only 41 fish of the control group, 33 of the methallibure group and 42 of the cyproterone acetate group remained in the tanks when precautions were taken to prevent further losses. The fishes were kept in 0.6m^2 fibre glass tanks and held at a stocking density of 1.0 kg/m^2 and a water flow rate of 22.7 dm^3/min.

4.2.1 (b) Experiment B

During March, 1978, 340 parr (sex unknown) were selected at random from the 0+ population of the previous year. 40 fish were killed and used

as initial controls. The rest were divided into three groups of 100 parr. One group acted as controls and the other two were treated with cyproterone acetate and methallibure respectively. They were held in 0.6m^2 fibre glass tanks at a stocking density of 1.3 kg/m^2 and a water flow rate of 22.7 dm^3/min.

4.2.2. Drugs

Methallibure (1$^{\alpha}$methylallyl-thiocarbamoyl-2-methyl-thiocarbamoylhydrazine) was kindly supplied by Dr. Walpole of I.C.I. (U.K.). Cyproterone acetate (1, 2α methylene-6-chlore-17-hydroxy-4-6-pregnadine-3; 20-dione-17-acetate) was donated by Dr. Clemens of Schering A.G. (G.F.R.). Both drugs were administered in a diet at a dosage rate of 250ppm.

4.2.3. Diet

The basic diet of the 1+ parr in experiment A was No. 4 pellets (B.P. Nutrition, U.K.). In experiment B (0+/1+ parr) No. 2 pellets (B.P. Nutrition, U.K.) were used initially and as the fish grew bigger the diet was changed to the larger No. 3 pellets (B.P. Nutrition, U.K.). The drugs were incorporated into the pellets in the following manner: the appropriate amount of methallibure and cyproterone acetate was dissolved in 20ml of ethanol. The ethanol solution was then thoroughly mixed with the fish pellets to ensure dispersion of the chemical agents through the food. The control diet was prepared in identical manner except for the exclusion of the chemosterilant agents.

4.2.4. Experimental regime

Experiment A lasted for 14 weeks and was carried out during the period 10th October to 30th December, 1976.

Experiment B was started on 13th March, 1978. The fishes were sampled in mid-August after 22 weeks of treatment. In order to obtain information on the effect of drug withdrawal on the subsequent gonad development, 30 fish in each treatment group and 40 fish in the control group were retained and fed the control ration. After 20 weeks (late December), the remaining experimental fish were sacrificed and examined.

The diets were prepared fresh each week, and the fish were fed four times a day during the week and once a day on Saturday and Sunday. Uneaten food, faeces, dead and moribund fishes were removed from the experimental tanks each day. The presence of external lesions such as fungal infection, eye loss, etc. on the dead fish was recorded.

In both experiments the methods used for capturing, anaesthetising the fish and collecting plasma, etc. were similar to those described in Section 2.2.

4.2.5. Histological techniques

Samples of skin, visceral organs, kidneys, gonads and pituitaries were collected from the 1+ parr in experiment A for histological studies. These were all fixed in 10% buffered formalin. $1cm^2$ pieces of skin were also collected and fixed in 5% formaldehyde in 0.6% Nacl. for quantitative studies of superficial mucous cell concentration. In experiment B histological examination was only performed on the gonads. These were fixed in either 10% buffered formalin or B.H.S.

The processing, staining and measuring procedures used for routine histological, mucohistochemical, and mucous cell quantitative studies have been described in section 2.2. Mucoid histochemical studies were carried out on five fish from each treatment group.

4.2.6. Radioimmunoassay

Plasma samples from the experiment A fish were assayed for cortisol and 11-ketotestosterone. These assay procedures have been reported in Section 2.2.

4.2.7. Statistics

Where appropriate, an analysis of variance (Campbell, 1967) was used to compare the population means.

Chi-squared analysis of the total numbers of Saprolegnia infected and non-infected fish from the different treatment groups were performed using a 2 x 2 contingency table.

4.3. Results

4.3.1 Experiment A

4.3.1 (a) General

Administration of methallibure and cyproterone acetate had no adverse effects on the intestines, liver, spleen and kidney of 1+ salmon parr. There were no marked differences between length, weight and condition factor of the treated fishes and the untreated controls (Table 4.3.1). No data were kept of the amount of food fed daily, but it appears from table 4.3.1 that the fish were only on a maintenance level as they did not increase in length or gain weight during the experimental period.

4.3.1. (b) Gonads

Examination of the testes of developing male parr at the beginning of the experiment showed spermatogenesis to be well advanced. Primary and secondary spermatocytes were present in many of the lobules but the majority of cysts contained spermatid and spermatozoa (Plate 27). By December, all the males in the control group were fully mature and all the lobules contained spermatozoa on microscopic examination (Plate 6). There was no difference in the testes of male parr treated with

methallibure and cyproterone acetate and all the lobules were at the mature spermatozoa stage (Plate 28). Table 4.3.2 illustrates that both these chemical agents markedly reduced circulating plasma 11-ketotestosterone levels in precocious males and that the GSI of both treatment groups was greater than the controls and in one case (cyproterone acetate), this was statistically significant ($p \leq 0.05$).

There was no statistical significance in the differences of the GSI and plasma androgen levels in the experimental immature parr. There was also no difference in the histological structure of the ovaries of the three groups as they were all in the early stages of oogenesis and contained oogonia, early and late perinucleolus stages.

4.3.1. (c) Interrenal

Fig. 30 illustrates that administration of methallibure and cyproterone acetate to 1+ parr did not affect the trend towards a greater nuclear diameter in the interrenal cells among precocious males compared with immature parr. Both precocious male experimental groups had a greater plasma cortisol level than the treated immature parr as shown in Table 4.3.3 but this difference was not statistically significant. These chemical agents also induced a slight but not statistically significant increase in corticosteroid secretion in both precocious males and immature parr.

4.3.1. (d) Pituitary

The staining reaction of the different cell types in the pituitaries of the untreated precocious males has been described in Section 2.3.7. The distribution and the staining reaction of the basophils, acidophils, chromophobes and undifferentiated cells in the pituitaries of precocious males fed methallibure and cyproterone acetate were the same as the untreated controls. Basophils were present in the pro and meso-adenohypophysis of the two treatment groups. In the pro-adenohypophysis they were present amongst the chromophobic layer separating the pro-adenohypophysis from the neurohypophysis. They were also scattered in the connective tissue between the acidophilic follicles and on the ventral border of the pro-adenohypophysis and meso-adenohypophysis. In the meso-adenohypophysis of the methallibure and cyproterone acetate groups as in the control fishes the basophils were most abundant along the lateral edges and in the rostro-ventral border. Individual basophils were also scattered amongst the somatotropes and undifferentiated cells in the fingerlike processes of the meso-adenohypophysis. All the basophils exhibited a strong affinity for the basophilic dyes. The number of PAS + ve basophils in the pituitaries of fish fed these chemical agents was similar to the untreated precocious males but as Table 4.3.4 shows they were significantly smaller. There were no changes in the amount or staining reaction of the NSM in the neurohypophysis of the two treatment groups.

4.3.1 (e) Skin

Epidermis

Methallibure and cyproterone acetate prevented some of the changes that occur in the skin during the later stages of spermatogenesis. Table 4.3.5 and Fig. 31 show that hyperplastic changes did not take place in the epidermis of the dorsum, pectoral and caudal peduncle areas of both treatment groups of precocious males. These chemical agents did not affect the skin changes in the pectoral fin and tail regions of precocious males (Table 4.3.8 and Fig. 31.) Tables 4.3.6, 4.3.7 and Fig. 31 illustrate that methallibure and cyproterone acetate increased the epidermal thickness of immature parr over the untreated controls and in three areas this was statistically significant, dorsum ($p \leq 0.05$) and caudal peduncle ($p \leq 0.01$) of the cyproterone acetate group and the pectoral fin in both the methallibure ($p \leq 0.001$) and the cyproterone acetate ($p \leq 0.05$) groups. The increase in the epidermal thickness of immature parr receiving these chemical agents was not accompanied by an increase in the number of cell layers in the epidermis. Figs 31 and 32 show that in general, the skin thickness of these treated immature parr was similar to that of the treated precocious males.

4.3.1 (f) Mucous cell concentration

Fig. 33 shows that methallibure caused a slight increase in the concentration of mucous cells in both precocious

and immature parr. This increase was statistically significant in the dorsum and pectoral fin areas of immature females (Fig. 33). Cyproterone acetate, on the other hand, markedly increased the apocrine cell concentration in both groups of 1+ parr and this was significantly different from the controls in the majority of areas examined (Fig. 33).

4.3.1 (g) Mucoid histochemistry

Methallibure and cyproterone acetate did not markedly change the staining reaction of the carbohydrate moiety of mucus in 1+ salmon parr. There were some differences between the treated and the control fishes (Tables 2.3.19 and 2.3.20) after the alcian blue/PAS and colloidal iron/PAS combination procedures were used.

Amongst the precocious male parr the one consistent feature after the alcian blue (pH 2.5)/PAS method was that there were no red cells containing neutral polysaccharides in the skin of the methallibure treated group. There were also some differences in the proportion of periodate non-reactive alcianophilic (blue) and periodate reactive alcianophilic (blue/purple) cells in the methallibure experimental group and the untreated controls (Tables 2.3.20 and 4.3.11). Methallibure induced an increase in the amount of blue cells in the pectoral and caudal peduncle areas and a decrease in the dorsum, pectoral and tail regions. There was also a fall in the number of blue/purple cells in the pectoral and caudal peduncle areas of

the methallibure group. Except for the absence of red cells, the proportion of the different staining cells in the caudal peduncle of the methallibure group was similar to the control group at 0 weeks (Table 2.3.18 and 4.3.11). The staining pattern of precocious males treated with cyproterone acetate after the AB (pH 2.5)/PAS combination was more or less similar to the control group (Tables 2.3.20 and 4.3.9).

Amongst the immature 1+ parr these drugs had the effect of decreasing the amount of periodate unreactive alcianophilic and increasing the number of periodate reactive alcianophilic cells in the dorsum, pectoral, caudal peduncle and pectoral fin areas when the AB (pH 2.5)/PAS technique was used. There was an increase in the number of red cells in the caudal peduncle of cyproterone acetate experimental group. In all the other areas examined both methallibure and cyproterone acetate decreased the number of red cells.

In all but two areas of the precocious males treated with methallibure and cyproterone acetate, there was an increase in blue/purple and a decrease in blue cells after the colloidal iron/PAS combination. The two exceptions were the caudal peduncle of the methallibure group and the pectoral fin of the cyproterone acetate group. In both groups of experimental ripe male parr the pectoral fin was the only region that had a substantial amount of cells with neutral polysaccharides, all the other areas had no red cells. The

staining pattern of both experimental groups of immature parr after the colloidal iron/PAS procedure was similar to the immature controls except that these chemical agents brought about a slight decrease in the neutral polysaccharide cells in most regions except the tail (Tables 2.3.19, 4.3.10 and 4.3.12). Tables 4.3.9, 4.3.10, 4.3.11, 4.3.12, 2.3.19 and 2.3.20 show that the reaction of the mucous cells in the four experimental groups to the rest of the staining procedures was similar to the control groups. Mild methylation did not markedly reduce alcianophilia whereas active methylation did. There were no sulphated mucins in the mucus secretion of these fish as the cells were AF-ve and the alcian blue/safranin combination stained them weakly blue and increasing ionic strength solutions of alcian blue markedly reduced alcianophilia. There were no blue or blue/purple staining cells after the AB (pH 1.0)/PAS procedure and all the mucous cells were stained either red or red/purple. Large amounts of sialic acid were present in the mucous cells of the treated precocious males as neuraminidase prevented alcianophilia but did not inhibit the PAS reaction.

4.3.1 (h) Saprolegniasis

Table 4.3.13 shows that both chemical agents significantly reduced the prevalence of Saprolegnia infection in precocious male parr. Methallibure appeared to be the most effective in this regard. Although there were a small number of immature parr amongst the control fish, the untreated precocious males were more frequently parasitised than the

immature fish. The areas most commonly affected were the tail, caudal peduncle, pectoral and pectoral fin.

4.3.1. (i) Summary

Table 4.3.14 summarises the major changes that take place in precocious males after treatment with methallibure and cyproterone acetate.

4.3.2. Experiment B

When the initial control group was sampled in March all the testes were immature and histological examination showed them to be at an early stage of testicular development with solitary spermatogonia and a number of small spermatogonial cysts (Plate 3).

In August, 49 parr were selected at random from the control group and of these, 42.9% were immature females and 57.1% were males with developing gonads. The testes were large white paired masses which filled about half the abdominal cavity. Histological examination of the testes from 10 individuals showed them to contain secondary spermatogonia, primary and secondary spermatocytes and spermatids (Plate 5). 56 parr were sampled from the methallibure experimental group and of these 42.8% were immature females and 57.2% were immature males. The gonads of the immature males were brown thread-like bodies running the full length of the abdominal cavity and microscopic

examination of 10 individuals showed that primary spermatogonia were the only germ cells present (Plate 29). 50 fish were also selected from the cyproterone acetate group and 42% had immature ovaries. Amongst the males there were two distinct groups; 34% of the males had immature gonads and 24% had developing testes. All the immature testes contained only primary spermatogonia whereas spermatogenesis was well advanced in the developing males where primary and secondary spermatogonia as well as spermatocytes were present with many of the lobules also containing spermatids (Plate 30).

The GSI and condition factor of the female and male experimental parr are shown in Table 4.3.15. The GSI of the developing males in the untreated control and cyproterone acetate groups were significantly larger than the immature males of the methallibure and cyproterone acetate groups (Table 4.3.15). This reflected the advanced stage of spermatogenesis present in the two former experimental groups. The ovaries of all three experimental groups were in an early stage of oogenesis and contained oogonia, early and late perinucleolus stages and there was no difference in the GSI of the untreated control females and the females fed methallibure and cyproterone acetate.

Table 4.3.15 also shows that administration of methallibure and cyproterone acetate to 1+ parr during the first half of the year slowed the growth rate. This decrease in the condition factor of the experimental parr was significantly different from the controls in three treatment groups (Table 4.3.15).

After sampling in August, the remaining fishes were fed the control diet for a further 20 weeks and no adverse effects were seen on withdrawal of the chemical agents. In December, all the surviving parr were sacrificed. The gonadosomatic indices and condition factors of the three experimental groups are shown in Table 4.3.16. 40 untreated control parr were examined; of these 47.5% were immature females and 52.5% were ripe sexually mature males. Examination of the male gonads showed that all the lobules contained fully mature spermatozoa. There were 27 survivors in the methallibure experimental group and 19 (70.4%) were immature females and 8 (29.6%) were immature males. Primary spermatogonia were the only germ cell stages present in the immature testes of these male parr. Only two fish in the cyproterone acetate experimental tank survived until December because of an accident due to a blocked inlet pipe in September. Of these two survivors, one was an immature female and the other was a fully mature precocious male, with all the lobules containing mature spermatozoa.

Mortalities, except for those arising from the blocked inlet pipe in September, were confined to the first few months. All the dead fish exhibited unilateral eye-loss which is thought to be a consequence of over-crowding. There was no evidence of Saprolegnia infection during the latter half of the experiment i.e. October to December.

Plasma 11-ketotestosterone levels in the experimental B fish were measured, but because of contamination of the solvents and failure of the scintillating counter, it was considered that the results were unreliable. Histological samples of the skin, interrenal and pituitary were also collected from these fish but they were not processed because of a lack of time.

4.4. Discussion

4.4.1. Effect of methallibure and cyproterone acetate on spermatogenesis, plasma androgen levels and gonadotrophic cells.

Methallibure and cyproterone acetate have two different modes of action on the reproductive system. Methallibure is a central inhibitor and prevents the synthesis and/or release of gonadotrophin, whereas cyproterone acetate is a true antiandrogen and competes with the androgens at the receptor sites within the target organs. Normally hypothalamus/gonadal feedback is held in balance by the anti-gonadotrophic activity of gonadal androgens, thus antiandrogens by preventing access of androgens to the neural receptor sites induce overstimulation of the gonadotrophic cells. In mammals, cyproterone acetate has a strong progestation effect due to the esterification of the 17 hydroxyl group (Neumann and Steinbeck, 1975), and this prevents oversecretion of gonadotrophin.

Morse, Leach, Rowley and Heller (1973) found no change in the plasma and urinary ICSH and FSH after treating normal men with cyproterone acetate. Johnson and Naqvi (1969) reported that

cyproterone acetate prevented the release of LH and thus increased the amount of this gonadotrophin in the pituitary. Lakshman and Issac (1973) showed that cyproterone acetate did not affect the size of gonadotrophic cells in male rats. Treatment of castrated catfish with cyproterone acetate did not produce any change in the number or activity of PAS + ve basophils in the meso-adenohypophysis (Sundararaj and Nayyar, 1969). It would have been difficult to notice any stimulatory action by cyproterone acetate on the neural/gonadal axis of these catfish as the gonadotrophic cells were already maximally stimulated due to castration. The observation reported here from experiment A suggests that cyproterone acetate inhibits the synthesis rather than the release of gonadotrophin in ripe salmon parr, as the basophilic cells of the meso-adenohypophysis were significantly smaller than the untreated controls.

The present investigation also showed that methallibure decreased gonadotrophic cell size in male salmon parr. This result agrees with Pandey and Leatherland (1970); van den Hurk and van de Kant (1975) and van den Hurk and Testerink (1975), who found that in the guppy and black molly methallibure blocked granulation of the gonadotropes and that the nuclear diameter of these basophilic cells were significantly smaller than the controls. Leatherland (1969) reported that methallibure caused a depletion of stainable and electron-dense granules in the gonadotrophic cells of <u>C. aggregata</u> and he suggested that methallibure blocked the synthesis of gonadotrophin. Singh, Raizada

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and Singh (1977) found that pituitary extracts of methallibure-treated catfish had a very low content of gonadotrophin and failed to induce uptake of ^{32}P in the ovary of hypophysectomised recipient fish.

The transformation of secondary spermatogonia to primary spermatocytes is gonadotrophin dependent but the earlier and later stages of spermatogenesis are not (Barr, 1963; Ahsan, 1966; Sundararaj and Nayyar, 1967; Pandey, 1969). Methallibure arrests spermatogenesis by inhibiting the differentiation of spermatogonia into spermatocytes but does not interfere with spermatogonial mitosis or the development of the spermatocytes (Hoar et al., 1967; Wiebe, 1968, 1969; Leatherland, 1969, Pandey and Leatherland, 1970; Martin and Bromage, 1970; Hyder, 1972; Hyder et al., 1974; van den Hurk and van de Kant, 1975). Both these chemical agents failed to stop spermatogenesis when fed during the last three months of the gonad maturation cycle of male salmon parr. This was due to the experiment being started in October when all the germ cells were either at or past the spermatogenic/spermatocyte transformation stage and so did not need any further pituitary stimulation. The slightly higher GSI in both methallibure and cyproterone acetate treated precocious males may have been due to the decrease in gonadotrophin secretion. This probably slowed down spermiation and absorption of the germ cells. Yamazaki and Donaldson (1968) have reported that gonadotrophin is necessary for spermiation in some fishes.

Both methallibure and cyproterone acetate treatment groups exhibited a decrease in plasma 11-ketotestosterone levels during the later stages of spermatogenesis. This is the first report that provides direct evidence that decreased gonadotrophic cell activity due to methallibure administration lowers circulating androgens in fish. Until now, the evidence has been indirect as methallibure has been shown to affect structures which are known to be dependent on gonadal androgens e.g. decrease in the height of kidney tubular epithelium of the guppy (Hoar et al, 1967), atrophy of secondary sexual characteristics in the sea perch (Wiebe, 1968). Methallibure has also been shown to decrease the Leydig cell size and to reduce enzyme activity in steroidogenic tissue (Wiebe, 1968; van den Hurk and van de Kant, 1975).

The decrease in the plasma androgen level of the cyproterone acetate treated males in this experiment agrees with Morse et al.(1973); Murray et al.(1975); Neumann and Steinbeck (1975) and Koch et al (1976) who found that the plasma and urinary testosterone fell in men treated with cyproterone acetate. This reduction in plasma 11-ketotestosterone levels of these experimental parr could be due to either a decrease in gonadotrophin secretion or to a local effect of cyproterone acetate in the testes. Neumann and Steinbeck (1975) in their review on antiandrogens stated that "incubation of rat testicular tissue homogenate

with several testosterone precursors revealed that in the presence of cyproterone acetate the strongest inhibition was exerted on the 3β hydroxysteroid dehydrogenase and the Δ 4 -5 isomerase system and that it also inhibited demolase (C_{17-20} side chain cleavage) activity".

Neither methallibure or cyproterone acetate reduced 11-ketotestosterone plasma levels to those of immature parr (Table 4.3.2 and 2.3.5). The nuclear diameter and cellular area of the meso-adenohypophyseal basophils are much larger in the methallibure and cyproterone acetate treated precocious males than in untreated immature parr (Tables 4.3.4 and 2.3.10). This suggests that these chemical agents did not completely inhibit gonadotrophin secretion, so that sufficient trophic hormone was released to stimulate a low level of androgen production. Pandey (1970) has shown that methallibure did not completely block the production or the release of gonadotrophin in adult guppies as the Sertoli and interstitial cells were not regressed. Van den Hurk and Testerink (1975) have also demonstrated incomplete inhibition of gonadotrophic cells by methallibure in the black molly, as the increase in the number of Leydig cells was prevented but the steroid synthesising capacity of the Leydig cells was not affected. No study could be made of the steroid synthesizing cells of the ripe male parr in this investigation as the spermatozoa were very densely stained in H. and E. sections and so obscured the lobule walls and the intralobular boundary cells.

The antiandrogenic effect of cyproterone acetate on the germ cells failed to prevent the development of mature spermatozoa in precocious males. This was because androgen concentration is higher in the testes than elsewhere in the body so that the ratio of antiandrogen/androgen is unfavourable for cyproterone acetate as far as the effect on the germinal epithelium is concerned. Neumann et al. (1969) said that "spermatogenesis may be normal when other androgen dependent systems are reduced to a castration-like state" (by antiandrogens) and they suggested that to inhibit spermatogenesis the dosage of antiandrogens must be about ten times as high as that necessary to cause atrophy of the accessory sexual glands.

In this study, despite decreased gonadotrophic cell activity in both methallibure and cyproterone acetate treatment groups and the antiandrogenic effect of cyproterone acetate on the germinal epithelium and its inhibition of testosterone synthesis, there was sufficient androgen produced to form mature spermatozoa.

Neumann and Steinbeck (1975) in their review have suggested that cyproterone acetate has also a local anti-spermatogenic action. This is independent of its effect on the neural/gonadal axis and its local androgenic antagonism of the germ cells. Morse et al. (1973) have described a large reduction in the sperm count and a decrease in germ cell numbers especially the later stages in cyproterone acetate treated

men. Rouse et al (1977) have reported inhibition of the later stages of spermatogenesis by cyproterone acetate in the stickleback. This anti-spermatogenetic effect did not occur in the precocious male salmon parr of this investigation as all the lobules in the gonads of cyproterone acetate treated fish contained fully mature spermatozoa.

The results of experiment B show that methallibure prevents precocious male sexual maturity when it is fed to immature parr early in the year. Pandey (1970) also found that methallibure prevented testes development in juvenile guppies. This may have been because the production/or release of gonadotrophin in these immature salmon parr was entirely blocked by methallibure. This was probably due to treatment being initiated before gonadotrophic cells had differentiated or before gonadotrophin secretion was great enough to partially overcome the action of methallibure.

Microscopic examination of the testes of developing male parr has shown that even in spring and early summer some of the gonads are more advanced than others (Section 2.3.2, Table 2.3.3). This may explain the partial success of cyproterone acetate in preventing precocious sexual maturity. During March, in about 40% of the male population the gonadotropes must have differentiated to a stage where they could secrete enough gonadotrophins to stimulate the steroidogenetic tissue in the testes to produce androgens

in sufficient amounts to overcome the effects of cyproterone acetate. Billard, Breton, Forstier, Jalabert and Weil (1978) have reported an increased gonadotrophin secretion in the brown and rainbow trout during the initial stages of spermatogenesis. This may have occurred in the salmon parr of this study and been sufficient to overcome the anti-androgenic and antigonadotrophic effects of cyproterone acetate. The gonadotropes in the rest of the male population may not have been differentiated at the beginning of this experiment and were prevented from doing so by the anti-gonadotrophin effect of cyproterone acetate.

4.4.2. Thyrotrophic cells

There is a lot of conflicting evidence about the role of the thyroid gland during sexual maturity. Methallibure has an anti-thyroid effect in guppies and completely blocked thyroxine secretion and caused degranulation of the thyrotrophic cells due to oversecretion (Pandey and Leatherland, 1970). There is no evidence that cyproterone acetate exerts anti-thyroid effects in fish but it does cause atrophy and regressive changes in the TSH cells of beagle bitches (El Etreby and Fath el Bab, 1978). In a previous section, it was shown that the basophils in the pro-adenohypophysis of precocious males were larger than those in immature parr (Section 2.3.7, Table 2.3.10) and it was suggested that this was due to increased storage of thyroid stimulating hormone (TSH) due to an

enhanced thyroid activity in the ripe males. In the present investigation the basophils in the pro-adenohypophysis of the methallibure ($p \leq 0.05$) and cyproterone acetate ($p \leq 0.001$) treated fishes were significantly smaller than the untreated ripe males. They did not exhibit any sign of degranulation. The reason for this may have been that the reduction of androgen secretion in the treated male parr may have decreased thyroid activity slightly with the result that a small amount of synthesised TSH was released and not stored as happened in the untreated control parr. The TSH cells in the methallibure and cyproterone acetate treated fish were still much larger than the TSH cells in the immature parr of the initial survey (Tables 2.3.10 and 4.3.4) so these chemical agents did not completely block thyroid activity. Leatherland (1969) also found partial depletion of thyrotrophic cells in methallibure treated C. aggregata.

4.4.3. Skin

4.4.3 (a) Epidermis

The prevention of the increase in epidermal thickness in ripe male parr by methallibure and cyproterone acetate adds further proof to the

theory that gonadal androgens are responsible for this change in precocious males. This reduction in the skin thickness is probably due to the decrease in androgen synthesis in both experimental groups. The skin may need a threshold level of circulating androgen for the initiation of the hyperplastic epidermal changes. Amongst the cyproterone acetate treated parr the decrease in skin thickness may also be due to competitive inhibition of 11-ketotestosterone at the androgen receptor sites in the skin. If this is the case, then these results provide evidence for the presence of androgen receptors in salmon parr skin. Androgen receptors have been shown to be present in the integument of lower vertebrates and Delrio and d'Istria (1973) and d'Istria, Delrio and Chieffi (1975) have found them in the thumb pad and skin of R. esculanta.

In mammals the androgenic activity of testosterone is believed to depend on its conversion to 5α DHT in the target organs by the enzyme 5α DTH reductase. Hay, Hodgins and Roberts (1976) have reported 5α DHT reductase action in the skin of rainbow trout. They also suggested that 11-ketotestosterone could be converted into an 11-keto-5α reduced metabolite. 5α DTH reductase may be present in the salmon skin and the mechanism of action of cyproterone acetate may have been competitive inhibition of the so called 11-keto-5α DHT at the androgen receptor site. Further studies on the isolation and substrate specifity of 5α reductase in salmon skin are needed to clarify this.

The results in Tables 4.3.6, 4.3.7 and Figs. 31 and 32 imply that cyproterone acetate and methallibure treatment increased the skin thickness of immature parr. This may not be the case and Figs. 31 and 32 may give a false impression because of the small number of untreated immature fish available for study. When the skin measurements of both immature parr treatment groups were compared with the immature parr in the initial survey (Section 2.3.8, Table 2.3.12) there was no difference between the treated and untreated fish and their skin measurements were more or less similar to both precocious male treated groups.

4.4.3 (b) Mucous cell concentration

Both drugs increased the concentration of superficial mucous cells in mature and immature parr. It is known that in man and various laboratory animals that androgens induce the growth and activity of sebaceous glands and treatment with cyproterone acetate leads to atrophy of these glands (Neumann and Steinbeck, 1975). The observations reported here suggest that mucus secretion in salmon parr is not under androgen control. Prolactin is known to increase mucus secretion in fish (Ball and Baker, 1969; Sage and Bern, 1971; Lemoine and Olivereau, 1973), so it is possible that the increased mucus cell concentration in the

experimental fish of this study could be due to methallibure and cyproterone acetate enhancing prolactin secretion. TSH and thyroxine have also been shown to play a part in mucus secretion in fish (Ball, 1969). In this study both cyproterone acetate and methallibure have been shown to decrease the size of thyrotrophic cells in the pituitary and it was suggested that this was due to increased secretion of TSH caused by a fall in thyroxine production. TSH secretion in these two groups of experimental parr may have induced the rise in the superficial mucous cell concentration. The increase in mucous cell numbers in these experimental parr may also be due to a secondary property of these drugs on mucus production in fish or to the small increase in the interrenal activity of the treated fish (Table 4.3.3 and Fig. 30). Increased cortisol levels have been shown in the previous section (Section 3.3.2, Fig. 32) to enhance mucus secretion in the dorsal fin, pectoral fin and tail.

4.4.3 (c) Mucohistochemistry

The mucohistochemical studies reported here show that gonadal androgens in general do not markedly affect the components of mucus. All the staining patterns in both immature and mature experimental groups were similar to the untreated controls.

The proportions of the different cell types after the AB (pH 2.5)/PAS method in the methallibure treated precocious male group differed slightly from the control group in that there was a fall in the number of red cells containing neutral polysaccharides. This was probably due to a direct action of methallibure on the mucous cells, and not to interference with androgen secretion, as it did not occur to any extent in the apocrine cells of the cyproterone acetate group.

4.4.4. Saprolegniasis

The untreated control group of precocious males were more frequently parasitised with Saprolegnia than were the two treatment groups of ripe males. Methallibure and cyproterone acetate treated precocious males differed from the untreated control group in that they had a thinner epidermal layer and a greater superficial mucous cell concentration. All the three groups exhibited a similar interrenal activity and mucohistochemical reactions, so possibly it is the skin changes during male sexual maturity that increase the susceptibility of ripe male parr to fungal infection. The control group of immature parr had a lower prevalence of fungal infection than the untreated precocious males and these fish also had a thinner epidermis but less mucous cell numbers.

Many disease conditions of the skin in man are androgen dependent and testosterone metabolism (formation of DHT) in the skin may at times be abnormally excessive and cause undesirable effects (Price, 1975). The immature parr and the precocious males treated with methallibure and cyproterone acetate all had lower plasma androgen levels than the control group of precocious males and it is possible that increasing androgen secretion and metabolism during sexual maturity may affect the skin and make salmon parr more vulnerable to fungal infections. However, none of the immature parr fed exogenous androgens (Section 3.3.6) were parasitised with Saprolegnia, whereas there was a high prevalence of fungal infection amongst the cortisol treated group. Thus the increased susceptibility of precocious males is more likely to be due to a combination of increased corticosteroid production allied to the changes in the skin brought about by increased androgen metabolism.

4.4.5. Growth

The growth of the experimental fish was not good, especially in experiment A where the fish did not exhibit any increase in length or weight. This may have been caused by many factors such as the unsuitability (due to overcrowding, etc.) of the

fibre-glass tanks for rearing parr, or to a miscalculation in the level of feeding so that the fish were only fed a maintenance diet. The high dosage rate of methallibure and cyproterone acetate used in these studies may have made the diet unpalatable. Treating the pellets with ethanol may have also made the diet inedible as it dissolves the outer fatty layer and causes the pellet to disintegrate faster in the water. In Experiment B both the methallibure and cyproterone acetate treated groups improved after the drugs were withdrawn in August and in December, after 20 weeks of a normal diet, they had a similar "K" factor as the untreated control group. Besides making the diet unpalatable, these drugs may have affected STH secretion but no evidence for this was found in the pituitary studies.

4.4.6. Conclusion

The experiments described here have shown that it is possible to use chemosterilant agents to prevent gonad development in salmon parr but it is essential that they be administered early in the maturation cycle. Cyproterone acetate may have been 100% successful if it had been fed earlier in the year, perhaps around the end of December and January before the increasing

photoperiod primes the reproductive system and the neural/gonadal axis. The central inhibitor methallibure was the most effective. In Experiment A, although both drugs failed to prevent sexual maturity, there was an important secondary effect in a lower prevalence of Saprolegnia infection amongst the two treatment groups.

Failure of the methallibure treated males in Experiment B to develop gonads once the drug was withdrawn in August may have been due to the lack of proper environmental stimulus. The photoperiod was decreasing at this time and de Vlaming (1972) has suggested that increasing day-length is very important for the initiation of gonadal development in salmonids. This suggests that the chemosterilants need not necessarily be fed all the year round to be effective and can be withdrawn after the longest day.

Methallibure or drugs with a similar mechanism of action (Δ 1-chlormadinone acetate etc.) would be the agents of choice for future experiments in the prevention of gonad maturation in aquaculture units. With further experimentation, it should be possible to refine the procedure and to find the lowest effective dose, metabolic clearance rates and residues as well as the optimum treatment period.

CHAPTER 5

SUMMARY AND CONCLUSIONS

This section summarises the results of the present investigations. The changes that occurred during precocious maturity are correlated with the effects of the exogenous steroids and the two chemosterilants on 0+ and 1+ salmon parr.

The initial survey showed that all the male 1+ population and a varying percentage of the 0+ male parr became sexually mature. None of the female parr developed precocious maturity. The gonadal cycle in the 1+ male parr was shown to be similar to adult males with the formation of normal spermatozoa, increased androgen secretion, and enhanced gonadotrophin synthesis and storage.

There appeared to be a seasonal cycle in cortisol production amongst 1+ parr, as both mature and immature parr had increased interrenal activity during late autumn. Precocious males had significantly greater corticosteroid production than immature parr. This was attributed to increased gonadal androgen production stimulating,either directly or indirectly, the adrenal gland, as administration of exogenous 17α methyltestosterone and testosterone to 0+ immature parr increased the interrenal nuclear diameter.

The thickness of the epidermis in Atlantic salmon parr depends on the season of the year. Both immature parr and

precocious males exhibited increased skin thickness during the period October to December. The epidermis of ripe males was also thicker and had significantly more cell layers than that of immature parr. It was shown that this was most probably due to increased plasma androgen levels. Administration of exogenous androgens induced a marked increase in the epidermal and dermal thickness of immature 0+ parr. The chemosterilants, methallibure and cyproterone acetate lowered plasma 11-ketotestosterone levels and also prevented the increase in the epidermal thickness of precocious males.

The effects of administration of androgens and chemosterilants suggest the presence of androgen receptors and 5α DHT reductase activity in the skin of salmon parr. Experiments using radioactive chemicals and biochemical purification techniques are needed to positively isolate cytoplasmic or nuclear androgen binding proteins and to identify androgen metabolic products in the skin of salmon.

A positive relationship was found to exist between the epidermal thickness, the total skin thickness and the body weight of 1+ parr. There was also a relationship between these skin measurements and the condition factor and depending on the time of year it was either a negative or positive correlation.

There was a definite seasonal cycle in mucus production in both groups of 1+ parr. The lowest concentration of mucous

cells occurred during the summer months and there was a gradual increase in apocrine cells during autumn and early winter. It was suggested that these seasonal changes in mucus production corresponded to changes in adrenal activity. The superficial mucous cell concentration increased in autumn at the same time as plasma cortisol levels rose. The apocrine cell numbers also increased when the fish were stressed due to handling and when they were subjected to bacterial infection. This hypothesis was confirmed when it was shown that administration of exogenous cortisol increased the superficial mucous cell concentration in pectoral fin, dorsal fin and tail of immature O+ parr.

Prolactin and thyroxine have also been found to enhance mucus production in fish. There is evidence to suggest that in salmonids thyroxine secretion increases during the winter and in this study the thyrotrophic cells in the pro-adenohypophysis have been shown to increase in size during late autumn suggesting storage of TSH due to an active thyroid. This increase in thyroid activity may also be a possible reason for the increase in the superficial mucous cell concentration in autumn. Another reason may be a seasonal cycle in prolactin secretion. Further studies using RIA techniques to monitor the annual cycle of prolactin and thyroxine, as well as ablation and replacement therapy experiments are needed to confirm this. However, it is unlikely that the seasonal increase in mucous cell numbers is due to oversecretion of one of the above mentioned hormones but is more probably due to a combination of all three.

Precocious males had a significantly greater number of mucous cells than immature parr during the later stages of spermatogenesis. This may be due to the greater interrenal or thyroid activity amongst the ripe parr at this time. Other workers have suggested that the increased mucus production in some sexually mature male salmonids may be due to enhanced androgen secretion. No evidence for this was found in these studies as exogenous androgens did not markedly increase the goblet cell numbers in immature parr nor did the compounds, methallibure and cyproterone acetate reduce the apocrine cell concentration in precocious 1+ male parr.

Biochemical analysis needs to be done to determine exactly whether or not sexual maturity changes the chemical composition of mucus. The results of the mucohistochemical studies reported here suggest that there is a possibility that the chemical composition of mucus may depend on the endocrinological status of the fish. Both androgens and corticosteroids affected the histochemical reaction of the goblet cells, and cortisol appeared to have had the strongest influence on mucus production and staining reaction in all three experiments.

In this report, there are conflicting results regarding the susceptibility of precocious males and immature parr to Saprolegnia infection. In the first experiment, when the fish were kept in the smolt release tank only a small percentage were infected and there was no significant

difference in the frequency of parasitism between immature and mature parr. There was a greater prevalence of fungus amongst the 1+ parr when they were held in $0.6m^2$ tanks in conditions similar to commercial production units. In this experiment the precocious males were more frequently infected than the immature parr. The difference between these two experiments may be explained by the fact that the salmon parr in the smolt release tank were held in much better husbandry conditions than those in the experimental tanks. The greater water flow rate probably flushed out most of Saprolegnia zoospores before they came in contact with the fishes' skin. The increased susceptibility of ripe males in conditions similar to commercial hatchery environments reported here agrees with other workers, and presents indirect evidence that slow water flow rates and increased stocking densities enhances the ability of fungal spores to parasitise fishes.

The evidence presented in these three investigations suggest a number of reasons why precocious males are more susceptible to fungal infections. The first is that increased gonadal androgen secretion causes proliferation of the epidermal tissue. The seasonal increase that takes place in the interrenal activity of 1+ parr during autumn is enhanced by the plasma androgens in ripe males. This produces higher plasma cortisol levels in precocious males. Mucus secretion and composition is also affected by the elevated plasma cortisol and 11-ketotestosterone levels in sexually mature males. This may provide an excellent growth medium for Saprolegnia spores at a time when the environmental

conditions are optimal for their propagation.

The report on the effects of chemosterilants on sexual maturity of salmon parr has shown that they can prevent precocious maturity if they are administered early in the reproductive cycle. Agents that act as central inhibitors (methallibure) by preventing gonadotrophin synthesis and/or release appear to be the more reliable. It has also been shown that it is not necessary to feed these drugs for the duration of the gonad maturation cycle and that there is a point when drugs can be withdrawn which appears to be around mid-summer. Also it may not be necessary to use dosages as high as 250ppm and future experiments designed to refine this technique in controlling sexual maturity in fishes should determine the lowest effective dose and the optimum treatment period.

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II

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Studies on precocious maturity in artificially reared 1+ Atlantic salmon parr Salmo salar L. Juv.

VOLUME II

CONTENTS

TABLE 1.1

The prevalence of Saprolegnia infection amongst 1+ Atlantic salmon parr at the Salmon Research Trust installations, Newport, Co. Mayo. (Modified from Salmon Research Trust of Ireland Annual Report, 1974).

Year	% Prevalence Jan./April	% Prevalence Oct./December
1968	0.8	5.3
1969	3.8	29.8
1970	1.8	11.1
1971	4.1	9.0
1972	2.2	7.9
1973	9.9	2.7

TABLE 2.1.1.

Hypothesis on the evolution of the pituitary-gonadotrophin system (after Haider and Blum, 1977).

Phase No.	No. of gonadotrophic cells in the pituitary (site of synthesis)	No. of types of gonadotrophin molecules	No. of gonadotrophic receptors in the gonad	Level of evolutionary change	Gonadotrophin system to be termed as
I	one	one	one	-	Primitive gonadotrophin system
II	one	one	two	Gonadotrophic receptors in the gonad.	Intermediate gonadotrophin system
III	two	one	two	Gonadotrophic cell type in the pituitary (site of synthesis)	Intermediate gonadotrophin system.
IV	two	two	one	Gonadotrophin molecule	Intermediate gonadotrophin system
V	two	two	two	All three levels	Differentiated gonadotrophin system

TABLE 2.1.2.

Comparison of histological alterations in spawning non-migratory and migratory rainbow trout and spawning Pacific salmon (modified from Robertson et al 1961 a).

	Rainbow Trout		Pacific Salmon
	Non-Migratory	Migratory (Steelhead)	
Post spawning mortality	Low	Circa 50%	100%
Pituitary degeneration	Slight	Moderate	Marked
Adrenocortical Tissue (Interrenal)			
(a) Hyperplasia	Mild	Marked	Marked
(b) Degeneration	Mild	Moderate	Marked
Skin thickness (increased)	Moderate	Marked	Marked
Scales absorption	Present	Present	Present
Stomach	Normal	Loss of villi only	Atrophy + degeneration
Liver degeneration	Slight	Moderate	Marked
Spleen			
(a) Lympocyte depletion	Moderate	Marked	Marked
(b) Increased connective tissue	Marked	Marked	Marked
Thymus lympocyte depletion	Moderate	Moderate	Marked
Kidney degeneration	None	Moderate	Marked
Pancreas islet hypertrophy	Marked	Marked	Marked
Thyroid	Normal	Normal	Atrophy + degeneration
Cardiovascular degeneration	Mild	Moderate	Marked

TABLE 2.2.1.

Staining reaction of carbohydrates (modified from Drury and Wallington, 1967)

	PAS	Alcian Blue	Methylation + Alcian Blue	Sialidase (Neuraminidase) + Alcian Blue	Dialysed Iron	Metachromasia
Polysaccharide (Glycogen)	+	-	-	-	-	-
Neutral Mucopolysaccharides	+	-	-	-	-	-
Simple Acid Mucopolysaccharide	±/-	+	-	+	+	+ or ±
Complex sulphated Mucopolysaccharides	±/-	+	-	+	+	+
Mucoproteins (Glycoproteins)	+	-	-	-	-	± or -
Sialic acid containing Mucoproteins	+	+	-	-	+	+ or ±

TABLE 2.2.2.

Histochemical Methods used in the Mucous Cell Study

Method	Specific Histochemical Result	Procedure Used
Alcian Blue (pH 2.5) + PAS	Acidic mucins - Blue. Neutral Polysaccharides - Red.	Pearse (1968) (Modified)
Alcian Blue (pH 1.0) + PAS	Sulphated acidic mucins - Blue. Sulphate-free sialic acid containing mucins - Red.	Pearse (1968) (Modified)
Hales' Colloidal Iron	Acidic mucins - Blue.	Spicer, Horn & Leppi (1967) (Modified)
Colloidal Iron + PAS	Sulphate-free acid muco polysaccharides - Blue or Blue/ Purple.	Spicer, Horn & Leppi (1967)
Mild methylation (in 0.1N HCL in ethanol) for 4 hours at 37°C and subsequently stained with alcian blue (pH 2.5)	Alcianophilia of non-sulphated mucins is inhibited.	Spicer, Horn & Leppi (1967)
Active methylation (in 0.1N HCL in ethanol) for 4 hours at 60°C and subsequently stained with alcian blue (pH 2.5)	Alcianophilia of both non-sulphated and sulphated mucins is inhibited.	Spicer, Horn & Leppi (1967)
Alcian blue + safranin	Very strong acidic sulphate mucins - Red. Other mucins - Blue.	Spicer, Horn & Leppi (1967)
Aldehyde Fuchsin	Sulphated mucins - Purple or Blue/Purple. Non- sulphated mucins stained weakly or not at all.	Pearse (1968)
Aldehyde Fuchsin + 1% alcian blue (pH 2.5)	Strongly acidic sulphated mucins - Purple or Blue/Purple. Non-sulphated mucins - Blue.	Spicer, Horn & Leppi (1967)
0.1% alcian blue + increasing concentrations of magnesium chloride ($Mg Cl_2$)	Hyaluronic acid, sialomucins and weak acidic sulphomucins not stained at or above 0.1M $Mg Cl_2$.	Pearse (1968)
Hyaluronidase treatment + alcian blue	Hydrolyses several common glycosaminoglycons (hyaluronic acid and chondroitin sulphate)	Drury & Wallington (1967)
Neuraminidase (250 units/ml) treatment + alcian blue/PAS	Inhibits alcianophilia of sialic acid containing glycoproteins.	Drury & Wallington (1967)

Suitable control staining procedures were performed where necessary.

See page 20A for explanation of the symbols.

TABLE 2.2.3.

Recovery of exogenous 11-ketotestosterone added to canine plasma

50 µl of canine plasma spiked with exogenous 11-ketotestosterone.	Mean Percentage Recovery
4ng	96.75 (n = 3)
2ng	97.6 (n = 2)
1ng	109 (n = 8)
500 pg	110 (n = 2)

TABLE 2.3.1.

Prevalence of sexual maturity amongst 1+ parr

December, 1976.

No. of Parr	% Precocious Males	% Immature Females
664	50.15	49.85

TABLE 2.3.2.

Prevalence of precocious maturity amongst 0+ parr and the gonadosomatic indices (G.S.I.)- March, 1978.

No. of Parr	Precocious Males		Immature Males		Immature Females.	
	%	G.S.I.	%	G.S.I.	%	G.S.I.
32	18.80	3.42 ± 0.30[x]	53.10	0.15 ± 0.02	28.10	0.44 ± 0.02

x = Mean ± standard error of the mean ($\bar{x}$ + S.E.)

TABLE 2.3.3.

Proportions (%) of the different types of cysts in the testes during the sexual cycle of precocious male 1+ parr

	Primary Spermatogonia	Secondary Spermatogonia	Primary Spermatocyte	Secondary Spermatocyte	Spermatid and Spermatozoa
April	100.00	-	-	-	-
May	100.00	-	-	-	-
June	74.00	26.00	-	-	-
August	1.00	60.75	14.5	8.90	14.9
September	0.20	32.00	26.7	23.20	17.9
October	-	1.00	35.9	36.10	27.5
November	-	-	25.9	36.60	37.5
December	-	-	-	-	100.0

TABLE 2.3.4.

Nuclear and cellular diameter (μm) of the early perinucleolus stage, late perinucleolus stage and early yolk vesicle stage oocytes in immature female 1+ parr sampled during October and December, 1976.

	Early Perinucleolus stage	Late Perinucleolus stage	Early Yolk Vesicle stage
Cell	$76.75 \pm 2.48^{(a)}_{(8)}$	$150.4 \pm 9.22_{(8)}$	$178.0 \pm 10.71_{(6)}$
Nucleus	$38.73 \pm 1.33_{(8)}$	$72.1 \pm 3.39_{(8)}$	$78.3 \pm 3.98_{(6)}$

a, $\bar{x} \pm$ S.E. (No. of fish)

TABLE 2.3.5.

Gonadosomatic index and plasma 11-ketotestosterone (µg/100 ml) levels in immature 1+ female parr (1976)

	G.S.I.(%)	11-ketotestosterone (µg/100 ml)
October	0.35 ± 0.03 (a)(10)	0.14 ± 0.03 (10)
December	0.41 ± 0.02 (15)	0.14 ± 0.02 (13)

(a) Values are $\bar{x}$ ± S.E. (No. of fish).

TABLE 2.3.6.

Plasma levels of cortisol (µg/100 ml) in 1+ parr sampled during Autumn and Winter (1976)

	October	December
Precocious Males	0.95 ± 0.17(a,b)(12)	4.03 ± 0.41(b,d)(18)
Immature Parr	0.57 ± 0.16(c)(12)	3.31 ± 0.56(c,d)(11)

a = $\bar{x}$ ± S.E. (No. of fish)
b, c: $p \leq 0.001$
d : $p \leq 0.01$

TABLE 2.3.7

Plasma levels of Na^+ (meq/l) and K^+ (meq/l) in 1+ parr December, 1975.

	Na^+ meq/l	K^+ meq/l
Precocious males	148.5 $\pm$ 4.28 (a) (12)	7.05 $\pm$ 0.46 (b) (12)
Immature Parr	151.75 $\pm$ 1.05 (9)	9.31 $\pm$ 0.48 (b) (8)

a, $\bar{x} \pm$ S.E. (No. of fish)

b, $p \leq 0.01$

TABLE 2.3.8.

Packed cell volume (PCV%) plasma levels of Na^+ (meq/l) K^+ (meq/l) Ca^{++} (mg/100 ml) and Mg^{++} (mg/100 ml) in 1+ parr sampled during October and December, 1976

	OCTOBER					DECEMBER				
	PCV%	Na^+ Meq/l	K^+ Meq/l	Ca^{++} Mg%	Mg^{++} Mg%	PCV%	Na^+ Meq/l	K^+ Meq/l	Ca^{++} Mg%	Mg^{++} Mg%
Precocious Males	39.07(a) ± 1.64(12)	123.14 ±12.73(6)	4.82(b) ±0.83(c)(7)	5.5 ±0.66(6)	2.02 ±0.4 (5)	42.55 ± 1.35(27)	110.59 ± 9.98(15)	2.17(d) ±0.35 (15)	7.20 ±0.66(12)	1.60 ±0.13(15)
Immature Parr	37.72 ± 0.98(12)	127.61 ± 6.97(8)	5.25(b) ±0.95(8)	6.83 ±0.40(8)	2.52 ±0.67(8)	41.41 ± 1.25(15)	107.06 ±16.51(7)	3.44(d) ±0.75(7)	6.06 ±1.34(7)	1.54 ±0.30(7)

a, $\bar{x} \pm$ S.E. (No. of fish)

b, $p \leq 0.01$

d, $p \leq 0.05$

TABLE 2.3.8.

Packed cell volume (PCV%) plasma levels of Na^+ (meq/l) K^+ (meq/l) Ca^{++} (mg/100 ml) and Mg^{++} (mg/100 ml) in 1+ parr sampled during October and December, 1976

	OCTOBER					DECEMBER				
	PCV%	Na^+ Meq/l	K^+ Meq/l	Ca^{++} Mg%	Mg^{++} Mg%	PCV%	Na^+ Meq/l	K^+ Meq/l	Ca^{++} Mg%	Mg^{++} Mg%
Precocious Males	39.07(a) ± 1.64(12)	123.14 ±12.73(6)	4.82(b) ±0.83(c)(7)	5.5 ±0.66(6)	2.02 ±0.4 (5)	42.55 ± 1.35(27)	110.59 ± 9.98(15)	2.17(d) ±0.35 (15)	7.20 ±0.66(12)	1.60 ±0.13(15)
Immature Parr	37.72 ± 0.98(12)	127.61 ± 6.97(8)	5.25(b) ±0.95(8)	6.83 ±0.40(8)	2.52 ±0.67(8)	41.41 ± 1.25(15)	107.06 ±16.51(7)	3.44(d) ±0.75(7)	6.06 ±1.34(7)	1.54 ±0.30(7)

a, $\bar{x} \pm$ S.E. (No. of fish)

b, $p \leq 0.01$

d, $p \leq 0.05$

TABLE 2.3.9

Staining reaction of cytoplasmic granules in the secretory cells of the 1+ salmon parr adenohypophysis.

Cell Types	1	2	3	4
Haematoxylin and eosin	-	Bright Red	Pale	Pale
Slidders	-	Orange Brown	Blue Green	Grey/ Green
Martius Scarlet Blue (Modified)	-	Maroon	Royal Blue	Light Blue
Masson's Trichrome	-	Light Brown	Dark Green	Light Green
Alcian Blue - PAS + orange G	-	Orange Yellow	Blue and Purple	-
PAS + orange G	-	Orange	Purple	-
Aldehyde fuchsin + PAS + orange G	-	Orange	Purple	-
Bargemann's chrome haematoxylin-phloxine	-	Bright Red/ Pink	Blue/Black	-
Gomori's aldehyde fuchsin without previous oxidation	-	-	Faintly Purple	-
Gomori's aldehyde fuchsin after oxidation.	-	-	-	-

TABLE 2.3.10.

Cellular area (μm^2) and nuclear diameter (μm) of the pro-adenohypopseal and meso-adenohypopyseal basophilic cells in 1+ parr sampled in October and December, 1976

	Pro-adenohypophysis				Meso-adenohypophysis			
	October		December		October		December	
	Cellular Area	Nuclear Diameter	Cellular Area	Nuclear Diameter	Cellular Area	Nuclear Diameter	Cellular Area	Nuclear Diameter
Precocious Males	38.63 ± 3.60(a,b,g) (5)	3.97 ± 0.17 (8)	47.87 ± 1.56(g,c) (8)	4.07 ± 0.09(8)	34.97 ± 2.81(i,d) (5)	3.96 ± 0.16(5)	47.42 ± 1.01(i,e) (8)	4.27 ± 0.08(f) (8)
Immature Parr	30.88 ± 1.67(b,h) (8)	3.83 ± 0.14(8)	36.27 ± 1.02(c,h) (8)	3.95 ± 0.09(8)	18.21 ± 1.29(d,j) (7)	3.23 ± 0.09(k) (6)	24.43 ± 1.40(e,j) (8)	3.76 ± 0.01(f,k) (8)

a) $\bar{x} \pm$ S.E. (No. of fish)
b) $p \leq 0.05$
c) $p \leq 0.001$
d) $p \leq 0.001$
e) $p \leq 0.002$
f) $p \leq 0.001$
g) $p \leq 0.05$
h) $p \leq 0.02$
i) $p \leq 0.001$
j) $p \leq 0.001$
k) $p \leq 0.01$

TABLE 2.3.11.

Skin measurements (epidermis (μm), dermis (μm) and number of cell layers in the epidermis) of the dorsum, pectoral and caudal peduncle areas of 1+ Atlantic salmon parr sampled during November and December, 1975

		DORSUM			PECTORAL			CAUDAL PEDUNCLE		
		Epidermis	No. of cell layers in epidermis	Dermis	Epidermis	No. of cell layers in epidermis	Dermis	Epidermis	No. of cell layers in epidermis	Dermis
NOV.	Precocious Males	74.25(a) ± 4.97(g) (5)	6.42(h) ±0.23(5)	109.20 ± 8.50(5)	79.00(b) ±5.72(5)	6.88 ±0.39(5)	78.20 ± 6.61(5)	83.32(d) ±6.18(5)	7.65(e) ±0.48(5)	189.28 ± 6.14(5)
	Immature Parr	51.35(g) ± 2.47(9)	5.10(h) ±0.13(9)	116.44 ± 4.63(9)	64.93(b) ±3.14(11)	6.14 ±0.25(11)	73.50 ±3.82(11)	57.32(d) ±5.22(9)	5.64(e) ±0.41(9)	165.52 ±11.21(9)
DEC.	Precocious Males	73.61(i) ± 3.83(18)	6.73(j) ±0.28(18)	114.59 ± 5.80(17)	92.69(k) ±2.65(18)	7.81(l) ±0.18(18)	78.71 ± 4.31(18)	86.07(m) ±4.66(18)	7.91(f) ±0.37(18)	182.06(c) ± 6.24(18)
	Immature Parr	54.44(i) ± 2.65(14)	5.82(j) ±0.18(14)	104.96 ± 4.70(14)	73.64(k) ±2.83(12)	6.77(l) ±0.17(12)	83.93 ± 2.66(12)	59.68(m) ±2.77(11)	6.29(f) ±0.29(11)	162.23(c) ± 0.89(11)

a,= $\bar{x}$ ± S.E. (No. of fish).
b,c, P ≤ 0.05
d,e,f, P ≤ 0.01
g,h,i,j,k,l,m, P ≤ 0.001

TABLE 2.3.12

Skin measurements (epidermis (μm), dermis (μm) and number of cell layers in the epidermis), in the dorsum, pectoral and caudal peduncle areas, of 1+ Atlantic salmon parr sampled during October and December, 1976

		DORSUM			PECTORAL (BELLY)			CAUDAL PEDUNCLE		
		Epidermis	No. of cell layers in epidermis	Dermis	Epidermis	No. of cell layers in epidermis	Dermis	Epidermis	No. of cell layers in epidermis	Dermis
OCT.	Precocious Males	55.90(a) ± 2.23(12)	6.60 ±0.29(12)	99.02(b) ± 4.02(12)	57.64 ± 3.28(11)	6.40(c) ±0.21(11)	68.60(d) ± 4.86(11)	51.17(e) ± 3.14(12)	6.15(f) ±0.23(12)	138.61(g) ± 7.61(12)
	Immature Males	51.01 ± 1.99(12)	5.48 ±0.10(12)	108.06(b) ± 6.77(12)	53.64 ± 4.09(9)	5.38(c) ±0.20(9)	92.70(d) ± 8.07(9)	36.72(e) ± 2.15(11)	4.50(f) ±0.15(11)	170.33(g) ± 8.76(11)
DEC.	Precocious Males	79.96(h) ± 2.16(16)	6.62(i) ±0.16(16)	112.12 ± 4.32(16)	91.36(j) ± 3.69(16)	7.23(k) ±0.21(16)	84.64 ± 2.93(16)	81.76(m) ± 2.72(16)	7.04(n) ±0.20(16)	181.33(f) ± 6.25(16)
	Immature Parr	67.20(h) ± 1.49(15)	5.71(i) ±0.13(15)	107.76 ± 3.49(15)	80.49(j) ± 3.11(8)	6.56(k) ±0.24(8)	89.20 ± 6.58(8)	57.23(m) ± 2.17(15)	5.35(n) ±0.15(15)	158.47(p) ± 6.44(15)

a, $\bar{x}$ ± S.E. (No. of fish)
k,j,b, P ≤ 0.05
p,g,d,c, P ≤ 0.02
m,i,h,f,e,c,n, P ≤ 0.001

TABLE 2.3.13

Skin measurements (epidermis (μm) number of cell layers in the epidermis) of the pectoral fin and tail of 1+ Atlantic salmon parr sampled during October and December, 1976

		PECTORAL FIN		TAIL	
		Epidermis	No. of cell layers in epidermis	Epidermis	No. of cell layers in epidermis
Oct.	Precocious Males	65.94 ± 1.02(a,b) (7)	7.56 ± 0.23(c) (7)	67.42 ± 3.94(d) (7)	7.41 ± 0.26(e) (7)
	Immature Parr	59.81 ± 2.25(b) (11)	6.64 ± 0.56(c) (11)	56.14 ± 3.19(d) (11)	5.87 ± 0.20(e) (11)
Dec.	Precocious Males	98.71 ± 3.01(15)	8.29 ± 0.23(15)	103.77 ± 3.53(15)	8.71 ± 0.28(15)
	Immature Parr	95.37 ± 3.01(15)	7.90 ± 0.16(15)	93.12 ± 5.06(15)	8.15 ± 0.37(15)

a, $\bar{x} \pm$ S.E. (No. of fish)
b,d, $p \leq 0.05$
c, $p \leq 0.01$
e, $p \leq 0.001$

TABLE 2.3.14.

Correlation coefficient of the dorsal, pectoral, caudal peduncle, pectoral fin and tail epidermis (μm) and the body weight minus the gonad weight in precocious male 1+ parr sampled during October and December 1976

	OCTOBER	DECEMBER
Area of skin	Precocious male parr	Precocious male parr
Dorsum	0.641 (a)(9)	0.182 (14)
Pectoral	0.574 (8)	0.565 (b)(14)
Caudal Peduncle	0.77(c) (9)	0.434 (14)
Pectoral Fin	0.225 (5)	0.24 (13)
Tail	0.335 (5)	0.357 (13)

a (degrees of freedom)
b, $p \leq 0.05$
c, $p \leq 0.01$

TABLE 2.3.15.

Correlation coefficient ('r') of the total skin (epidermis + dermis) measurements (μm) in the dorsal, pectoral, caudal peduncle areas with the body weight minus the gonad weight in precocious 1+ Atlantic salmon parr sampled during October and December, 1976

Area of skin	October	December
Dorsum	0.509(a)(9)	0.017(14)
Pectoral	0.794(c)(8)	0.346(14)
Caudal peduncle	0.647(b)(9)	0.73 (c)(14)

a (Degrees of freedom)
b, $p \leq 0.05$
c, $p \leq 0.01$

TABLE 2.3.16

Superficial mucous cell concentration (No. of mucous cells/mm^2) in the epidermis of 1+ salmon parr sampled during October, November and December 1978

		Head	Dorsum	Pectoral	Caudal Peduncle	Tail	Pectoral Fin
October	Immature Parr	± 531.80(a) ± 31.60(8)	± 360.10(b) ± 21.13(9)	± 390.70 ± 30.58(8)	± 345.70(c) ± 11.62(9)	480.10(d) ± 26.65(9)	± 540.70(e) ± 37.06(9)
	Precocious Males	592.30 ± 53.66(10)	± 450.60(b) ± 27.58(9)	± 435.70 ± 24.27(10)	± 402.20(c) ± 8.76(10)	± 601.50(d) ± 30.83(9)	± 675.90(e) ± 31.55(10)
November	Immature Parr	± 504.70(f) ± 17.72(9)	± 379.50(g) ± 27.80(9)	± 368.40(h) ± 33.59(9)	± 403.90 ± 23.13(9)	± 514.80 ± 26.90(9)	± 620.20(i) ± 23.39(9)
	Precocious Males	± 571.30(f) ± 17.41(9)	± 498.70(g) ± 19.61(9)	± 487.60(h) ± 43.70(9)	± 448.00 ± 19.33(9)	± 602.10 ± 38.98(9)	± 716.50(i) ± 30.87(8)
December	Immature Parr	± 610.80(j) ± 27.43(10)	± 465.70(k) ± 29.24(10)	± 431.50(l) ± 17.42(9)	± 440.30(m) ± 17.63(8)	± 572.90(n) ± 13.57(10)	± 645.70(p) ± 24.26(10)
	Precocious Males	± 778.20(j) ± 55.16(8)	± 562.50(k) ± 24.91(10)	± 548.10(l) ± 34.44(10)	± 554.90(m) ± 23.08(10)	± 640.40(n) ± 24.96(10)	± 738.10(p) ± 24.61(10)

a, $\bar{x}$ ± S.E. (No. of fish)

b,e,f,j,p $p \leq 0.02$

c $p \leq 0.002$

h, k, n,p ≤ 0.05

d,g,i,l,m $p \leq 0.01$

TABLE 2.3.17

Mucohistochemical reaction of the epidermal mucous cells of immature 1+ parr sampled in October, 1976.

Stain	Dorsum	Pectoral	Caudal Peduncle	Tail	Pectoral Fin
P.A.S.	++ Red	++ Red	++ Red	++ Red	++ Red
Hales Colloidal Iron	++ Blue	+++ Blue	+++ Blue	+/++ Blue	+++ Blue
Hales Colloidal Iron + PAS	92% Blue/ Purple +++ 3.6% Blue + 4.4% Red +	98.5% Blue/ Purple +++ 1.5% Blue ++	98.4% Blue/ Purple +++ 1.6% Blue ++	98.4% Blue/ Purple +++ 0.3% Blue ± 1.1% Red ±	91% Blue/ Purple +++ 1.2% Blue ++ 7.8% Red ±
Alcian Blue (pH 2.5)	+/+++ Blue	++ Blue	++ Blue	++ Blue	++ Blue
Alcian Blue (pH 1.0)	-	-	-/±	-/±	-
Alcian Blue (pH 2.5) + PAS	57.4% Blue +++ 40.1% Blue/ Purple + 2.5% Red +	61% Blue +++ 35.9% Blue/ Purple +/++ 3.1% Red +	53.5% Blue +++ 42.7% Blue/ Purple ±/+ 4.3% Red +	51.7% Blue ++ 43.7% Blue/ Purple + 4.6% Red +	33.7% Blue + 51% Blue/ Purple ++ 15.3% Red ++
Alcian Blue (pH 1.0) + PAS	96.5% Red/ Purple +/+++ 3.5% Red +	50% Red/ Purple ++ 50% Red ++	100% Red/ Purple +/++	100% Red, Purple +/+++	96.1% Red/ Purple ++ 3.9% Red +
Mild Methylation	±/+	±/+	±/+	±/+	±/+
Active Methylation	±	±	±	±/+	±
Alcian Blue + 0.1M $MgCl_2$	±/+	±	+	±/+	±
Alcian Blue + 0.2M $MgCl_2$	±	±	+	+	±
Alcian Blue + 0.5M $MgCl_2$	±	-/±	±/±	±	±
Alcian Blue + 0.6M $MgCl_2$	±/±	±	±	±/±	±/±
Alcian Blue + 0.8M $MgCl_2$	±	±	±	±	±
Alcian Blue + Safranin	± Blue	±/+ Blue	±/+ Blue	+ Blue	++ Blue
Aldehyde Fuchsin	-	-	-	-	-
Aldehyde Fuchsin + Alcian Blue	+/++ Blue	±/+/++ Blue	±/+/++ Blue	±/+ Blue	+/++ Blue
Hyaluronidase (pH 6.9) + Alcian Blue (pH 2.5)	+/+++	++	+/++	+/++	+/++
Neuraminidase Alcian Blue (pH 2.5)					±

See page 20A for explanation of the symbols.

TABLE 2.3.18

Mucohistochemical reaction of the epidermal mucous cells of precocious 1+ male Atlantic salmon parr sampled in October, 1978.

Stain	Dorsum	Pectoral	Caudal Peduncle
P.A.S.	+++ Red	+++ 100% Red	+++ 100% Red
Hales Colloidal Iron	++/+++ Blue	+/+++ Blue	++ Blue
Hales Colloidal Iron + PAS	80.5% Blue/ Purple +++ 19.5% Blue +++	99% Blue/ Purple +++ ve 1% Blue + ve	91.7% Blue/Purple ++/+++ 2.8% Blue + ve 5.5% Red + ve
Alcian Blue (pH 2.5)	Blue ++	Blue +/+++	Blue ++
Alcian Blue (pH 1.0)	-/± Blue	-/± Blue	-/± Blue
Alcian Blue (pH 2.5) + PAS	74.5% Blue +++ 16.1% Blue/ Purple +++ 9.4% Red ++	84.7% Blue +++ 12.1% Blue/ Purple ++ 3.2% Red ++	68.4% Blue +++ 28.3% Blue Purple ++ 2.3% Red ++
Alcian Blue (pH 1.0) + PAS	93.3% Purple/ Red +++ 6.7% Red ++	90.1% Purple/ Red +++ 9.9% Red ++	100% Purple/ Red +++
Mild Methylation	+/++	±/+	±/+
Active Methylation		±	-/±
Alcian Blue + 0.1M $MgCl_2$	±/+	±	±/+
Alcian Blue + 0.2M $MgCl_2$	±	±	±/+
Alcian Blue + 0.5M $MgCl_2$	±	±	±/+
Alcian Blue + 0.6M $MgCl_2$	±/±	±/±	±
Alcian Blue + 0.8M $MgCl_2$	±	±	±
Alcian Blue (pH 2.5) + Safranin	+ Blue	+ Blue	++ Blue
Aldehyde Fuchsin	-	-	-
Aldehyde Fuchsin + Alcian Blue	+ Blue	+ Blue	+ Blue
Hyaluronidase (pH 6.9) + Alcian Blue (pH 2.5)	+/++ Blue	+ Blue	+ Blue
Neuraminidase + Alcian Blue (pH 2.5)		±	

See page 20A for explanation of the symbols

TABLE 2.3.19

Mucohistochemical reaction of the epidermal mucous cells in immature 1+ parr sampled in December, 1976.

Stain	Dorsum	Pectoral	Caudal Peduncle	Tail	Pectoral Fin
PAS	++ Red	++ Red	++ Red	++ Red	++ Red
Hales Colloidal Iron	+/++ Blue	+/++ Blue	++ Blue	+/++ Blue	+/++ Blue
Hales Colloidal Iron + PAS	89.2% Blue/ Purple +++ 7.8% Blue + 3% Red +	91.3% Blue/ Purple +++ 8% Blue ++ 0.7% Red +	96.4% Blue/ Purple +++ 3.6% Blue	98.1% Blue/ Purple +++ 0.5% Blue + 1.4% Red +	87% Blue/ Purple +++ 3.5% Blue + 9.5% Red +
Alcian Blue (pH 2.5)	++ Blue	++ Blue	++ Blue	++ Blue	++ Blue
Alcian Blue (pH 1.0)	-/±	-	-	-	-
Alcian Blue (pH 2.5) + PAS	75% Blue +++ 25% Blue/ Purple ++	80.2% Blue +++ 16.4% Blue/ Purple + 3.4% Red ++	69.6% Blue ++ 29.7% Blue/ Purple +/++ 0.7% Red ++	24.3% Blue ++ 75.7% Blue/ Purple ++	66.7% Blue ++ 23.5% Blue/ Purple +/± 9.8% Red +
Alcian Blue (pH 1.0) + PAS	100% Red/ Purple ++	98.2% Red/ Purple + 1.8% Red ±	100% Red/ Purple ++/+++	100% Red/ Purple ++/+++	88.6% Red/ Purple +++ 11.4% Red ±/+
Mild Methylation	±/+	+/++	+/++	±/+	+/++
Active Methylation	±/-	-/±	±	±	±
Alcian Blue + 0.1M $MgCl_2$	+	±/+	±	±	±
Alcian Blue + 0.2M $MgCl_2$	+	±	±	±	±
Alcian Blue + 0.5M $MgCl_2$	±	±/±	±	±/±	±/±
Alcian Blue + 0.6M $MgCl_2$	±	-ve	±	±	±
Alcian Blue + 0.8M $MgCl_2$	±	-	±	-/±	-/±
Alcian Blue + Safranin	+	±	±	±	+
Aldehyde Fuchsin	-	-	-	-	-
Aldehyde Fuchsin + Alcian Blue	+/++ Blue	+/Blue	+/++ Blue	+/++ Blue	+/++ Blue
Hyaluronidase (pH 6.9) + Alcian Blue (pH 2.5)	+/++	+/++	+/++	+/++ -	+/++
Neuraminidase + Alcian Blue (pH 2.5)			±		

See page 20A for explanation of the symbols.

TABLE 2.3.20

Mucohistochemical reactions of the epidermal mucous cells in precocious 1+ male parr sampled in December, 1976.

Stain	Dorsum	Pectoral	Caudal Peduncle	Tail	Pectoral Fin
P.A.S.	++ Red	++ Red	++ Red	++ Red	++ Red
Hales Colloidal Iron	++ Blue	++/+++ Blue	++/+++ Blue	++/+++ Blue	+/++ Blue
Hales Colloidal Iron + Pas	78.4% Blue/ Purple +++ 20.5%Blue + 1.1% Red ±	90.1% Blue/ Purple ++/+++ 8.3% Blue ++ 0.8% Red +	89.1% Blue/ Purple ++/+++ 10.9% Blue ++	98.6% Blue/ Purple ++/+++ 1.4% Blue +	81% Blue/ Purple ++ 2.7% Blue + 16.3% Red +
Alcian Blue (pH 2.5)	++	++	++	++	++
Alcian Blue (pH 1.0)	-	-	-/+	-/±	-/±
Alcian Blue (pH 2.5) + PAS	54.7% Blue +++ 45.3% Blue/ Purple ++	53.3% Blue + 45.7% Blue/ Purple +/++ 1% Red	34.2% Blue + 64.2% Blue/ Purple +/++ 1.6% Red +	30% Blue + 68.75% Blue/ Purple +/++ 1.25% Red +	50.7% Blue ++ 24.2% Blue/ Purple + 24.1% Red +/++
Alcian Blue (pH 1.0) + PAS	100% Red/ Purple +/+++	100% Red/ Purple ++/+++	100% Red/ Purple ++ 3.7% Red ±	100%Red/ Purple +++	100% Red/ Purple ++
Mild Methylation	++/+++	++/+	+/++	+	+/++
Active Methylation		±	±/-		±/±
Alcian Blue + 0.1M $MgCl_2$	±	±	±	±	±
Alcian Blue + 0.2M Mg Cl_2	-/±	±	±	±	±
Alcian Blue + 0.5M $MgCl_2$	±	±	±	±	±
Alcian Blue + 0.6M $MgCl_2$	-/±	±	-/±	±	-/±
Alcian Blue + 0.8M $MgCl_2$	-	±	-	±	-
Alcian Blue + Safranin	± Blue	± Blue	± Blue	± Blue	± Blue
Aldehyde Fuchsin	-	-	-	-	-
Aldehyde Fuchsin + Alcian Blue (pH 2.5)	+/++ Blue	+/++ Blue	+/++ Blue	±/+ Blue	+/++ Blue
Hyaluronidase (pH 6.9) + Alcian Blue (pH 2.5)	+/++	±/++	+/++	+/++	+
Neuraminidase + Alcian Blue (pH 2.5)			±		

See page 20A for explanation of the symbols.

TABLE 2.3.21

Prevalence of Saprolegnia infection in 2,000 1+ parr from October, 1975, to February, 1976.

	No. of fungussed Parr	% parr affected
Total	89	4.45
Precocious Males	52	2.60
Immature Parr	37	1.85

TABLE 2.3.22

Number of 1+ parr infected with Saprolegnia fungus in the dorsum, dorsal fin, pectoral, pectoral fin, caudal peduncle and tail regions of the body (October, 1975 to February, 1976)

	Precocious Males	Immature Parr	χ^2 test probability.
Dorsum	7	9	NS*
Dorsal fin	9	5	NS
Pectoral	18	11	NS
Pectoral Fin	30	18	NS
Caudal Peduncle	35	14	0.01
Tail	44	18	0.001

* Not significant.

TABLE 3.3.1.

The effect of exogenous steroids, methyltestosterone, testosterone, cortisol, cortisone and β oestradiol, on the epidermal thickness(μm), the number of cell layers in the epidermis and on the dermal thickness (μm) of the dorsum of 0+ parr

Treatment	Epidermis	No. of cells in the epidermal layer	Dermis
Control (after)	54.11 ± 2.73(a) (7)	5.04 ± 0.11(7)	67.76 ± 3.49(7)
Methyltesto-sterone	84.45 ± 3.13(d) (10)	7.12 ± 0.7(d) (10)	80.28 ± 2.23(b) (10)
Testosterone	74.67 ± 3.76(d) (6)	6.2 ± 0.23(d) (6)	83.85 ± 8.18(b) (6)
Cortisol	53.32 ± 1.83(10)	5.14 ± 0.12(10)	46.83 ± 1.88(d) (10)
Cortisone	54.25 ± 2.28(10)	5.13 ± 0.10(10)	84.64 ± 4.38(c) (10)
β oestradiol	55.68 ± 2.63(9)	5.24 ± 0.19(9)	74.40 ± 3.89(9)

(a) $\bar{x}$ ± S.E. (No. of fish)
(b) Significantly different from control $p \leq 0.05$
(c) Significantly different from control $p \leq 0.01$
(d) Significantly different from control $p \leq 0.001$

TABLE 3.3.2.

The effect of exogenous steroids, methyltestosterone, testosterone, cortisol, cortisone and β oestradiol on the epidermal thickness (μm), the number of cell layers in the epidermis and on the dermal thickness (μm) of the pectoral area of 0+ parr

Treatment	Epidermis	No. of cells in the epidermal layer	Dermis
Control(after)	63.29 ± 2.40(a) (7)	5.37 ± 0.12(7)	64.11 ± 3.77(7)
Methyltesto-sterone	96.11 ± 4.27(d) (10)	7.8 ± 0.27(d) (10)	55.45 ± 3.02(c) (10)
Testosterone	79.58 ± 4.15(b) (5)	6.98 ± 0.36(d) (5)	54.28 ± 1.62(b) (5)
Cortisol	58.26 ± 3.19(d) (10)	5.2 ± 0.19(10)	47.19 ± 2.05(d) (10)
Cortisone	68.01 ± 1.05(9)	5.91 ± 0.09(9)	59.87 ± 1.92(9)
β oestradiol	61.78 ± 2.51(d) (9)	5.63 ± 0.15 (9)	47.53 ± 1.73(a) (9)

(a) $\bar{x} \pm$ S.E. (No. of fish)

(b) Significantly different from control, $p \leq 0.05$

(c) Significantly different from control, $p \leq 0.01$

(d) Significantly different from control, $p \leq 0.001$

TABLE 3.3.3.

The effect of exogenous steroids, methyltestosterone, testosterone, cortisol and cortisone on the thickness (μm) and number of cell layers in the epidermis of the pectoral fin in 0+ parr

	Epidermis	No. of cell layers in the epidermis
Control (after)	64.04 ± 2.90 (a) (7)	6.23 ± 0.15 (7)
Methyltestosterone	88.08 ± 2.71 (b) (10)	8.10 ± 0.17 (b) (10)
Testosterone	92.25 ± 2.70 (b) (6)	7.90 ± 0.19 (b) (6)
Cortisol	67.68 ± 2.60 (10)	6.65 ± 0.16 (10)
Cortisone	69.24 ± 1.6 (9)	6.70 ± 0.14 (9)

(a) $\bar{x}$ ± S.E. (No. of fish)

(b) Significantly different from control $p \leq 0.001$

TABLE 3.3.4.

The effect of exogenous steroids, methyltestosterone, testosterone, cortisol, cortisone and β oestradiol on the epidermal thickness(μm), no. of cell layers in the epidermis and on the dermal thickness (μm) of the caudal peduncle of 0+ parr

Treatment	Epidermis	Dermis	No. of cell layers in the epidermis
Control (after)	46.05 ± 3.05 (a) (2)	90.25 ± 3.26(2)	4.7 ± 0.30(2)
Methyltesto-sterone	77.03 ± 2.30 (10)	93.36 ± 4.39(10)	6.58± 0.13(10)
Testosterone	65.18 ± 5.21 (4)	104.38 ± 8.98(4)	6.23± 0.32(4)
Cortisol	49.24 ± 1.75(10)	71.43 ± 3.43(10)	4.99± 0.20(10)
Cortisone	49.77 ± 1.17(10)	103.77 ± 3.12(10)	4.96± 0.10(10)
β oestradiol	41.22 ± 2.42(9)	82.96 ± 4.94(9)	4.29± 0.13(9)

(a) $\bar{x}$ ± S.E. (No. of fish)

TABLE 3.3.5.

The effect of exogenous steroids, methyltestosterone, testosterone, cortisol, cortisone and β oestradiol on the epidermal thickness (μm) and on the number of cell layers in the epidermis of the tail region of 0+ parr

	Epidermis	No. of cell layers in the epidermis
Control (after)	69.35 ± 8.67(a)(2)	6.65 ± 0.25(2)
Methyltestosterone	93.93 ± 3.71(10)	8.11 ± 0.24(10)
Testosterone	93.66 ± 7.52(5)	8.04 ± 0.41(5)
Cortisol	69.38 ± 2.69(10)	7.05 ± 0.28(10)
Cortisone	78.73 ± 5.89(9)	7.43 ± 0.38(9)
β oestradiol	76.76 ± 7.88(5)	7.28 ± 0.82(5)

a) $\bar{x}$ ± S.E. (No. of fish)

TABLE 3.3.6

Changes in the epidermal and dermal thickness (μm) and the no. of cell layers in the epidermis of the dorsum, pectoral and caudal peduncle areas of 0+ parr during the period mid-September to mid-December, 1976

		Epidermis	No. of cell layers in the epidermis	Dermis
Dorsum	Control (Before)	31.43 ± 1.01(a,b) (8)	4.90 ± 0.2(8)	51.38 ± 2.69(c) (8)
	Control (After)	54.11 ± 2.73(b) (7)	5.04 ± 0.11(7)	67.76 ± 3.49(c) (7)
Pectoral	Control (Before)	28.0 ± 4.34(d) (7)	4.50 ± 0.23(e) (7)	38.30 ± 6.03(f) (7)
	Control (After)	63.29 ± 2.40(d) (7)	5.37 ± 0.12(e) (7)	64.11 ± 3.77(f) (7)
Caudal Peduncle	Control (Before)	28.39 ± 4.34(8)	4.70 ± 0.36(8)	62.73 ± 5.73(8)
	Control (After)	46.05 ± 3.05(2)	4.70 ± 0.30(2)	90.25 ± 3.26(2)

a, $\bar{x} \pm$ S.E. (No. of fish)
b,d, $p \leq 0.005$
c,f, $p \leq 0.0025$
e, $p \leq 0.01$

TABLE 3.3.7

Histochemical reaction of the epidermal mucous cells in the dorsum, pectoral and caudal peduncle areas of 0+ parr during September, 1976.

Stain	Dorsum	Pectoral	Caudal Peduncle
P.A.S.	++ Red	++ Red	++ Red
Hales Colloidal Iron	+/++ Blue	+/++	+/++ Blue
Hales Colloidal Iron + P.A.S.	16% Blue + 84% Blue/Purple ++	17.4% Red + 82.6% Blue/ Purple +++	14.3% Red + 2.4% Blue + 83.3% Blue/Purple +++
Alcian Blue (pH 2.5)	++	++	++
Alcian Blue (pH 1.0)	-ve	-ve	-ve
Alcian Blue (pH 2.5) + P.A.S.	60% Blue ++ 36% Blue/Purple ++ 4% Red +	65% Blue ++ 31% Blue/Purple + 4% Red	50% Blue ++ 40% Blue/Purple + 10% Red +
Alcian Blue (pH 1.0) + P.A.S.	100% Red/Purple ++	100% Red/Purple +	100% Red/Purple ++
Mild Methylation	+	+	±
Active Methylation	±	±	±
Alcian Blue + 0.1M $MgCl_2$	+/±	+	+
Alcian Blue + 0.2M $MgCl_2$	±	±	±
Alcian Blue + 0.5M $MgCl_2$	±	±	±
Alcian Blue + 0.6M $MgCl_2$	±	±	±
Alcian Blue + 0.8M $MgCl_2$	±	±/-	±/-
Alcian Blue + 0.9M $MgCl_2$	±/-	-	-
Alcian Blue + Safranin	± (Blue)	± (Blue)	± (Blue)
Aldehyde Fuchsin	-ve	-ve	-ve
Aldehyde Fuchsin + Alcian Blue	+/++ (Blue)	+ (Blue)	+/++ (Blue)
Hyaluronidase (pH 6.9) + Alcian Blue	+/++	+/++	+/++

See page 20A for explanation of the symbols.

TABLE 3.3.8.

Histochemical reaction of epidermal mucous cells in the dorsum, pectoral, caudal peduncle, tail and pectoral regions of 0+ parr during December, 1976.

Stain	Dorsum	Pectoral	Caudal Peduncle	Tail	Pectoral Fin
P.A.S.	++	++	++	++	++
Hales Colloidal Iron	+	+	+	++	+/++
Hales Colloidal Iron + PAS	89.9% Blue/ Purple ++ 7.5% Blue + 2.6% Red +	92.3% Blue/ Purple ++ 7% Blue + 0.3% Red ±	93.5% Blue/ Purple ++ 6% Blue + , 0.5% Red ±	98.8% Blue/ Purple +++ 1.2% Blue ++	93.2% Blue/ Purple +++ 1.6% Blue + 5.2% Red ±/+
Alcian Blue (pH 2.5)	+/++	++/+++	++	++	++/+++
Alcian Blue (pH 1.0)	-	-	-	-	-
Alcian Blue (pH 2.5) + PAS	86.5. Blue + 12.3% Blue/ Purple + 1.2% Red ±	74.7% Blue ++ 25.3% Blue/ Purple +	47.8% Blue +/++ 52.2% Blue/ Purple ±/+	75.8% Blue ++ 24.2. Blue/ Purple ±/+	43.1% Blue ++ 33.3% Blue/ Purple + 23.6% Red ++
Alcian Blue (pH 1.0) + PAS	100% Red/ Purple ++	100% Red/ Purple ++	100% Red/ Purple ++	100% Red/ Purple +/++	99.2% Red/ Purple ++ 0.8% Red ±
Mild Methylation	±/+	+	+	+	+
Active Methylation	±	±	±	±	±
Alcian Blue + 0.1M $MgCl_2$	±/+	+	+	+	±
Alcian Blue + 0.2M $MgCl_2$	±	+/±	±	±	±
Alcian Blue + 0.5M $MgCl_2$	±/-	±	±	±	±
Alcian Blue + 0.6M $MgCl_2$	-	-	-	-	-
Alcian Blue + 0.8M $MgCl_2$	-	±	±/-	±/-	±/-
Alcian Blue + 0.9M $MgCl_2$	-	±/-	-	-	-
Alcian Blue + Safranin	± (Blue)	±/+ (Blue)	± (Blue)	± (Blue)	±/+ (Blue)
Aldehyde Fuchsin	-	-	-	-	-
Aldehyde Fuchsin + Alcian Blue	+/++ (Blue)	+ (Blue)	+/++ (Blue)	+/++ (Blue)	+ (Blue)
Hyaluronidase (pH 6.9) + Alcian Blue (pH 2.5)	+/++	+/++	++	++	+/++
Neuraminidase + Alcian Blue (pH 2.5) + PAS		100% Red/ Purple + ve			

TABLE 3.3.9.

Histochemical reaction of epidermal mucous cells in the dorsum, pectoral, caudal peduncle areas of O+ parr treated with oestrogen.

Stain	Dorsum	Pectoral	Caudal Peduncle
P.A.S.	++	++	++
Hales Colloidal Iron	+/++	+	+
Hales Colloidal Iron + P.A.S.	85.8% Blue/Purple ++ 12.8% Blue ++ 14% Red ±	87.5% Blue/Purple ++ 11.9% Blue + 0.6% Red ±	95.6% Blue/Purple + 0.7% Blue ± 3.7% Red ±
Alcian Blue (pH 2.5)	+/++	+/++	++/+++
Alcian Blue (pH 1.0)	-	-	-
Alcian Blue (pH 2.5) + P.A.S.	52.7% Blue ++ 45.3% Blue/Purple ++ 2% Red ±	45.3% Blue ++ 54.7% Blue/Purple ++	40% Blue + 56.8% Blue/Purple + 3.2% Red ±
Alcian Blue (pH 1.0) + P.A.S.	100% Red/Purple ++	100% Red/Purple ++	100% Red/Purple ++
Mild Methylation	+	±/+	+
Active Methylation	±	±	±
Alcian Blue +0.1M $MgCl_2$	±	±/+	±
Alcian Blue + 0.2M $MgCl_2$	±	±	±
Alcian Blue + 0.5M $MgCl_2$	±	±	±
Alcian Blue + 0.6M $MgCl_2$	±	±	±
Alcian Blue + 0.8M $MgCl_2$	±/-	-	-
Alcian Blue + 0.9M $MgCl_2$	-	-	-
Alcian Blue + Safranin	± (Blue)	±/+ (Blue)	± (Blue)
Aldehyde Fuchsin	-	-	-
Aldehyde Fuchsin + Alcian Blue	(Blue)	(Blue)	±/+ (Blue)

See page 20A for explanation of the symbols.

TABLE 3.3.10

Histochemical reaction of epidermal mucous cells in the dorsum, pectoral, caudal peduncle, tail, pectoral fin areas of 0+ parr treated with testosterone.

Stain	Dorsum	Pectoral	Caudal Peduncle	Tail	Pectoral Fin
P.A.S.	++	++	++	++	++
Hales Colloidal Iron	+/++	+	+/++	++	+/++
Hales Colloidal Iron + PAS	2.8% Blue ++ 92.2% Blue/ Purple ++ 5% Red +	4.2% Blue + 94.6% Blue/ Purple +++ 1.2% Red ±	6.1% Blue + 92.9% Blue/ Purple ++ 1% Red ±	1.2% Blue + 97.1% Blue/ Purple +++ 1.7% Red ±	73.4% Blue/ Purple +++ 26.6% Red +
Alcian Blue (pH 2.5)	++/+++	+/++	+/++	++	++
Alcian Blue (pH 1.0)	-	-	-	-	-
Alcian Blue (pH 2.5) + PAS	73.5% Blue +++ 18% Blue/ Purple ++ 8.5% Red ±	73.9% Blue +++ 26.1% Blue/ Purple ±	37.2% Blue +++ 58.6% Blue/ Purple +/++ 4.2% Red ±	100% Blue/ Purple +/++	17.2% Blue ++ 63.4% Blue/ Purple ++/+++ 19.3% Red ±
Alcian Blue (pH 1.0) + PAS	100% Red/ Purple ++	100% Red/ Purple ++	100% Red/ Purple ++	100% Red/ Purple	100% Red/ Purple
Mild Methylation	±/+	+	±/+	±/+	±/+
Active Methylation	±	±	±	±	±
Alcian Blue + 0.1M $MgCl_2$	±	±	±	±	±
Alcian Blue + 0.2M $MgCl_2$	±	±	±	±	±
Alcian Blue + 0.5M $MgCl_2$	±	±	±	±	±
Alcian Blue + 0.6M $MgCl_2$	±/-	±	±	±/-	±/-
Alcian Blue + 0.8M $MgCl_2$	-	-	-	-	-
Alcian Blue + 0.9M $MgCl_2$	-	-	-	-	-
Alcian Blue + Safranin	± (Blue)	± (Blue)	± (Blue)	± (Blue)	± (Blue)
Aldehyde Fuchsin	-	-	-	-	-
Aldehyde Fuchsin + Alcian Blue	+/++ (Blue)	+ (Blue)	+/++ (Blue)	+ (Blue)	+/+++ (Blue)
Hyaluronidase (pH 6.9) + Alcian Blue	+/++	+	+/++	+/++	+/++

See page 20A for explanation of the symbols.

TABLE 3.3.11

Histochemical reaction of epidermal mucous cells in the dorsum, pectoral, caudal peduncle, tail, pectoral fin areas of 0+ parr treated with methyltestosterone.

Stain	Dorsum	Pectoral	Caudal Peduncle	Tail	Pectoral Fin
P.A.S.	++	++	++	++	++
Hales Colloidal Iron	+/++	++	+	+	+/++
Hales Colloidal Iron + PAS	6.7% Blue + 92.6% Blue/ Purple +++ 0.7% Red +	12.3% Blue +++ 87.7% Blue/ Purple +++	100% Blue/ Purple +++	8.4% Blue ++ 91.6% Blue/ Purple +/++	8.1% Blue ++ 79.4% Blue/ Purple +++ 12.5% Red ±
Alcian Blue (pH 2.5)	+/++	++/+++	++	+++	+++
Alcian Blue (pH 1.0)	-	-	-	±	-
Alcian Blue (pH 2.5) + PAS	77.5% Blue ++ 19.4% Blue/ Purple ++ 3.1% Red ±	42.9% Blue +++ 60.9% Blue/ Purple ++	65.7% Blue ++ 34.3% Blue/ Purple +	36.2% Blue+ 63.8% Blue/ Purple ±	30.8% Blue ++ 56.9% Blue/ Purple + 12.3% Red ±
Alcian Blue (pH 1.0) + PAS	100% Red/ Purple ++	100% Red/ Purple ++	100% Red/ Purple ++	100% Red/ Purple ++	100% Red/ Purple ++
Mild Methylation	±/-	±/+	±	±	+
Active Methylation	±	±	±	±	±
Alcian Blue + 0.1M $MgCl_2$	±/+	±	±	±	±
Alcian Blue + 0.2M $MgCl_2$	±	±	±	±	±
Alcian Blue + 0.5M $MgCl_2$	-	±/-	±/-	±	±
Alcian Blue + 0.6M $MgCl_2$	-	-	-	±/-	-
Alcian Blue + 0.8M $MgCl_2$	-	-	-	-	-
Alcian Blue + 0.9M $MgCl_2$	-	-	-	-	-
Alcian Blue + Safranin	±/+ (Blue)	± (Blue)	± (Blue)	± (Blue)	± (Blue)
Aldehyde Fuchsin	-	-	-	-	-
Aldehyde Fuchsin + Alcian Blue	+/++ (Blue)	+/++ (Blue)	+/++ (Blue)	+/++ (Blue)	+/++ (Blue)
Hyaluronidase (pH 6.9) + Alcian Blue	+/++	++	+/++	+/++	+/++
Neuraminidase + Alcian Blue + PAS			100% Red/ Purple ±		

See page 20A for explanation of the symbols.

TABLE 3.3.12

Histochemical reaction of the epidermal mucous cells in the dorsum, pectoral, caudal peduncle, tail and pectoral fin areas of 0+ parr treated with cortisone.

Stain	Dorsum	Pectoral	Caudal Peduncle	Tail	Pectoral Fin
P.A.S.	++	++	++	++	++
Hales Colloidal Iron	+/++	+	+	+/++	++
Hales Colloidal Iron + PAS	93% Blue/ Purple ++ 3.5% Blue ++ 4.5% Red +	96.8% Blue/ Purple +++ 1.8% Blue + 1.4% Red ±	94.4% Blue/ Purple ++ 3.4% Blue ++ 2.2% Red +	80.9% Blue/ Purple ++ 13.5% Blue + 5.6% Red +	71.5% Blue/ Purple +++ 3.2% Blue ++ 25.3% Red++
Alcian Blue (pH 2.5)	+/++	+/++	+/++	+/++	+/++
Alcian Blue (pH 1.0)	-	-	-	-	-
Alcian Blue (pH 2.5) + PAS	75.6% Blue ++ 24.4% Blue/ Purple ++	73.7% Blue ++ 26.3% Blue/ Purple +	27.6% Blue ++ 69.8% Blue/ Purple ++ 2.5% Red ±	48.4% Blue +/++ 45.3% Blue/ Purple + 9.4% Red ±	52.5% Blue ++ 47.5% Blue/ Purple ++
Alcian Blue (pH 1.0) + PAS	100% Red/ Purple ++	100% Red/ Purple ++	100% Red/ Purple ++	100% Red/ Purple +/++	100% Red/ Purple ++
Mild Methylation	±/+	±/+	+	±/+	+/++
Active Methylation	±	±	±	±	±
Alcian Blue + 0.1M $MgCl_2$	±	±/+	+	±/+	±
Alcian Blue + 0.2M $MgCl_2$	±	±	±	±	±
Alcian Blue + 0.5M $MgCl_2$	-	±	±	±	±
Alcian Blue + 0.6M $MgCl_2$	-	±	±	±	±/-
Alcian Blue + 0.8M $MgCl_2$	-	±/-	±/-	±/-	±/-
Alcian Blue + 0.9M $MgCl_2$	-	±/-	-	-	-
Alcian Blue + Safranin	± (Blue)	± (Blue)	± (Blue)	+ (Blue)	± (Blue)
Aldehyde Fuchsin	-	-	-	-	-
Aldehyde Fuchsin + Alcian Blue	±/+ (Blue)	+/++ (Blue)	+ (Blue)	+ (Blue)	+ (Blue)
Hyaluronidase (pH 6.9%) + Alcian Blue	+/++	+	+	+/++	++

See page 20A for explanation of the symbols

TABLE 3.3.13.

Histochemical reaction of epidermal mucous cells in the dorsum, pectoral, caudal peduncle, tail and pectoral fin areas of 0+ parr treated with cortisol.

Stain	Dorsum	Pectoral	Caudal Peduncle	Tail	Pectoral Fin
P.A.S.	++	++	++	++	++
Hales Colloidal Iron	++	++/+++	+/++	++	++/+++
Hales Colloidal Iron + PAS	87.7% Blue/ Purple ++ 12.3% Blue +	93.2% Blue/ Purple ++ 6.1% Blue +	83.3% Blue/ Purple +++ 16.7% Blue ++	97.4% Blue/ Purple +++ 2.6% Red±	97.9% Blue/ Purple +++ 2.1% Blue ±
Alcian Blue (pH 2.5)	++	+/++	+/++	++/+++	++/+++
Alcian Blue (pH 1.0)	-	-	-	-	-
Alcian Blue (pH 2.5) + PAS	48.2% Blue +++ 49.8% Blue/ Purple ++ 2% Red +	42.5% Blue ++ 57.5% Blue/ Purple +	31.7% Blue + 66.8% Blue/ Purple ++ 1.5% Red ±	22.9% Blue ++ 77.1% Blue/ Purple +	50.5% Blue +++ 35.3% Blue/ Purple ++ 14.2% Red +
Alcian Blue (pH 1.0) + PAS	100% Red/ Purple ++/+++	100% Red/ Purple ++/+++	100% Red/ Purple +++	100% Red/ Purple ++	100% Red/ Purple +++
Mild Methylation	±/+	+	+	+/++	±/+
Active Methylation	±	±	±	±	±
Alcian Blue + 0.1M $MgCl_2$	±/+	±	±	±	±
Alcian Blue + 0.2M $MgCl_2$	±	±	±	±	±
Alcian Blue + 0.5M $MgCl_2$	±	±	±	±	±
Alcian Blue + 0.6M $MgCl_2$	±	±/-	±	±	±/-
Alcian Blue + 0.8M $MgCl_2$	±/-	-	±/-	±/-	-
Alcian Blue + 0.9M $MgCl_2$	-	-	-	-	-
Alcian Blue + Safranin	± (Blue)	± (Blue)	± (Blue)	± (Blue)	± (Blue)
Aldehyde Fuchsin	-	-	-	-	-
Aldehyde Fuchsin + Alcian Blue	+ (Blue)	+/++	+	+/++	+
Hyaluronidase (pH 6.9) + Alcian Blue	+/++	++/+++	++/+++	++/+++	+/++

See page 20A for explanation of the symbols.

TABLE 3.3.14

The nuclear diameter(μm) of the interrenal cells of O+ parr treated with methyltestosterone, testosterone, cortisol, cortisone and β oestradiol.

Treatment	Interrenal Nuclear Diameter
Control	$4.69 \pm 0.1 \overset{(a,b,c,d)}{(5)}$
Methyltestosterone	$5.07 \pm 0.14 \overset{(b)}{(7)}$
Testosterone	$5.39 \pm 0.85 \overset{(c)}{(4)}$
Cortisol	$4.50 \pm 0.14_{(7)}$
β oestradiol	$4.91 \pm 0.06_{(8)}$
Cortisone	$5.34 \pm 0.07 \overset{(d)}{(7)}$

a, $\overset{-}{+}$ $\pm$ S.E. (No. of fish)
b, $p \leq 0.05$
c, $p \leq 0.01$
d, $p \leq 0.001$

TABLE 3.3.14

The nuclear diameter(μm) of the interrenal cells of 0+ parr treated with methyltestosterone, testosterone, cortisol, cortisone and β oestradiol.

Treatment	Interrenal Nuclear Diameter
Control	$4.69 \pm 0.1 \,{}^{(a,b,c,d)}_{(5)}$
Methyltestosterone	$5.07 \pm 0.14 \,{}^{(b)}_{(7)}$
Testosterone	$5.39 \pm 0.85 \,{}^{(c)}_{(4)}$
Cortisol	$4.50 \pm 0.14_{(7)}$
β oestradiol	$4.91 \pm 0.06_{(8)}$
Cortisone	$5.34 \pm 0.07 \,{}^{(d)}_{(7)}$

a, $\bar{+}$ $\pm$ S.E. (No. of fish)
b, $p \leq 0.05$
c, $p \leq 0.01$
d, $p \leq 0.001$

TABLE 3.3.15

Mortalities due to Saprolegnia amongst 0+ parr fed methyltestosterone, testosterone, cortisol, cortisone and β oestradiol.

Treatment	Control	β oestradiol	Cortisone	Cortisol	Methyltestosterone	Testosterone
No. of fish at start of experiment	47 (a,b)	49 (a)	42	62 (b)	42	49
Mortalities due to Saprolegnia	-	28 (a)	-	23 (b)	1	-

a,b, $p \leq 0.001$

TABLE 3.3.16

Patterns of Saprolegnia infection in O+ parr fed cortisol and 17 β oestradiol.

Treatment	Head	Dorsum + Dorsal Fin	Caudal peduncle + tail	Pectoral + pectoral fin
Cortisol	-	1	8	15[(a)]
β oestradiol	-	2	9	26[(a)]

a, $p < 0.025$

Table 4.3.1.

The changes in the length (cm), weight (g) and the condition factor ('K') of 1+ Atlantic salmon parr fed methallibure and cyproterone acetate during the period October to December, 1976.

	Precocious Males			Immature Parr		
	Length	Weight	'K'	Length	Weight	'K'
Control (0 weeks)	13.36(a) ± 0.37(11)	31.07 ± 2.23(11)	1.10 ±0.02(11)	14.93 ± 0.36(12)	38.68 ± 2.63(12)	1.13 ±0.02(10)
Control (14 weeks)	13.74 ± 0.29(17)	28.14 ± 1.89(17)	1.00 ±0.02(17)	13.82 ± 0.58(6)	27.70 ± 3.84(6)	1.01 ±0.03(6)
Methallibure	14.28 ± 0.28(24)	32.23 ± 1.78(24)	1.01 ±0.02(24)	15.47 ± 0.28(6)	36.97 ± 2.45(6)	0.99 ±0.03(6)
Cyproterone acetate	13.84 ± 0.30(21)	28.97 ± 1.66(21)	0.99 ±0.01(20)	15.03 ± 0.43(12)	34.51 ± 2.79(12)	1.01 ±0.03(12)

a, $\bar{x} \pm$ S.E. (No. of fish)

Table 4.3.2.

Gonadosomatic indices (G.S.I.) and plasma levels of 11-ketotestosterone (µg/100 ml), in 1 + parr treated with methallibure and cyproterone acetate during the period October to December 1976

	Precocious Males		Immature Parr	
	11-Keto-testosterone	G.S.I.	11-Keto-testosterone	G.S.I.
Control (0 weeks)	$0.72 \pm 0.05^{(a)}_{(9)}$	$13.54 \pm 1.40_{(11)}$	$0.14 \pm 0.03_{(10)}$	$0.35 \pm 0.03_{(10)}$
Control (14 weeks)	$1.01 \pm 0.12^{(b)(c)}_{(13)}$	$6.71 \pm 0.31^{(d)}_{(16)}$	$0.10 \pm 0.02_{(6)}$	$0.46 \pm 0.03_{(7)}$
Methallibure	$0.35 \pm 0.06^{(b)}_{(15)}$	$7.41 \pm 0.43_{(24)}$	$0.15 \pm 0.03_{(6)}$	$0.37 \pm 0.02_{(6)}$
Cyproterone Acetate	$0.47 \pm 0.06^{(c)}_{(17)}$	$8.05 \pm 0.43^{(d)}_{(20)}$	$0.10 \pm 0.03_{(10)}$	$0.43 \pm 0.03_{(11)}$

a, $\bar{x} \pm$ S.E. (No. of fish)
b, $p \leq 0.001$
c, $p \leq 0.001$
d, $p \leq 0.05$

Table 4.3.3.

Plasma levels of cortisol (µg/100 ml) in 1+ parr treated with methallibure and cyproterone acetate during the period October to December 1976

Treatment	Precocious Males	Immature Parr
Controls (0 weeks)	$0.95 \pm 0.17^{(a)}_{(12)}$	$0.57 \pm 0.16_{(12)}$
Controls (14 weeks)	$3.97 \pm 0.58_{(11)}$	$3.64 \pm 0.65_{(6)}$
Methallibure	$4.47 \pm 0.28_{(13)}$	$3.86 \pm 1.06_{(4)}$
Cyproterone acetate	$5.00 \pm 0.54_{(15)}$	$3.78 \pm 0.86_{(9)}$

a = $\bar{x} \pm$ S.E. (No. of fish).

Table 4.3.4.

Nuclear diameter (μm) and cellular area (μm^2) of the basophils in the pro-adenohypophysis and meso-adenohypophysis of 1 + parr treated with methallibure and cyproterone acetate during the period October to December 1976

	Pro-adenohypophysis		Meso-adenohypophysis	
	Nuclear Diameter	Cellular Area	Nuclear Diameter	Cellular Area
Control (14 weeks)	$4.07 \pm 0.09^{(a)}_{(8)}$	$47.87 \pm 1.56_{(8)}$	$4.27 \pm 0.08_{(8)}$	$47.42 \pm 1.01_{(8)}$
Methallibure	$4.21 \pm 0.10_{(6)}$	$40.22 \pm 2.13^{(b)}_{(6)}$	$4.12 \pm 0.05_{(7)}$	$40.93 \pm 1.00^{(c)}_{(7)}$
Cyproterone Acetate	$3.92 \pm 0.09_{(7)}$	$38.16 \pm 1.66^{(c)}_{(7)}$	$4.13 \pm 0.05_{(7)}$	$38.60 \pm 1.55^{(d)}_{(7)}$

a, $\bar{x} \pm$ S.E. (No. of fish)
b, Significantly different from the control ($p \leq 0.05$)
c, Significantly different from the control ($p \leq 0.01$)
c, Significantly different from the control ($p \leq 0.001$).

TABLE 4.3.5.

The effects of administration of methallibure, and cyproterone acetate on the epidermis (μm), and dermis (μm) and no. of cell layers in the epidermis of the dorsum, pectoral and caudal peduncle areas of precocious 1+ male Atlantic salmon parr during the period October to December 1976.

	DORSUM			PECTORAL			CAUDAL PEDUNCLE		
Treatment	Epidermis	No. of cell layers in epidermis	Dermis	Epidermis	No. of cell layers in Epidermis	Dermis	Epidermis	No. of cell layers in Epidermis	Dermis
Control	79.96 (a) ±2.16 (b) (16)	6.62 ±0.16 (c) (16)	112.12 ± 4.32 (16)	91.36 (d) ±3.69 (16)	7.23 (e) ±0.21 (16)	84.64 ±2.93 (16)	81.76 (f) ±2.72 (16)	7.04 (g) ±0.20 (16)	181.33 ± 6.25 (16)
Cyproterone Acetate	71.30 (b) ±2.70 (14)	6.10 (c) ±0.23 (14)	119.04 ± 7.65 (14)	76.36 (d) ±2.46 (14)	6.29 (e) ±0.15 (14)	83.75 ±3.78 (14)	68.51 (f) ±1.29 (14)	6.08 (g) ±0.10 (14)	171.24 ± 7.17 (14)
Methallibure	71.60 (b) ±1.60 (15)	5.87 (c) ±0.13 (15)	111.4 ± 7.61 (15)	73.22 (d) ±1.60 (15)	6.19 (e) ±0.13 (15)	87.07 ±4.07 (15)	68.72 (f) ±2.12 (14)	5.96 (g) ±0.22 (14)	172.58 ± 7.81 (14)

a, $\bar{x} \pm$ S.E. (No. of fish)
b,c Significantly different from the control ($p \leq 0.01$)
d,e,f,g, Significantly different from the control ($p \leq 0.001$)

Table 4.3.6.

The effects of administration of methallibure, and cyproterone acetate on the epidermis (μm), and dermis (μm) and no. of cell layers in the epidermis of the dorsum, pectoral and caudal peduncle areas of 1+ immature female Atlantic salmon parr during the period October to December 1976

	DORSUM			PECTORAL			CAUDAL PEDUNCLE		
Treatment	Epidermis	No. of cell layers in Epidermis	Dermis	Epidermis	No of cell layers in Epidermis	Dermis	Epidermis	No. of cell layers in Epidermis	Dermis
Control	68.56 (a) ±2.34 (b) (7)	5.89 ±0.19 (7)	110.9 ± 6.52 (7)	71.98 ± 4.48 (6)	6.43 ±0.22 (6)	78.88 ±7.83 (6)	59.26 (c) ±3.53 (7)	5.47 ±0.28 (7)	155.16 ± 5.28 (7)
Cyproterone acetate	74.7 (b) ±1.33 (10)	6.33 ±0.10 (10)	107.86 ± 5.58 (10)	77.93 ±2.95 (10)	6.49 ±0.21 (10)	94.08 ±5.34 (10)	69.31 (c) ±1.69 (10)	6.03 ±0.17(10)	173.58 ±10.46 (10)
Methallibure	71.33 ±1.47 (7)	5.91 ±0.18 (7)	117.47 ± 4.32 (7)	81.30 ±3.11 (8)	6.56 ±0.24 (8)	89.20 ±6.58 (8)	64.65 ±2.05 (7)	5.74 ±0.13 (7)	184.16 ± 7.31 (7)

a, $\bar{x} \pm$ S.E. (No. of fish)

b, Significantly different from the control ($p \leq 0.05$)

c, Significantly different from the control ($p \leq 0.01$)

Table 4.3.7.

The effects of administration of methallibure and cyproterone acetate on the epidermis (μm) and number of cell layers in the epidermis of the pectoral fin and tail regions of immature 1+ Atlantic salmon parr during the period October to December 1976

	PECTORAL FIN		TAIL	
	Epidermis	No. of cell Layers in Epidermis	Epidermis	No. of cell Layers in Epidermis
Control	94.73(a,b,c) ± 1.80(7)	8.09 ±0.17(7)	99.91 ±9.56(7)	8.77 ±0.67(7)
Cyproterone acetate	107.87(b) ± 3.87(9)	8.53 ±0.26(9)	111.71 ±8.59(10)	8.79 ±0.42(10)
Methallibure	114.24(c) ± 4.46(8)	9.30 ±0.28(8)	111.33 ± 5.39(7)	9.14 ±0.47(7)

a, $\bar{x} \pm$ S.E. (No. of fish)
b, Significantly different from the control ($p \leq 0.05$)
c, Significantly different from the control ($p \leq 0.001$)

Table 4.3.8.

The effects of administration of cyproterone acetate and methallibure on the epidermis (μm) and number of cell layers in the epidermis of the pectoral fin and tail regions of precocious 1+ male Atlantic salmon parr during the period October to December 1976

	PECTORAL FIN		TAIL	
	Epidermis	No. of cell layers in epidermis	Epidermis	No. of cell layers in epidermis
Control	98.71 (a) ± 3.01 (15)	8.29 ±0.23 (15)	103.77 ± 3.53	8.71 ±0.28 (15)
Cyproterone acetate	103.13 ± 4.54 (14)	8.71 ±0.32 (14)	111.8 ± 6.05 (13)	9.58 ±0.43 (13)
Methallibure	97.39 ± 2.40 (15)	8.37 ±0.25 (15)	100.77 ± 4.23 (15)	8.80 ±0.37 (15)

a, $\bar{x} \pm$ S.E. (No. of fish)

TABLE 4.3.9.

Histochemical reactions of the epidermal mucous cells in the dorsum, pectoral, caudal peduncle, tail and pectoral fin regions of precocious 1+ male parr treated with cyproterone acetate during the period October to December, 1976.

Stain	Dorsum	Pectoral	Caudal Peduncle	Tail	Pectoral Fin
P.A.S.	++	++	++	++	++
Hales Colloidal Iron	+	+	+	+	+
Hales Colloidal Iron + PAS	11.4% Blue + 88.6% Blue/ Purple +++	3.7% Blue + 90.6% Blue/ Purple +++	2.8% Blue + 97.2% Blue/ Purple +/+++	100% Blue/ Purple ++/+++	0.5% Blue + 70% Blue/ Purple ++ 29.5% Red+
Alcian Blue (pH 2.5)	++	++	++/+++	++	++/+++
Alcian Blue (pH 1.0)	-	-	-	-	-
Alcian Blue (pH 2.5) + PAS	59.1% Blue +++ 39.8% Blue/ Purple ++ 1.1% Red ±	64.4% Blue +++ 35.6% Blue/ Purple +/+++	32.2% Blue ++ 65.7% Blue/ Purple ++/+++ 2.1% Red±/+	32.5% Blue ++ 66% Blue/ Purple +/+++ 1.5% Red +	42% Blue ++ 34.1% Blue/ Purple ± 23.9% Red+
Alcian Blue (pH 1.0) + PAS	100% Dark Purple +++	100% Dark Purple +++	100% Dark Purple +++	100% Dark Purple +++	100% Dark Purple +++
Mild Methylation	+/++	+/++	+	+	+
Active Methylation	±	±	±	±	±
Alcian Blue + 0.1M $MgCl_2$	±	±	±	±	+/±
Alcian Blue + 0.2M $MgCl_2$	±	±	±	±	±
Alcian Blue + 0.5M $MgCl_2$	-	±	-	-	-
Alcian Blue + 0.6M $MgCl_2$	-	-	-	-	-
Alcian Blue + 0.8M $MgCl_2$	-	-	-	-	-
Alcian Blue + 0.9M $MgCl_2$	-	-	-	-	-
Alcian Blue + Safranin	± (Blue)	± (Blue)	± (Blue)	± (Blue)	±/+ (Blue)
Aldehyde Fuchsin	-	-	-	-	-
Aldehyde Fuchsin + Alcian Blue	+ (Blue)	+ (Blue)	+/++ (Blue)	+ (Blue)	+ (Blue)
Hyaluronidase (pH 6.9) Control	++	++	++	++	++
Hyaluronidase (pH 6.9)	++	++	++	++	++
Neuraminidase (250 units/ml) + AB (pH 2.5) + PAS (Control)			40.5% Blue ++ 58.6% Blue/Purple ++ 1.9% Red ±		
Neuraminidase (250 Units/ml) + Alcian Blue + PAS			100% Purple ±		

See page 20A for explanation of the symbols.

TABLE 4.3.10

Histochemical reactions of the epidermal mucous cells in the dorsum, pectoral, caudal peduncle, tail and pectoral fin regions of immature 1+ parr treated with cyproterone acetate during the period October to December, 1976.

Stain	Dorsum	Pectoral	Caudal Peduncle	Tail	Pectoral Fin
P.A.S.	++	++	++	++	++
Hales Colloidal Iron	+	+	+	+	+
Hales Colloidal Iron + PAS	93.7% Blue/ Purple +++ 6.3% Blue ++	94.7% Blue/ Purple +++ 5.3% Blue +	91.2% Blue/ Purple ++/+++ 9.5% Blue ++ 0.3% Red+	90.6% Blue/ Purple ++ 0.7% Blue + 8.7% Red ±+	96.3% Blue/ Purple +++ 3.7% Blue ++
Alcian Blue (pH 2.5)	+++	++	++/+++	+/++	++
Alcian Blue (pH 1.0)	-	-	-	-	-
Alcian Blue (pH 2.5) + PAS	35.7% Blue ++ 64.3% Blue/ Purple ++	54.2% Blue ++ 45.8% Blue/ Purple ++	33.7% Blue ++ 64% Blue/ Purple ++ 2.3% Red ±	22.5% Blue + 77.5% Blue/ Purple +/++	21.8% Blue ++ 77.6% Blue/ Purple ++/++ 0.6% Red ±
Alcian Blue (pH 1.0) + PAS	100% Dark Purple +++	100% Dark Purple +++	100% Dark Purple +++	100% Dark Purple +/++	100% Dark Purple +
Mild Methylation	+	+/++	+	+	+
Active Methylation	±	±	±	±	±
Alcian Blue + 0.1M $MgCl_2$	±/+	±	±	+	±
Alcian Blue + 0.2M $MgCl_2$	±	±	±	±	±
Alcian Blue + 0.5M $MgCl_2$	±	±	±	±	±
Alcian Blue + 0.6M $MgCl_2$	-	±	-	-	-
Alcian Blue + 0.8M $McCl_2$	-	-	-	-	-
Alcian Blue + 0.9M $MgCl_2$	-	-	-	-	-
Alcian Blue + Safranin	± (Blue)	± (Blue)	± (Blue)	± (Blue)	± (Blue)
Aldehyde Fuchsin	-	-	-	-	-
Aldehyde Fuchsin + Alcian Blue	+ (Blue)	+ (Blue)	+ (Blue)	+ (Blue)	+ (Blue)
Hyaluronidase (pH 6.9) Control	+++	++	++/+++	++/+	++
Hyaluronidase (pH 6.9)	++	++	++	++	++

See page 20A for explanation of the symbols.

TABLE 4.3.11

Histochemical reactions of the epidermal mucous cells in the dorsum, pectoral, caudal peduncle, tail and pectoral fin regions of precocious male 1+ parr treated with methallibure during the period October to December, 1976.

Stain	Dorsum	Pectoral	Caudal Peduncle	Tail	Pectoral Fin
P.A.S.	++	++	++	++	++
Hales Colloidal Iron	+	+	+	+	+
Hales Colloidal Iron + PAS	100% Blue/ Purple ++/+++	94% Blue/ Purple ++/+++ 6% Blue +	75.5% Blue/ Purple ++/+++ 24.5% Blue ++	100% Blue/ Purple ++/+++	92.3% Blue/ Purple ++ 1.4% Blue + 6.3% Red++
Alcian Blue (pH 2.5)	+++	+++	++/+++	++/+++	+++
Alcian Blue (pH 1.0)	-	-	-	-	-
Alcian Blue (pH 2.5) + PAS	38.3% Blue ++ 61.7% Blue/ Purple ++	65.1% Blue ++ 34.9% Blue/ Purple +	70.5% Blue + 29.5% Blue/ Purple +	14.7% Blue ++ 85.3% Blue/ Purple +	2.5% Blue ++ 97.5% Blue/ Purple +/++
Alcian Blue (pH1.0) + PAS	100% Dark Purple ++	100% Dark Purple ++	100% Dark Purple ++	100% Dark Purple	100% Dark Purple
Mild Methylation	+/++	+/++	±/+	+/++	+/++
Active Methylation	±	±	±	±	±
Alcian Blue + 0.1M $MgCl_2$	±	±	±	±	±
Alcian Blue + 0.2M $MgCl_2$	-	±	±	-	±/-
Alcian Blue + 0.5M $MgCl_2$	-	±	±	-	-
Alcian Blue + 0.6M $MgCl_2$	-	-	-	-	-
Alcian Blue + 0.8M $MgCl_2$	-	-	-	-	-
Alcian Blue + 0.9M $MgCl_2$	-	-	-	-	-
Alcian Blue + Safranin	± (Blue)	± (Blue)	± (Blue)	±/+ (Blue)	± (Blue)
Aldehyde Fuchsin	-	-	-	-	-
Aldehyde Fuchsin + Alcian Blue	+/++ (Blue)	+/++ (Blue)	+ (Blue)	+ (Blue)	+/++ (Blue)
Hyaluronidase (pH 6.9) Control	+++	++/+++	+/+++	++/+++	+/+++
Hyaluronidase (pH 6.9)	++	++	+/++	+/++	+/++
Neuraminidase (250 units/ml) + Alcian Blue + PAS (Control)			26.5% Blue ++ 73.5% Blue/ Purple +/++		
Neuraminidase (250 units/ml) Alcian Blue + PAS			100% Purple ±		

See page 20A for explanation of the symbols.

TABLE 4.3.12

Histochemical reaction of epidermal mucous cells in the dorsum, pectoral, caudal peduncle, tail and pectoral fin regions of immature 1+ parr treated with methallibure during the period October to December, 1976.

Stain	Dorsum	Pectoral	Caudal Peduncle	Tail	Pectoral Fin
P.A.S.	++	++	++	++	++
Hales Colloidal Iron	++	++	++	++	++
Hales Colloidal Iron + PAS	96.6% Blue/ Purple +++ 3.4% Blue +	95.7% Blue/ Purple ++ 3.4% Blue + 0.9% Red +	97% Blue/ Purple ++ 3% Blue +	90.5% Blue/ Purple +++ 7% Blue + 2.5% Red ±	94.8% Blue/ Purple +++ 1.7% Blue ++ 3.5% Red ±
Alcian Blue (pH 2.5)	++	++	++	++	++
Alcian Blue (pH 1.0)	-	-	-	-	-
Alcian Blue (pH 2.5) + PAS	51.5% Blue ++ 48.5% Blue/ Purple ++	46.8% Blue + 49.4% Blue/ Purple + 3.8% Red +	37.1% Blue + 62.9% Blue/ Purple +	53.7% Blue + 46.3% Blue/ Purple +	34.1% Blue + 62.9% Blue/ Purple ++ 3.1% Red
Alcian Blue (pH 1.0) + PAS	100% Dark Purple ++	100% Dark Purple ++	100% Dark Purple ++	100% Dark Purple ++	100% Dark Purple ++
Mild Methylation	+/++	+/++	+/++	+	+/++
Active Methylation	±	±	±	±	±
Alcian Blue + 0.1M $MgCl_2$	±	±	±	±	±
Alcian Blue + 0.2M $MgCl_2$	+	±/-	±	±	±
Alcian Blue + 0.5M $MgCl_2$	±/-	-	±	±	±
Alcian Blue + 0.6M $MgCl_2$	-	-	-	-	±
Alcian Blue + 0.8M $MgCl_2$	-	-	-	-	-
Alcian Blue + 0.9M $MgCl_2$	-	-	-	-	-
Alcian Blue + Safranin	± (Blue)	± (Blue)	± (Blue)	+ (Blue)	± (Blue)
Aldehyde Fuchsin	-	-	-	-	-
Aldehyde Fuchsin + Alcian Blue	+/++ (Blue)	+ (Blue)	+/++ (Blue)	+ (Blue)	+/++ (Blue)
Hyaluronidase (pH 6.9) Control	++	++	++	++	++
Hyaluronidase (pH 6.9)	++	++	+/++	++	+/++

See page 20A for explanation of the symbols.

Table 4.3.13

Number of mortalities due to Saprolegnia in 1+ Atlantic salmon parr treated with methallibure and cyproterone acetate during the period October to December, 1976

		No. of fish in each experimental group	No. of fish parasitised with Saprolegnia
Control	Immature Parr	7	- (a)
	Precocious Males	34	18 (a,b,c)
Methallibure	Immature Parr	7	1
	Precocious Males	26	2 (b)
Cyproterone Acetate	Immature Parr	12	-
	Precocious Males	30	8 (c)

a, c, $p \leq 0.05$
b, $p \leq 0.001$

TABLE 4.3.14

Summary of the effects of methallibure and cyproterone acetate on 1+ precocious male salmon parr during the period October to December.

	Methallibure	Cyproterone acetate
Spermatogenesis	Continued normally	Continued normally
Plasma 11-ketotestosterone levels	Decreased	Decreased
Gonadotrophic cells	Decreased in size	Decreased in size
Interrenal nuclear diameter	No change	No change
Plasma cortisol	Slight increase	Slight increase
Epidermis	Prevented the increase in epidermal thickness	Prevented the increase in epidermal thickness
Superficial mucous cell concentration	Slight increase	Marked increase
Saprolegnia infection	Significantly lower	Significantly lower

Table 4.3.15

The gonadosomatic indices (G.S.I.) and condition factor ("K") of 1+ Atlantic salmon parr treated with methallibure and cyproterone acetate during the period March to August 1978

		G.S.I.	"K"
Control	Males	$6.32 \pm 0.89^{(a)}_{(28)}$	$1.30 \pm 0.01^{(c)}_{(28)}$
	Females	$0.42 \pm 0.03_{(14)}$	$1.31 \pm 0.01^{(b)(d)}_{(20)}$
Methallibure	Males	$0.04 \pm 0.002_{(32)}$	$1.26 \pm 0.01_{(32)}$
	Females	$0.48 \pm 0.03_{(24)}$	$1.24 \pm 0.02^{(b)}_{(24)}$
Cyproterone acetate	Males (i)	$0.07 \pm 0.01_{(15)}$	$1.19 \pm 0.03^{(c)}_{(17)}$
	Males (ii)	$5.21 \pm 0.98_{(12)}$	$1.24 \pm 0.05_{(12)}$
	Females	$0.48 \pm 0.02_{(20)}$	$1.25 \pm 0.01^{(d)}_{(20)}$

a, $\bar{x} \pm$ S.E. (No. of fish)
b, Significantly different from control ($p \leq 0.005$)
c, Significantly different from control ($p \leq 0.0005$)
d, Significantly different from control ($p \leq 0.0005$)

Table 4.3.16

The effects of administration and withdrawal of methallibure and cyproterone acetate on the subsequent gonadosomatic indices (GSI) and the condition factor ('K') of 1+ Atlantic salmon parr in December 1978

		G.S.I.	'K'
Control	Females	$0.32 \pm 0.03_{(18)}$ [a]	$1.24 \pm 0.02_{(18)}$
	Males	$8.28 \pm 0.36_{(21)}$	$1.14 \pm 0.02_{(21)}$
Methallibure	Females	$0.40 \pm 0.03_{(19)}$	$1.21 \pm 0.02_{(19)}$
	Males	$0.05 \pm 0.01_{(7)}$	$1.20 \pm 0.03_{(7)}$
Cyproterone Acetate	Females	$0.53_{(1)}$	$1.09_{(1)}$
	Males	$10.22_{(1)}$	$1.21_{(1)}$

a, $\bar{x} \pm$ S.E. (No. of fish).

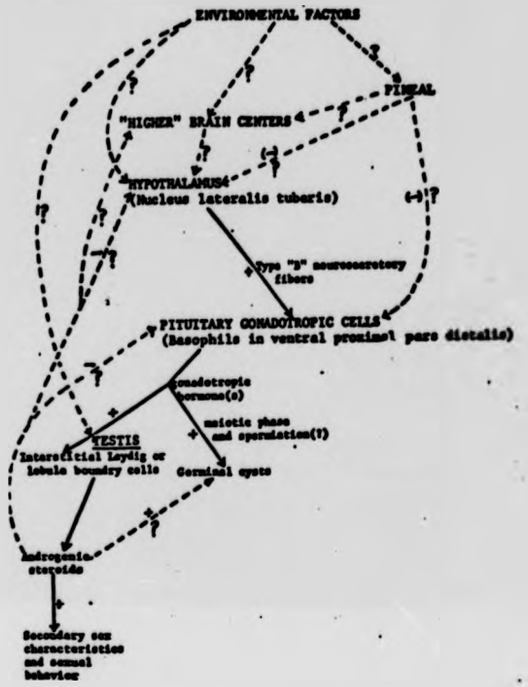


Fig. 1. Schematic diagram of the control mechanisms involved in teleost reproduction. Solid lines represent pathways which are relatively well established, dashed lines indicate possible control pathways which lack definitive experimental proof. (After de Vlaming, 1974).

Fig. 2. (a) Testosterone (b) 11-Ketotestosterone.

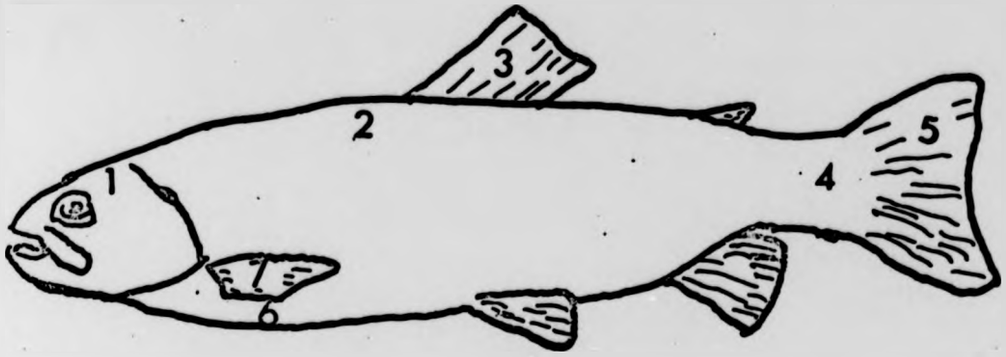


Fig. 3. Areas of salmon parr sampled for skin studies.

1. Head
2. Dorsum
3. Dorsal fin
4. Caudal peduncle
5. Tail
6. Pectoral (Belly)
7. Pectoral fin

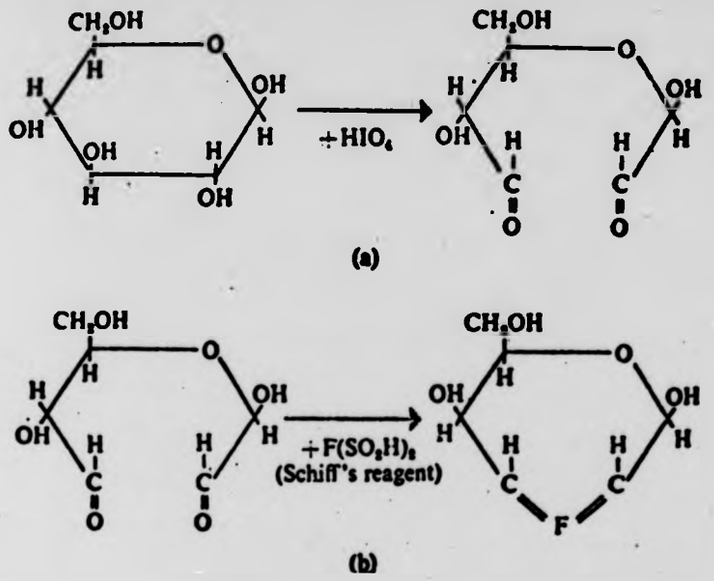


Fig. 4. The chemical changes in the periodic acid-Schiff reaction.

a) Periodic acid oxidation

b) Recolouration of Schiff's reagent by aldehyde groups (After Drury and Wallington, 1967).

Fig. 5. a) $R\text{-}COOH + CH_3OH \overset{HCL}{\rightleftharpoons} R\text{-}COOCH_3 + H_2O$

b) $R\text{-}CH_2OSO_3H + CH_3OH \overset{HCL}{\rightleftharpoons} R\text{-}CH_2OCH_3 + H_2SO_4$

Active and Mild Methylation

a) Esterification of carboxyl groups in mucopolysaccharides.

b) Desulphation of mucopolysaccharides.

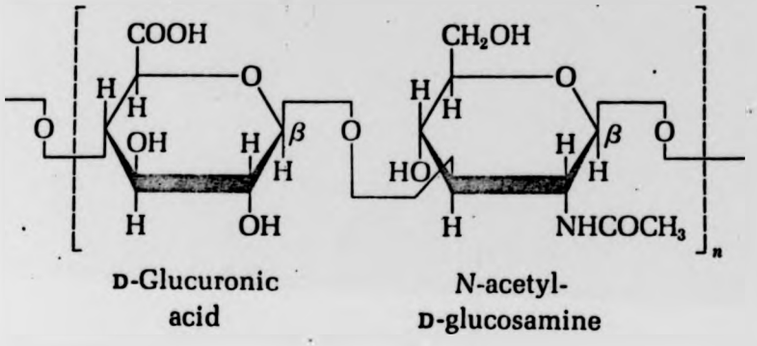


Fig. 6. Repeating unit of hyaluronic acid (After Lehninger, 1975).

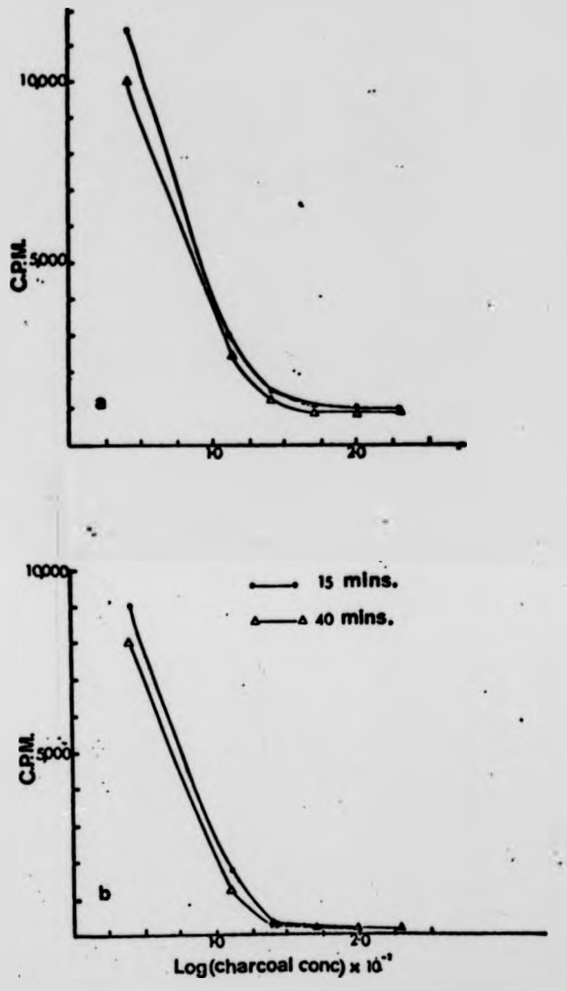


Fig. 7. Effects of different concentrations of charcoal and incubation times on

a) the binding of 11-ketotestosterone to its antiserum.

b) the non-specific binding.

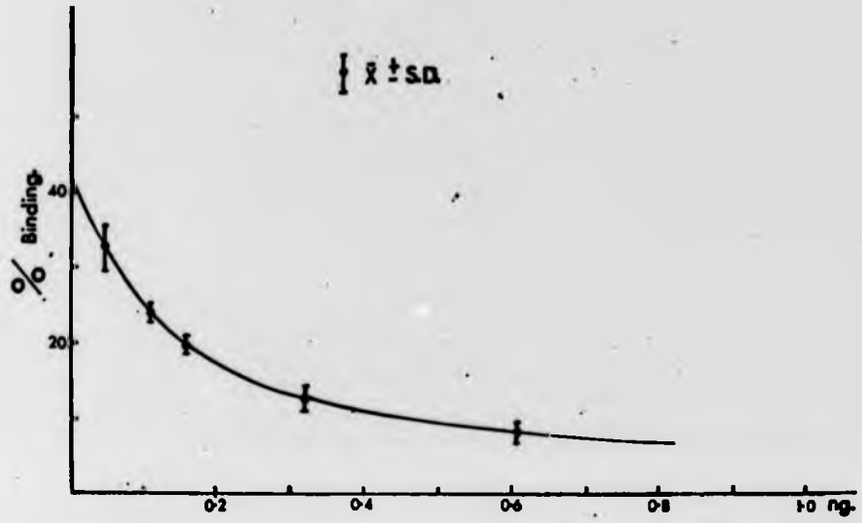


Fig. 8. Intraassay variation of 14 standard curves for the cortisol radioimmunoassay.

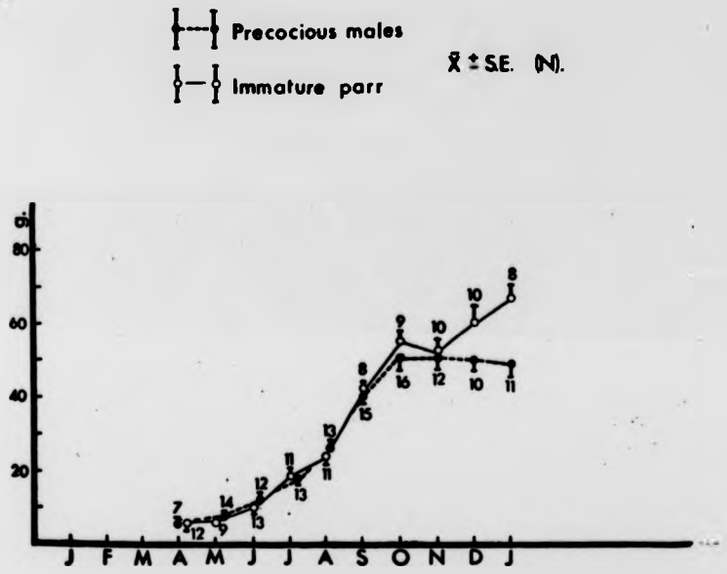


Fig. 9. Changes in the mean monthly weight of 1+ Atlantic salmon parr during the period April, 1978 to January, 1979. Vertical bars show the standard error of the mean (± S.E.), the number of fish sampled is indicated for each mean.

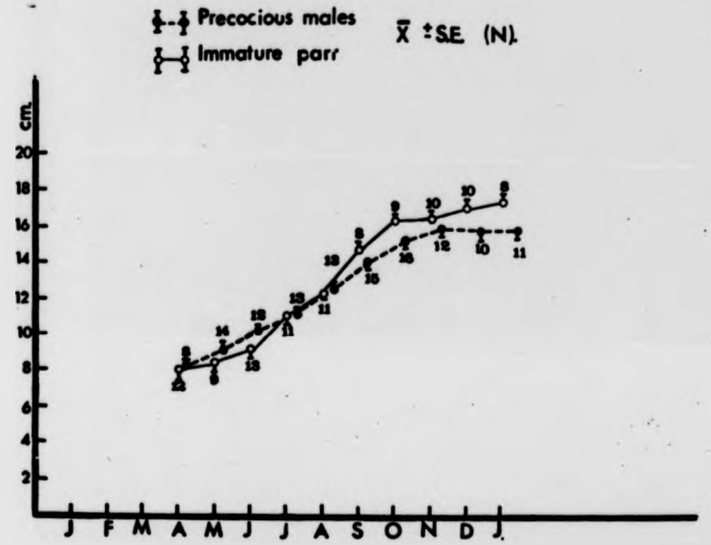


Fig. 10. Changes in the mean monthly length of 1+ Atlantic salmon parr during the period April, 1978 to January, 1979. Vertical bars show the S.E, the number of fish sampled is indicated for each mean.

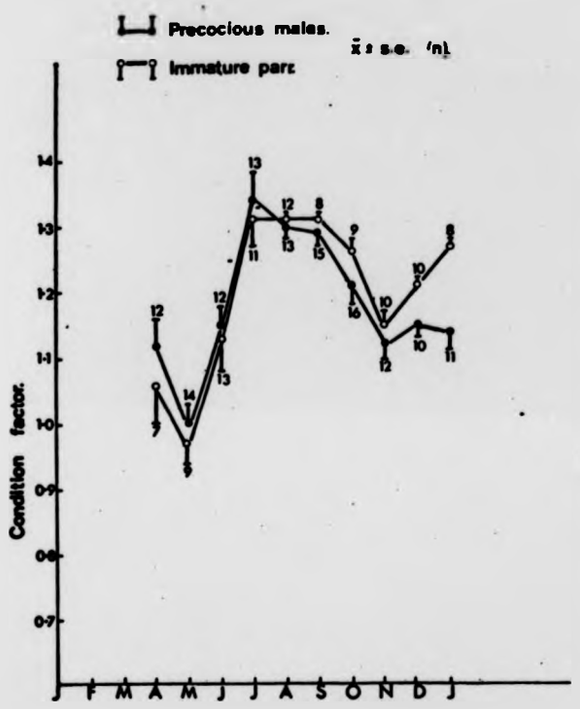


Fig. 11. Changes in the condition factor of 1+ Atlantic salmon parr during the period April, 1978 to January, 1979. Vertical bars show the S.E., the number of fish sampled is indicated for each mean.

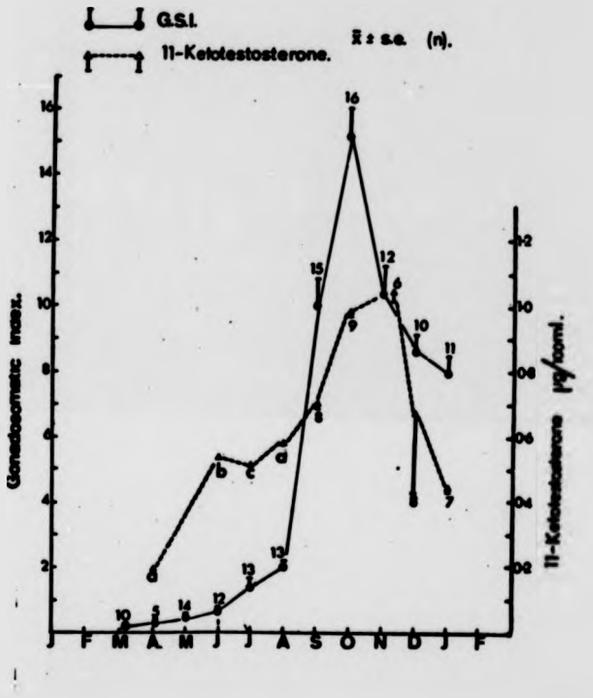


Fig. 12. Changes in the GSI (% body weight) and plasma 11-ketotestosterone (µg/100 ml) level during the gonad maturation cycle of precocious male 1+ Atlantic salmon parr. Data expressed as mean ± S.E., the number of fish sampled for each mean is also indicated above the vertical bar.

a,b,c,d. One determination of pooled plasma from 12 developing male parr.

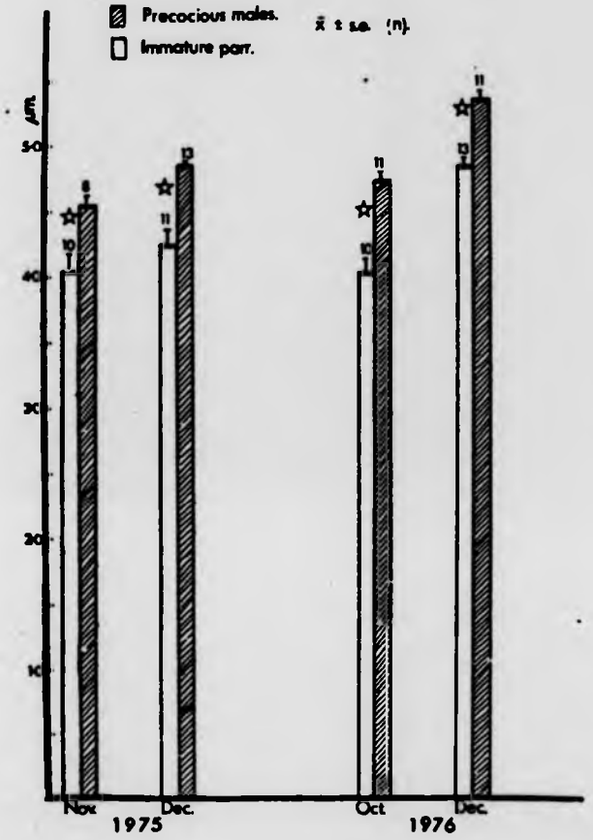
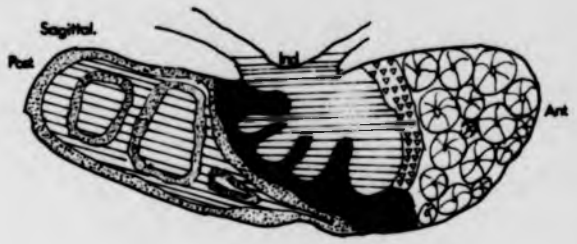


Fig. 13. Mean nuclear diameter of the interrenal cells of 1+ salmon parr sampled during late autumn and early winter (1975 and 1976). Vertical bars show the S.E., the number of fish sampled is indicated for each mean.

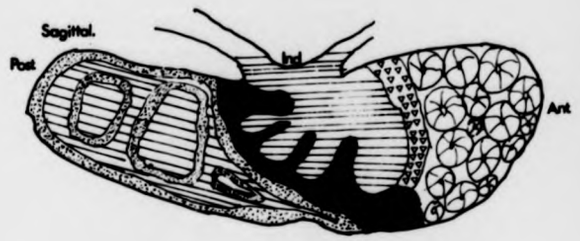
☆ Significantly different from each other ($p \leq 0.001$).

Fig. 14. Diagrammatic representation of the mid-sagittal and transverse section of the pituitary of 1+ Atlantic salmon parr.



Ind. Infundibulum.

Pro-adenohypophysis.
Meso- " "
Meta- " "
Neurohypophysis.
Chromophobic cells.



Ind. Infundibulum.

Pro-adenohypophysis.
Meso- " "
Meta- " "
Neurohypophysis.
Chromophobic cells.

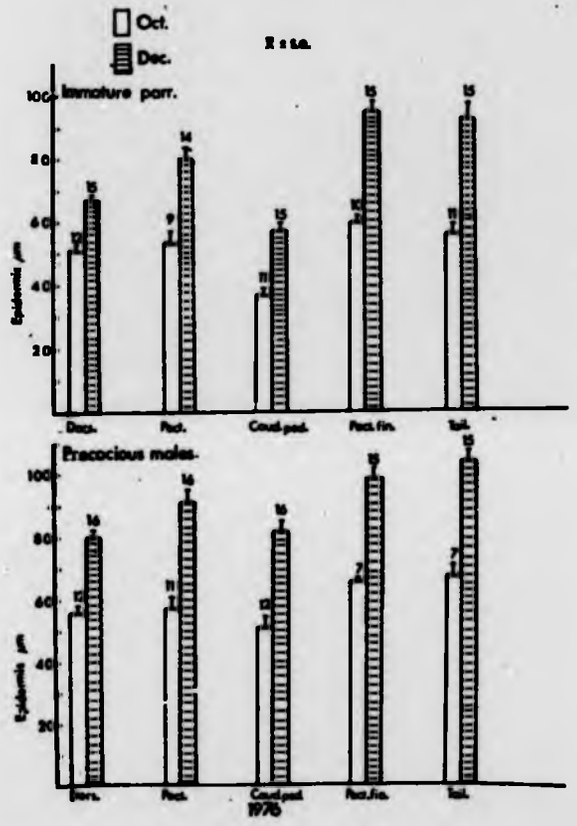


Fig. 15. Changes in the thickness of the epidermis of 1+ Atlantic salmon parr during the period late autumn to mid winter, 1976. Data expressed as mean ± S.E. No. of fish examined is indicated over the vertical bars.

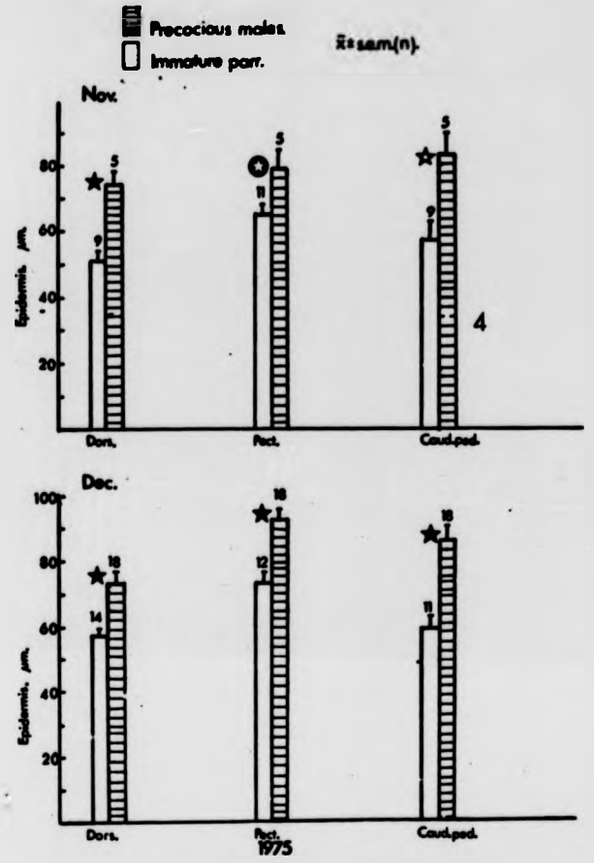
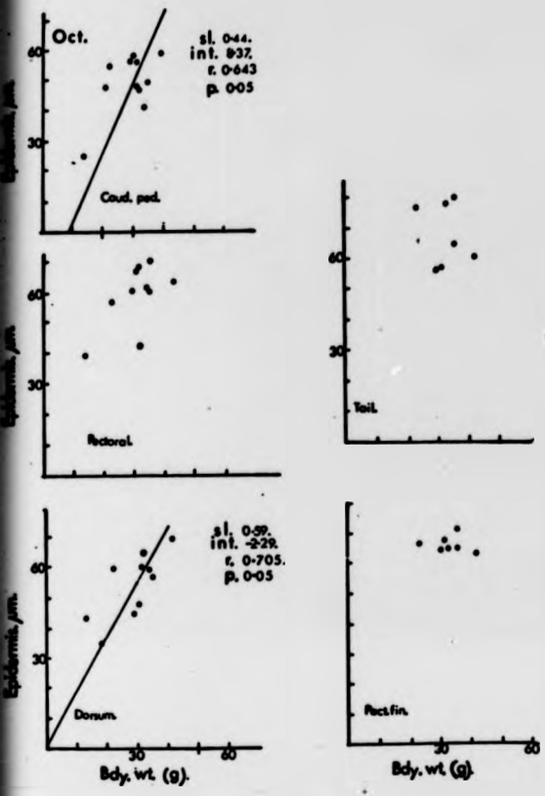


Fig. 16. The mean thickness of the epidermis of 1+ salmon parr examined during November and December, 1975. Vertical bars show the S.E. No. of fish sampled for each mean is indicated over the vertical bars.

✪ Significantly different from each other ($p \leq 0.05$).

☆ Significantly different from each other ($p \leq 0.01$)

★ Significantly different from each other ($p \leq 0.001$).



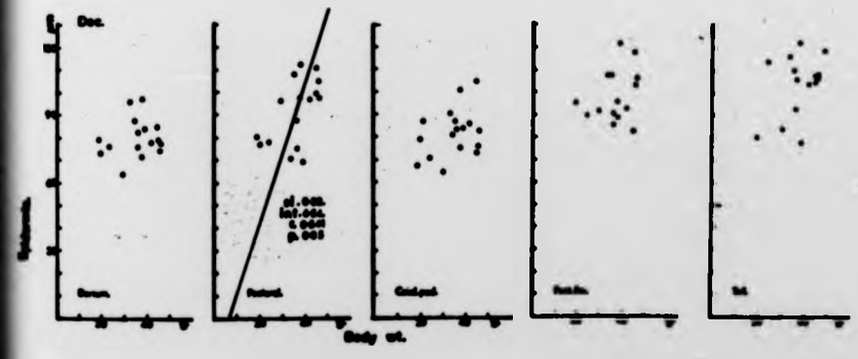


Fig. 17.

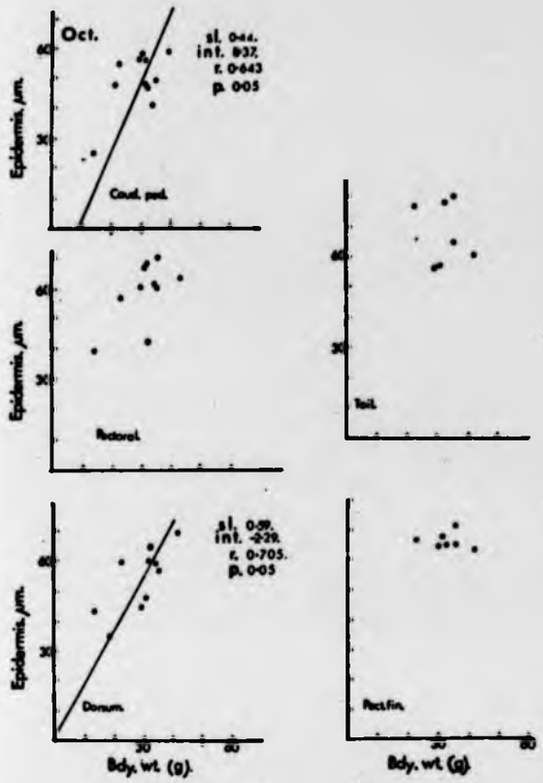
The relationship of the epidermal thickness and the body weight in precocious male 1+ salmon parr sampled during (a) October (b) December, 1976.

sl. : slope

int. : intercept

r : correlation coefficient

p : probability



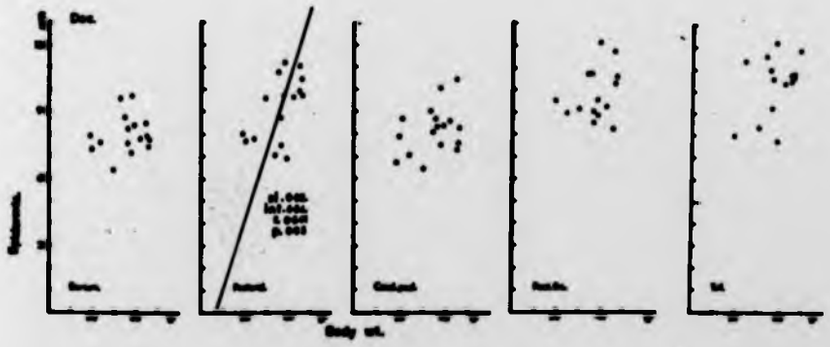


Fig. 17.

The relationship of the epidermal thickness and the body weight in precocious male 1+ salmon parr sampled during (a) October (b) December, 1976.

sl. : slope

int. : intercept

r : correlation coefficient

p : probability

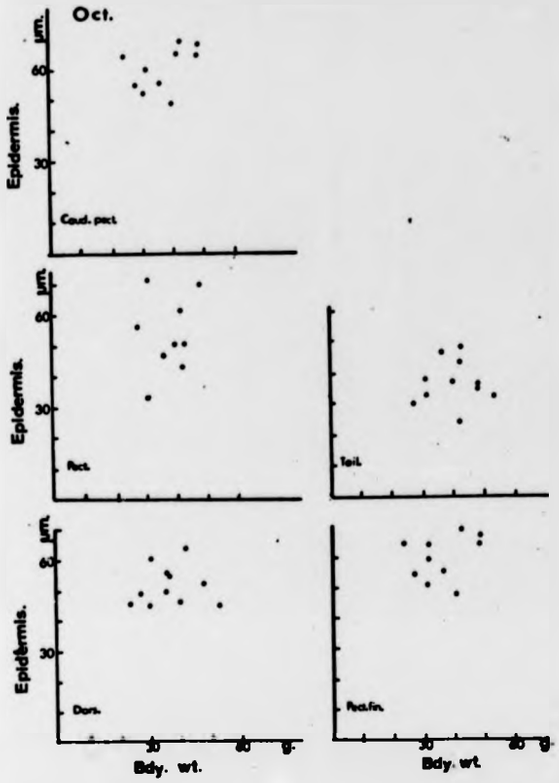


Fig. 18

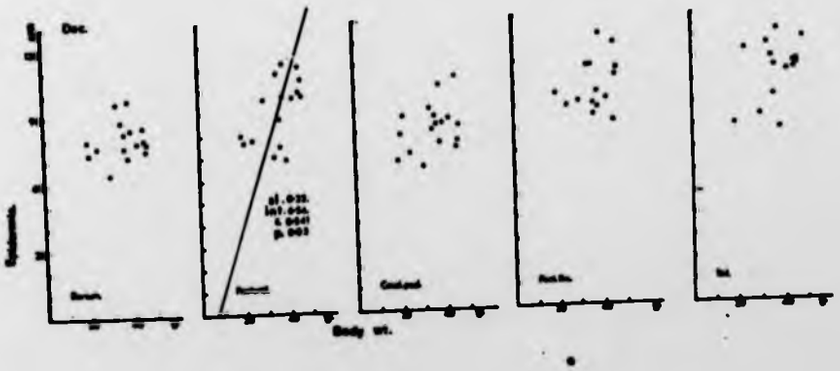
Relationship of the epidermal thickness and body weight in immature female 1+ salmon parr sampled during (a) October (b) December, 1976.

sl. : slope

int. : intercept

r : correlation coefficient

p : probability



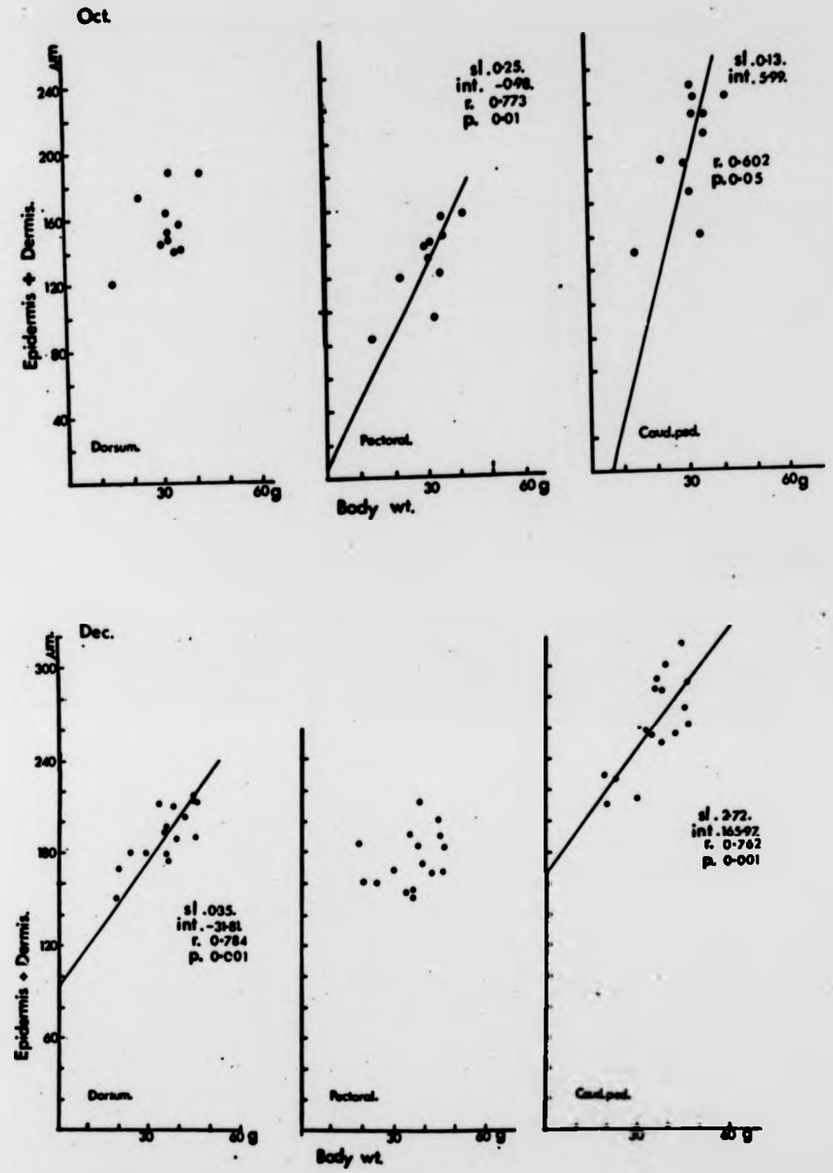


Fig. 19. The relationship of the total skin thickness (epidermis + dermis) with body weight in precocious 1+ male salmon parr sampled during October and December, 1976.

sl. : slope.

int. : intercept

r : correlation coefficient

p : probability

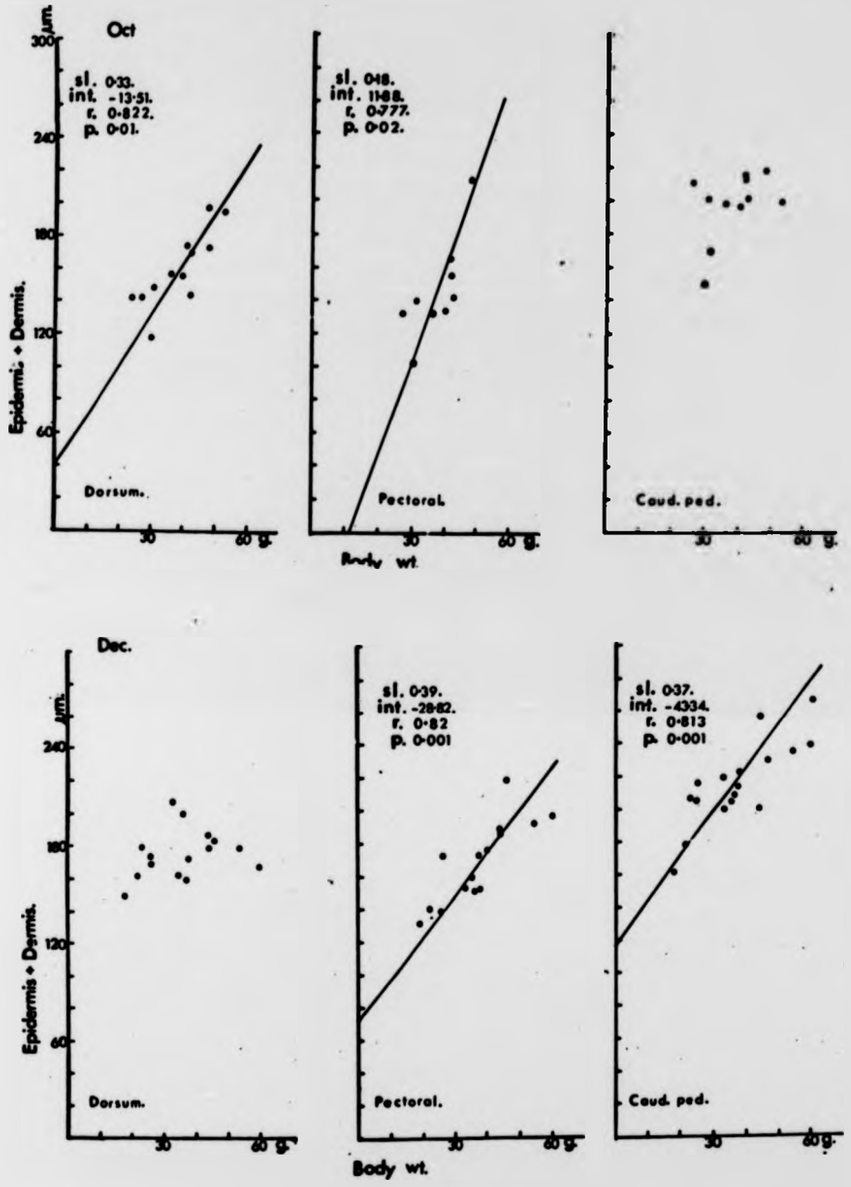


Fig. 20. The relationship of the total skin thickness with the body weight in immature 1+ female salmon parr sampled during October and December, 1976.

sl. : slope

int. : intercept

r : correlation coefficient

p : probability.

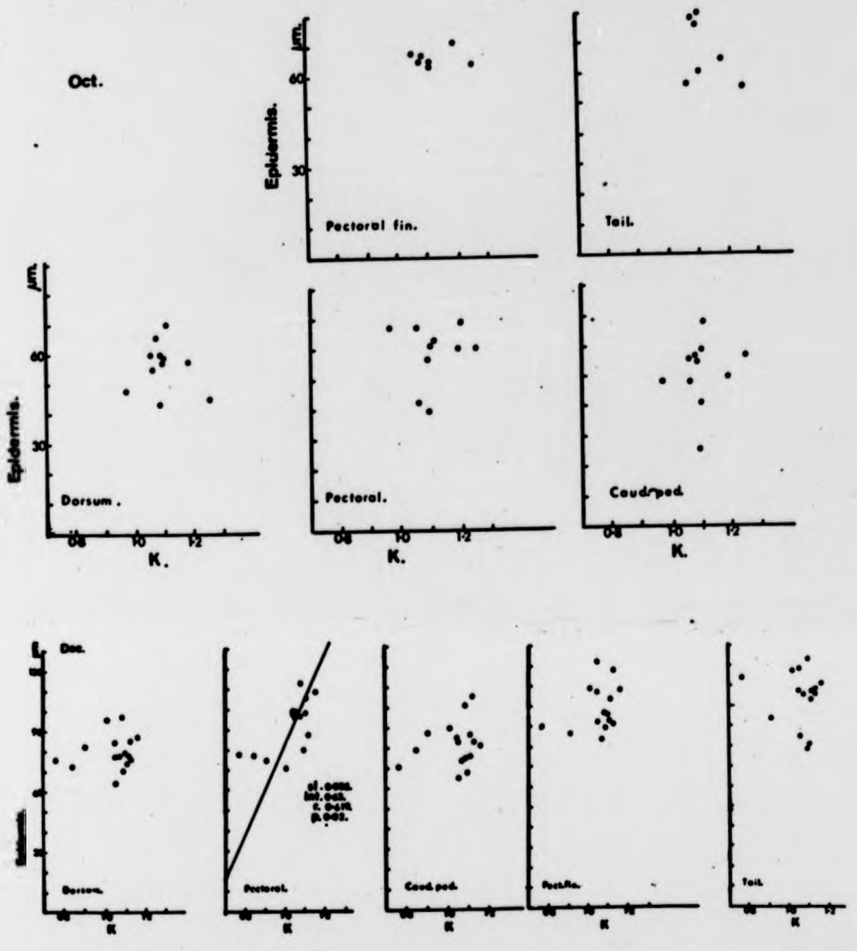


Fig. 21. The relationship of the epidermal thickness with the condition factor ('K') in precocious male 1+ salmon parr sampled during October and December, 1976.

sl. : slope
int. : intercept
r : correlation coefficient
p : probability.

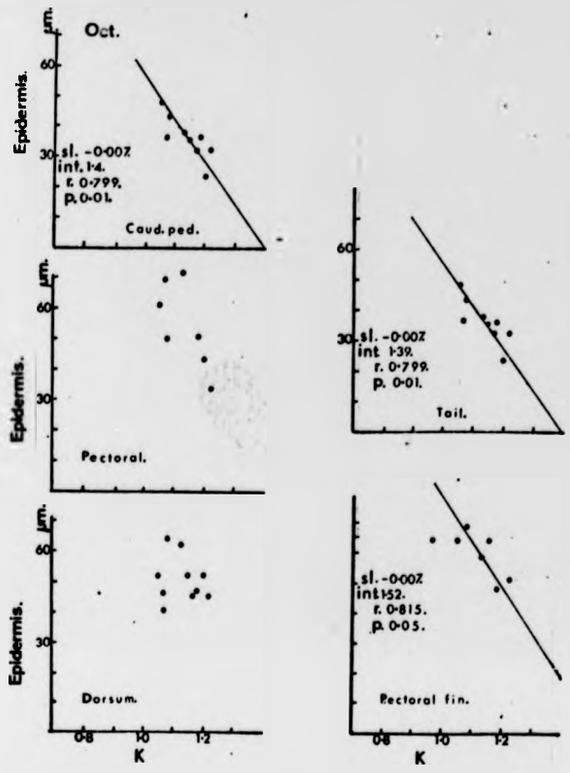


Figure 22

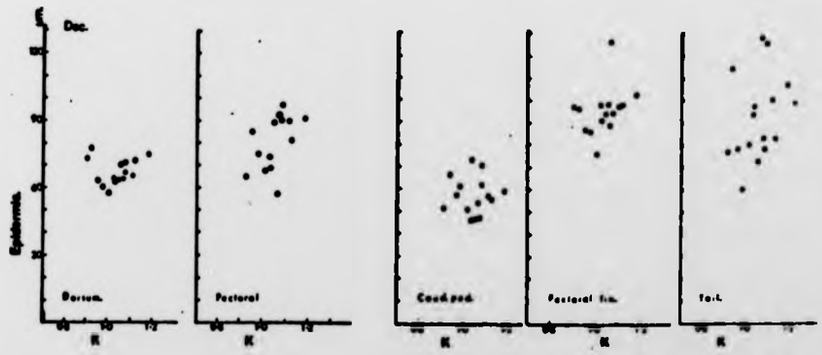
The relationship of the epidermis with the condition factor 'K' in immature female 1+ salmon parr sampled during October and December, 1976.

sl. : slope

int. : intercept

r : correlation coefficient

p : probability



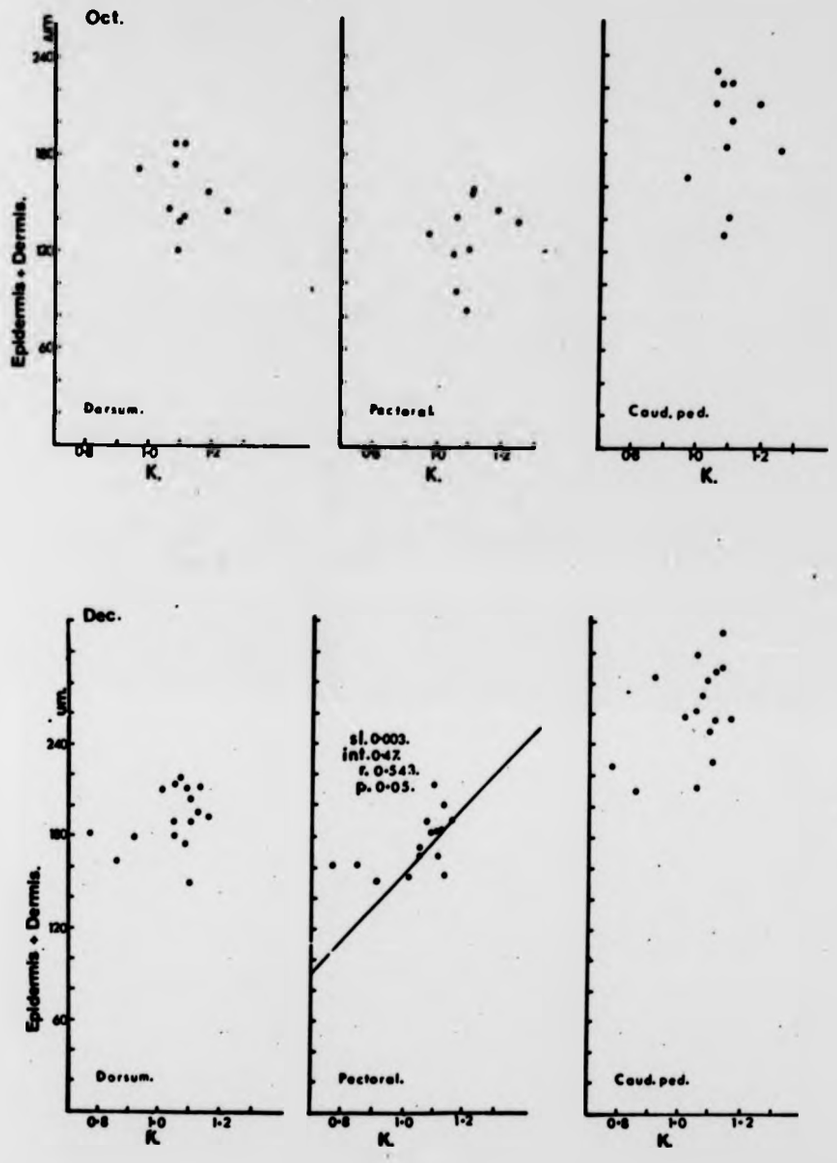


Fig. 23. The relationship of the total skin thickness (epidermis + dermis) with the condition factor ('K') in precocious male 1+ salmon parr sampled during October and December, 1976.

sl. : slope

int. : intercept

r : correlation coefficient

p : probability

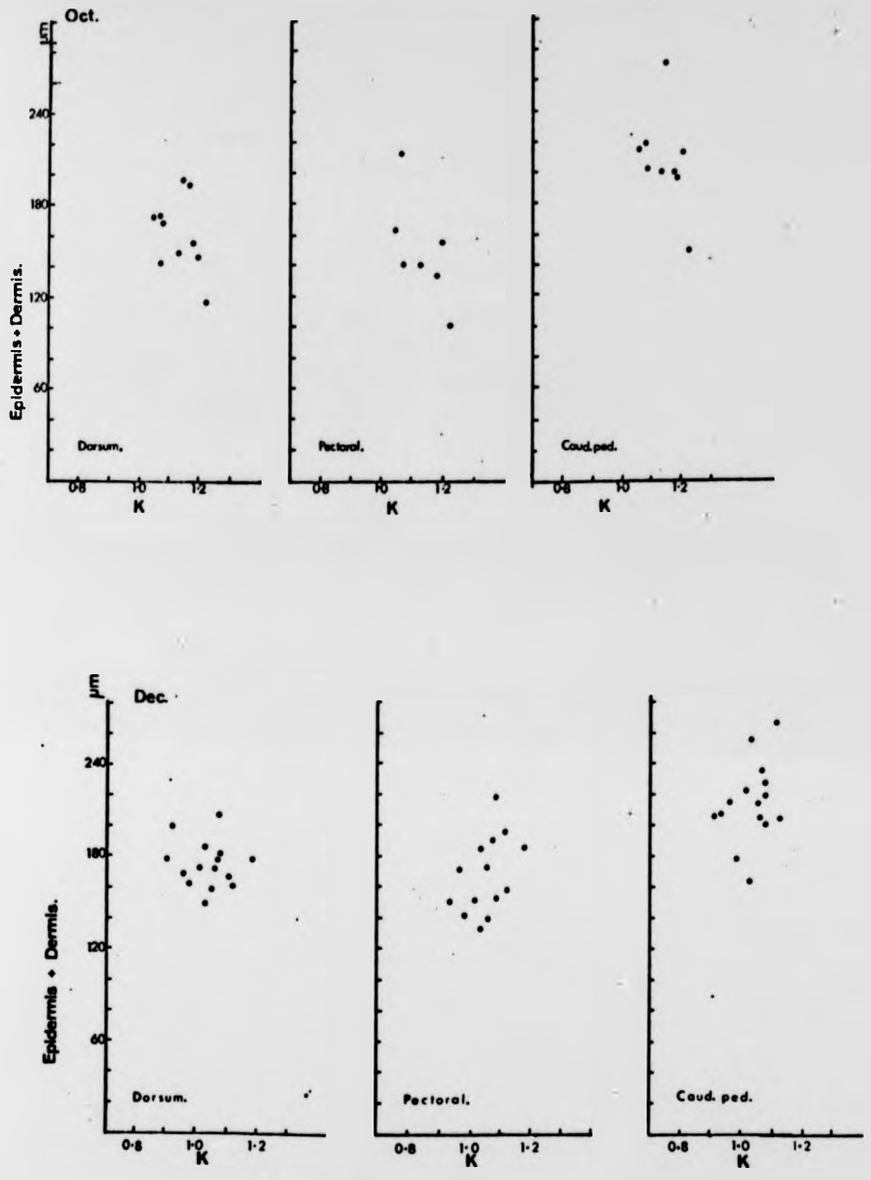


Fig. 24. The relationship of the total skin thickness (epidermis + dermis) with the condition factor 'K' in immature female 1+ salmon parr sampled during October and December, 1976.

sl. : slope

int. : intercept

r : correlation coefficient

p : probability.

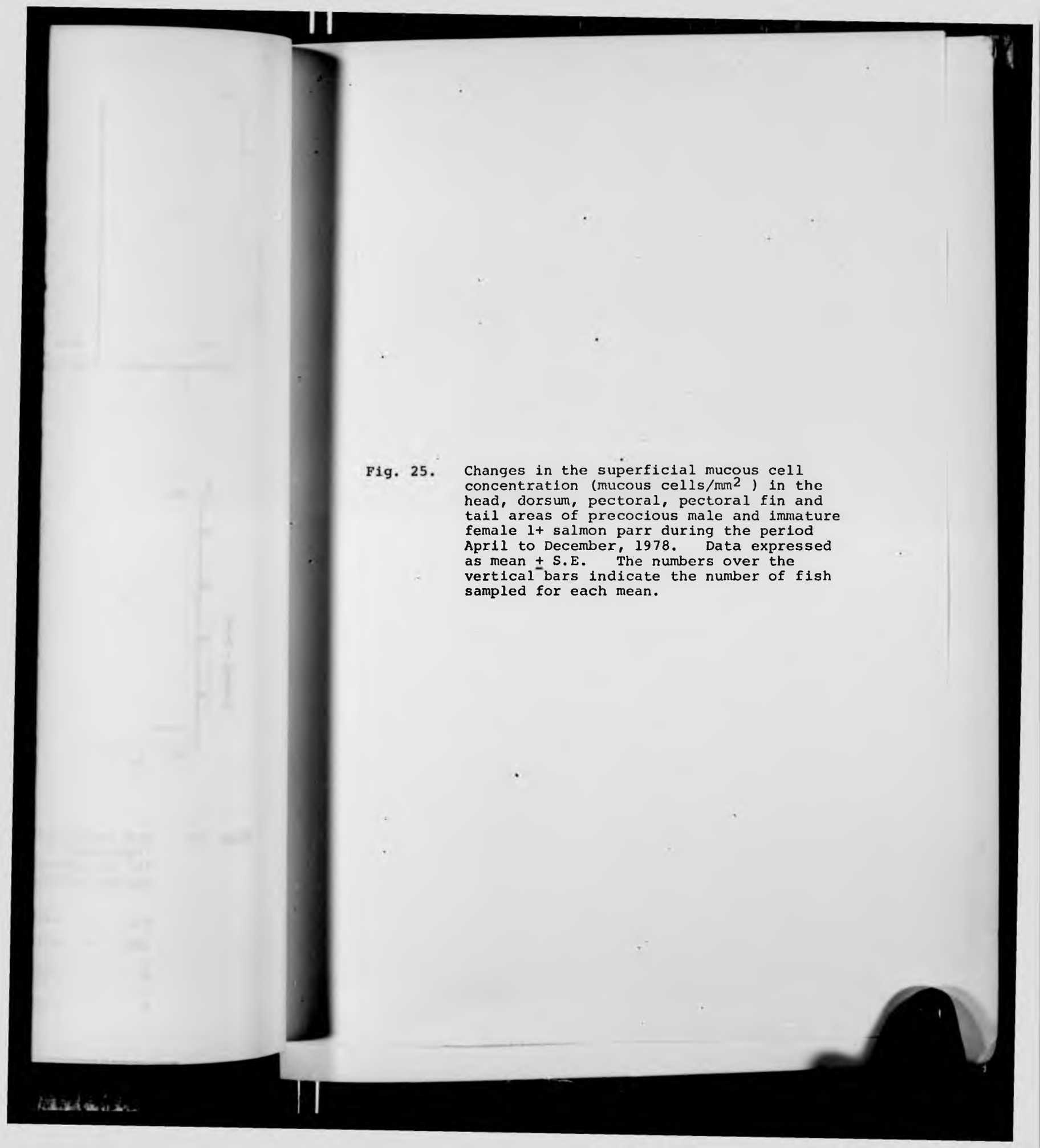


Fig. 25. Changes in the superficial mucous cell concentration (mucous cells/mm^2) in the head, dorsum, pectoral, pectoral fin and tail areas of precocious male and immature female 1+ salmon parr during the period April to December, 1978. Data expressed as mean $\pm$ S.E. The numbers over the vertical bars indicate the number of fish sampled for each mean.

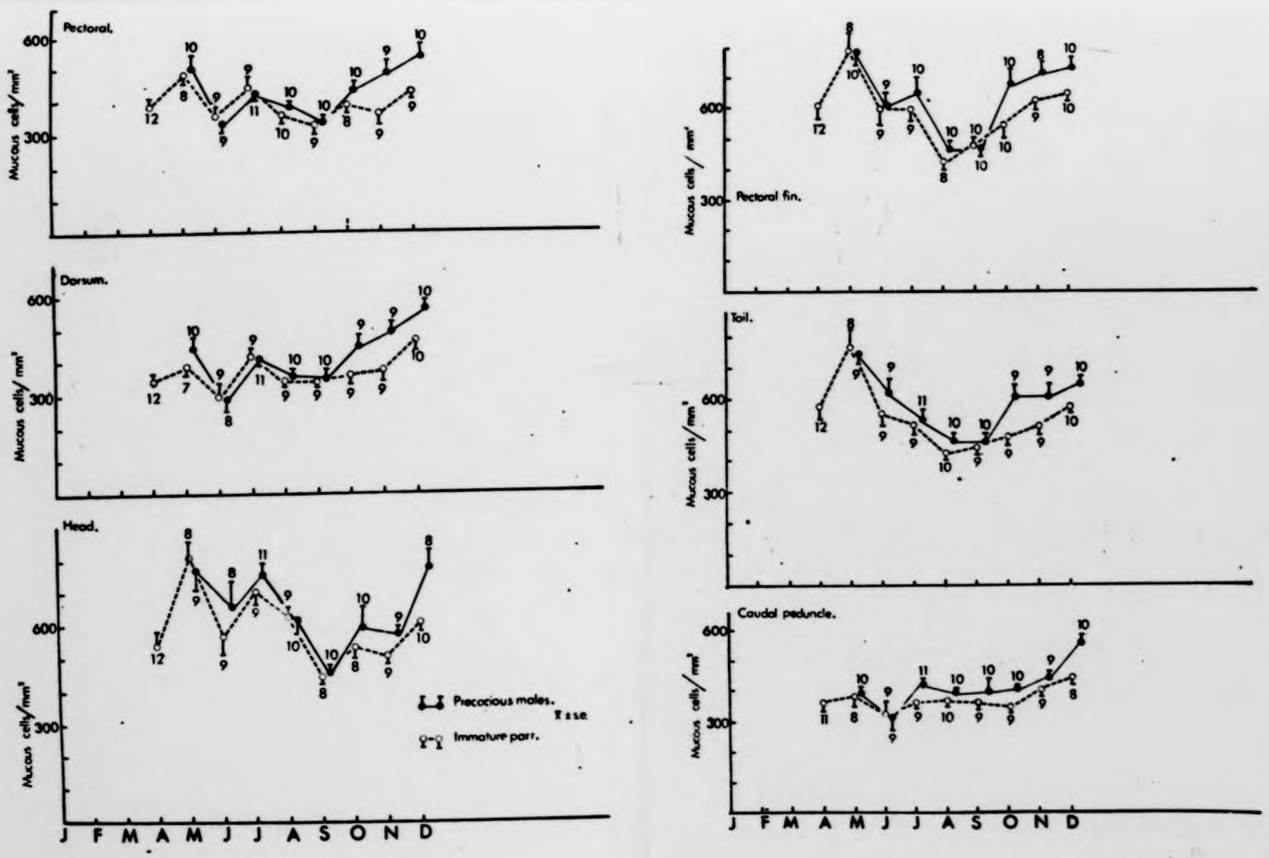

Pectoral.
Dorsum.
Head.
Pectoral fin.
Tail.
Caudal peduncle.
Mucous cells/mm²
600
300
J F M A M J J A S O N D
Precocious males.
Immature parr.
x̄ ± s.e.

Fig. 26. The concentration of superficial mucous cells (mucous cells/mm^2) in the head, dorsum, pectoral, pectoral fin and tail areas of 1+ salmon parr sampled in October and December, 1976. Data expressed as mean $\pm$ S.E., the number of fish sampled for each mean is also indicated.

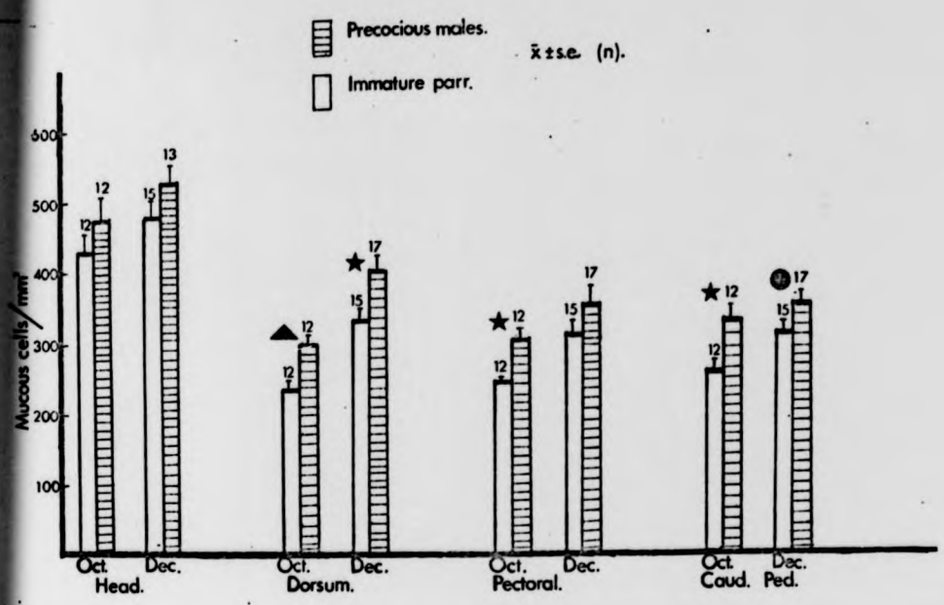
✪ Significantly different from each other ($p \leq 0.001$)

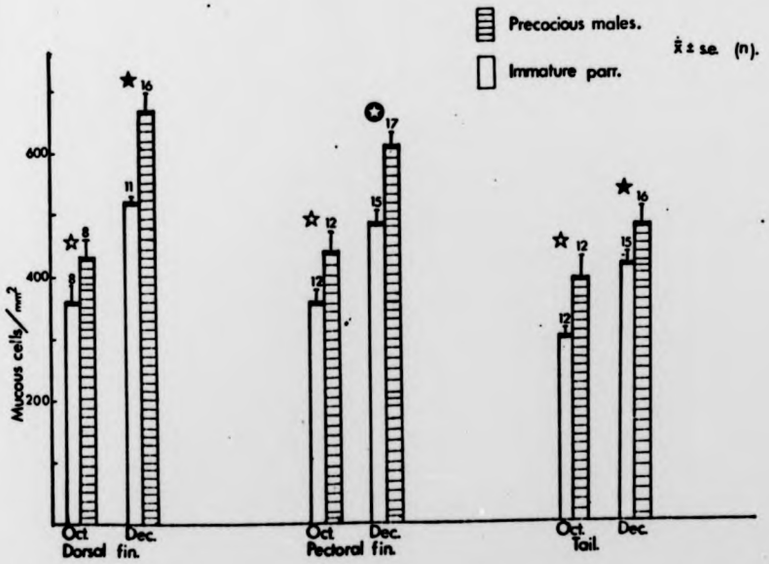
☆ Significantly different from each other ($p \leq 0.05$).

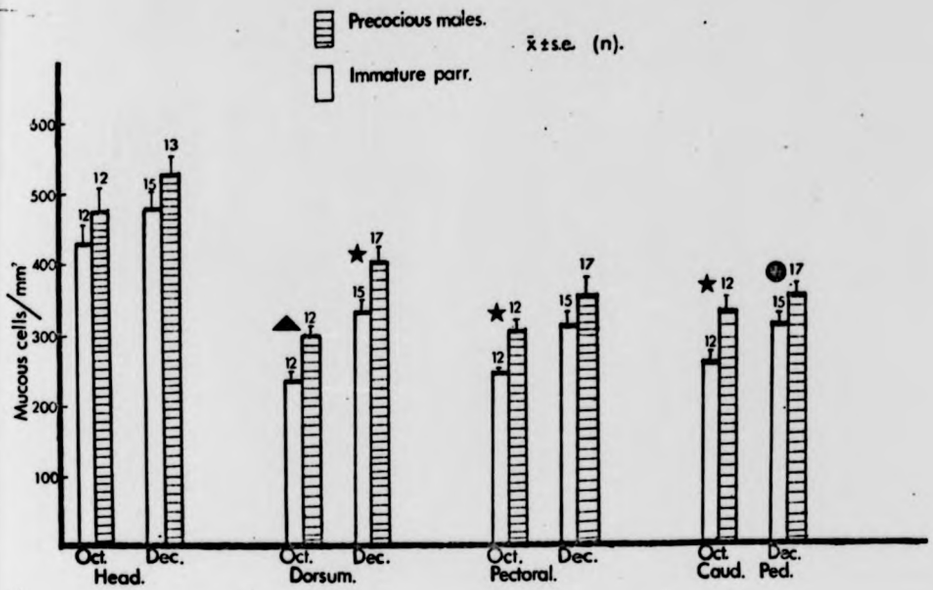
★ Significantly different from each other ($p \leq 0.01$).

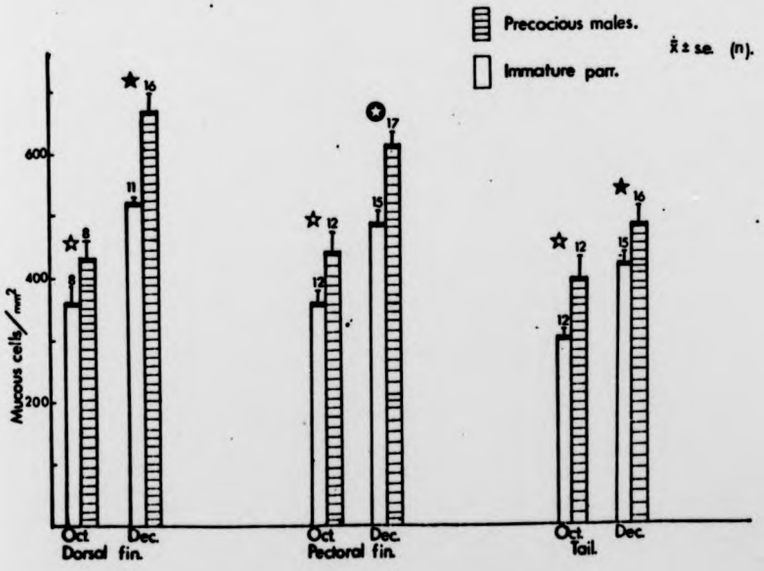
● Significantly different from each other ($p \leq 0.02$).

▲ Significantly different from each other ($p \leq 0.002$)


Precocious males.
Immature parr.
x̄ ± s.e. (n).
Mucous cells/mm²
600
500
400
300
200
100
Oct.
Dec.
Head.
Dorsum.
Pectoral.
Caud. Ped.


Precocious males.
Immature parr.
x̄ ± se. (n).
Mucous cells/mm²
600
400
200
Oct
Dec.
Dorsal fin.
Pectoral fin.
Tail.


Precocious males.
Immature parr.
x̄ ± s.e. (n).
Mucous cells/mm²
Oct.
Dec.
Head.
Dorsum.
Pectoral.
Caud. Ped.


Precocious males.
Immature parr.
x̄ ± se. (n).
Mucous cells/mm²
Oct.
Dec.
Dorsal fin.
Pectoral fin.
Tail.

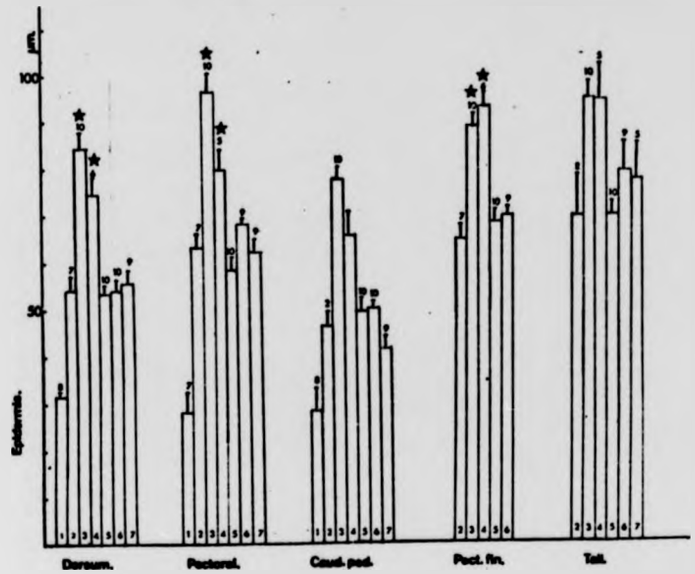


Fig. 27. The mean epidermal thickness of 0+ parr treated with 17 β oestradiol, 17α methyltestosterone, testosterone, cortisol and cortisone. Vertical bars show the S.E, and the number of fish sampled for each mean is shown above the vertical bar.

1. Control (0 weeks)
2. Control (14 weeks)
3. 17α methyltestosterone
4. Testosterone
5. Cortisol
6. Cortisone
7. 17 β oestradiol

★ Significantly different from the control (14 weeks) ($p \leq 0.001$).

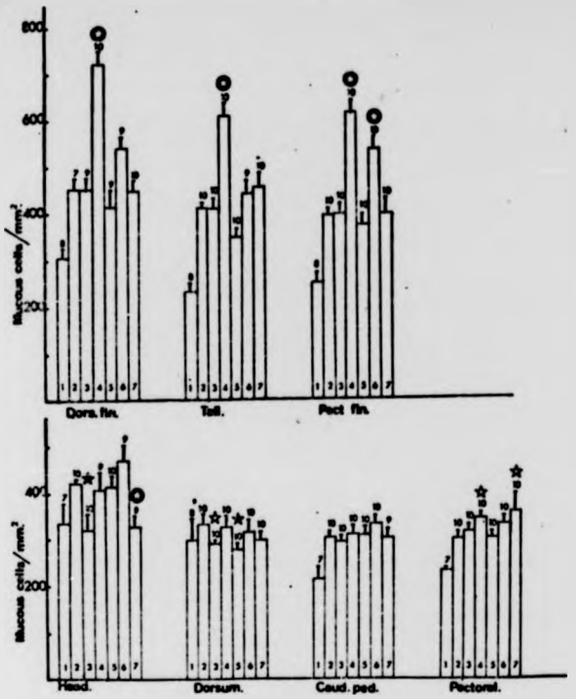


Fig. 28. The superficial mucous cell concentration in the head, dorsum, caudal peduncle, pectoral, dorsal fin, pectoral fin and tail areas of 0+ parr treated with 17β oestradiol, 17α methyltestosterone, testosterone, cortisol and cortisone. Data expressed as mean ± S.E., the number of fish sampled is indicated over the vertical bars.

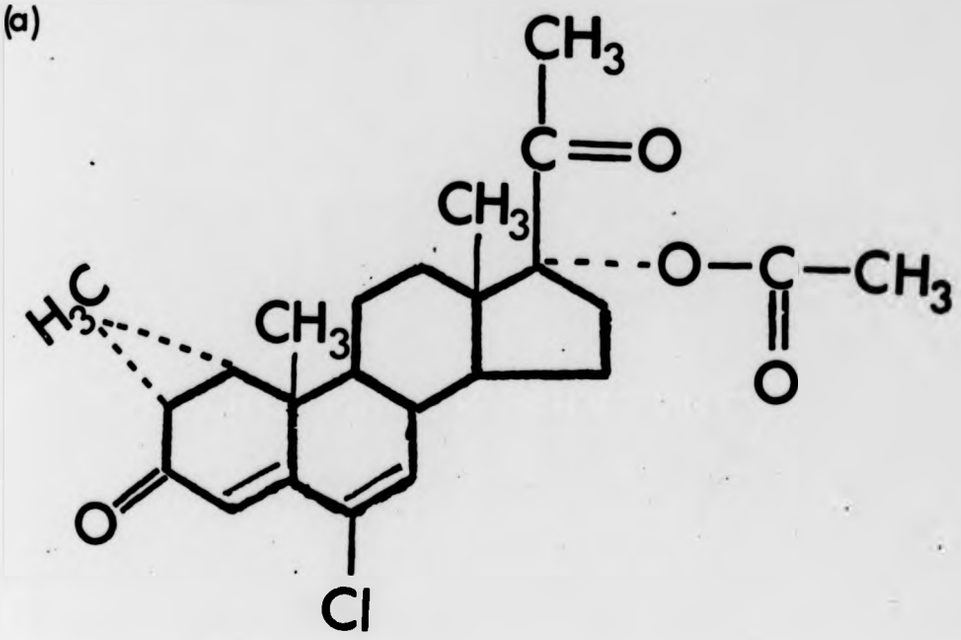
1. Control (0 weeks)
2. Control (14 weeks)
3. Cortisone
4. Cortisol
5. 17α methyltestosterone
6. Testosterone
7. 17 β oestradiol

☆ Significantly different from control group (14 weeks) ($p \leq 0.05$)

★ Significantly different from control group (14 weeks) ($p \leq 0.01$)

✪ Significantly different from control group (14 weeks) ($p \leq 0.001$)

(a)



(b) $CH=CH.\underset{\underset{CH_3}{|}}{CH}.NH.CS.NH.NH.CS.NH.CH_3$

Fig. 29. a) Cyproterone acetate

b) Methallibure

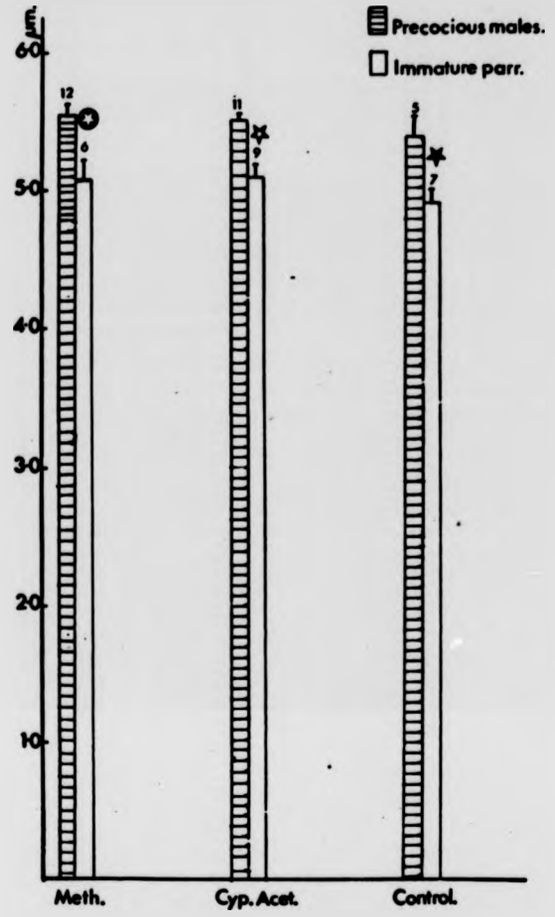


Fig. 30. Interrenal nuclear diameter of immature and precocious male 1+ parr treated with methallibure and cyproterone acetate during the period October to December, 1976. Data expressed as mean ± S.E., the numbers above the vertical bars indicate the number of fish sampled for each mean.

★ Significantly different from precocious males ($p \leq 0.05$).

✪ Significantly different from precocious males ($p \leq 0.002$).

☆ Significantly different from precocious males ($p \leq 0.005$).

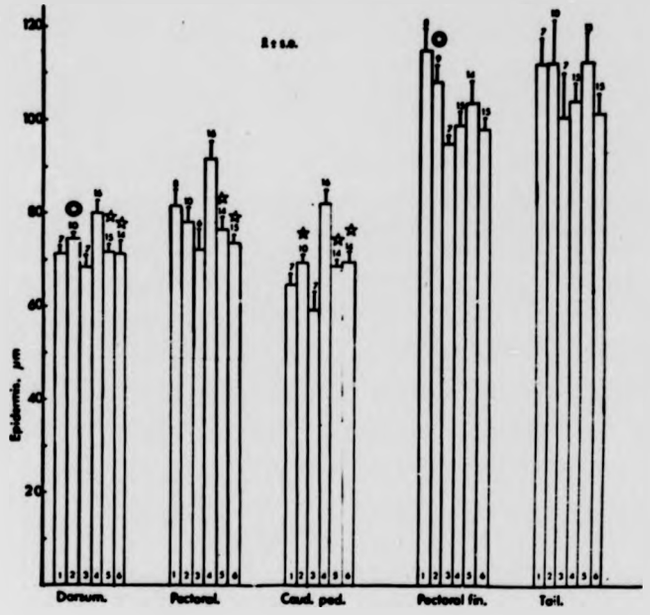


Fig. 31. The effects of methallibure and cyproterone acetate on the epidermal thickness of the dorsum, pectoral, caudal peduncle, pectoral fin and tail regions of immature and precocious 1+ parr. Data expressed as mean ± S.E., the numbers above the vertical bars indicate the number of fish sampled for each mean.

1. Immature parr treated with methallibure.
2. Immature parr treated with cyproterone acetate.
3. Immature parr control group.
4. Precocious male parr control group.
5. Precocious male parr treated with cyproterone acetate.
6. Precocious male parr treated with methallibure.

Significantly different from the controls ($p \leq 0.05$)

Significantly different from the controls ($p \leq 0.01$)

Significantly different from the controls ($p \leq 0.001$)

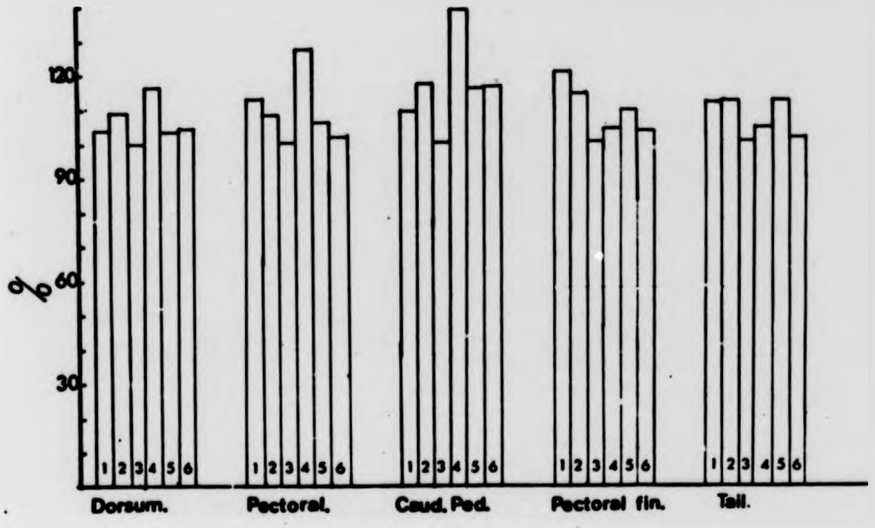


Fig. 32 Changes in the epidermal thickness of immature and precocious male 1+ parr treated with methallibure and cyproterone acetate expressed as a percentage of the epidermis of the immature parr control group.

1. Immature parr treated with methallibure.
2. Immature parr treated with cyproterone acetate.
3. Immature parr control group (100%).
4. Precocious male parr control group.
5. Precocious male parr treated with cyproterone acetat
6. Precocious male parr treated with methallibure.

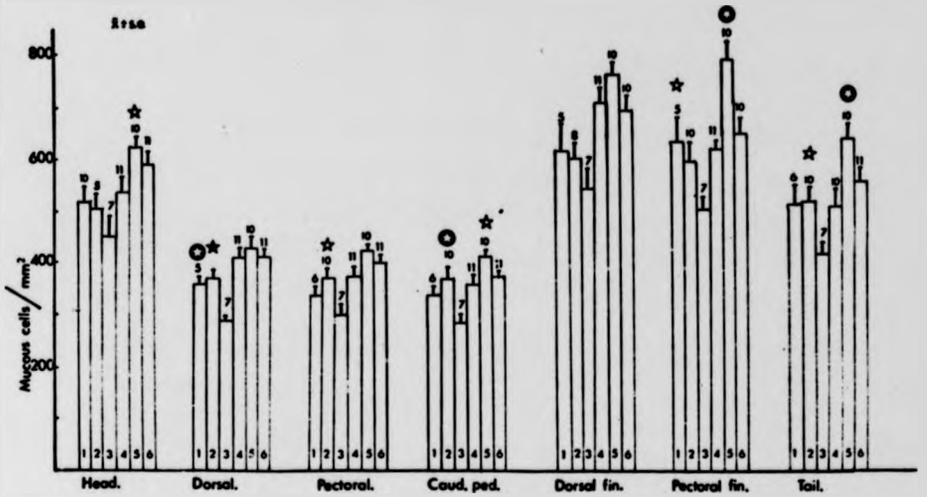


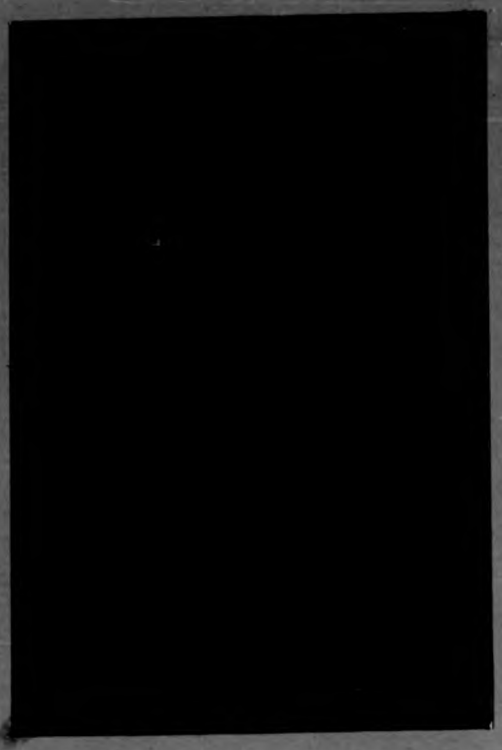
Fig. 33. The superficial mucous cell concentration of the head, dorsum, pectoral, caudal peduncle, dorsal fin, pectoral fin and tail of immature parr and precocious male parr treated with methallibure and cyproterone acetate. Data expressed as mean + S.E., the number of fish above the vertical bars represent the number of fish sampled for each mean.

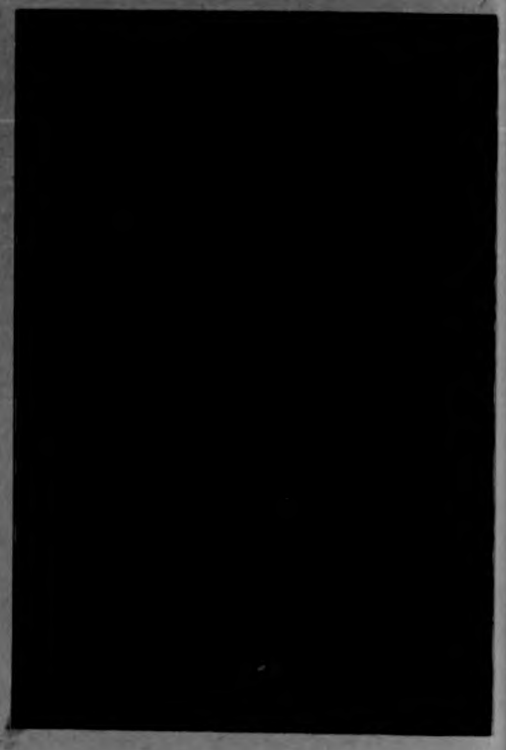
1. Immature parr treated with methallibure.
2. Immature parr treated with cyproterone acetate.
3. Immature parr control group.
4. Precocious male parr control group.
5. Precocious male parr treated with cyproterone acetate.
6. Precocious male parr treated with methallibure.

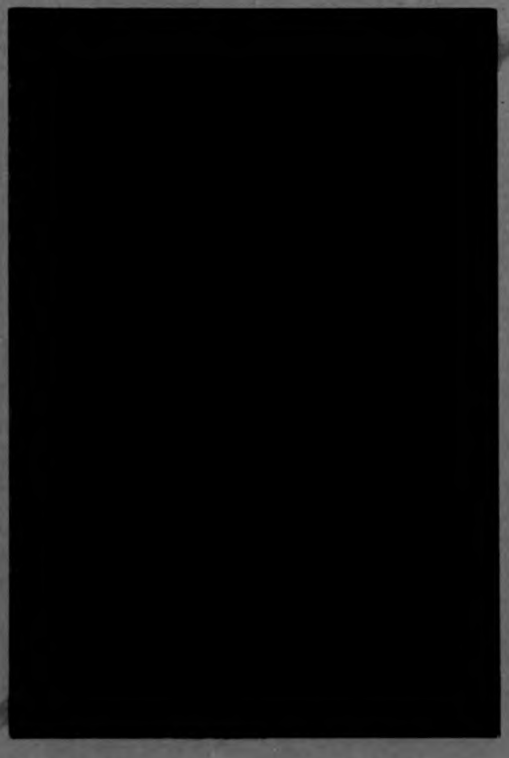
☆ Significantly different from the controls ($p \leq 0.05$)

✪ Significantly different from the controls ($p \leq 0.01$)

★ Significantly different from the controls ($p \leq 0.001$)







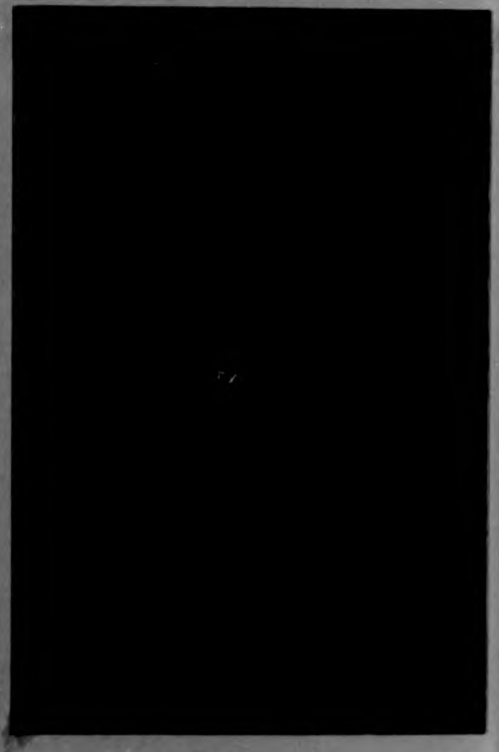
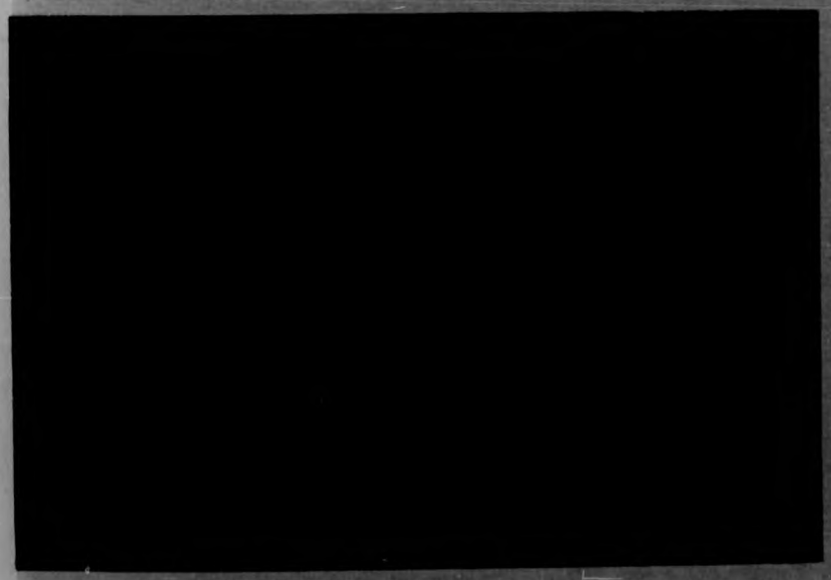


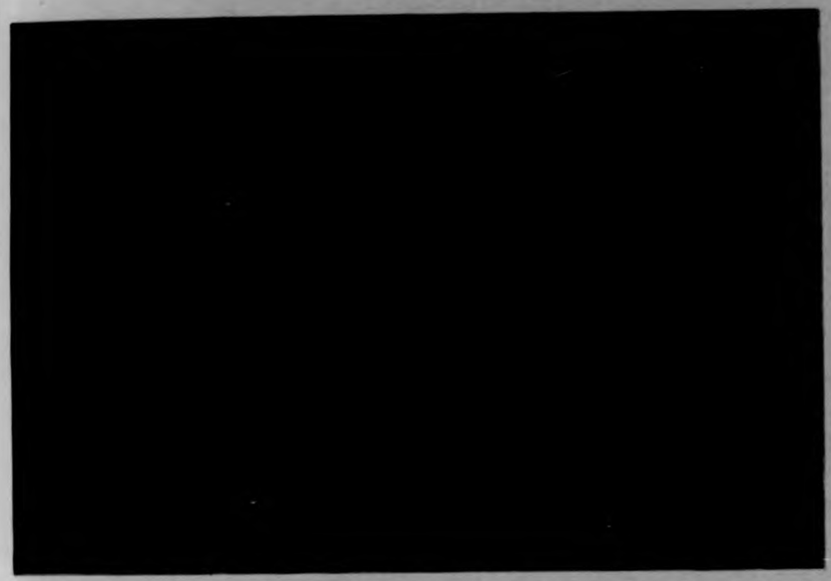
Plate 2. Precocious male 1+ salmon parr.

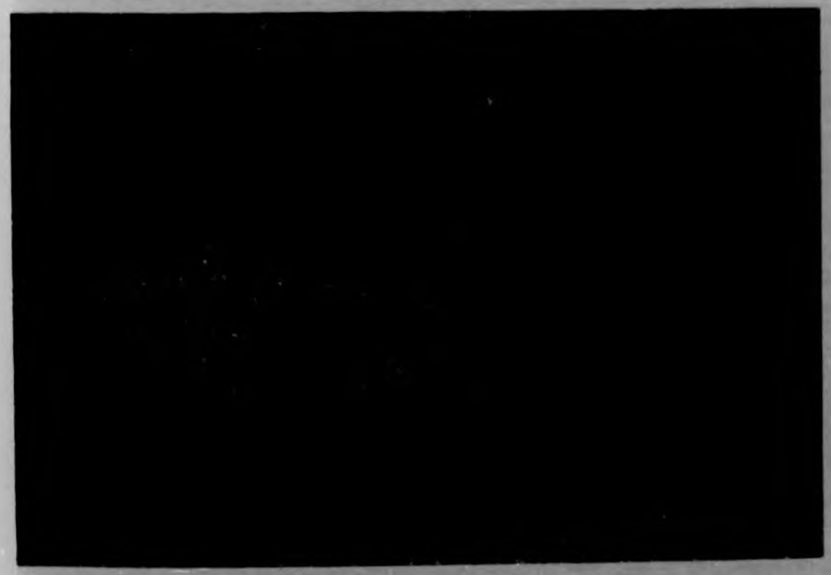
(a) Spleen

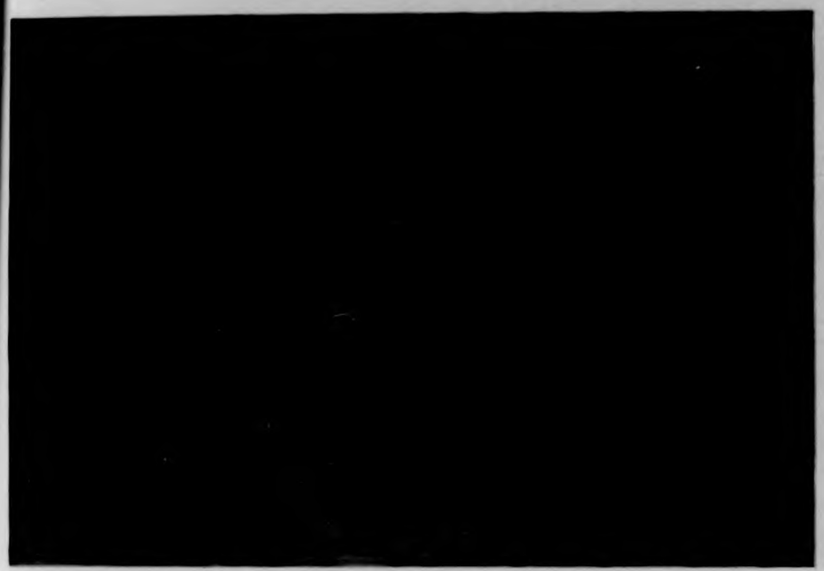
(b) Heart

(H. & E. x 80)









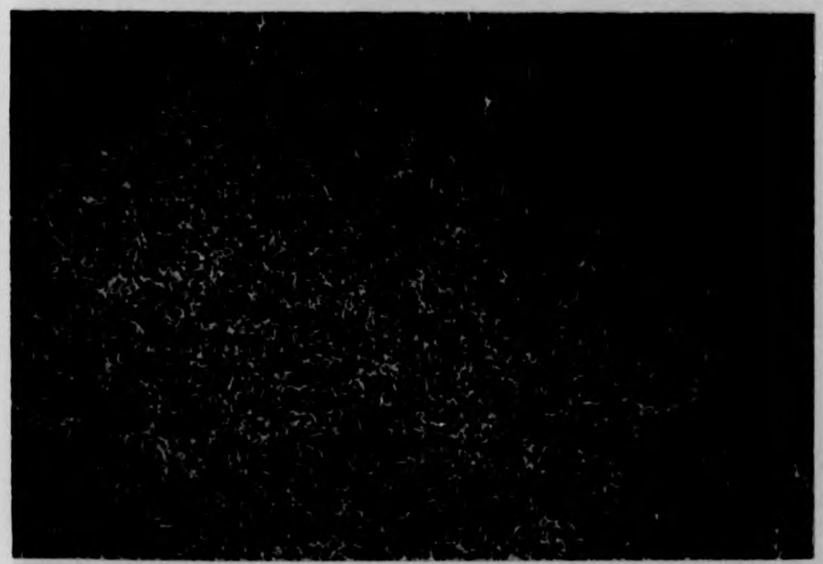
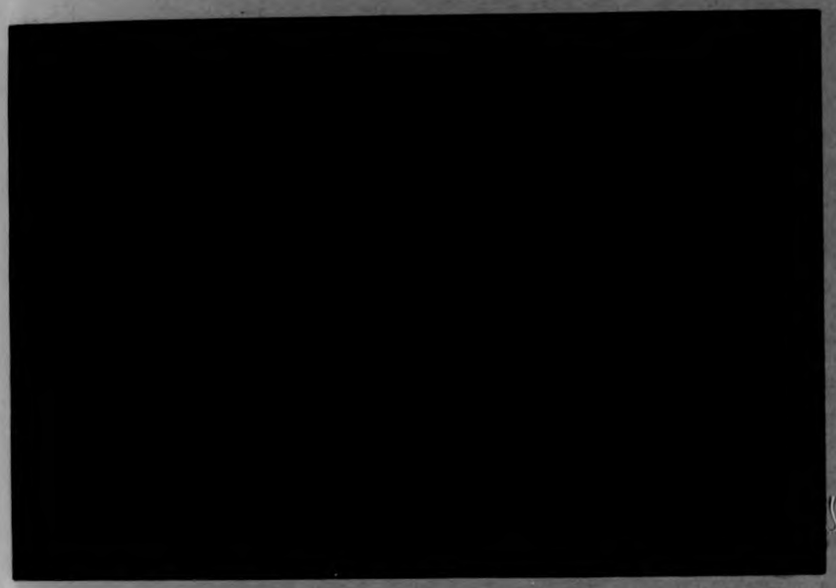


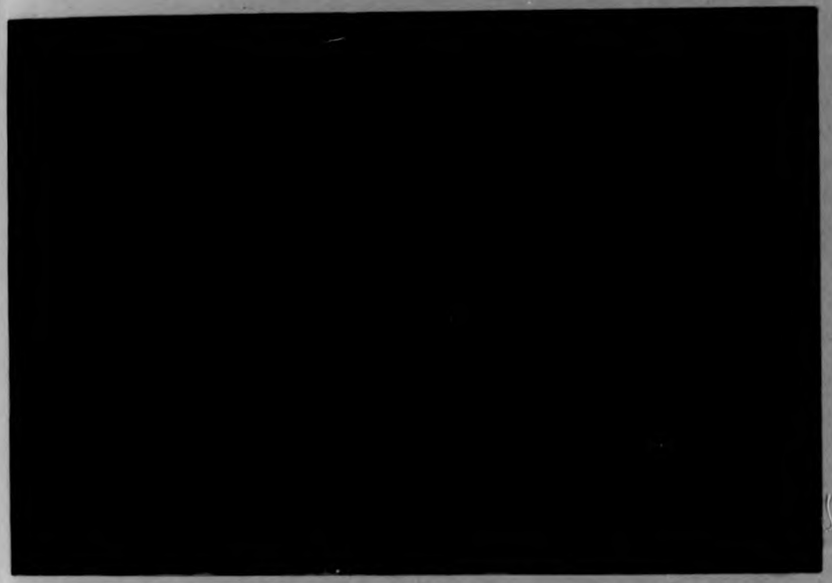
Plate 3. Immature testis containing cysts of primary spermatogonia.

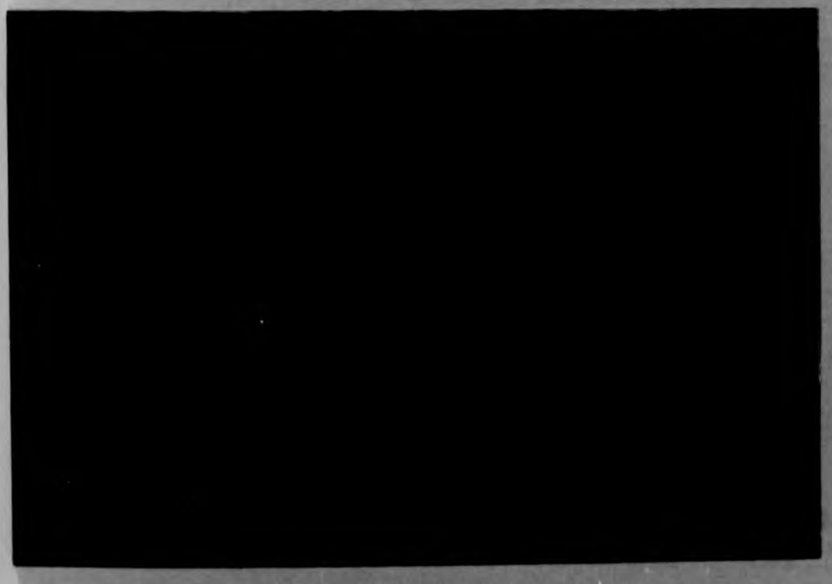
(H. & E. x 320)

Plate 4. Testis of a male salmon parr that had previously undergone a sexual cycle. Note the clumps of spermatozoa amongst the developing spermatogonia.

(H. & E. x 320)







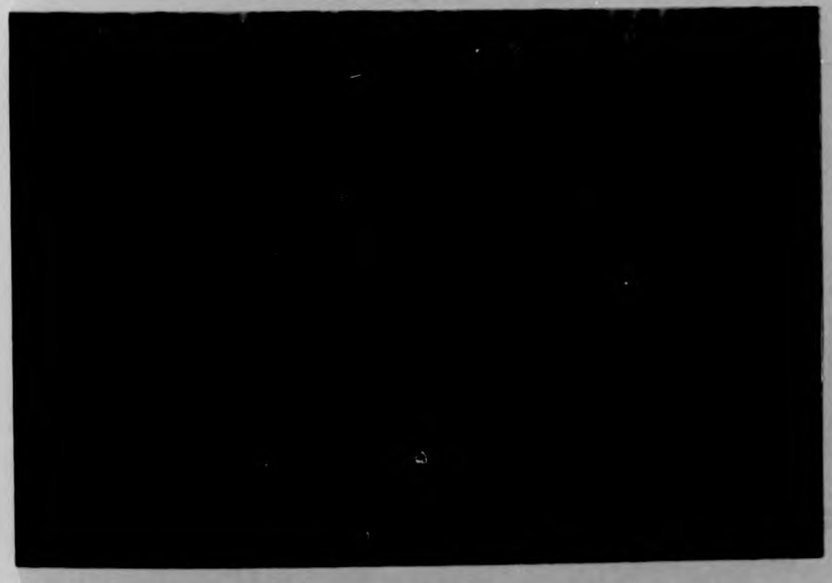
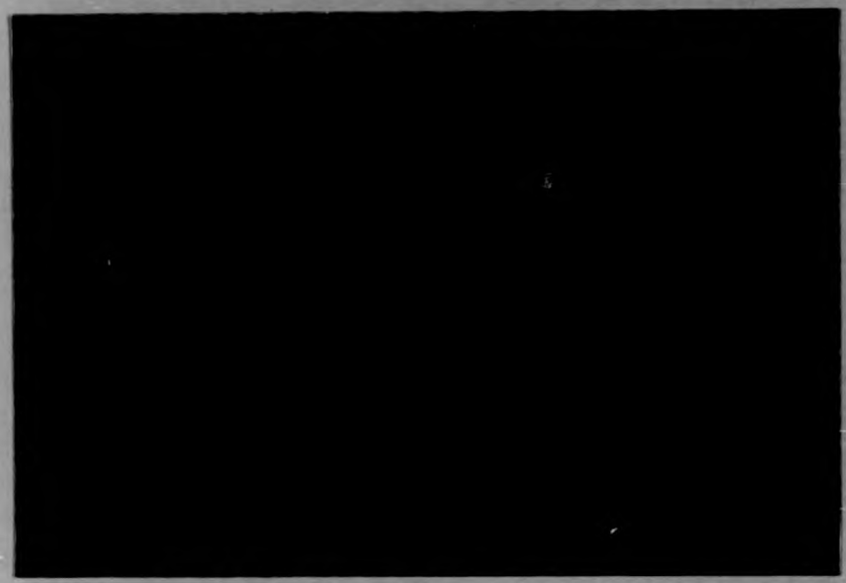


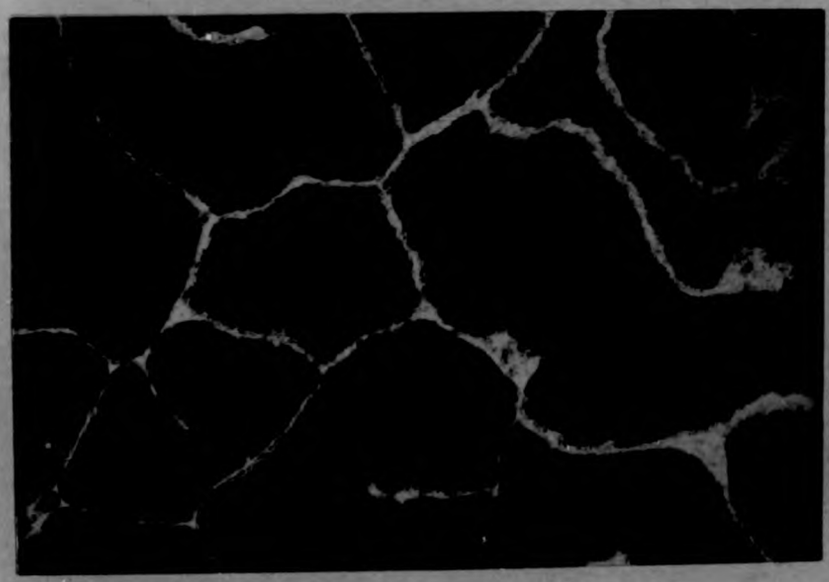
Plate 5. Testis of developing male parr sampled during August showing that all the stages of spermatogenesis are present and that some lobules are more advanced than others.

(H. & E. x 320)

Plate 6. Testis of precocious male 1+ parr sampled in December. All the lobules contain mature spermatozoa.

(H. & E. x 320)





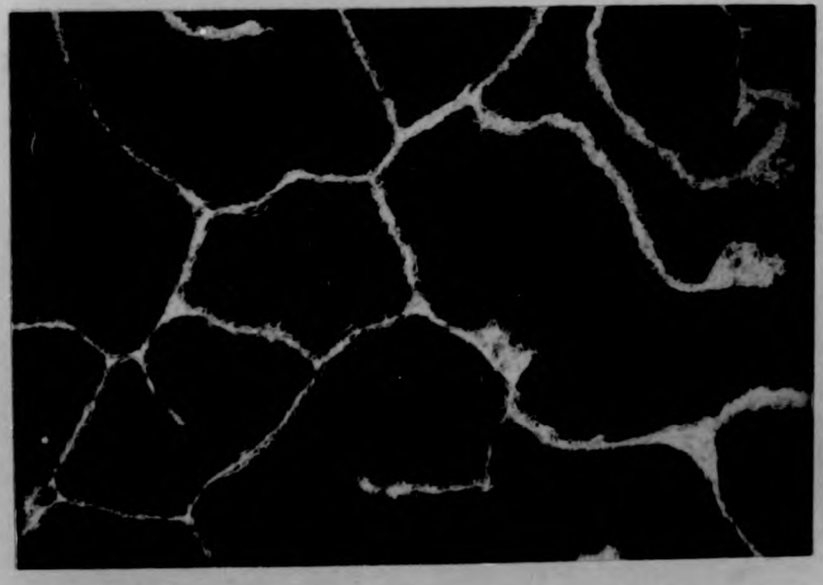


Plate 7. Ovary of immature 1+ female parr.

(a) Early perinucleolus stage

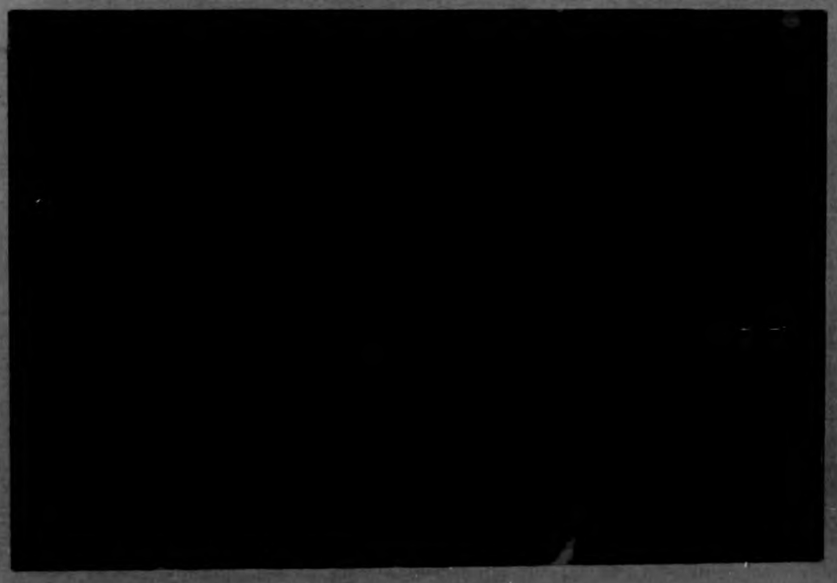
(b) Late perinucleolus stage

(c) Early yolk vesicle stage

(H. & E. x 80)

Plate 8. Interrenal tissue of 1+ precocious male parr surrounding a blood vessel (b). The haematopoietic tissue (h) of the head kidney with melanin macrophage centres (m) scattered through it is also shown.

(H. & E. x 320)



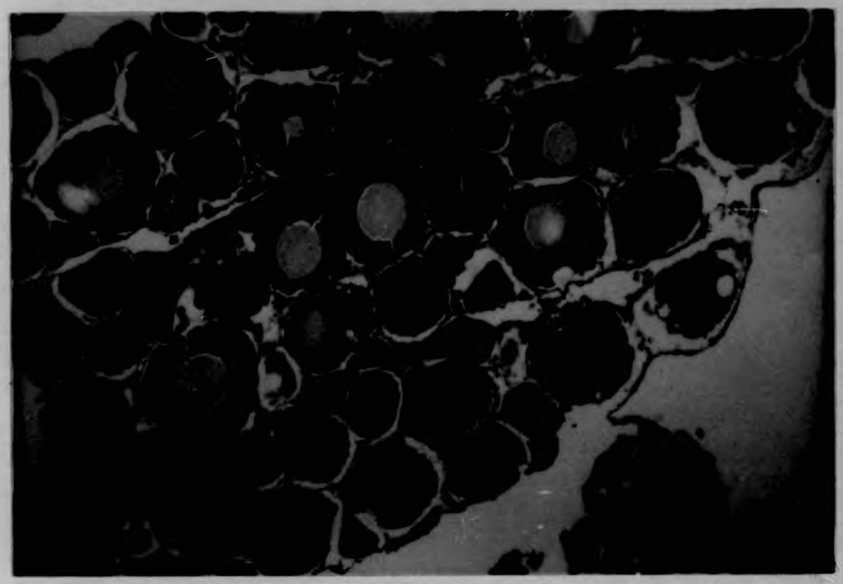
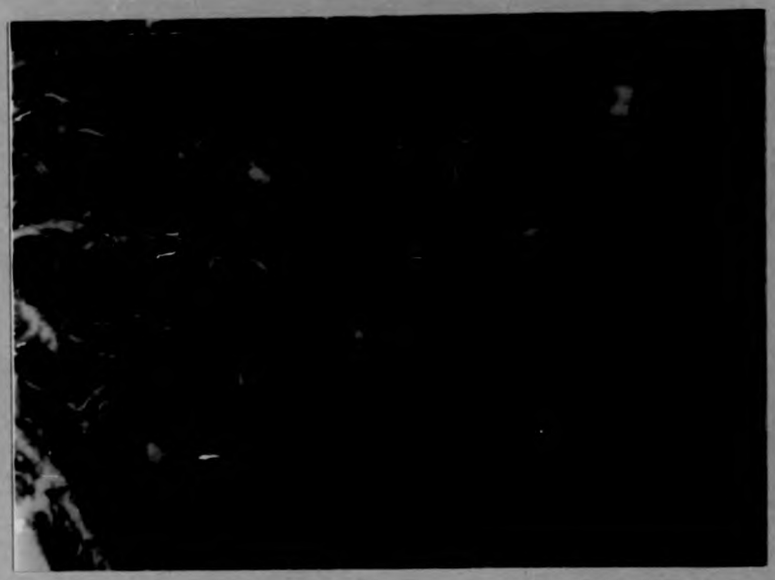
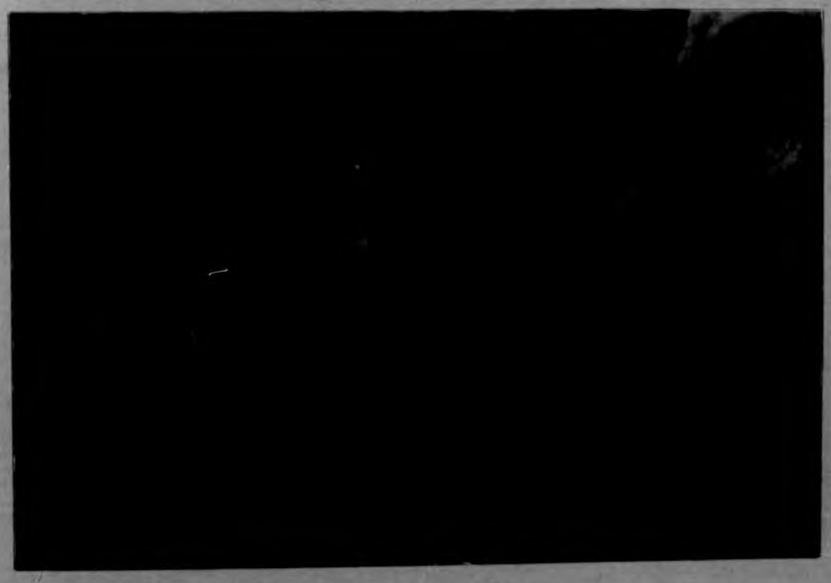


Plate 9 (a) & (b).

Pro-adenohypophysis of precocious male 1+ parr. Acidophilic (yellow) follicles with colloid material in their lumina. PAS + ve (purple) cells scattered between the follicles.

(AB - PAS - OG x 320)





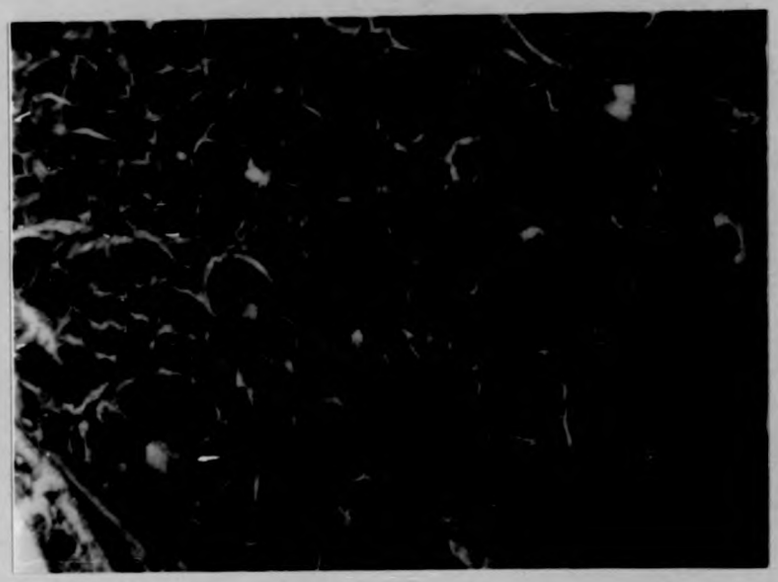


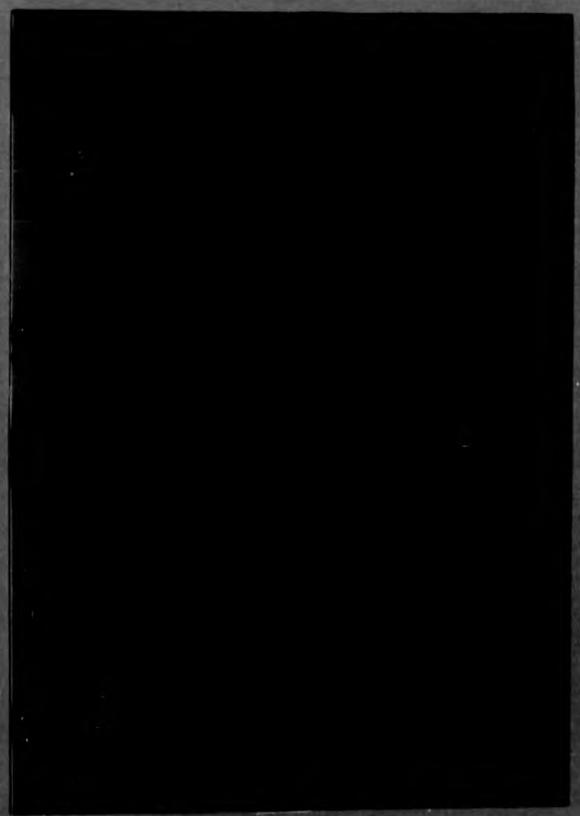
Plate 10. (a) Transverse section of the pituitary of precocious male 1+ parr showing the hypothalamus, meso-adenohypophysis and meta-adenohypophysis. A group of basophilic cells are present on the lateral edge of the meso-adenohypophysis (arrow).

(x 320)

(b) High power of the meso-adenohypophysis of precocious male 1+ parr containing basophilic cells. The basophilic gonadotrophic cells are stained green and somatotrophic cells (S) are stained light brown. Part of the neurohypophysis (N) is also shown.

(x 800)

((a) and (b) stained with Masson's trichrome technique)



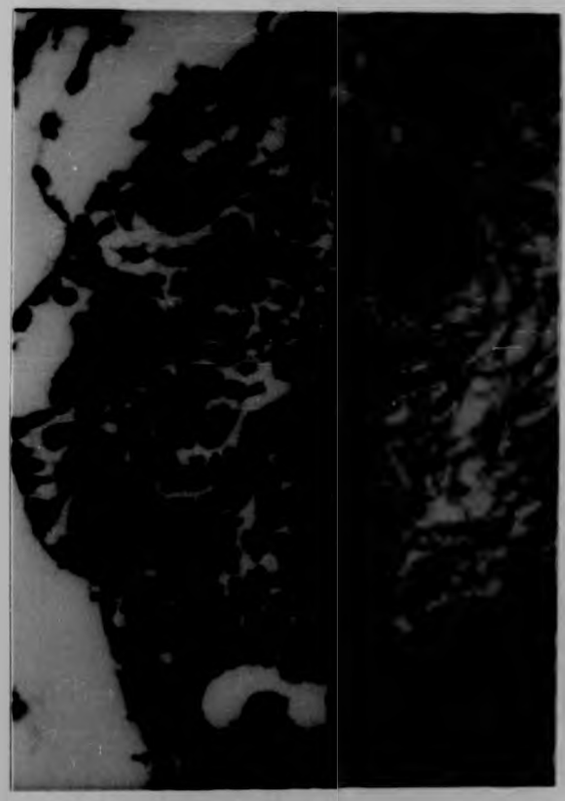
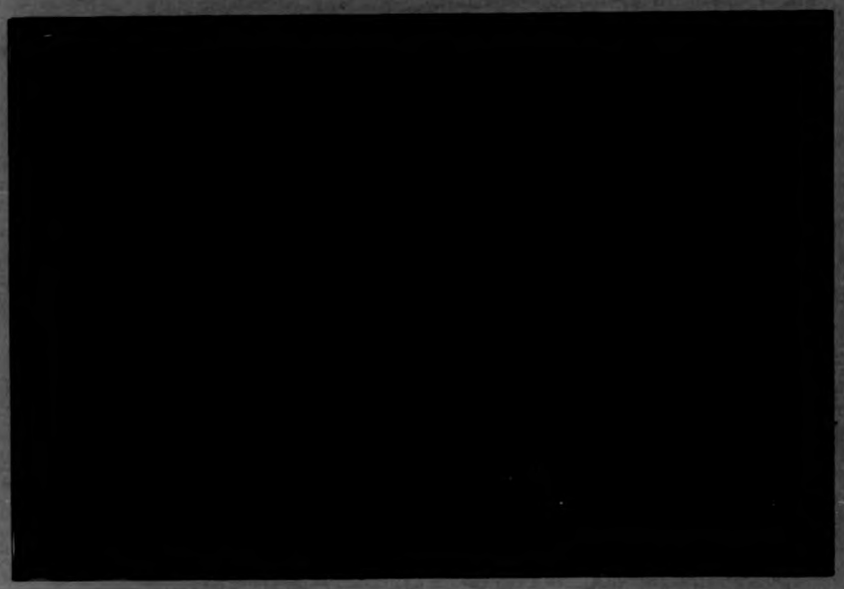
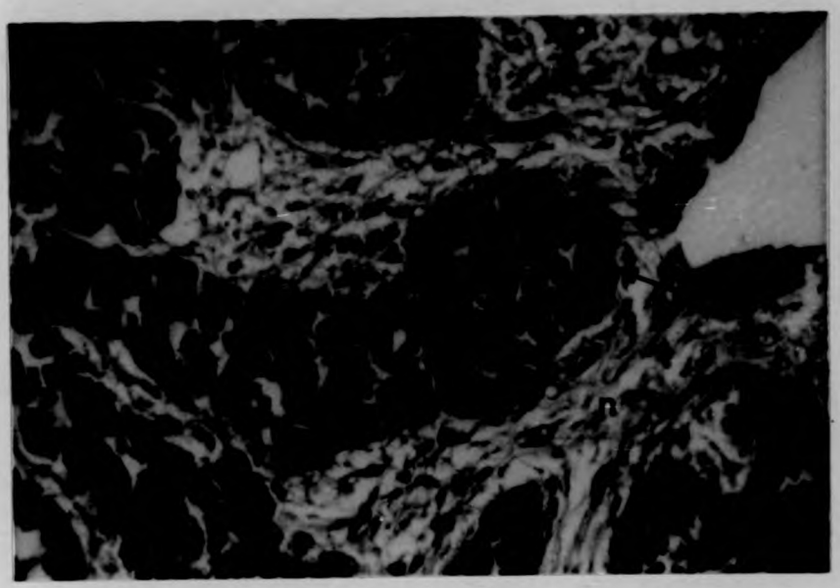


Plate 11. (a) Meso-adenohypophysis of precocious male 1+ parr, showing individual basophilic cells (green) amongst the undifferentiated cells at the tips of the fingerlike processes.
(Masson's trichrome x 80)

(b) High power of a meso-adenopophyseal process showing large basophilic cells (green) on the border with the neurohypophysis.

N, neurohypophysis
S, somatotrophic cells
(Masson's trichrome x 320)

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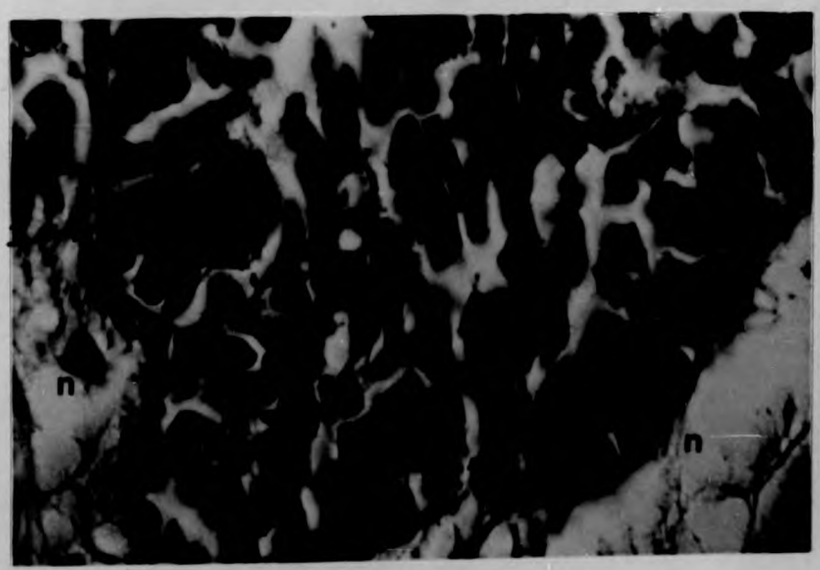

n
n

Plate 12. Meta-adenohypophysis of precocious 1+ male parr showing PAS + ve granules in some of the chromophobic cells.

(AB - PAS - OG x 800)

[redacted]

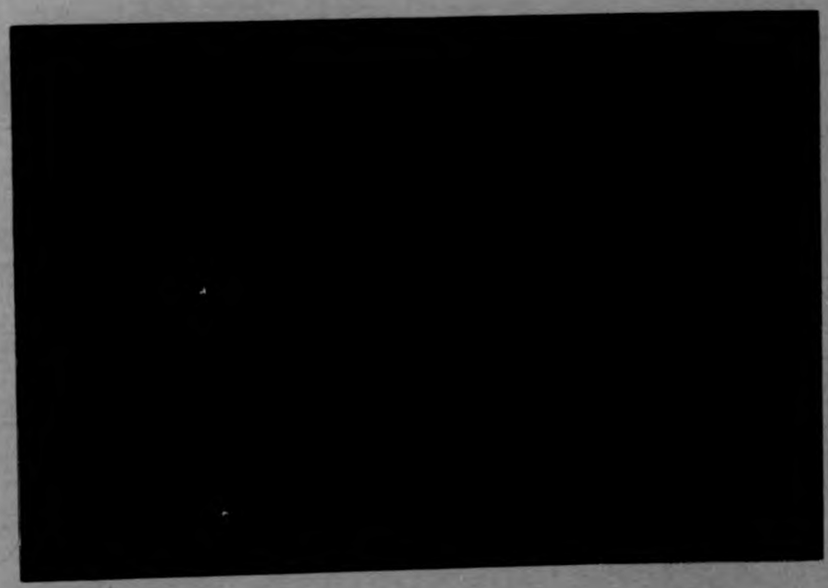
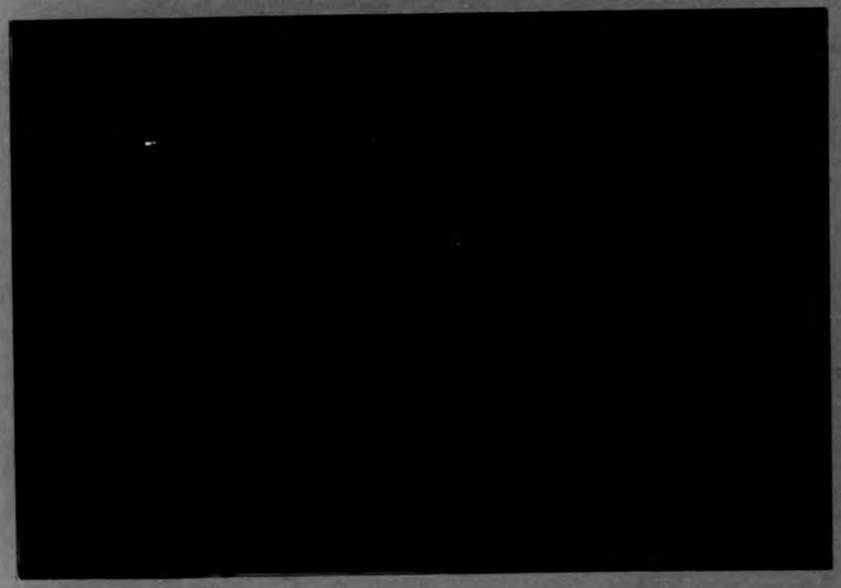


Plate 13. Neurosecretory material (NSM) in the neurohypophysis in the meta-adenohypophyseal area of the pituitary of 1+ parr.

(a) NSM stained black with Bargemann's technique.(Bargemann x 320)

(b) NSM stained blue with aniline blue when stained with the Slidder's procedure.(Slidder's x 80)



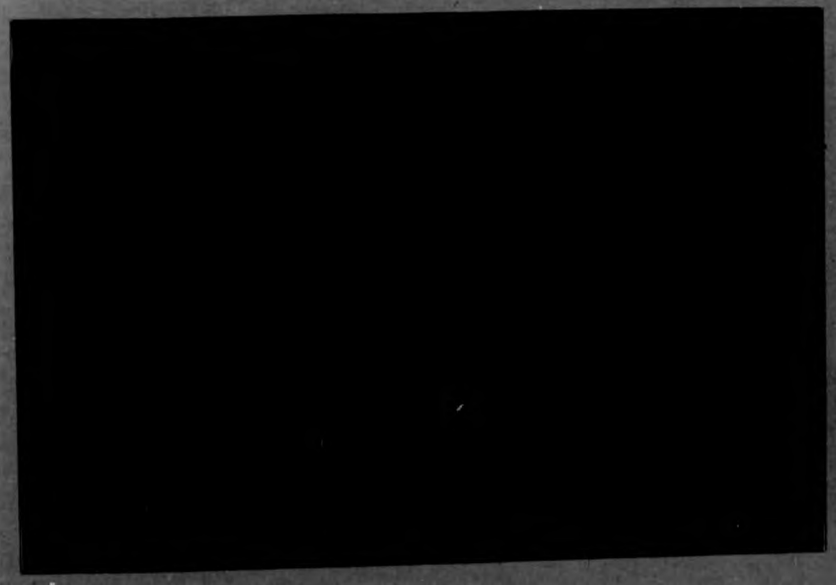


Plate 14. Neurohypophysis of precocious male 1+ parr. Some of the nerve fibres in the neurohypophysis around the infundibulum of the pituitary of precocious male 1+ parr are PAS + ve. The NSM in this area is also PAS + ve.

(AB - PAS - OG x 800)

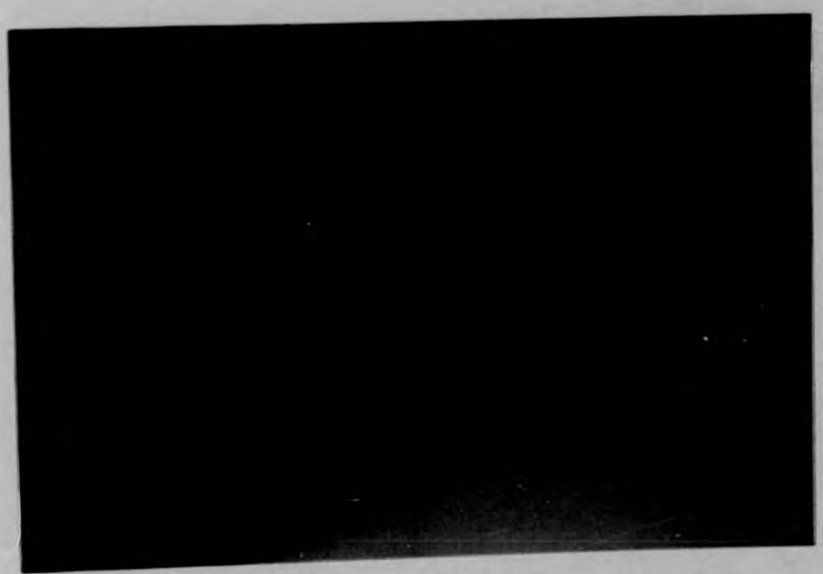
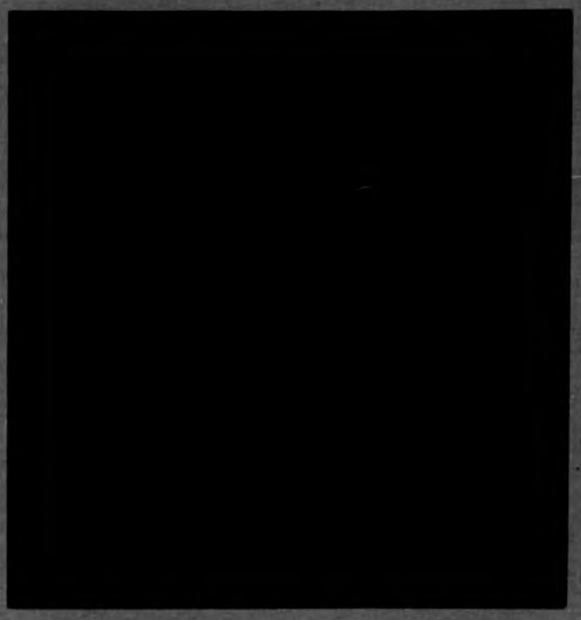
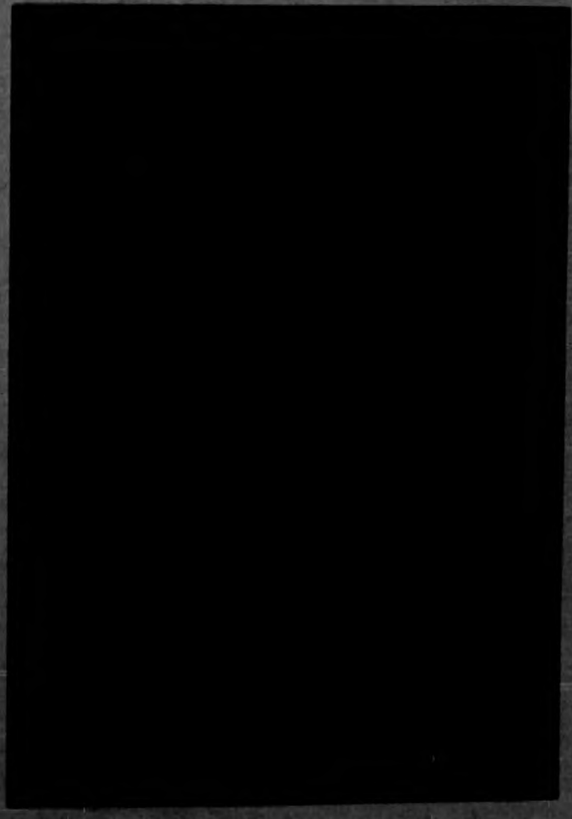


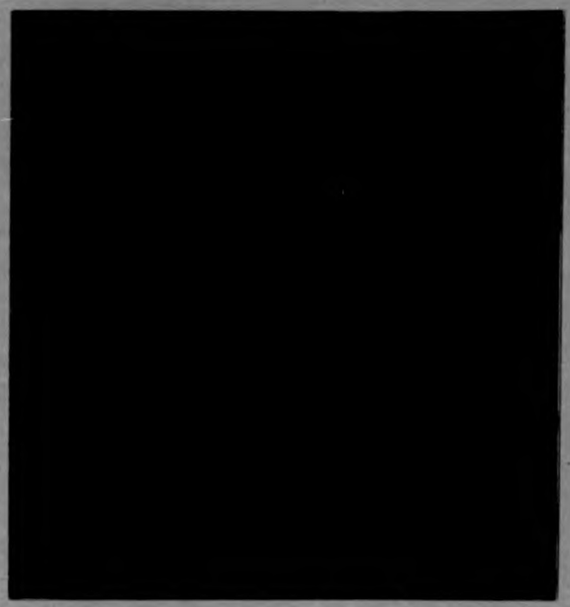
Plate 15. (a) Transverse section of the pituitary of an immature female 1+ parr showing the meso-adenohypophysis (MES) and meta-adenohypophysis (MET). There are no basophilic cells on the lateral edge of the meso-adenohypophysis. Compare with plate 10 (a).
(AB - PAS - OG x 80)

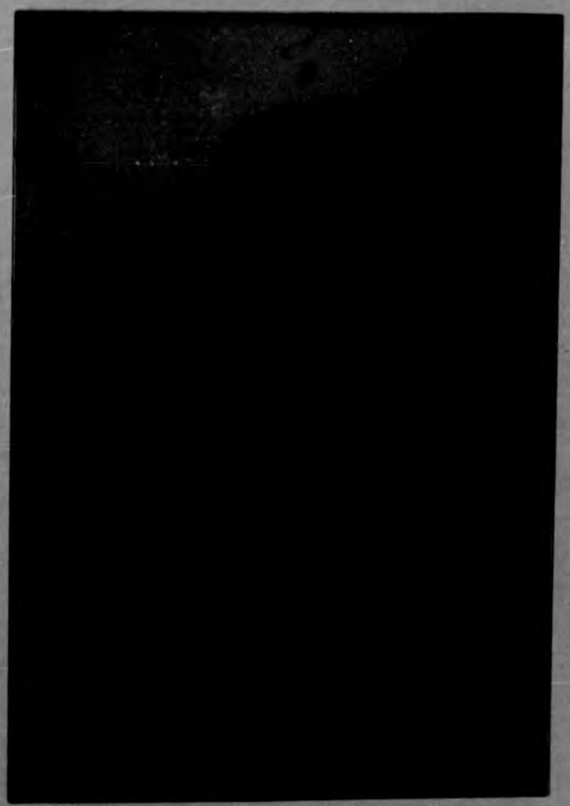
(b) High power of the lateral edge of the meso-adenohypophysis of an immature 1+ female parr. There are no PAS + ve cells present. Compare with Plate 10 (b) and Plate 15 (c). A few PAS + ve cells can be seen in the extension of the meta-adenohypophysis into the neurohypophysis. The acidophilic somatotrophic cells are stained yellow (S).
(AB - PAS - OG x 320)

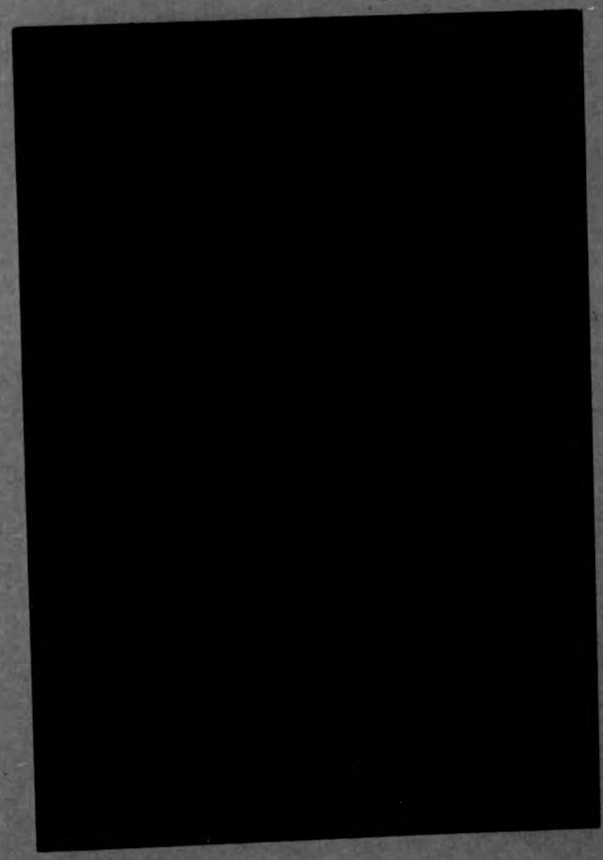
(c) Transverse section of a pituitary of a 1+ precocious male parr. High power of the lateral edge showing numerous PAS + ve cells in the meso-adenohypophysis.
(AB - PAS - OG x 320)











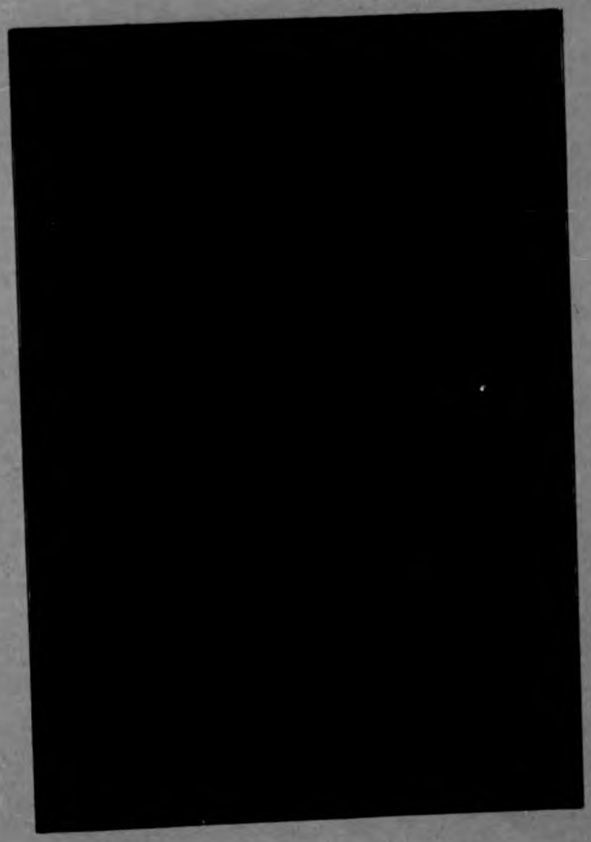
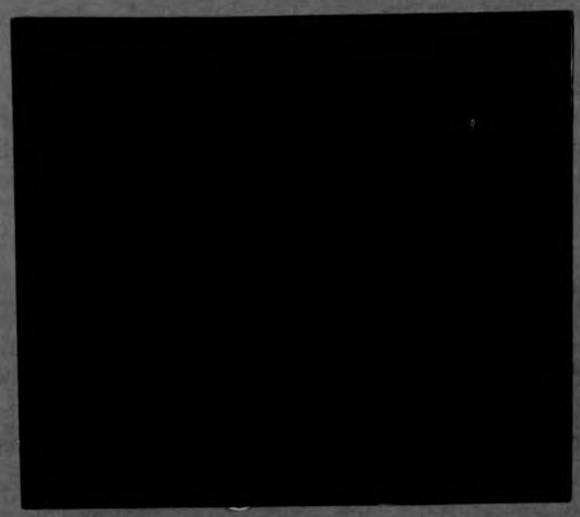
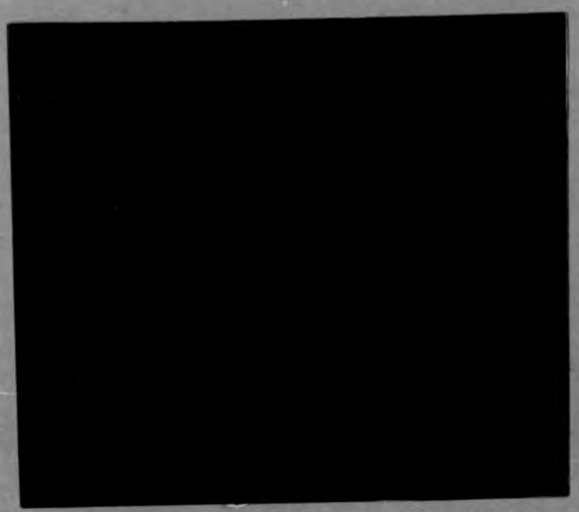


Plate 16. Epidermis of the pectoral area of precocious 1+ salmon parr showing breaks in the continuity of the outer layer of cells (arrowed).
(H. & E. x 320)





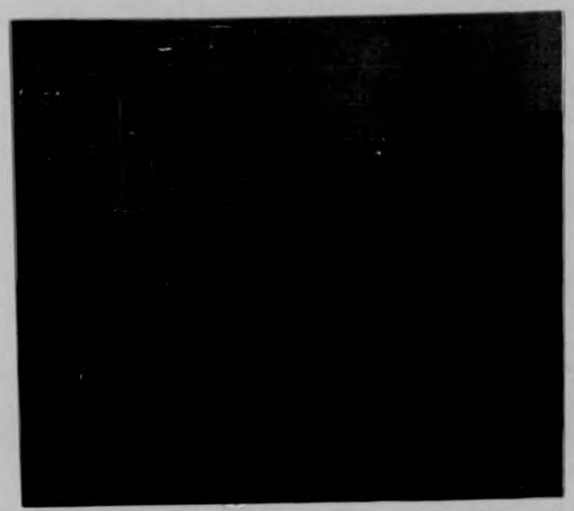
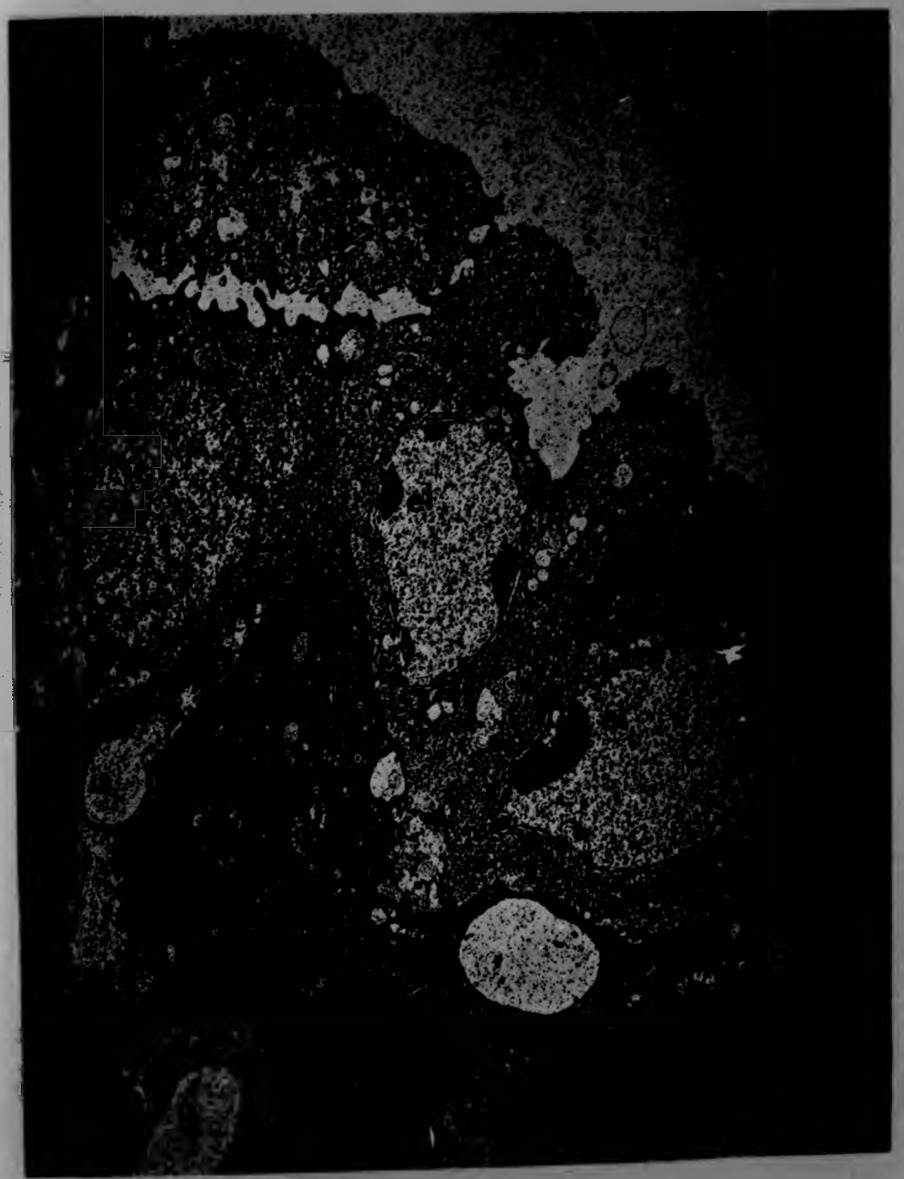
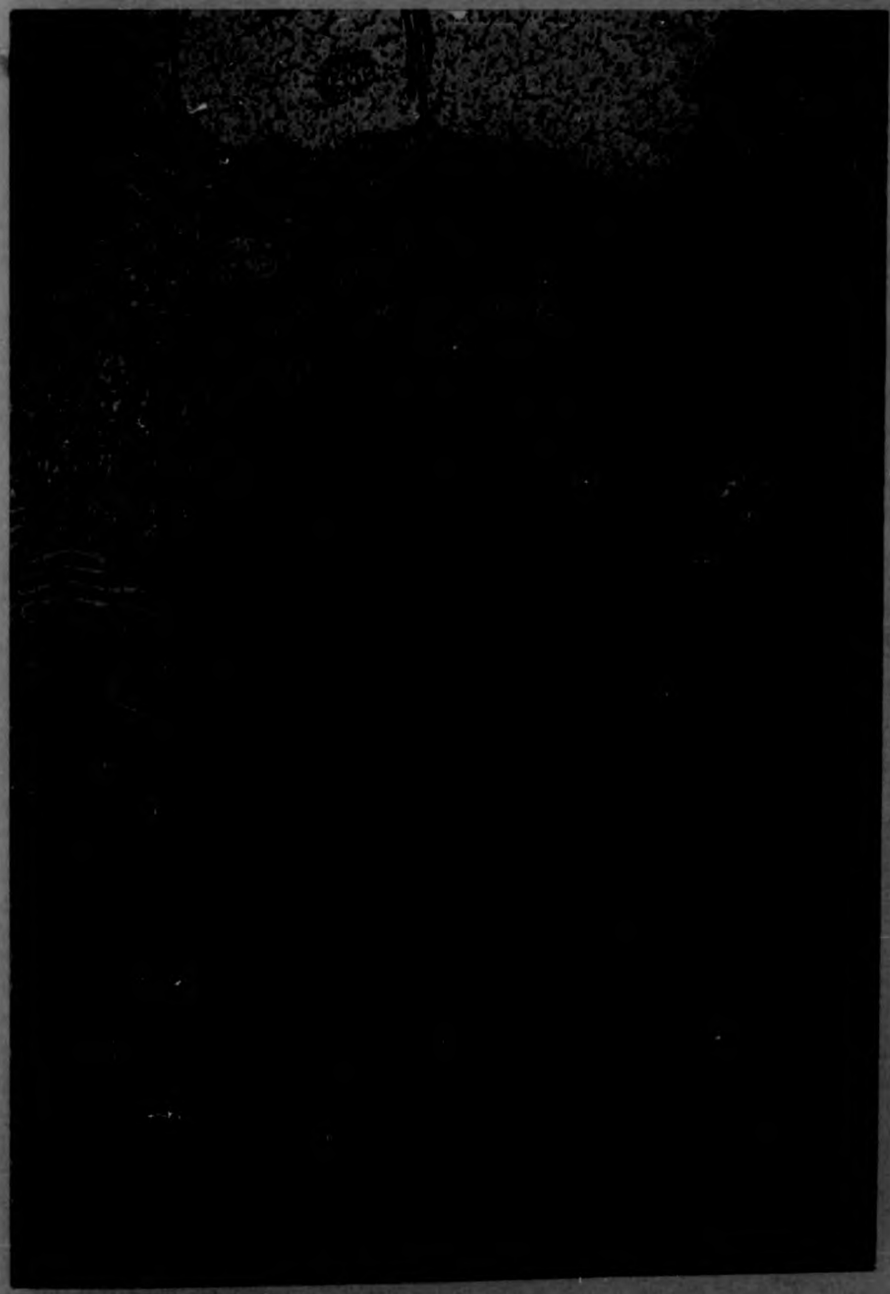


Plate 17. Malpighian filament cells with indented nuclei and prominent nucleoli. Note the numerous intracellular spaces (is), some of them contain filamentous material.

(x 8000)





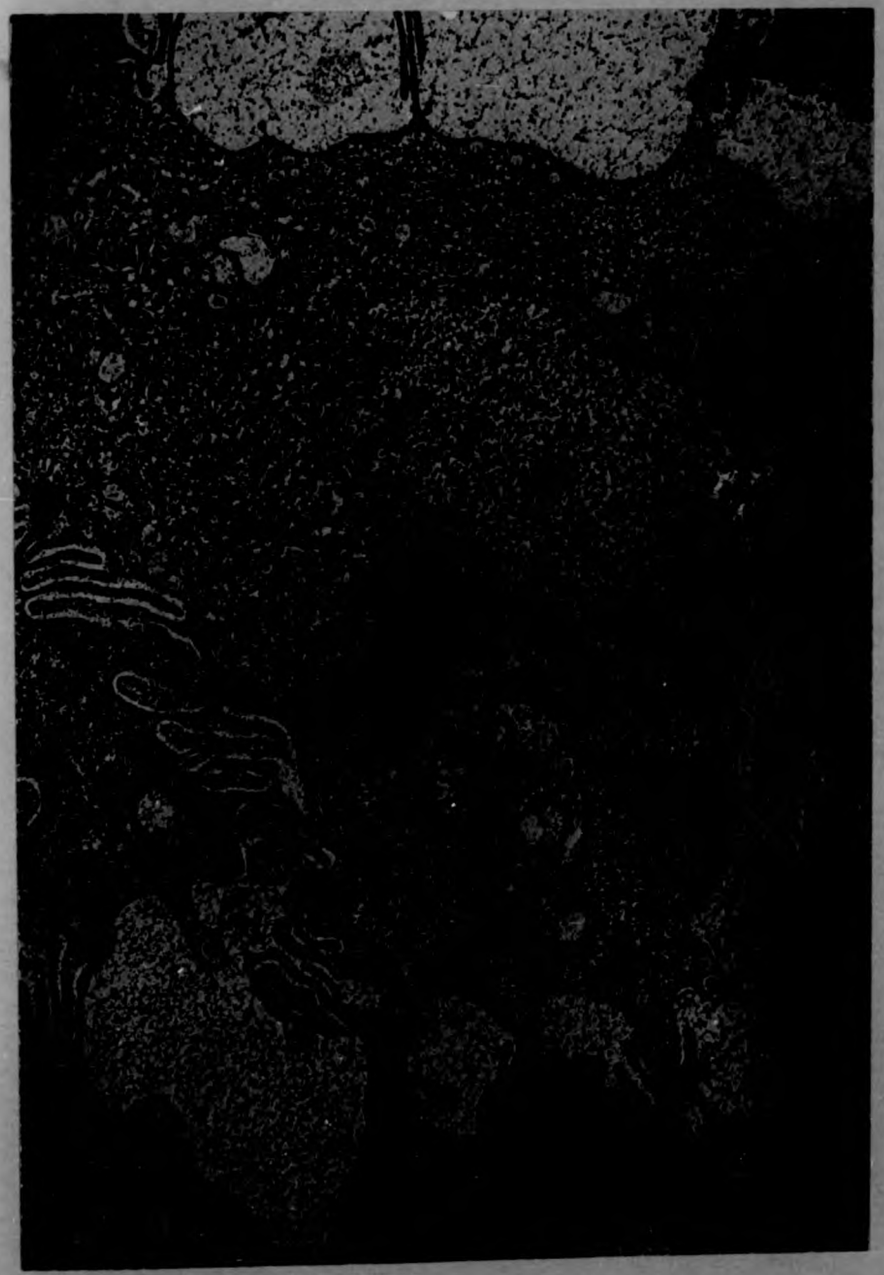
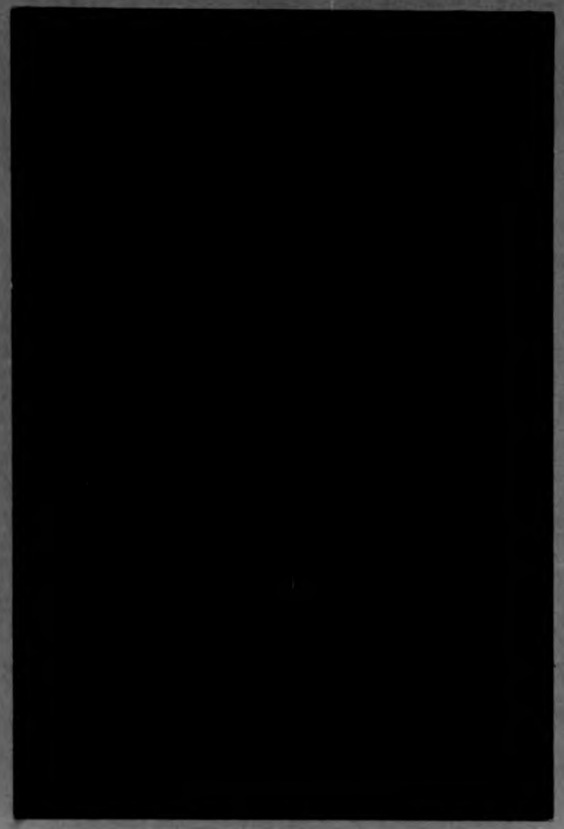


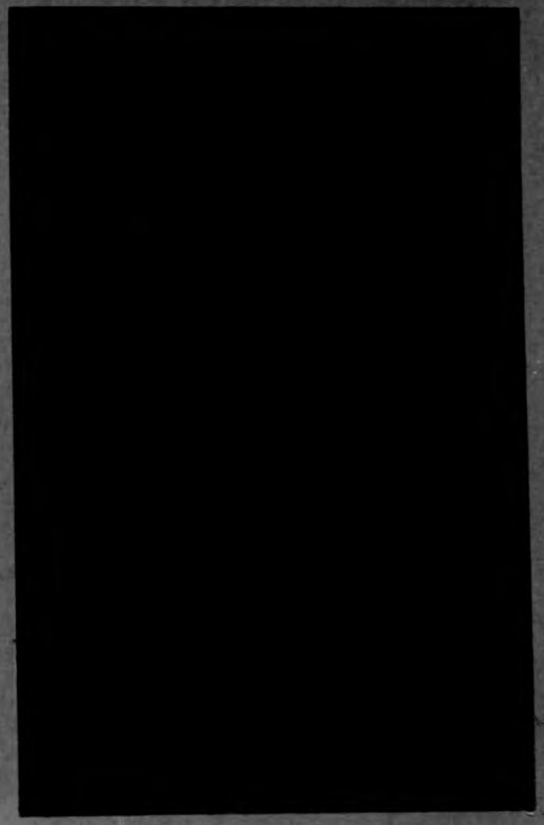
Plate 20. Alcian blue (pH 2.5)/PAS

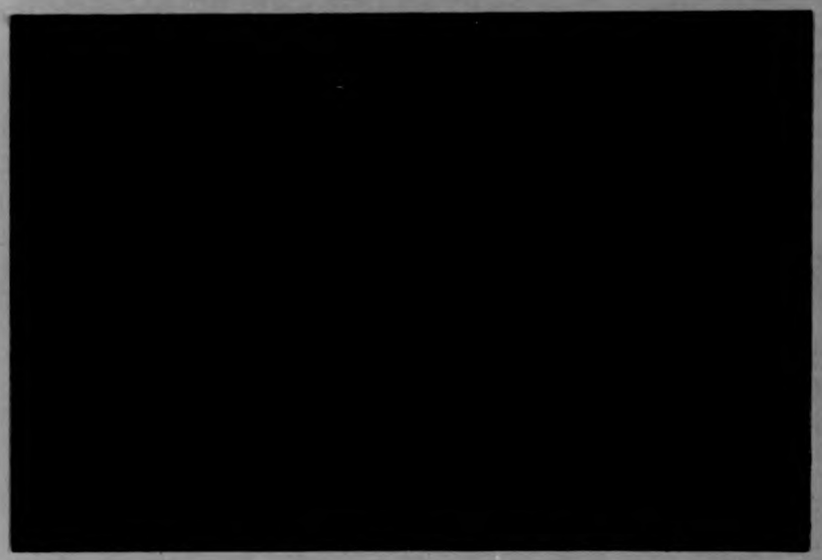
(a) Caudal peduncle of precocious 1+ male parr sampled in December.

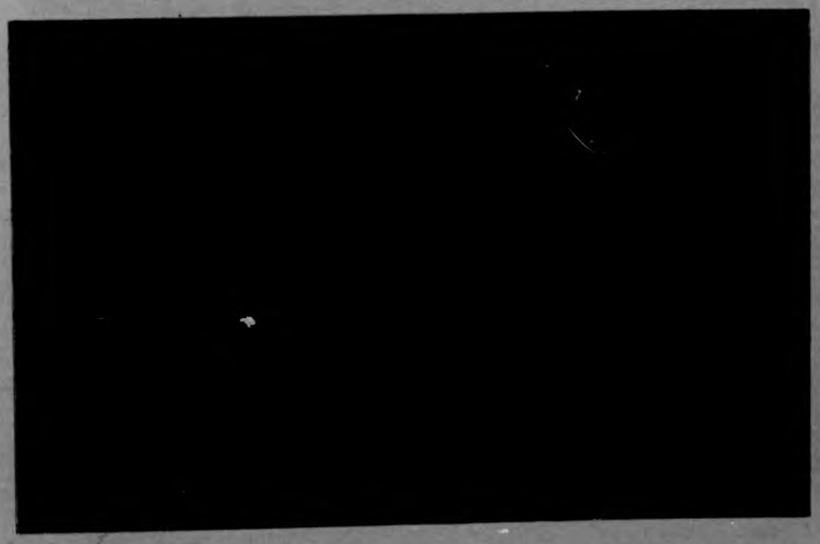
(b) Caudal peduncle of immature 1+ female parr sampled in December.

(x 80)









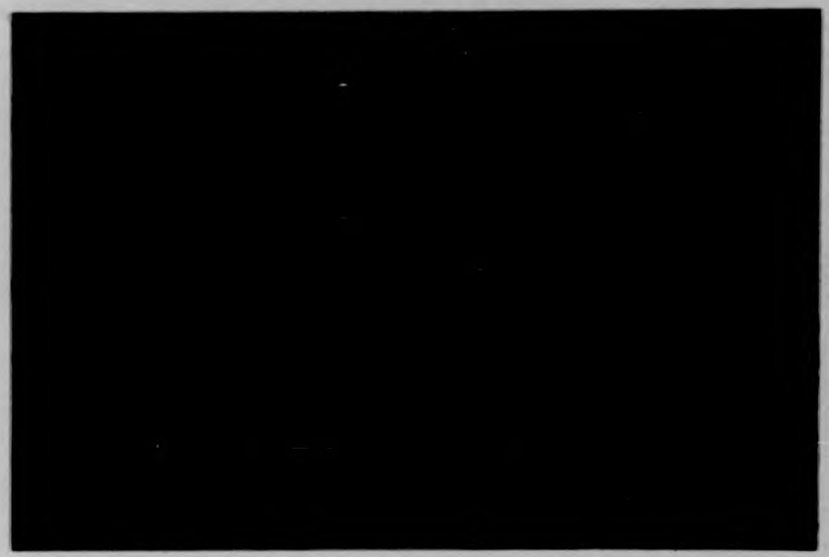


Plate 21. Colloidal Iron/PAS

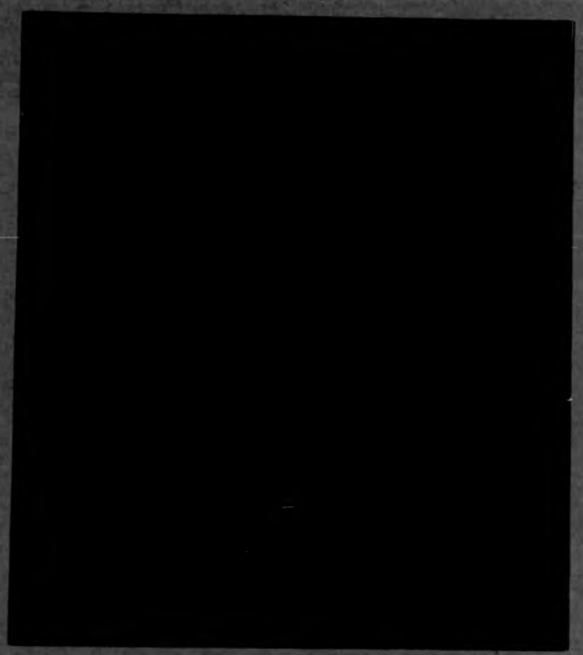
(a) Pectoral area of precocious male 1+ parr

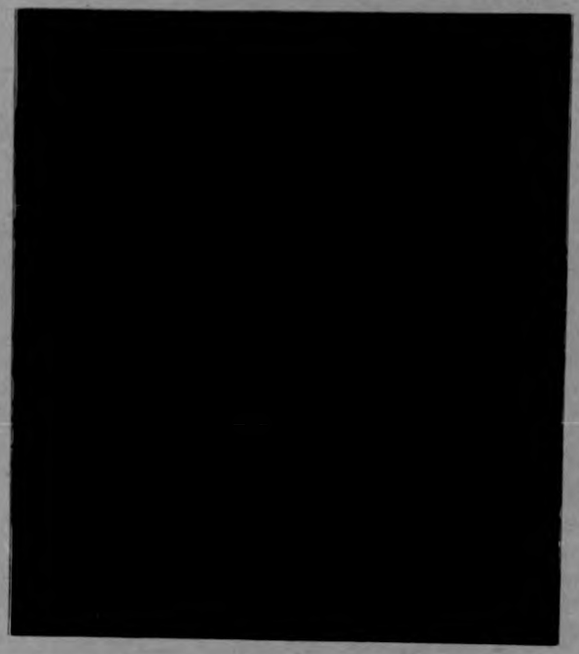
(b) Pectoral area of immature female 1+ parr

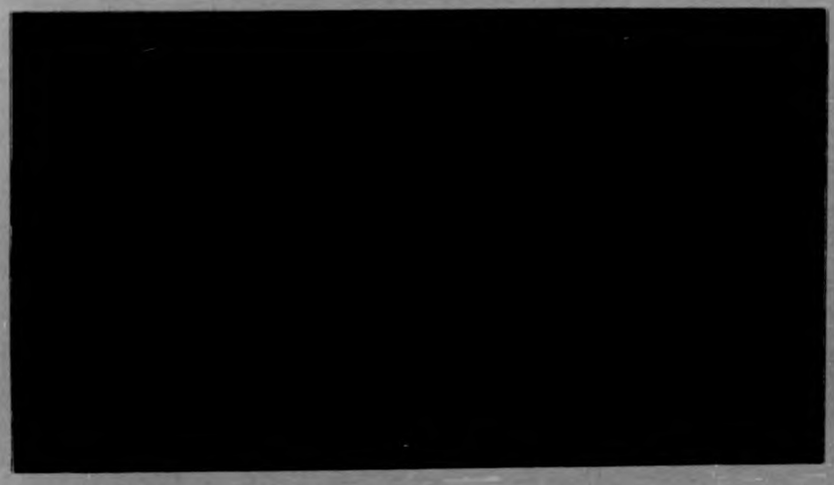
The majority of mucous cells in both sexes are stained blue/purple.

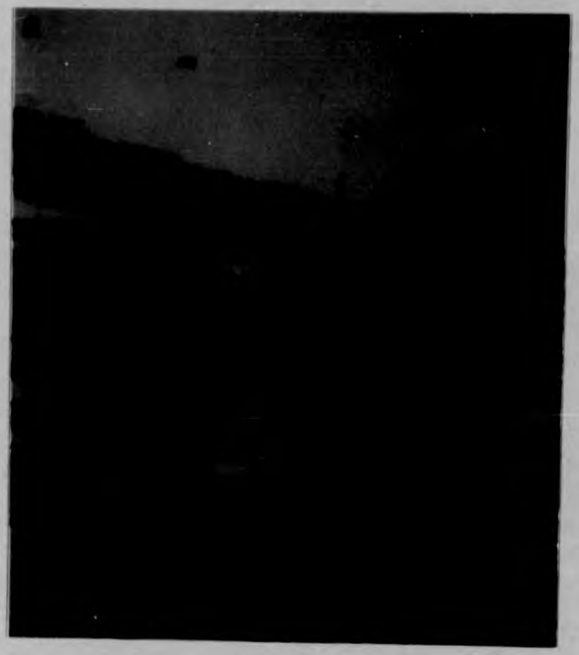
The remains of the labile cuticle layer can be seen in (a) (arrow).

(x 80)









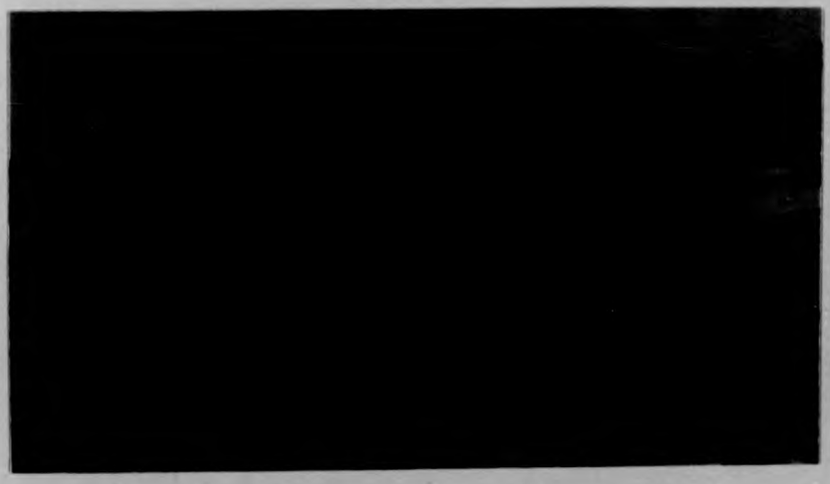


Plate 22. Alcian Blue (pH 1.0)/PAS

(a) Pectoral area of precocious male 1+ parr

(b) Pectoral area of immature female 1+ parr

All the cells are stained red or red/purple.

(x 80)

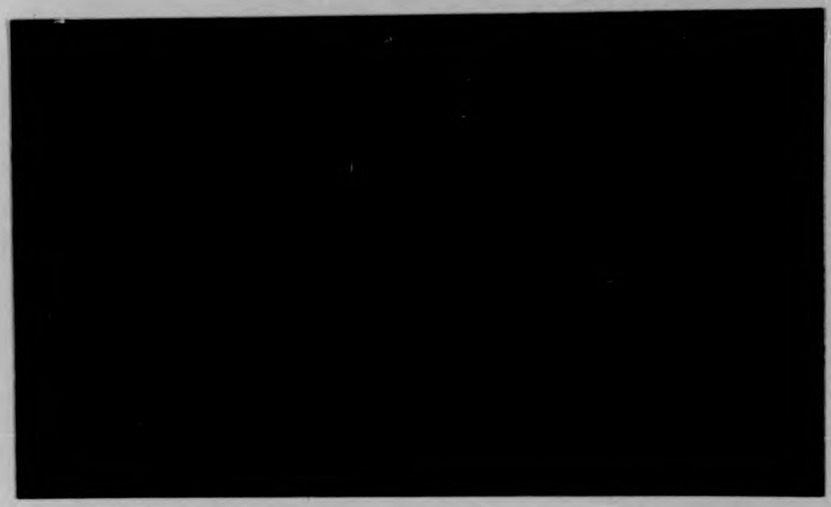
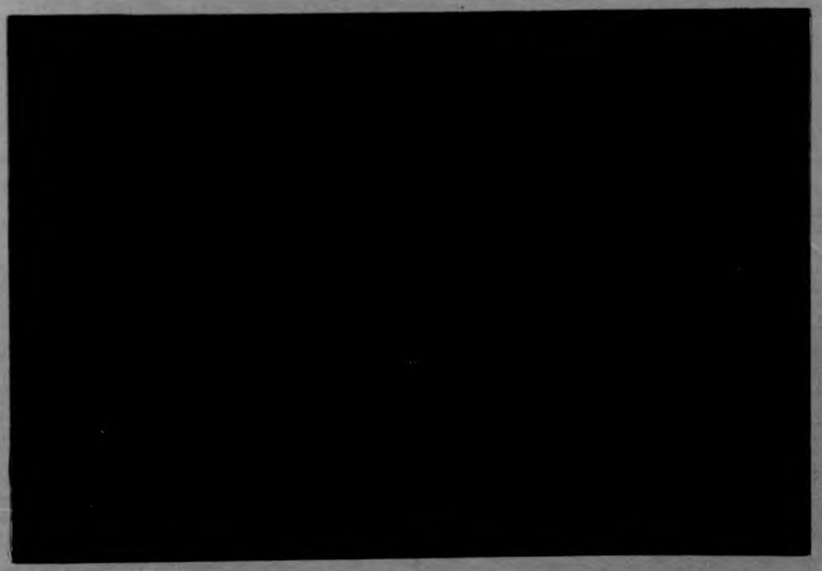


Plate 23. Pectoral area of precocious male 1+ parr.

(a) Mild methylation/alcian blue.

(x 80)

[redacted]



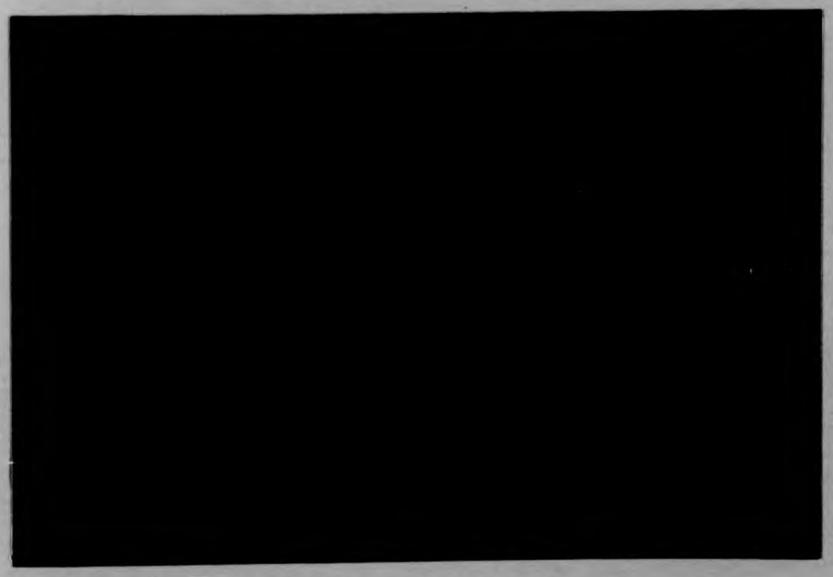


Plate 24. Alcian Blue/Safranin

Pectoral fin of immature 1+ female parr. All the cells are stained blue.

(x 80)

[redacted]

Plate 25. Saprolegnia infection in the pectoral area of precocious 1+ male parr. Note that the epidermis has been completely necrotised by the fungus. Dermis and hypodermis are intact and there is a small amount of lymphocytic infiltration in the hypodermis. The underlying subcutaneous muscle layer is undergoing degeneration.

(H. & E. x 80)

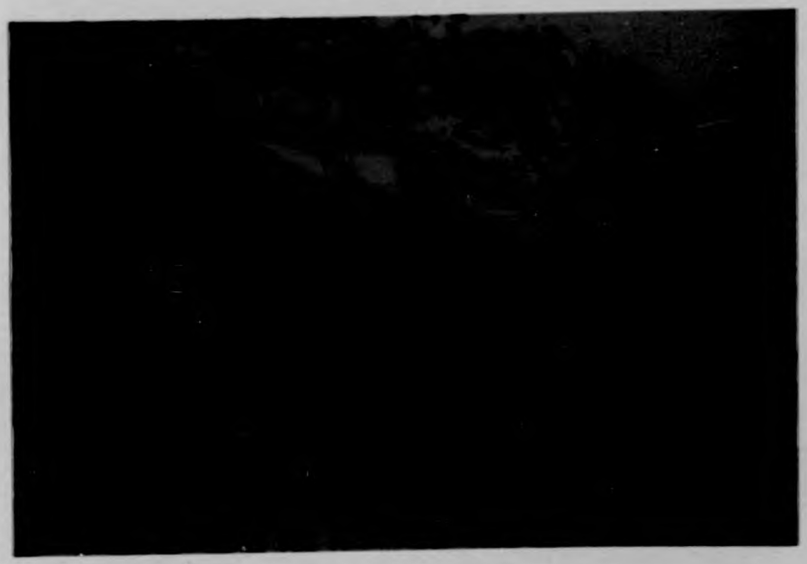
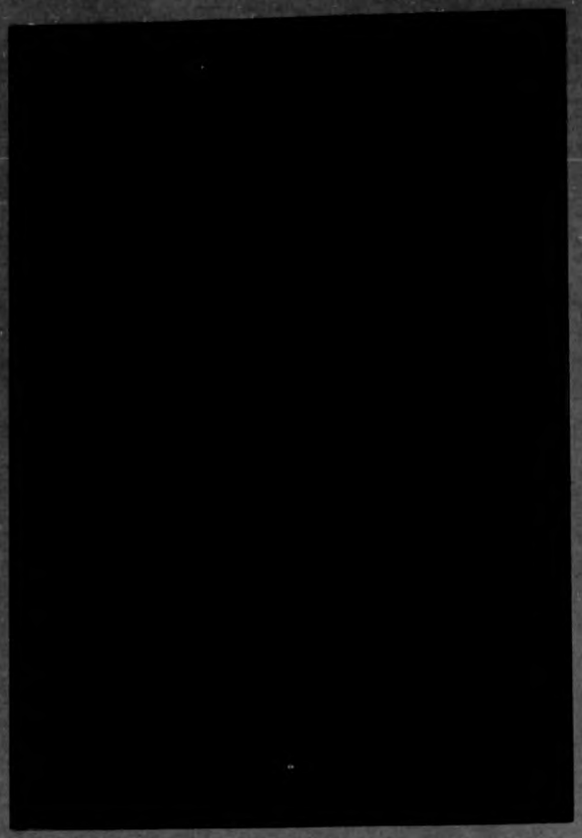


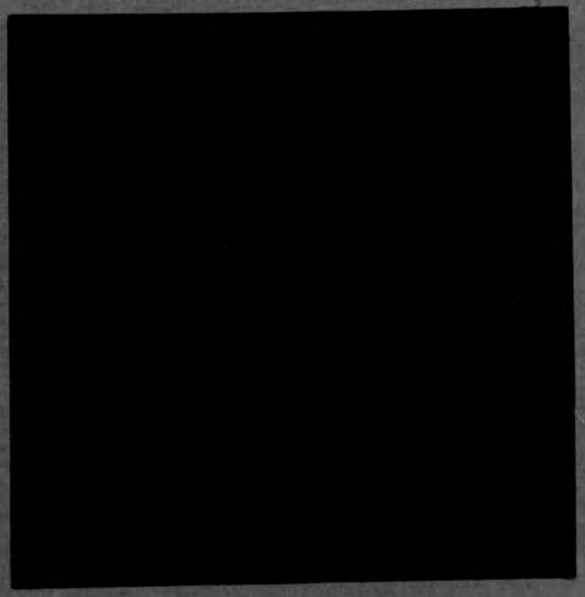
Plate 26. (a) Epidermis of the caudal peduncle of 0+ salmon parr (control group).

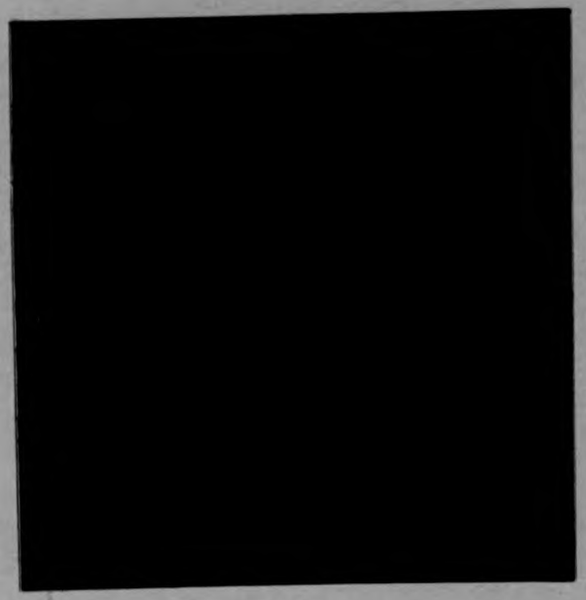
(b) Epidermis of the caudal peduncle of 0+ parr treated with 17α methyltestosterone.

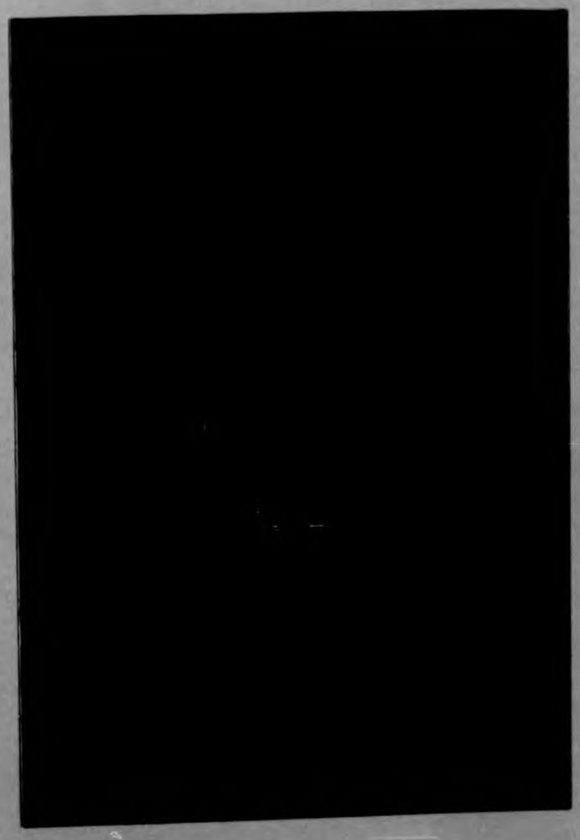
Note the increase in the number of cell layers in the epidermis of the androgen treated fish.

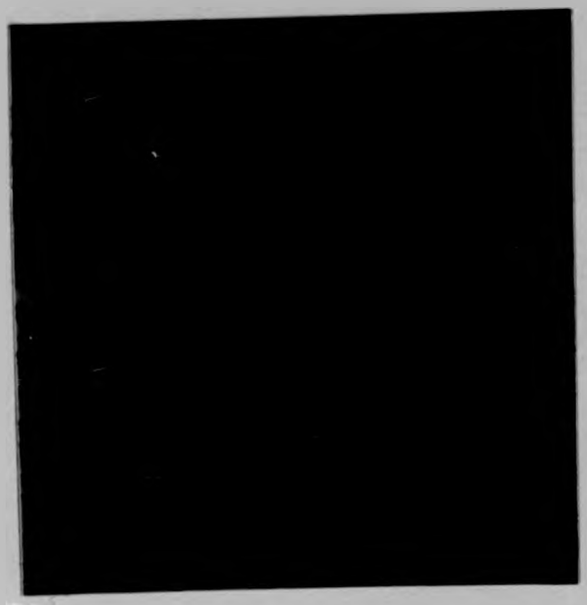
(H. & E. x 320)











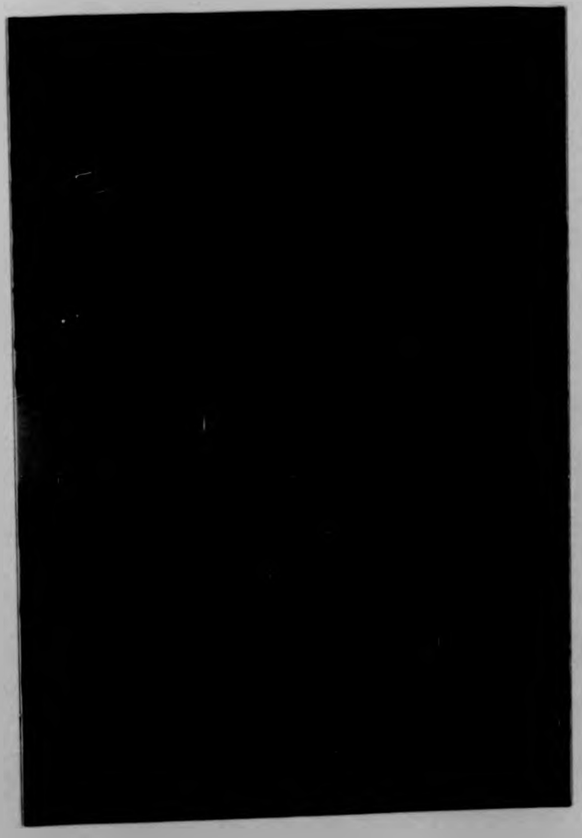


Plate 27. Testis of precocious male 1+ parr at the beginning of experiment A. Some of the lobules had spermatozoa present. All the other lobules contained spermatocytes or spermatogonia dividing into spermatocytes.

(H. & E. x 80)

Plate 28. Testis of precocious 1+ male parr (experiment A) treated with cyproterone acetate. Note all the lobules contain spermatozoa. Compare with Plate 6.

(H. & E. x 80)

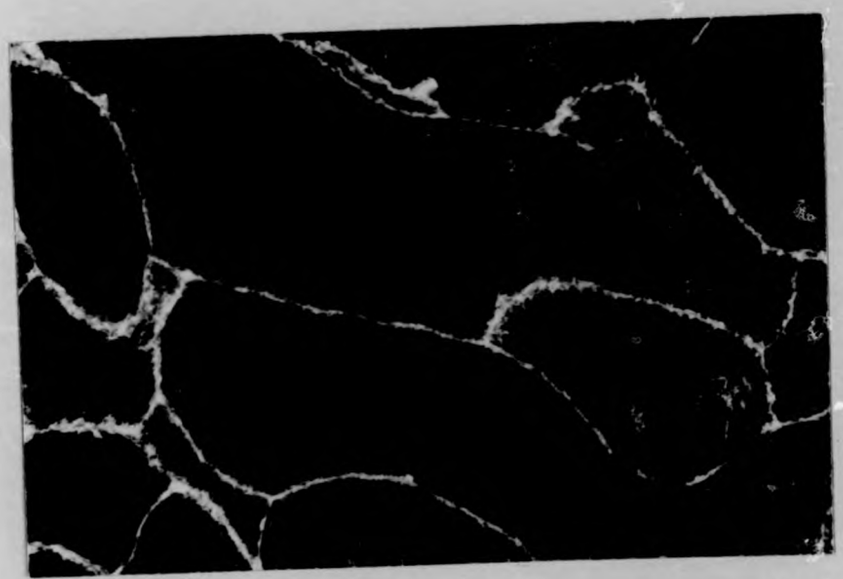
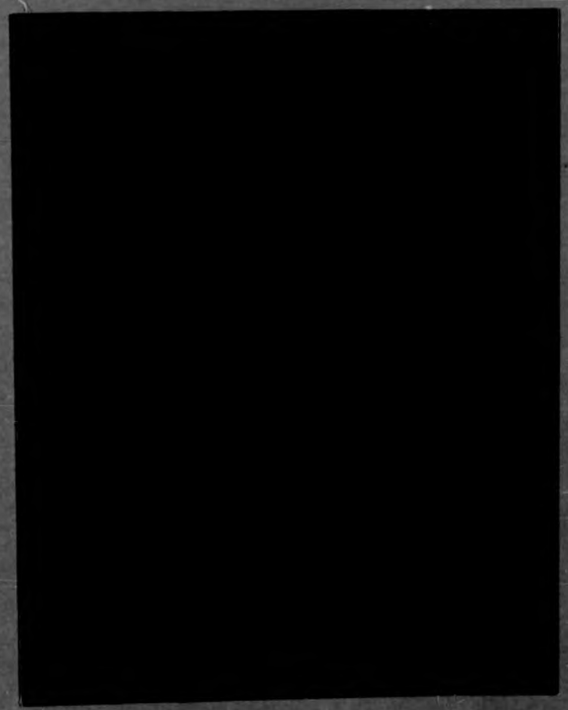


Plate 29. Testis of 1+ male parr treated with methallibure during the period March to August (experiment B). No development has occurred as all the cysts contain primary germ cells.

(H. & E. x 320)



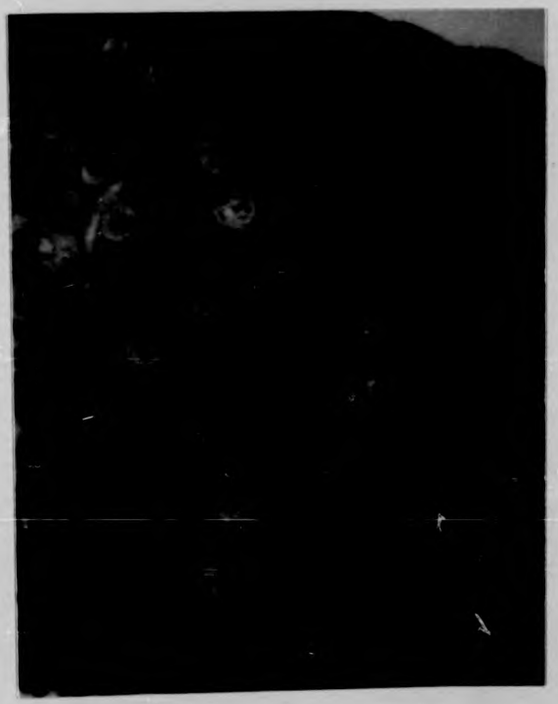
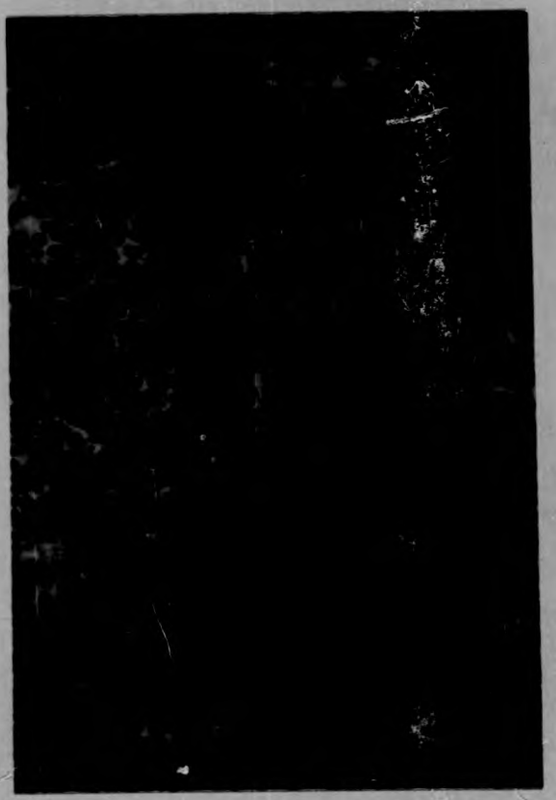


Plate 30. Testis from developing 1+ male parr treated with cyproterone acetate during the period March to August (experiment B).

(H. & E. x 320)

[redacted]



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