



National Library of Canada

Cataloguing Branch  
Canadian Theses Division

Ottawa, Canada  
K1A 0N4

Bibliothèque nationale du Canada

Direction du catalogage  
Division des thèses canadiennes

## NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter, ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

**THIS DISSERTATION  
HAS BEEN MICROFILMED  
EXACTLY AS RECEIVED**

## AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

**LA THÈSE A ÉTÉ  
MICROFILMÉE TELLE QUE  
NOUS L'AVONS REÇUE**



UNIVERSITÉ D'OTTAWA  
UNIVERSITY OF OTTAWA

BIOGRAPHICAL INFORMATION

Name: Martin Paul Komourdjian

Date and Place of Birth: Berlin, W. Germany, October 7, 1946.

Education: University of Ottawa, B.Sc.(Hons.) 1971.

Positions held:

Demonstratorships in Physiology, Comparative Physiology  
and General Endocrinology, 1969-1974.

Publications:

Komourdjian, M.P., R.L. Saunders and J.C. Fenwick.  
1976. Evidence for the role of growth hormone as  
a part of a "light-pituitary axis" in growth and  
smoltification of Atlantic salmon (Salmo salar).  
Can. J. Zool. In Press.

Komourdjian, M.P., R.L. Saunders and J.C. Fenwick.  
1976. The effect of porcine somatotropin on growth,  
and survival in seawater of Atlantic salmon (Salmo  
salar) parr. Can. J. Zool. In Press.

Awards:

National Research Council of Canada, Postgraduate  
Scholarship, 1972-1975.

ABSTRACT

The effect of photoperiod and the role of the pituitary in the growth and the parr-smolt transformation of Atlantic salmon, Salmo salar, were studied.

Salmon parr exposed to a reciprocal photoperiod in December were, by February, larger, had lower condition factors, lower muscle lipid, greater salinity tolerance and behaved more like smolts than another group exposed to a simulated natural photoperiod. However, in June the situation was reversed. Longer daylight periods in either regimen coincided with hypertrophy and hyperplasia of pituitary somatotrops. Adrenocorticotrops and prolactin cells were also judged to be active under long daylight conditions. Porcine growth hormone treatment mimicked the growth and salinity tolerance promoting effects of long daylight periods on parr, held under short daylight conditions, and elicited various xanthophore and melanophore responses.

Electrophoresis of fragments of salmon pituitary regions and specific staining of the bands resulted in a preliminary identification of prolactin and growth hormone. These identifications made on the basis of PAGE were confirmed by radioimmunoassay, and in the case of prolactin, by the sodium retaining activity bioassay in the hypophysectomized killifish, Fundulus heteroclitus.

In an alkaline PAGE support medium, the putative salmon

growth hormone had an Rf of 0.39. Prolactin had an Rf of 0.47 and consisted of a fast and a slow moving component.

Quantitation of pituitary prolactin and growth hormone by polyacrylamide gel electrophoresis (PAGE) and densitometry indicated a depletion in these fractions during the rapid growth characteristic under long daylight periods. Conversely, the periods of slow growth under short daylight periods were associated with an increase in these fractions. Constant daylight accelerated growth, precipitated the onset of parr-smolt transformation and produced a generalized but temporary pituitary activation. Photoperiod manipulation also affected ionic regulation. The concentration of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Cl}^-$  could not be related to either the onset or the subsequent events of the parr-smolt transformation. However, a possible link between ion cycles and growth patterns was indicated.

These data suggest that photoperiod plays a role as an environmental cue in the endocrine regulation of growth and parr-smolt transformation of Atlantic salmon parr.

RESUME

L'effet de la photopériode et le rôle de la glande pituitaire dans la croissance et la transformation de parr en smolt furent étudiés chez le saumon de l'Atlantique, Salmo salar.

Les saumons parr exposés à une photopériode réciproque en décembre étaient, deux mois plus tard, plus longs, possédaient des coefficients de condition et un taux de lipides musculaires plus faibles, exhibaient une résistance accrue à la salinité et ressemblaient plus à des smolts qu'un second groupe exposé à une photopériode naturelle artificielle. Cependant, en juin, la situation était renversée. Des périodes de lumière du jour plus longues coïncidèrent dans les deux groupes avec une hypertrophie et une hyperplasie des cellules somatotropes. Les cellules adrénocorticotropes et les cellules lactotropes furent jugées actives sous ces conditions photopériodiques. Le traitement à l'hormone somatotrope porcine produisit des effets similaires à la lumière du jour prolongée sur des saumons parr soumis à des périodes de lumière du jour courtes, ainsi que divers effets xanthophoriques et mélanophoriques.

L'électrophorèse verticale (disc electrophoresis) de fragments des régions hypophysaires du saumon, ainsi que la coloration spécifique des disques permirent l'identification préliminaire de la prolactine et de l'hormone somatotrope. Ces identifications basées sur l'électrophorèse furent ensuite

confirmées par des essais radioimmunologiques et, dans le cas de la prolactine, par un essai biologique basé sur la rétention du sodium chez le fondule, Fundulus heteroclitus, hypophysectomisé maintenu en eau douce.

Dans un milieu électrophorétique alcalin, l'hormone somatotrope putative du saumon avait un Rf de 0.39. La prolactine avait un Rf de 0.47 et consistait de deux composants, l'un rapide et l'autre plus lent.

La détermination de la prolactine et de l'hormone somatotrope par l'électrophorèse verticale révéla un épuisement de ces fractions pendant la croissance rapide caractérisant les périodes de lumière du jour prolongées. Réciproquement, les périodes de croissance lente furent associées avec une augmentation de ces fractions.

Le régime de lumière continue stimula la croissance, précipita la smoltification et produisit une activation hypophysaire généralisée mais temporaire. La manipulation photopériodique affecta également la régulation ionique. Aucun rapport ne put être établi entre la concentration des éléments Na, K, Ca et Cl et soit le début de la smoltification, soit les événements postérieurs à cette transformation. Cependant, un lien paraît possible entre les cycles ioniques et les rythmes de croissance.

Les données indiquent que la photopériode joue un rôle dans la régulation endocrinienne de la croissance et de la smoltification du parr du saumon de l'Atlantique.

TABLE OF CONTENTS

	<u>Page</u>
BIOGRAPHICAL INFORMATION .....	i
ABSTRACT .....	ii
RESUME .....	iv
TABLE OF CONTENTS .....	vi
LIST OF TABLES .....	ix
LIST OF FIGURES .....	xi
ACKNOWLEDGEMENTS .....	xv
PREFACE .....	1
GENERAL INTRODUCTION .....	3
SECTION I - Some Effects of Photoperiod on Growth and Smoltification of Atlantic Salmon, <u>Salmo salar</u> .	
INTRODUCTION .....	6
MATERIALS AND METHODS .....	12
Animals and Initial Holding Conditions .....	12
Experimental Regimen .....	13
Histological Procedures .....	13
RESULTS .....	16
Growth and Condition Factor .....	16
Morphological Changes .....	16
Lipid Content .....	22
Adenohypophyseal Histology .....	22
Salinity Tolerance .....	24
DISCUSSION .....	29

SECTION II - Some Effects of Porcine Growth Hormone  
in Atlantic Salmon Parr in Relation to  
Growth and Salinity Tolerance.

INTRODUCTION .....	34
MATERIALS AND METHODS .....	36
RESULTS .....	39
Effects of Porcine Growth Hormone on Growth and Morphology .....	39
Effects of Porcine Growth Hormone on Salinity Tolerance .....	39
DISCUSSION .....	44

SECTION III<sup>3</sup> - Electrophoresis and Identification of  
Growth Hormone and Prolactin.

INTRODUCTION .....	48
MATERIALS AND METHODS .....	51
Polyacrylamide Disc Gel Electrophoresis (PAGE) .....	52
Isolation of Putative Prolactin (PRL) and Growth Hormone .....	54
Bioassay of Atlantic Salmon Prolactin .....	58
Radioimmunoassay (RIA) of Salmon PRL and GH on PAGE .....	60
Vasopressor Activity of PAGE Extracts .....	61
Photoperiod Effects on Pituitary Electropho- retograms .....	62
RESULTS .....	64
Extraction of Putative PRL .....	66
Prolactin Bioassay .....	67

	<u>Page</u>
Radioimmunoassay of PAGE Eluates .....	69
Vasopressor Activity of Gel Eluates .....	69
Effect of Constant Daylight on Pituitary Electro- phoretograms .....	70
Effect of a 4h Increase or Decrease in Photoperiod on Pituitary Electrophoretograms .....	70
DISCUSSION .....	72
 SECTION IV - Some Effects of Photoperiod on Plasma Ionic Levels and Growth of Atlantic Salmon.	
INTRODUCTION .....	81
MATERIALS AND METHODS .....	84
Histological Procedures .....	85
Plasma Ion Analysis .....	85
Disc Electrophoretic Quantitation .....	85
RESULTS .....	87
Effect of Photoperiod on Growth and Condition Factor .....	87
Effect of Different Photoperiods on Disc-Gel Electrophoresable PRL and GH .....	98
Effect of Photoperiod on Blood Electrolytes .....	98
DISCUSSION .....	103
GENERAL DISCUSSION .....	111
SUMMARY .....	122
SYNOPSIS .....	124
APPENDIX .....	126
LITERATURE CITED .....	129

LIST OF TABLES

Page

SECTION I

- Table 1. Effect of photoperiod on lipid and moisture content in epaxial musculature of Atlantic salmon at different times of the year ..... 23
- Table 2. Effect of photoperiod on tolerance of Atlantic salmon to 40 ‰ seawater at different times of the year ..... 27

SECTION II

- Table 1. Effect of porcine growth hormone injected on alternate days for four weeks on the instantaneous growth rates in length and weight of Atlantic salmon parr ..... 42

SECTION III

- Table 1. Effect of PAGE fractions of Salmo salar pituitary rostral pars distalis on the plasma sodium concentration in hypophysectomized seawater adapted Fundulus heteroclitus following transfer to fresh water ..... 68
- Table 2. Effect of increasing or decreasing the daily light exposure by 4h for 18 days on electrophoresable pituitary GH and PRL levels in Atlantic salmon acclimated to 12h daily light periods ..... 71

APPENDIX

Table 1. Relative mobilities (Rf's) of disc gel electrophoretic bands from pituitary extracts, serum, and brain tissue of Atlantic salmon .....	126
Table 2. Disc-gel electrophoretic mobilities (Rf's) of fish growth hormones and prolactins at an alkaline pH .....	127.
Table 3. Effects of photoperiod on disc-gel electrophoresable pituitary prolactin (PRL) and growth hormone (GH) in Atlantic salmon .....	128

LIST OF FIGURES

	<u>Page</u>
SECTION I	
Fig. 1. Instantaneous growth rates in length of Atlantic salmon under natural and reciprocal photoperiods .....	17
Fig. 2. Instantaneous growth rates in weight of Atlantic salmon under natural and reciprocal photoperiods .....	18
Fig. 3. Mean length of Atlantic salmon under natural and reciprocal photoperiod regimens at different times of the year .....	19
Fig. 4. Mean weight of Atlantic salmon under natural and reciprocal photoperiod regimens at different times of the year .....	20
Fig. 5. Mean condition factors of Atlantic salmon under natural and reciprocal photoperiod regimens at different times of the year ...	21
Fig. 6. Sagittal section through pituitary from Atlantic salmon after 6 weeks exposure to a natural photoperiod regimen .....	25
Fig. 7. Sagittal section through pituitary from Atlantic salmon after 6 weeks exposure to a reciprocal photoperiod regimen .....	25
Fig. 8. Mid-sagittal section through an Atlantic salmon smolt pituitary gland .....	26

	<u>Page</u>
SECTION II	
Fig. 1. Effect of porcine GH injection on length, weight and condition factors in the parr of Atlantic salmon .....	41
Fig. 2. Effect of porcine growth hormone therapy on pigmentation of Atlantic salmon parr ...	43
SECTION III	
Fig. 1. Components of disc gel fraction extraction apparatus .....	56
Fig. 2. Assembly steps of disc gel extraction apparatus .....	57
Fig. 3. Assembled disc gel extraction apparatus ...	57
Fig. 4. Polyacrylamide disc gel of homogenate of Atlantic salmon pituitary gland .....	65
SECTION IV	
Fig. 1. Instantaneous growth rate in length of Atlantic salmon under natural, reciprocal and constant daylight photoperiod regimens at different times of the year .....	88
Fig. 2. Instantaneous growth rates in weight of Atlantic salmon under natural, reciprocal and constant daylight photoperiod regimens at different times of the year .....	89
Fig. 3. Mean length of Atlantic salmon under natural, reciprocal and constant daylight photoperiod regimens at different times of the year .....	90

Fig. 4.	Mean weights of Atlantic salmon under natural, reciprocal and constant daylight photoperiod regimens at different times of the year .....	91
Fig. 5.	Mean condition factor of Atlantic salmon under natural, reciprocal and constant daylight photoperiod regimens at different times of the year .....	92
Fig. 6.	Proximal pars distalis from Atlantic salmon after 6 weeks exposure to continuous illumination .....	94
Fig. 7.	Proximal pars distalis from Atlantic salmon after 6 weeks exposure to a reciprocal photoperiod regimen .....	94
Fig. 8.	Rostral pars distalis from Atlantic salmon after 9 weeks exposure to continuous illumination .....	95
Fig. 9.	Follicular region of rostral pars distalis from Atlantic salmon after 4 months exposure to continuous illumination .....	95
Fig. 10.	Mid-sagittal section through pituitary of Atlantic salmon parr .....	97
Fig. 11.	Enlarged portion of proximal pars distalis from pituitary of Atlantic salmon parr .....	97
Fig. 12.	Plasma sodium concentrations of Atlantic salmon under natural, reciprocal and continuous daylight photoperiod regimens, at 10°C, at different times of the year .....	99

Fig. 13.	Plasma potassium concentrations of Atlantic salmon under natural, reciprocal and continuous daylight photoperiod regimens, at 10°C, at different times of the year .....	100
Fig. 14.	Plasma calcium concentrations of Atlantic salmon under natural, reciprocal and continuous daylight photoperiod regimens, at 10°C, at different times of the year .....	101
Fig. 15.	Plasma chloride concentrations of Atlantic salmon under natural, reciprocal and continuous daylight photoperiod regimens, at 10°C, at different times of the year .....	102

## ACKNOWLEDGEMENTS

Gratitude is due to my supervisors Drs. James C. Fenwick and Richard L. Saunders for their assistance throughout this investigation.

The author would also like to express his thanks to Dr. Madeleine Olivereau and Mr. Edward Byard for their invaluable guidance in histological matters. I am also indebted to Dr. Brian McKeown for his assistance with radioimmunological analyses and to Dr. Arthur H. Houston, Dr. H.J. Koch, Dr. Thomas W. Moon and Dr. C.S. Nicoll for their stimulating suggestions during the investigation.

I would like to express my appreciation to Mr. Eugene B. Henderson for his enthusiastic technical assistance, to Mrs. Julie Fenwick for her editorial help and to Mrs. Nicole Voyer for typing the manuscript.

This study was made possible by the financial support offered by the National Research Council and the Fisheries Board of Canada in the form of grants to Dr. Fenwick and by a National Research Council of Canada Postgraduate Scholarship (1972-1975) to the author. Experimental facilities were generously supplied by the Directors of the St. Andrews Biological Station, Drs. John M. Anderson and Ralph O. Brinkhurst. The author also expresses his appreciation to Mr. Ralph Black, manager of the St. John Hatchery, and to Dr. B. Kinter of the Mt. Desert Island Biological Station for their supply of excellent specimens of Atlantic salmon and killifish, respectively.

Finally, I would like to thank my wife, Cathy, for her help and patience during the preparation of this manuscript.

PREFACE

In spite of the numerous publications on the effect of photoperiod on reproduction, and its endocrine control in fishes, only a few studies have related photoperiod manipulation to growth. And fewer still have concerned themselves with the effects of photoperiod on the preadaptation of seawater which occurs during the transformation from parr to smolt stages of some salmonids. Nonetheless, a mediation of these effects by the pituitary has been surmised. But, in spite of extensive studies on salmonid pituitary cytology and histophysiology, no direct evidence for this relation has been found. This gap is a direct result of the paucity of experimentation concerning the effects of photoperiod manipulation on endocrine function during growth and smoltification.

The Atlantic salmon is an important commodity in Canada and elsewhere and much effort and money has been invested in the aquaculture of this animal. But little of the available information on photoperiod control of growth concerns the Atlantic salmon. Furthermore, relatively few reports on the effects of exogenous pituitary hormones on this species have been published. Also, no decisive reports exist on the identification, purification and quantitation of any of the salmonid pituitary hormones.

The aim of this study, then, has been to investigate the possible effects of different photoperiod regimens on

growth and parr-smolt transformation of Atlantic salmon and to explore the possible role of the pituitary hormones, especially growth hormone and prolactin, in this process. Attention was concentrated on these hormones as they have been the object of major speculation in earlier studies.

## GENERAL INTRODUCTION

Although effects of photoperiod on parameters such as sexual maturation and colour changes have long been known, the study of its effects on the growth of teleosts has developed only in the last few decades.

Temperature has also been related to growth rates (Brown, 1957). But attempts to correlate annual temperature cycles and growth cycles of teleosts (Deason and Hile, 1947; Ball and Jones, 1960; Gerking, 1966) failed to establish temperature as a primary factor in the control of seasonal growth cycles. The length of growing season has been related to latitude (Beckman, 1943), but this relation involves a variety of environmental factors. Other experiments have suggested that seasonal growth cycles are more closely related to photoperiod than to temperature (Ball and Jones, 1960). The use of simulated photoperiods showed that growth of several species of teleosts was enhanced by long daylight regimens (Eisler, 1957; Gross et al., 1965; Saunders and Henderson, 1970; Bilton and Robins, 1971). However, some conflicting results have been reported in various species. Total darkness was reported to stimulate growth, presumably as a result of decreased activity (Bjorklund, 1958; Anderson, 1959), and increased light was found to reduce growth (Brown, 1946).

Considerable interest has focused on the effect of photoperiod on salmonids since light was shown to influence sali-

nity tolerance development (Wagner, 1974), salinity preference (Baggerman, 1960) and growth (Saunders and Henderson, 1970). Salinity tolerance is indicative of the preadaptation to seawater occurring during the transformation from the parr to smolt stage of the Atlantic salmon, which occurs one or more years after hatching. Salmon hatched in fresh water (alevin stage) lose their yolk sac (fry stage) and remain in fresh water as parr. Animals in this stage are characterized by dark vertical pigmentary bars, the parr marks, on their integument until they are prepared to migrate to the sea as smolts. This transformation of parr into smolts has been linked to growth (Parry, 1958).

The function of the pituitary of mammals (Belkin, 1972; Wurtman, 1975) and some teleosts (Rasquin and Rosenbloom, 1954) has been shown to be influenced by light. In the case of the fish this is supported by the observation that some show seasonal endocrine related rhythms reflected in osmoregulation (Lam and Hoar, 1967; Lam and Leatherland, 1969; Oide, 1971) and reproduction (Atz, 1957; Donaldson, 1973). A role for the pituitary in mediating light related effects in smoltification and growth (Ball, 1961, 1969; Hoar, 1963, 1965; Saunders and Henderson, 1970) has also been suggested. It seems possible that the complex of morphological changes associated with smoltification might be mediated by alterations in the production and release of pituitary hormones. But concrete evidence for such a mediation has not been presented.

In pursuance of this supposition the following hypotheses were tested in the ensuing studies:

- 1) Photoperiod is capable of altering the timing of the onset of smoltification as indicated by increased salinity tolerance, sharply lower condition factors, and morphological changes.
- 2) The rapid growth phase associated with smoltification occurs in response to long daylight periods and can be mimicked by synthetic growth hormone.
- 3) Continuous daylight exaggerates the effects of long (simulated natural) daylight periods, but is unable to override existent biological rhythms of the animal.
- 4) That the induction of increased production increased release or both of growth hormone and prolactin would be reflected in the histological appearance of the pituitary and in the quantitative amount of these hormones as estimated by disc-gel electrophoresis of the pituitary peptides.

The biology of the Atlantic salmon and its parr-smolt transformation have been reviewed (Fontaine, 1965; Hoar, 1965). An extensive review of the pituitary cytology of teleosts including salmonids is also available (Ball, 1969b).

## INTRODUCTION

Growth is known to vary seasonally in fishes (Brown, 1946; Swift, 1955, 1961; Hoar, 1965; Gerking, 1966) and has been shown to be sensitive to photoperiod manipulation. Longer daylight periods resulted in faster growth of green sunfish, Lepomis cyanellus, (Gross et al., 1965) and Atlantic salmon, Salmo salar (Saunders and Henderson, 1970). Long daylight periods have also been shown necessary to induce salinity preference (Baggerman, 1960) and salinity tolerance (Wagner, 1974a) in several diadromous salmonids. Moreover, both growth and salinity tolerance increase at the time of parr-smolt transformation of Atlantic salmon (Fontaine, 1960; Koch, 1968) which occurs under lengthening daylight periods. The transformation is indicated by a sharp decline in the condition factor and the development of hypo-osmoregulatory mechanisms (Hoar, 1939; Fontaine, 1965; Pinder and Eales, 1969; Wagner, 1974a).

Light effects on pituitary function have been demonstrated in the rat (Relkin, 1972a, b) and in the Mexican cavefish, Astyanax mexicanus (Rasquin and Rosenbloom, 1954). Astyanax held in darkness showed "cytoplasmic deposition" in growth hormone cells and this coincided with slower growth of the fish. Furthermore, the role of the pituitary as mediator of photoperiod control of reproduction is well established (Wurtman, 1975). In light of these findings, the pituitary has been surmised to mediate the light-related effects in the parr-smolt transformation

(Hoar, 1958, 1963, 1965; Ball, 1961, 1969; Saunders and Henderson, 1970). However, such a mediation has not yet been demonstrated.

The Atlantic salmon pituitary gland exhibits extensive gross morphological changes between the parr and adult stages (Woodman, 1939). The major of these changes is a shift from a disc-like structure (Fig. 6), flattened against the brain, through a knob-like structure, to the dorsoventrally protruding tongue-shaped gland of the adult (Fig. 8). Changes in cytology such as an increase in the follicular (prolactin) cell area (Woodman, 1939), and a shift of the pars intermedia from a caudal to a ventral position in the post-smolt (Olivereau, 1954) also have been suggested to be age related. Further, Fontaine and Olivereau (1949) noted a decrease in pituitary basophilia during the parr-smolt transformation and this was thought to reflect the absence or decrease in sexual maturity which occurs at this time. \*

The cytology of the Atlantic salmon pituitary was first described by Woodman (1939). Most cell types found in other teleost pituitaries have now been described in the Atlantic salmon (Ball, 1969). The identification of these types has been facilitated by similarities in pituitary morphology and in the location of the various pituitary cell types in eels and salmonids. But the positive identification of some cell types has yet to be made.

The pituitary of salmonids, like that of other teleosts,

SECTION I

SOME EFFECTS OF PHOTOPERIOD ON GROWTH AND SMOLTIFICATION  
OF ATLANTIC SALMON, SALMO SALAR

consists of a neurohypophysis and an adenohypophysis. The adenohypophysis is divisible into three topographically distinguishable areas: rostral pars distalis (RPD), proximal pars distalis (PPD) and the pars intermedia (Fig. 8). Tinctorially, the adenohypophyseal chromophiles are generally divided along the classical distinction of acidophiles, or orangeophiles (prolactin cells and somatotrops) and basophiles or periodic acid-Schiff +ve cells (thyrotrops, gonadotrops and at least one type of pars intermedia cells).

Prolactin secreting cells form the follicles characteristic of primitive teleosts (Olivereau, 1954). As in other species, the granules are concentrated in the basal part of the cell and are acidophilic and erythrosinophilic (Ball and Baker, 1969). Their identity was recently confirmed by immunofluorescence studies (Aler, 1971). Prolactin cells are fusiform in transverse or longitudinal section with their nuclei near the base. The nucleus becomes basal in active prolactin cells with a well developed endoplasmic reticulum while the opposite transformation and an increased size of the follicular cavity are interpreted as signs of decreased activity (Olivereau, 1969). The functions of teleost prolactin are varied (Ball, 1969a) but its principal role appears to be in osmoregulation (Olivereau, 1969). Olivereau (1969) found that Atlantic salmon prolactin cells were more active in fresh water, degranulated slightly preceding seaward migration and exhibited signs of much

reduced activity after a couple of weeks in seawater. Further, transfer of salmon from fresh to seawater results in a marked decrease in activity of these cells (Olivereau, 1969). It has been suggested that the hormone acts to limit the sodium outflux, probably at the gill level, thus altering the sodium turnover rate (Ball, 1969a). Effects on other target tissues such as the kidney, or on mucous cells which have been demonstrated in several other species (Ball, 1969) have yet to be found in salmonids. No evidence exists as yet for a reproductive function of this hormone in salmon. Neither has prolactin been found to promote growth in teleosts. Woodman (1939), however, noted that the follicular region of the Atlantic salmon increases in size with age but found no evidence of seasonal change in this area of the pituitary.

The thyrotrops are found interspersed among the prolactin cell follicles and are the basophiles of the rostral pars distalis of the Atlantic salmon (Ball and Baker, 1969). Histologically they are distinguishable by their relatively large size and angular shapes. Radiothyroidectomy of the European eel, Anguilla anguilla, and steelhead trout, Salmo gairdneri, (Olivereau, 1962; Olivereau et al., 1964) leads to a strong hypertrophy of this cell type. Addition of thyroxine to the medium of cultured S. gairdneri pituitary cells leads to the specific repression of the thyrotrops. In Atlantic salmon, an increased thyrotrop activation occurs prior to migration (Olivereau, 1968).

The third type of rostral pars distalis cells, the corticotrops, are typical chromophobes arranged in parallel cords or sheets of cells with their long axes perpendicular to the neurohypophyseal border. The interrenal metabolic inhibitor SU 4885, which activates this type of cell, was used to identify it as the adrenocorticotropin secreting type in A. anguilla (Olivereau and Ball, 1963).

The proximal pars distalis consists of somatotrops and gonadotrops. Gonadotrops appear inactive in fast growing parr about to smoltify (Fontaine and Olivereau, 1949).

The somatotrops are the characteristic, orange G +ve, acidophiles of the proximal pars distalis. They are generally elliptical or oval with a similarly shaped nucleus at one end of the cell. Their identity was postulated by the process of elimination after all the other main types of cells had been recognized (Ball and Baker, 1969). Recent immunofluorescence studies on the salmonid O. nerka (McKeown and Van Overbeeke, 1971) have shown that fluorescent anti-ovine growth hormone binds specifically to this type of cell. A somatotropic action still remains to be demonstrated in Atlantic salmon for growth hormone from any source.

At least two types of cells have been postulated in the pars intermedia of most teleosts. They are tinctorially distinguishable with one showing affinity for lead hematoxylin (PbH) and the other for PAS. The PbH+ve type is thought to be responsible for melanophore stimulating hormone

(MSH) whereas the PAS +ve type has been linked to osmoregulation (Schreibman et al., 1973). However, in eels it is the PAS +ve type that is activated when the fish are kept against a black background for several weeks (Knowles and Vollrath, 1966). Baker (1963) did not find evidence of PAS +ve cells in the pars intermedia of salmonid pituitaries. All cells appeared alike and stained with PbH. Follenius (1963, 1965) suggested that two tinctorially distinct cell types were present in salmon but that these represent different stages in the functional cycle of a single cell type.

The implication of a photoperiod axis in the process of smoltification of Atlantic salmon requires the demonstration that photoperiod affects the process and that changes in pituitary cytology occur that can be related to the transformation. Therefore, the present study was designed to test the effect of different photoperiod regimens on the parr-smolt transformation of Atlantic salmon held at constant temperature. In addition, the pituitary cytology was studied in relation to the metamorphosis. Further, groups of fish were examined for salinity tolerance and body moisture and lipid content at different times of the year.

## MATERIALS AND METHODS

### Animals and Initial Holding Conditions

Salmon parr (River Phillip stock), hatched in the spring of 1970, were obtained from the Cobequid Fish Culture Station, Nova Scotia and were brought to the Biological Station, St. Andrews, New Brunswick, on November 29, 1971. They were held in round fiberglass tanks, measuring 1.8m in diameter and filled to a depth of 0.6m. Each tank was equipped with a submersible pump which provided a water current of approximately 14cm/sec at the periphery. This current dragged waste materials to a center drain. Cleaning was supplemented by scrubbing tank walls and bottoms at regular intervals. Fresh dechlorinated water was supplied from header tanks by gravity feed at the rate of approximately 900 l/hr. Oxygen supersaturation was prevented by splashing of water in the header tanks. The temperature was maintained by mixing heated and chilled water in the header tanks and, when necessary, by adding extra cooling units to the header tank system. The tanks, enclosed in a lightproof compartment, were illuminated by two fluorescent "daylight type" tubes (92 watts each) which were centered 94 cm over the water surface. The lighting units were activated through automatic on - off timers. The timers were adjusted by 15 min every three days to simulate changes in the respective photoperiod regimen.

### Experimental Regimen

Initially 200 fish were held under a simulated natural photoperiod for 15 days. During this period the animals were acclimated to  $10^{\circ}\text{C}$  by gradually increasing the temperature from  $6^{\circ}\text{C}$ .

Following acclimation the fish were tagged and fork lengths to the nearest millimeter and weights to the nearest gram were recorded. One group of 100 fish was exposed to a simulated natural photoperiod (NP) and another group of 100 was placed under a reciprocal photoperiod (RP). As December 16 corresponded to the shortest daylight period in the NP regimen, the RP group was exposed to its longest daylight period (16h) at this time. Both groups were held at  $10 \pm 2^{\circ}\text{C}$  and were fed dry pellet food (Ewos F-159, 4P and 5P salmon grower, Ewos, A.B.) to satiety three times daily. Both groups were fed during the light period at the same time each day. Total food consumed was not recorded.

At three week intervals all the fish were rapidly anesthetized (1 : 80 tert-amyl alcohol), weighed and measured. At this time seven fish from each group were selected at random. The pituitaries of these fish were removed, fixed in Bouin's picric-formol-acetic fixative and embedded in Tissuemat (Fisher) for sectioning. Carcasses were promptly frozen and stored below  $-20^{\circ}\text{C}$  for lipid analysis.

Mid-sagittal and 1/3 depth (from left) 4-5  $\mu\text{m}$  sections of the pituitaries were stained by the Cleveland-Wolfe

method or with periodic acid-Schiff (PAS)-hematoxylin (Groat's)-orange G (2% orange G in 2% aq. acetic acid). A minimum of six sections per pituitary were examined by these methods. Serial sections were stained with methyl green-pyronin (Kurnick, 1955) for demonstration of RNA. Chloroform extracted pyronin was included to prevent the nonspecific pyroninophilia exhibited by DNA after Bouin fixation. Control sections for RNA were treated with 10% perchloric acid at 4°C for 12 hours (Pearse, 1961). The state of activity of cells was judged by the relative extent of the microscopic field occupied by particular cell types, by nuclear size and by their degree of pyroninophilia.

A piece of epaxial muscle of about 1 cm<sup>3</sup> was excised from alongside the dorsal fin for total lipid determination. These samples were extracted three times by the chloroform-methanol-water method of Bligh and Dyer (1959) and the filter paper used was extracted once. A contralateral piece of epaxial muscle of similar size was removed and dried to constant weight at 105°C for moisture determination.

Instantaneous (or specific) growth rates were calculated according to the formula  $100 \left[ \frac{\log_e Y_T - \log_e Y_t}{T - t} \right]$  where  $Y_T$  and  $Y_t$  are mean lengths or mean weights at time  $T$  and  $t$ .  $T$  being later than  $t$  (Brown, 1957). In computing the value for the interval preceding each sampling date, the fish

sampled at the beginning of that interval were not considered in the mean  $Y_T$ . However, they were considered in calculating  $Y_T$  for the preceding interval. The condition factor was determined according to the formula  $K = 100 W/L^3$  (Hoar, 1939; Brown, 1957). Significant differences between the two experimental means for either the length or weight data were computed by the Student's 't' test.

Fish from a replicate experiment started in December 1972 were tested for salinity tolerance in February, April and June of 1973 (Table 2 of results). For this, five of the fresh water acclimated fish from each photoperiod group were placed in seawater (SW) concentrated to 40 ‰ salinity with artificial sea salts (Instant Ocean, Aquarium Systems Inc.) in recirculating tanks. Water from these tanks was pumped through filters (Aqua-Pure) to remove waste material. The water was then circulated through the tungsten heat exchange coils of a cooling system and finally splashed back into the tanks. The temperature was kept at  $10.5 \pm 0.5^\circ\text{C}$ . The tests were conducted under continuous daylight to facilitate observations. The state of the animals was recorded at 1-2 h intervals from 8 a.m. to 2 a.m. and at 2-3 h intervals thereafter. Time to 50% mortality was determined from probit plots (Finney, 1952).

## RESULTS

### Growth and Condition Factor

Fish exposed to longer daylight periods in December (RP) showed higher growth rates (Fig. 1, 2) than those under the simulated natural photoperiod (NP) and had become significantly longer ( $P < 0.01$ ) after five weeks (Fig. 3, 4). Concomitantly, the condition factor of the RP fish decreased rapidly. In contrast, however, the condition factor of the fish under the simulated NP continued to increase (Fig. 5).


In early March the growth rate of NP fish increased markedly and by late June their length approximated that of the RP fish (Fig. 3). The condition factor of NP fish started to decline less than a month after the other group but this drop was more gradual (Fig. 5).

In general, there was a positive relationship between the length of the daylight periods and instantaneous growth rate in length of either group (Fig. 1).

### Morphological Changes

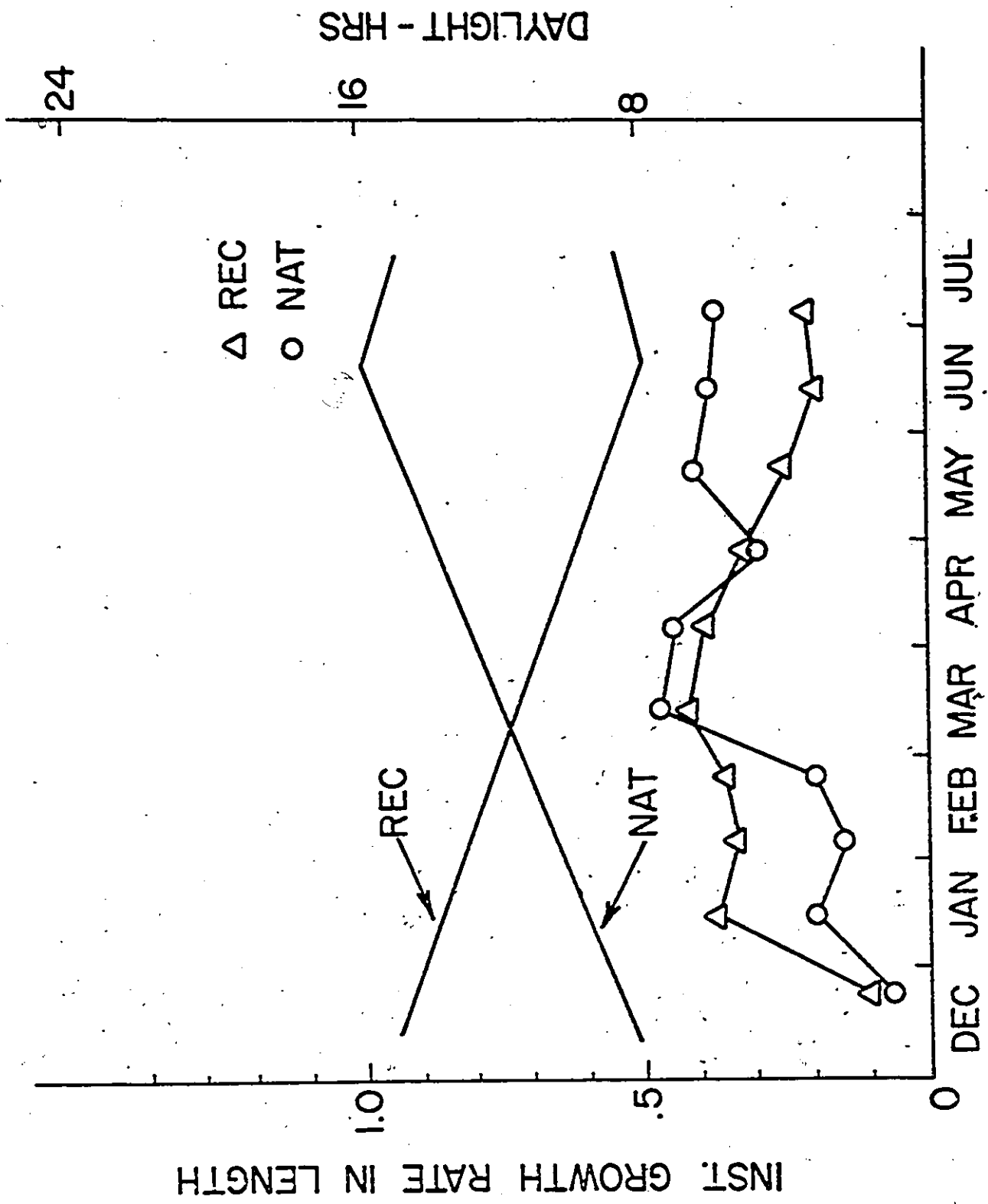
Several morphological changes were clearly related to photoperiod regimens. Fish silvered and parr markings disappeared coincident with a decrease in condition factor in either group. This occurred in February in the RP fish and almost three months later in the NP group. Together with this silvering, tail and pectoral fin margins darkened

Figure 1



Instantaneous growth rates in length of Atlantic salmon  
under natural and reciprocal photoperiod regimens.

(Nat-natural photoperiod; Rec-reciprocal photoperiod).



INST. GROWTH RATE IN LENGTH

DAYLIGHT - HRS

△ REC  
○ NAT

REC

NAT

DEC JAN FEB MAR APR MAY JUN JUL

Figure 2

Instantaneous growth rates in weight of Atlantic salmon  
under natural and reciprocal photoperiod regimens.

(Nat-natural photoperiod; Rec-reciprocal photoperiod).

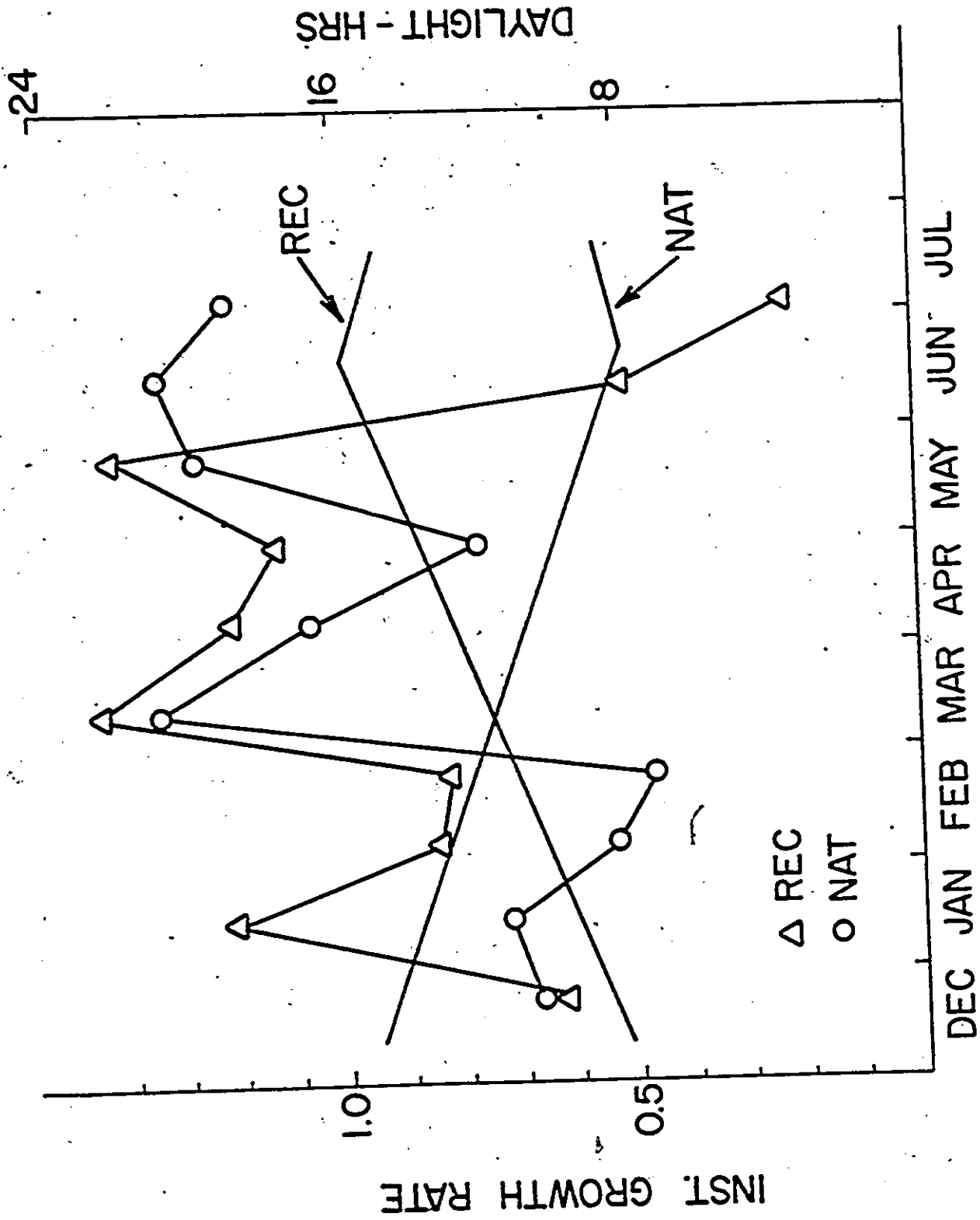
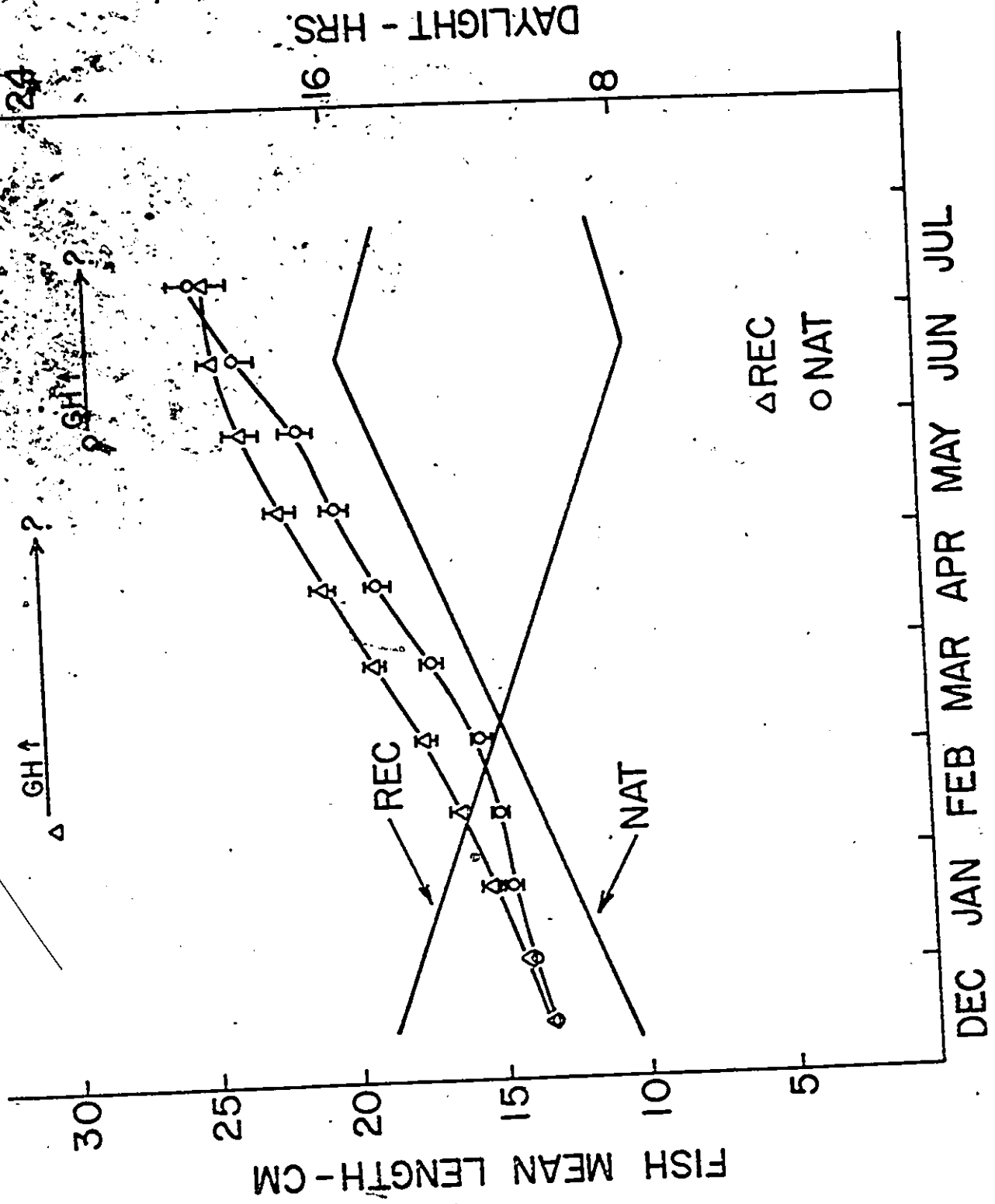


Figure 3

Mean length of Atlantic salmon under natural and reciprocal photoperiod regimens at different times of the year (99% confidence intervals indicated). Arrows indicate approximate duration of observed increase in somatotropic function (Nat-natural photoperiod; Rec-reciprocal; GH-growth hormone).



FISH MEAN LENGTH - CM

DAYLIGHT - HRS

Δ REC  
○ NAT

DEC JAN FEB MAR APR MAY JUN JUL

GH↑

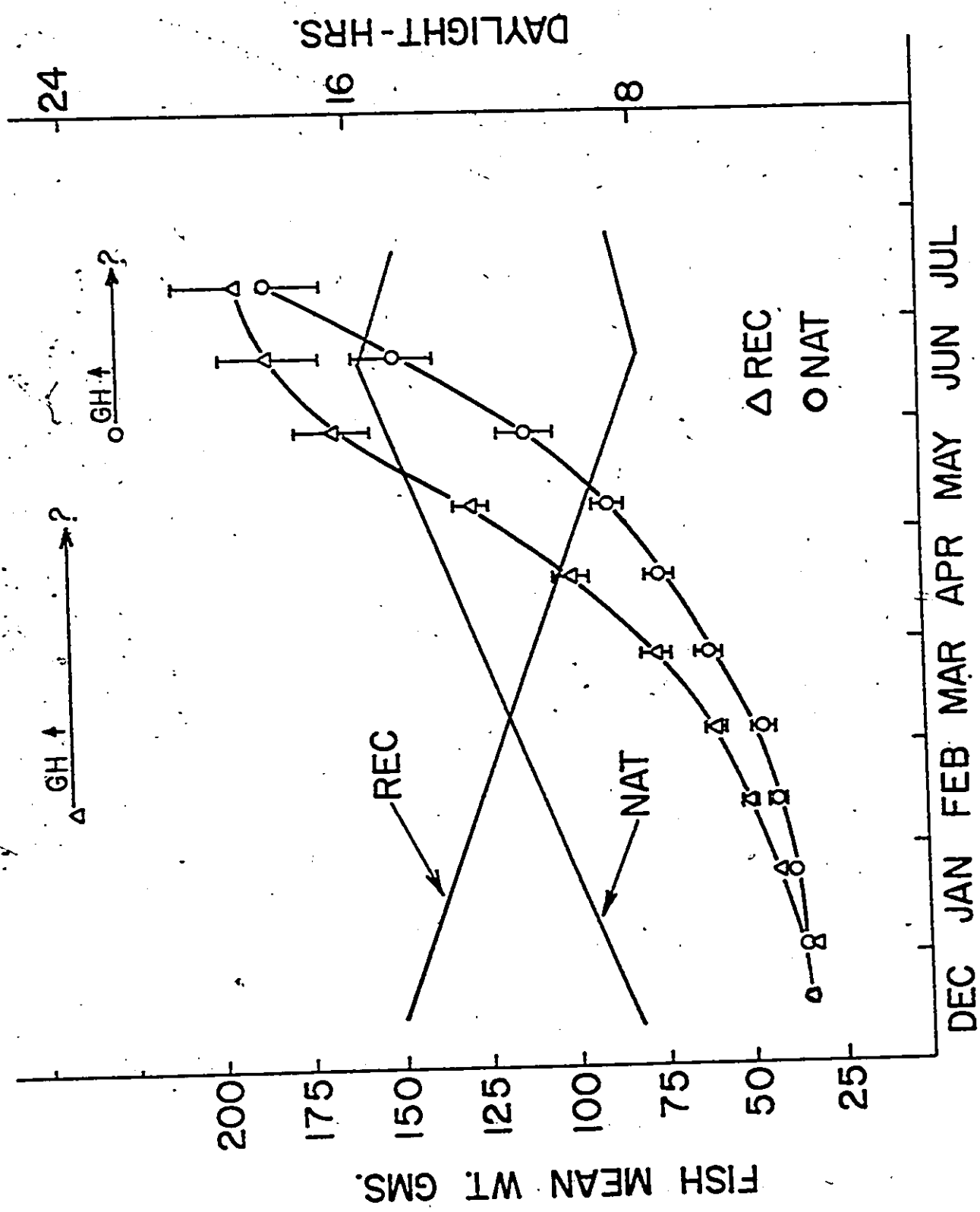
GH↑

REC

NAT

Figure 4

Mean weight of Atlantic salmon under natural and reciprocal photoperiod regimens at different times of the year. (99% confidence intervals indicated). Horizontal arrows indicate approximate duration of observed increase in somatotrop function. (Nat-natural photoperiod; Rec-reciprocal photoperiod; GH-growth hormone).



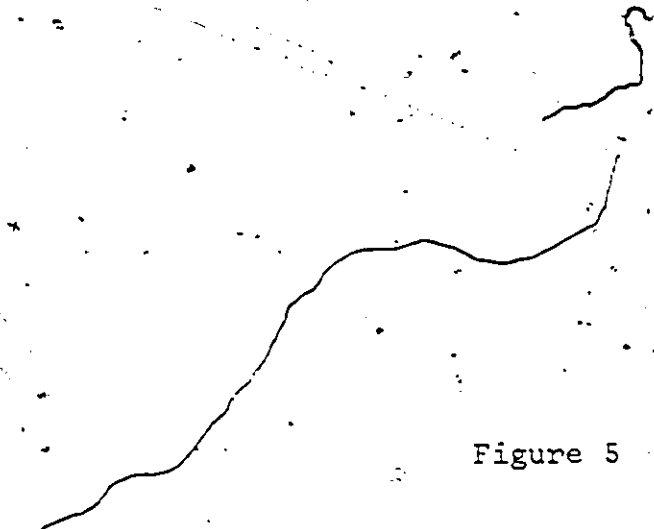
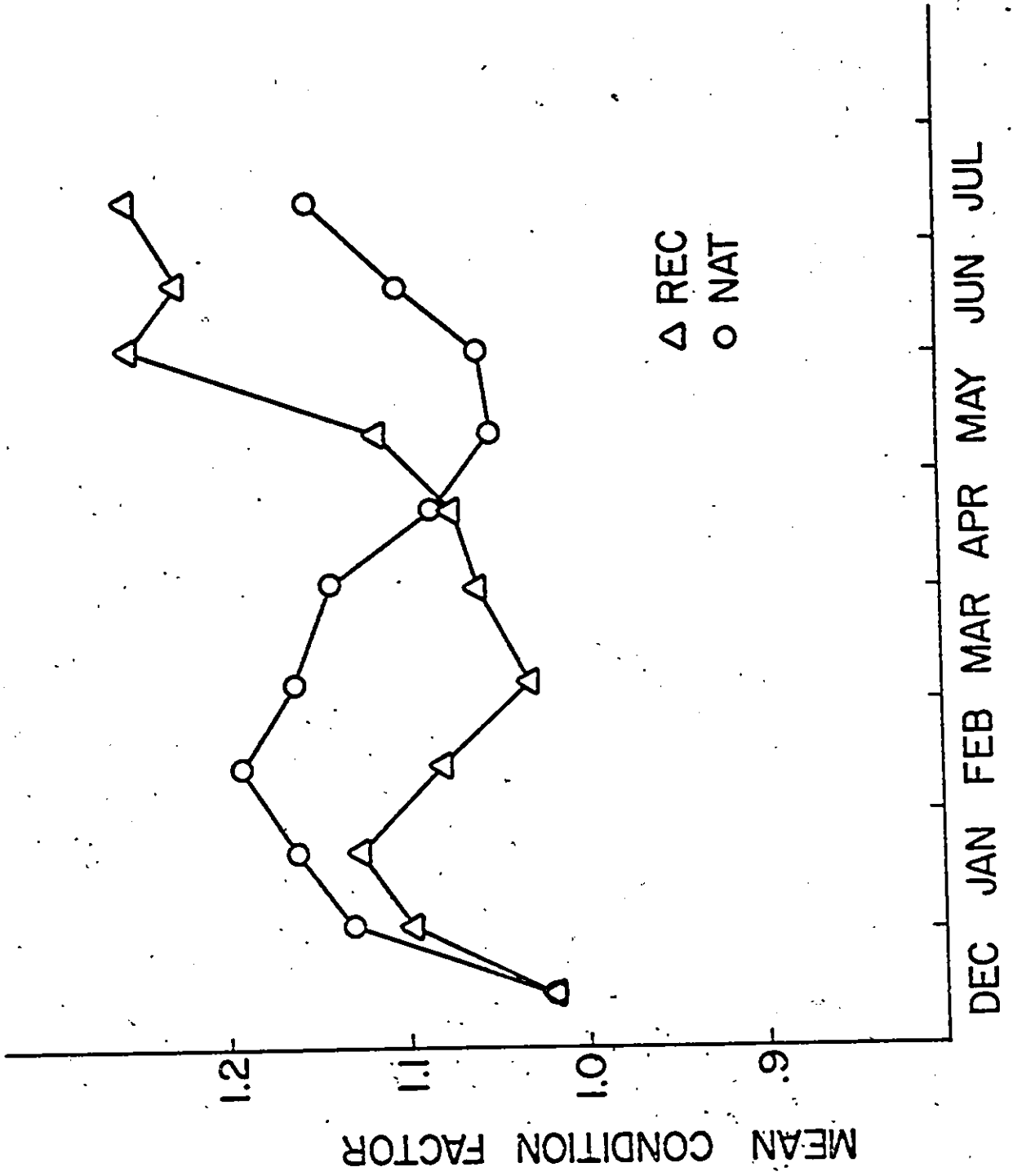


Figure 5

Mean condition factor of Atlantic salmon under natural and reciprocal photoperiod regimens.

$$\text{Condition factor} = \frac{100 \cdot W}{L^3}$$





markedly. This dark colour faded towards the month of June in RP fish. By this time, NP fish had silvered and showed darker tail and fin margins.

#### Lipid Content

Analyses of dorsal muscle samples showed that total lipid content was significantly lower ( $P < 0.05$ ) in the smoltifying fish than in those of the other group at the same date (Table 1). That is, low muscle lipid content coincided with low condition factors.

No significant differences were found between the body moisture content of smoltifying and non-smoltifying salmon at the same date (Table 1). Lower values were obtained for both body moisture and muscle lipid content of parr in December (Table 1) but these differences may have reflected the stress induced by transporting the fish to the laboratory.

#### Adenohypophyseal Histology

Periodic study of pituitaries from animals from either photoperiod regimen showed that higher growth rates were accompanied by an activation of the pituitary somatotrops. This activation was evidenced by hyperplasia, hypertrophy and an increased pyroninophilia of these cells. The increase in pyroninophilia appeared to precede any noticeable hyperplasia but otherwise these two indicators were in good agreement. The increase in GH cell number was esti-

TABLE 1

Effect of photoperiod on lipid and moisture content in epaxial musculature of Atlantic salmon at different times of the year.<sup>a</sup>

	Muscle Lipid (% dry wt.)		% Muscle Moisture	
	<u>Natural Photoperiod</u>	<u>Reciprocal Photoperiod</u>	<u>Natural Photoperiod</u>	<u>Reciprocal Photoperiod</u>
Dec. 15	3.85 ± .31		74.32 ± .30	
Feb. 17	6.74 ± .30 <sup>bd</sup>	4.88 ± .27	76.54 ± .53	78.36 ± .28
June 22	4.95 ± .53 <sup>bc</sup>	7.71 ± .67 <sup>cd</sup>	76.93 ± .21	75.64 ± .27

- a Values are means ± SE of means. N = 7
- b Significantly different from reciprocal photoperiods at same date P < .05
- c Significantly different from the Feb. 17 group under the same photoperiod regimen P < .05
- d Significantly different from samples on Dec. 15, P < .05

mated to be about 20-30% (Fig. 6, 7). The increase in nuclear size was of about 15 to 20% in volume. The first site where new cells arose was in the area immediately adjacent to the neurohypophysis. This was followed by sites caudal to the hypophyseal stalk previously occupied exclusively by cells of the pars intermedia (Fig. 8). Eventually, all available space in the proximal pars distalis was filled with GH cells and scattered immature chromophobes; i.e. immature gonadotrops. Coinciding with somatotrop activation, the adrenocorticotrops and the pars intermedia cells were also judged to be active. Morphological and behavioural smolt characteristics were also manifest at this time. Prolactin cells, however, showed no consistent state of activation other than a uniformity of appearance within individual follicles.

#### Salinity Tolerance

Exposure of salmon parr to the different photoperiod regimens altered the chronological development of salinity tolerance with an increase in salinity tolerance being associated with an increased daily light exposure (Table 2). RP fish tolerated concentrated seawater as early as mid-February and this corresponded to a period of long daily light exposure. However, when daily light exposure was short these same fish did not show a high degree of salinity tolerance. The NP fish failed to tolerate concentrated seawater in February but showed good tolerance later in the

Figure 6

Sagittal section through pituitary from Atlantic salmon after 6 weeks exposure to a natural photoperiod regimen starting in mid-December. NH, neurohypophysis; PI, pars intermedia; RPD, rostral pars distalis; PPD, proximal pars distalis. Dark cells in PPD are somatotrops. PAS-hematoxylin-OG. X320.

Figure 7

Sagittal section through pituitary from Atlantic salmon after 6 weeks exposure to a reciprocal photoperiod regimen starting in mid-December. NH, neurohypophysis; PI, pars intermedia; PPD, proximal pars distalis. Note increase in somatotrops (dark grey cells in PPD) as compared to Fig. 6 above. PAS-hematoxylin-OG. X320.

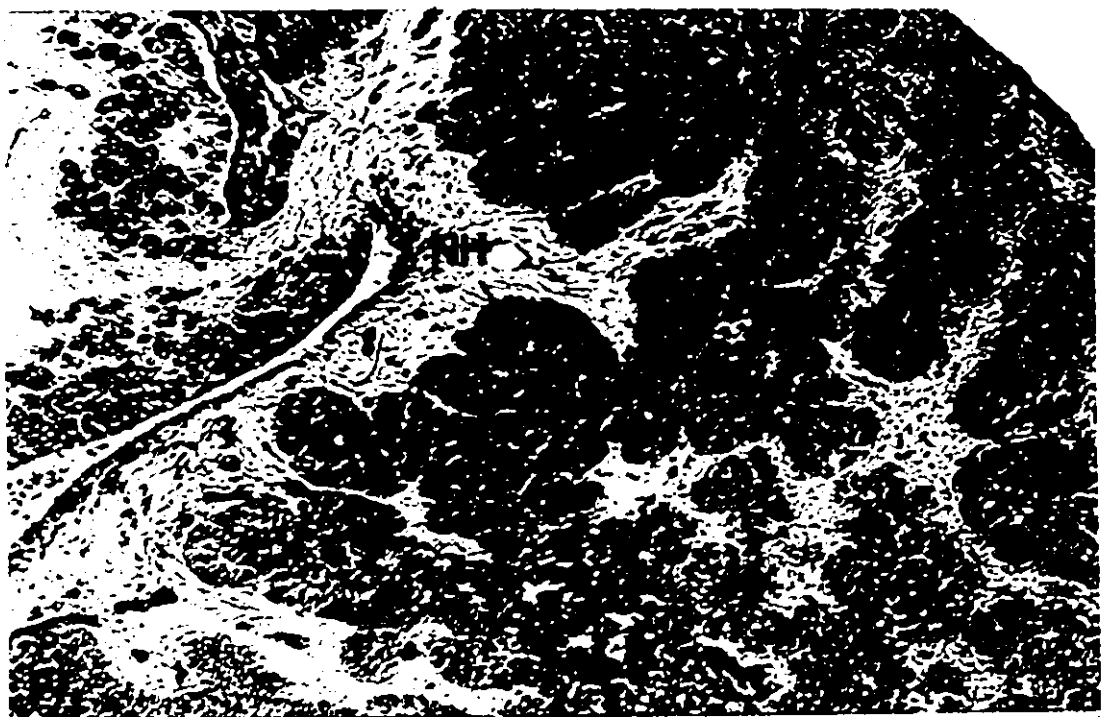


Figure 8

Mid-sagittal section through a characteristically tongue-shaped Atlantic salmon smolt pituitary gland attached to floor of brain. The main regions (RPD) rostral pars distalis, (PPD) proximal pars distalis, (PI) pars intermedia and (NH) neurohypophysis are indicated. (S) shows the region of the gland occupied by pars intermedia cells in the parr but replaced by somatotrops in fast growing smolts. PAS-hematoxylin-OG. X80.



C

TABLE 2

Effect of photoperiod on tolerance of Atlantic salmon to 40°/oo seawater at different times of the year.<sup>a</sup>

---

TIME TO 50°/oo MORTALITY.  
IN 40°/oo SW<sup>a</sup>.

---

<u>Date</u>	<u>Natural Photoperiod</u>	<u>Reciprocal Photoperiod</u>
	<u>Hours</u>	
Feb. 13	12.5	>100
April 2	>100	>100
June 7	>100	18.5

---

<sup>a</sup> Values calculated from probit plots; N = 5 in all cases.

year when daily light exposure was longer. Groups that showed failures in seawater in less than 100 hours attained 100% mortality before the end of the experiment. There was a considerable difference between the time from the first overt signs of stress, as shown by increased pigmentation or loss of righting reflex, to the time of death. But this time interval appeared shorter in those fish developing stress within 48 hours of the start of the experiment. When deaths occurred at night, the time of death could often only be approximated within the span of several hours that elapsed between observations.

## DISCUSSION

This study indicates that photoperiod influenced growth and, at least on the basis of the development of salinity tolerance and characteristic changes in the morphology and behaviour, altered the timing of the onset of smolting in Atlantic salmon. Further, these changes coincided with alterations in the histology of the anterior pituitary which suggested a direct involvement of this gland in the photoperiodic induction of growth and possibly some other aspects of smoltification.

Oliverreau (1954) observed that smolts exhibit a greater number of fuchsinophiles in the pituitary pars distalis than do parr. Further, these fuchsinophiles are tinctorially and topographically equatable with the orangeophilic cells of the proximal pars distalis which are believed to produce growth hormone (Oliverreau, personal communication). The present study suggests that this increase in growth hormone cells actually occurs during the early stages of the parr-smolt transformation and before the morphological traits of the smolt are well developed. Further, this activation of growth hormone cells appeared to be induced by long daylight periods. Moreover, it is possible that the slight decrease in the percentage of somatotrops in RP fish towards the end of the summer, when daylight exposure under this regimen became shorter, is indicative of a seasonal photoperiod rhythm. These results corroborate the

earlier work by Swift and Pickford (1962) who found that the growth-promoting activity of perch pituitary glands was highest during the spring or early summer and that this corresponded to the rapid growth period. It is likely, therefore, that in the perch, as in the salmon, the somatotrops are under the influence of photoperiod.

Although smoltification is associated with profound biochemical (Lovern, 1934; Malikova, 1957) and physiological changes (Fontaine, 1960) the possible role of the endocrines (Saunders and Henderson, 1970) remains to be elucidated. Certainly, work on other teleosts suggests that salinity tolerance and colour changes may have an endocrinological basis. For example, recent work on salinity adaptation in teleosts (Kamiya, 1972; Forrest et al., 1973) indicated the existence of a pituitary-interrrenal axis in gill Na-K-ATPase activation. Further, an MSH involvement in fin darkening (Abbott, 1973) and a possible influence of cortisol on silvering (Olivereau, 1972) have also stimulated speculation about a pituitary involvement in smoltification. It is not clear what, if any, interrelationships exist between the various aspects of smoltification. The size of the fish (Elson, 1957) and the rate of growth (Parry, 1958) have been suggested as permissive factors in the onset of the parr-smolt transformation. Since growth rate seems to be an important parameter in the transformation it seems reasonable to speculate that growth hormone is also involved. But GH may have functions

additional to that of growth promotion. This is suggested by the observations that GH promoted salinity tolerance in brown trout, Salmo trutta (Smith, 1956), and affected body electrolyte regulation in steelhead trout, Salmo gairdneri (Chartier-Baraduc, 1959).

The tendency for somatotrop expansion along the neurohypophyseal border during the periods of stimulation may be indicative of an intimate control over these cells by the neurohypophysis. A close proximity of somatotrops and the neurohypophysis has also been reported for other species (Ball and Baker, 1969) and may also be related to hormone release directly into the neurohypophysis (Olivereau, 1954; McKeown and Leatherland, 1973). Unfortunately, light microscopy presents serious limitations to the study of the somatotrops. Although the granules of these cells, as well as the somatotrops of other species (Schreibman et al., 1973), are larger than those of the prolactin cells, they are nevertheless poorly detectable because of technical limitations. The use of acetic acid in the application of orange-G in this study greatly enhanced the staining quality and reproducibility offered by the commonly used phosphotungstic acid solvent. However, further improvements in reproducibility will be necessary to precisely judge granule depletion.

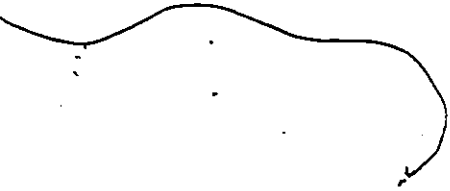
Prolactin is another hormone which plays an important role in hydromineral regulation (Ball, 1969; Olivereau, 1969). Although the prolactin cells remain active in sea-

water they are much more active in freshwater and this suggests that prolactin is mainly a fresh water hormone (Olivereau, 1969). It might be expected, therefore, that the prolactin cells might show some modification during the process of smoltification. However, in the present investigation the prolactin cells did not show the expected signs of regression during this transformation. It is possible that a continued release of prolactin is needed to maintain osmotic equilibrium in fresh water. This it might do by countering the effects of Na-K-ATPase activation by the pituitary-interrenal axis (Kamiya, 1972). Another possibility is that prolactin intervenes in lipid metabolism. Recent findings that prolactin mobilizes lipid during long daylight periods (de Vlaming, et al., 1974) make it a logical target for the investigation of seasonal changes of body lipids. Evidence for such seasonal changes in the current study is in agreement with the findings of Pinder and Eales (1969). Data from these workers also suggests that the lipid content of Atlantic salmon describes an annual cycle.

A suitable combination of photoperiod and temperature probably determines the onset of smoltification in the Atlantic salmon and can therefore be used to manipulate it. However, Baggerman (1960), though able to induce salinity preference in Pacific salmon by exposure to long daylight periods, was unable to ~~prevent~~ a loss of this preference. These data indicate that a limit to photoperiod manipulation

may be imposed by the operation of endogenous cycles. It is possible that maintenance of preadaptation to seawater may require exposure of salmonid smolts to seawater (Zaugg and Wagner, 1973).

The acceleration of preadaptation to seawater by exposure to long daylight periods in winter is indicated by this study. More recently, Wagner (1974a) made similar observations in steelhead trout. It is not yet known whether other parameters of smoltification, such as growth, are influenced by photoperiod in salmonids other than Atlantic salmon. But it is likely that the commercially valuable potential of advancing smoltification by photoperiod manipulation may be universally applicable to other species. The key to such a successful enterprise lies in the better understanding of a postulated "photoperiod-endocrine axis".



SECTION II

SOME EFFECTS OF PORCINE GROWTH HORMONE IN RELATION TO  
GROWTH AND SALINITY TOLERANCE OF ATLANTIC SALMON PARR.

## INTRODUCTION

Seasonal growth patterns have been shown in several species of fishes with the patterns exhibiting slowest growth rate in the winter (Le Cren, 1951; Ball, 1961). In brown trout, Salmo trutta, (Ball, 1961) this seasonal growth pattern was more closely associated with photoperiod than with temperature. Further, in Atlantic salmon, Saunders and Henderson (1970) reported that the pattern could be altered by changes in daily light exposure. As linear growth in several species of teleosts stops following hypophysectomy (Pickford, 1957), and can be partially restored by exogenous mammalian growth hormone in killifish, Fundulus heteroclitus (Pickford, 1953, 1954) it seems reasonable to expect some role for growth hormone in the photoperiodic mediation of growth patterns.

In the previous section of this thesis a correlation between the growth patterns in Atlantic salmon and the pituitary cytology of growth hormone cells was reported. Briefly, these cells exhibited hyperplasia and hypertrophy during the rapid growth phase of salmon parr which occurs during long daylight periods. Previously, mammalian growth hormone injections have also been reported to promote the development of salinity tolerance in brown trout (Smith, 1956), and to induce salinity preference in coho salmon, Oncorhynchus kisutch (McInerney cited in Hoar, 1966). In Atlantic salmon, salinity tolerance normally develops during

the fast growth phase of smoltifying parr (Brown, 1957). Similar observations led Parry (1958) to suggest that growth and salinity development might be causally related. These findings suggest the presence of a somatotropic hormone in fishes which can be partially mimicked by mammalian growth hormone and indicate that this hormone may exert effects beyond simple growth promotion.

This section reports on the effects of porcine growth hormone on linear growth, morphology and salinity tolerance in Atlantic salmon parr. The effects of the hormone are studied under photoperiod conditions earlier determined to minimize the endogenous hormonal contribution, that is, under a short daylight regimen.

## MATERIALS AND METHODS

Atlantic salmon parr, aged 2+ of  $11.5 \pm 0.2$  cm fork length, reared at the Saint John Hatchery, Saint John, New Brunswick, were transported to the Fisheries Research Board Biological Station at Saint Andrews, New Brunswick on June 4, 1972. The fish were kept in running dechlorinated fresh water at  $11.5 \pm 0.2^{\circ}\text{C}$  under a photoperiod with a light to dark period opposite in length to the natural conditions which prevail at the time at that latitude. At the start of the experiment this consisted of 9 hr of light and 15 hr of darkness. Temperature was controlled by means of thermosensory electronic relays at the level of the header tanks. The relays activated pumps which added chilled or warm water as needed to balance out any fluctuations in temperature in the header tanks. Illumination was provided by two 40CW fluorescent lights positioned 91 cm over the tanks and controlled by a timer. The timer was adjusted by 15 minutes every three days to produce the desired light to dark ratio. The adjustment was made, alternately, at the start or at the end of the light phase.

The fish were divided into two experimental groups of 14 fish each. One group was injected intraperitoneally with 0.1 ml/g body weight of 0.6% saline containing 10 $\mu\text{g}/\text{ml}$  porcine growth hormone (Somatotropin No. S-1501, Lot 28B-2350, 0.7U/mg, Sigma Chemical Co.). The other group received the carrier only. All fish were injected on alternate

days for a period of four weeks starting on June 27th. The injections were given via a 26 ga. needle attached to a 1cc disposable syringe.

All handling of fish was preceded by anesthesia with 1:80 tert-amyl alcohol to the stage of response loss. Morphometric determinations were made only four times during the experiment (Fig. 1) to keep stress to a minimum. Fork length was determined to the nearest millimeter and weight was determined to the nearest 0.1 g after blotting the fish with moist absorbent paper.

Fish were fed Ewos salmon pellet food 4P (Astra-Ewos AB) thrice daily to satiation on injection free days and twice daily, morning and evening on the days of injection. The relative amounts of food required by each group during these findings was recorded. After 28 days, and on an injection-free day, fresh water was gradually replaced by seawater (30 ‰ salinity) over a period of 22 hours. Temperature was kept at  $11.5 \pm 0.2^{\circ}\text{C}$  throughout. Fish were held under these conditions for five weeks. The surviving fish were then transferred to a routine holding tank under the prevailing conditions for further observation during a nine month period.

Instantaneous (or specific) growth rates were calculated according to the formula  $100 \cdot \frac{\log_e Y_T - \log_e Y_t}{T - t}$  where  $Y_T$  and  $Y_t$  are lengths or weights at time  $T$  and  $t$ ,  $T$  being later than  $t$  (Brown, 1957). Condition factor was determined according to the formula  $K = 100 W/L^3$ ;  $W$  = weight in grams,  $L$  = fork

length in centimeters (Hoar, 1939; Brown, 1957).

The Student's 't' test was used to determine significant differences in lengths and weights between the two experimental means.

RESULTS

Effects of Porcine Growth Hormone on Growth and Morphology

Growth hormone injected fish grew more in length than the saline injected controls and were significantly longer ( $P < .05$ ) after four weeks (Fig. 1). This was a reflection of the higher instantaneous growth rate in length recorded for the hormone treated fish (Table 1). Growth hormone treated fish also gained more weight but this difference was not significant ( $P > .05$ ) after four weeks. During the same interval the condition factors of the growth hormone injected fish decreased considerably while that of the controls increased. The hormone treated fish consumed about one third more food than the controls.

Although parr marks remained visible in both groups, the black fin margins characteristic of salmon smolts became very evident in the hormone injected fish after two weeks. The hormone injected fish also exhibited an intense "yellowing" around their operculae and on their fins (Fig. 2).

Effects of Porcine Growth Hormone on Salinity Tolerance

After one week in seawater more than 50% of the controls had died. Most of the remaining fish in this group died before the end of the second week. The incidence of mortality did not appear to be size related. None of the growth-hormone injected fish died over the observation period or during a nine month post-experimental observation period.

when the hormone injected fish grew well, continued to silver and lost their parr marks.

Figure 1

Effect of porcine GH injection on length, weight and condition factors in the parr of Atlantic salmon, Salmo salar, L. Values are means with the 95% confidence intervals indicated. (Saline injected - o ; GH injected - Δ).

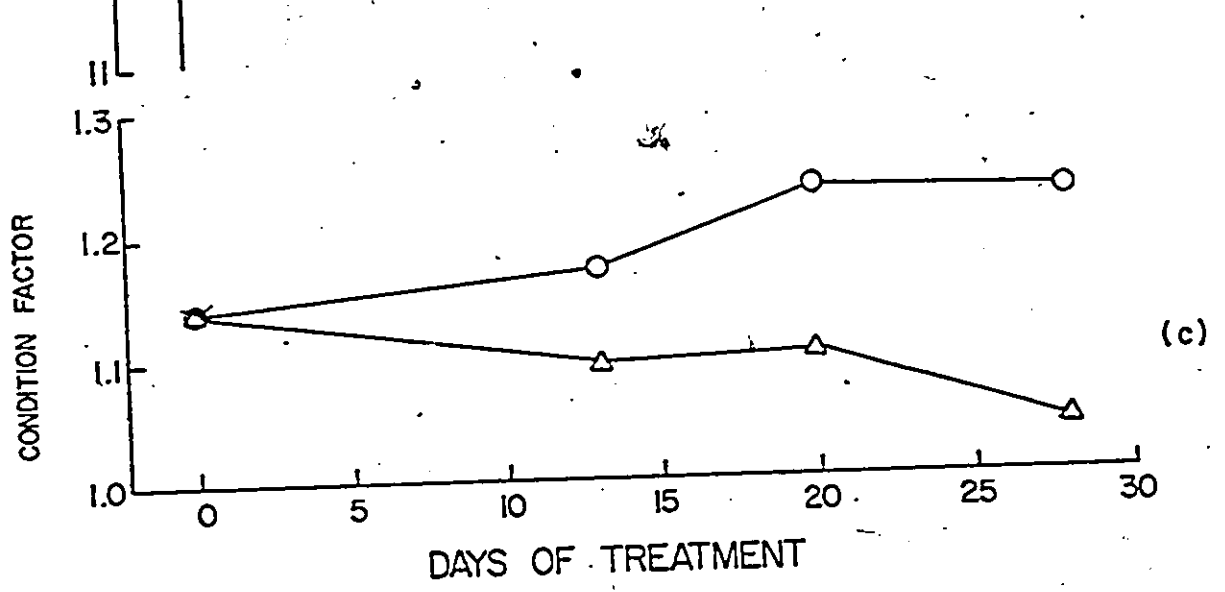
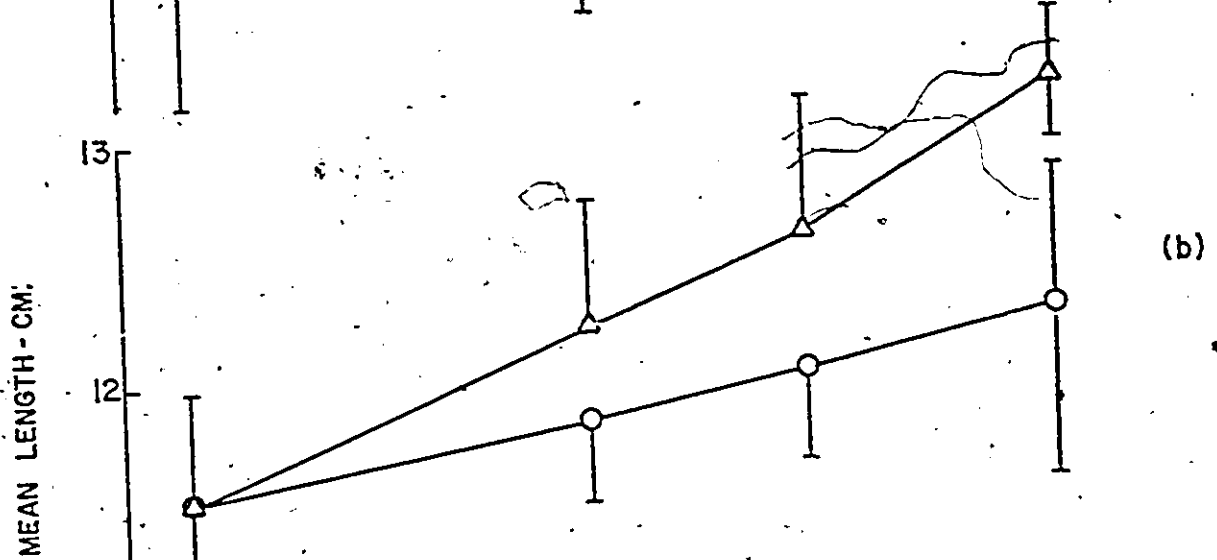
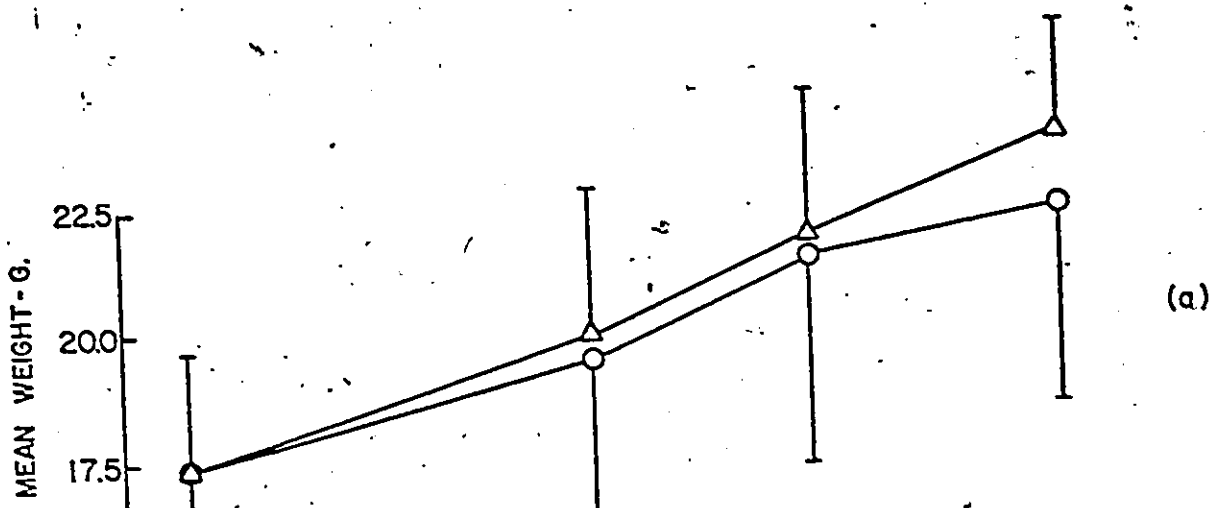


TABLE 1

Effect of porcine growth hormone injected on alternate days for four weeks on the instantaneous growth rates in length and weight of Atlantic salmon parr.<sup>a</sup>

---

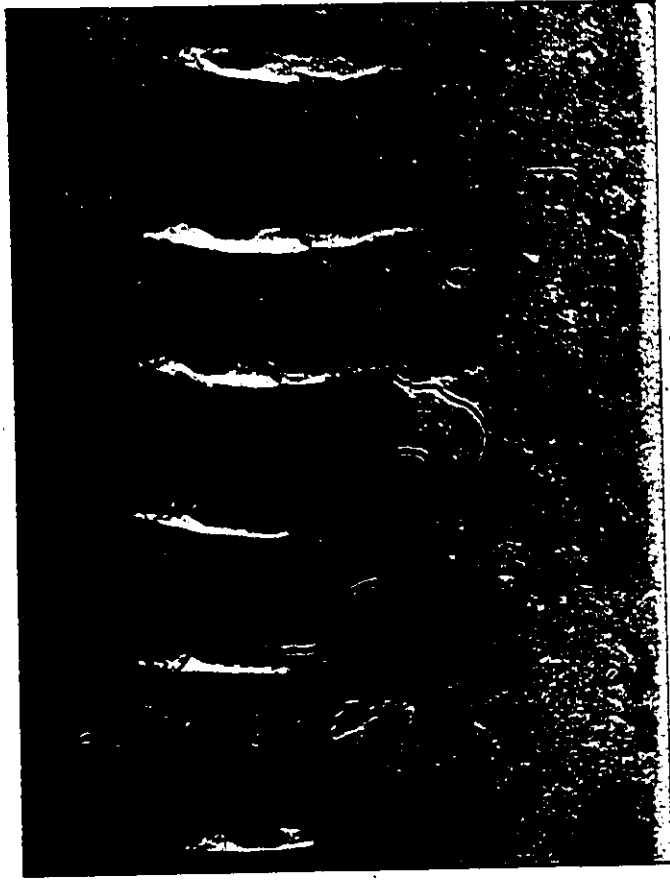
Injection Period	0.6% Saline		Porcine Growth Hormone	
	<u>length</u>	<u>weight</u>	<u>length</u>	<u>weight</u>
June 27-July 10	0.1	0.86	0.46	1.11
July 10-July 17	0.22	1.35	0.41	1.45
July 17-July 25	0.23	0.64	0.59	1.01

---

a Instantaneous growth rates were calculated according to the formula  $100 \cdot \frac{\log_e Y_T - \log_e Y_t}{T - t}$ , where  $Y_T$  and  $Y_t$  are mean lengths or mean weights at times T and t.

Figure 2

Effect of porcine growth hormone therapy on pigmentation of Atlantic salmon parr. Top three fish are saline injected controls. Note yellow pigmentation and darkening of fins of hormone treated animals.



## DISCUSSION

This study indicates that porcine GH promoted linear growth in Atlantic salmon parr. This finding is in agreement with similar studies using purified beef GH on S. trutta (Swift, 1954) and on intact and hypophysectomized F. heteroclitus (Pickford and Thompson, 1948; Pickford, 1957). It is probable that the differences in growth rates between saline injected and GH treated fish in the present study were maximized by using short daylength conditions which presumably decrease endogenous GH production (Section I). Similar findings have been reported by Swift (1954) who found that S. trutta injected with beef GH grew faster than saline injected controls in March but that untreated controls matched the GH treated fish during the longer daylight conditions in July. It is possible, therefore, that reports of a lack of effect of GH injections in some instances (Pickford, 1957) may have been due to experimental photoperiod conditions. That is, fish may have been tested during periods of maximal endogenous GH production.

The observed decrease in condition factor of GH injected fish is similar to that found in fast growing salmon and at the time of parr-smolt transformation (Hoar, 1939) and corroborates earlier reports in this thesis. This decrease in condition factor may be a consequence of accelerated linear growth but could also be affected by possible fat mobilization by GH as has been suggested for mammals (Russell, 1955; Raben, 1973).

Survival in seawater of GH injected parr is in agreement with Smith's (1956) findings in brown trout. It is significant that in the present experiment survival was possible under photoperiod conditions that had failed to favour development of smolt characteristics during an earlier study in this thesis.

The mode of action of GH is not yet clear nor has it been shown whether GH from other species, fish in particular, has a similar effect. The resolution of this problem will have to await the purification of conspecific GH. Smith (1956) suggested that GH promotes survival in seawater by increasing the metabolic rate of the fish. It is not likely, however, that GH plays an exclusive role in the process of preadaptation to seawater. Preadaptation of eels to seawater appears to involve adrenocorticotropin and cortisol (Kamlya, 1972; Forrest et al., 1973) but the role of GH in this species has not been studied. Since the process in salmonids involves similar mechanisms, such as gill Na-K-ATPase activation, the pituitary-interrenal axis might be expected to be involved in the seawater preadaptation of these species as well. However, this remains to be demonstrated.

It is notable that accelerated growth and salinity tolerance were induced in the absence of overt silvering, an event normally associated with the parr-smolt transformation. Hormone injected fish were transferred to seawater

with conspicuous parr marks and did not silver in that medium until several months after the conclusion of this experiment. This finding suggests that silvering is not an obligatory component of the process of preadaptation to seawater. The lack of silvering is also significant since it suggests that the GH preparation was not contaminated with TSH. Elevated levels of TSH would presumably have resulted in a stimulation of the thyroid and thyroid hormones are known to promote silvering in salmonids (Landgrebe, 1941; Robertson, 1949).

The intense yellowing of the fins, the operculae and to a lesser degree some other body parts may have been caused by GH itself. Several reports attributed a xanthophore response to prolactin (Sage and Bern, 1972) and human GH (Ball and Ingleton, 1973). However, a more recent study indicates some uncertainty about the xanthophore dispersing activity of prolactin (Farmer *et al.*, 1975). No explanation can be offered for the cause of fin darkening observed in the GH treated fish but the possibility of ACTH contamination cannot be ruled out. Preliminary work has not suggested an effect of porcine GH on pituitary cytology that would account for the observed effects. Thus, it appears that the effects of the GH preparation in the current study were not mediated by that gland.

This study also suggests that the receptors for growth hormone do not fail to respond to increased levels of the hormone under conditions of short daylength. Therefore,

photoperiod effects are probably mediated by the pituitary and are expressed as changes in the production, the release, or both, of the hormone.

SECTION III

ELECTROPHORESIS AND IDENTIFICATION OF GROWTH HORMONE  
AND PROLACTIN

## INTRODUCTION

The preceding studies reported in this thesis suggested a role for growth hormone in growth and smoltification of Atlantic salmon. Histological observations also suggested that production of the hormone could be altered by photoperiod manipulation. It appeared reasonable, therefore, to attempt to measure changes in hormone production by the more direct approaches of electrophoresis and biological or immunological assay.

Pituitary hormones have been successfully separated by the polyacrylamide disc gel electrophoresis (PAGE) method of Davis (1964). Electrophoretic separation has been accomplished on pituitary extracts from rats (Jones et al., 1965; Kragt and Meites, 1966; Hodges and McShan, 1970; Ben-David and Chrambach, 1963), mice (Cheever et al., 1969), eels, Anguilla anguilla (Knight et al., 1970), flounder, Pleuronectes flesus (Chadwick, 1970), mollies, Poecilia latipinna (Ball and Ingleton, 1973), cichlids, Tilapia mossambica (Clarke, 1973a; Farmer et al., 1975), mudfish, Labeo umbratus (Hattingh and du Toit, 1973), and blue shark, Prionace glauca (Lewis et al., 1972). Prolactin and growth hormone have each been assumed to yield single discrete bands in most of these gel studies on teleost pituitaries and these bands have been tentatively identified. A partial purification and characterization of prolactin has, however, only been accomplished in the cichlid Tilapia

mossambica (Clarke, 1973b; Farmer et al., 1975) using bioassay to identify the hormone as the Na-retaining principle. The positive identification of a teleost growth hormone on disc gels has not yet been reported. This can be attributed to the absence of a suitable assay for this hormone.

Changes in the putative prolactin band density of Poecilia latipinna (Ball and Ingleton, 1973) and Tilapia species pituitaries (Clarke, 1973a), following transfer of the fish between waters of different salinity, have been successfully studied with the aid of scanning densitometry. Abrupt exposure of seawater adapted animals to fresh water resulted in a transient decrease of electrophoresable prolactin levels in the pituitary.

More recently, electrophoretic techniques have also been applied to obtain hormone fractions by preparative electrophoresis (Yadley et al., 1973). However, the applications and limitations of qualitative and quantitative disc-gel electrophoresis in the study of teleost endocrinology have not been investigated in depth.

Evidence for the actions of salmonid prolactin is limited. Donaldson et al. (1968) reported that injections of a crude extract of chinook salmon, Oncorhynchus tshawytscha, presumed to contain prolactin, reversed a drop in plasma osmolarity in hypophysectomized goldfish, Carassius auratus.

The following experiments were undertaken to separate hormonal principles present in Atlantic salmon pituitary

electrophoretograms and to verify the identities of prolactin and growth hormone by a variety of assays. In addition, this study attempts to establish whether the size of the electrophoresable pool of Atlantic salmon pituitary hormones can be modified by photoperiod manipulation.

## MATERIALS AND METHODS

Atlantic salmon parr and smolts were obtained from various sources at the St. Andrews Biological Station. However, only material from fish from the same source, and held under identical conditions, was used in any one experiment. No attempt was made to separate sexes but none of the fish used in this study showed overt signs of sexual maturity.

Fish in any one experiment were killed by rapid over-anesthesia in tert-amyl alcohol (1:50) or by a blow on the head. The brain was exposed by cutting off the top of the head. The lateral nerves, optic nerves and medulla were transected and the brain, with the pituitary still attached, was gently lifted out of the head. Subsequently, the pituitary was carefully detached from the brain by severing the stalk and blood was blotted off with filter paper. All operations which involved handling of tissues were carried out at 4°C.

One group of pituitaries was dissected into RPD, PPD, PI and pars nervosa fragments. Several pituitaries were stained for 12 hours in 2% orange G in 2% acetic acid and destained in 70% aq. amyl alcohol. This procedure stained the rostral pars distalis a bright orange. In the fresh pituitary this part usually appears as a distinct white cap. The rest of the brain remained pale. The rostral pars distalis was then easily separated as a sheet by blunt dis-

section. An attempt was also made to separate the proximal pars distalis from the pars intermedia and the pars intermedia from the neurohypophysis. All the fragments of each area were immediately frozen at  $-30^{\circ}\text{C}$  until they were homogenized prior to electrophoresis.

A group of pituitaries was also perfused to eliminate contaminating blood. This was done to study any possible contribution to the electrophoretic pattern of whole pituitary homogenates by blood peptides. To this effect six salmon smolts were anesthetized in 1:80 tert-amyl alcohol and their hearts were removed. A polyethylene cannula was then inserted into the bulbus arteriosus and a 0.6% NaCl-sucrose solution, adjusted to 320 mOsm, was pumped through the fish at the rate of 5 ml/min. The perfusate was allowed to drain through the cut end of the portal vein. Within 30 minutes all tissues, except the spleen and the saccus vasculosus became blanched. After 60 minutes of perfusion the pituitaries were removed from the fish in routine manner. Four of the pituitaries were frozen at  $-30^{\circ}\text{C}$  for electrophoresis and the other two were placed in Bouin's fixative for histological examination.

#### Polyacrylamide Disc Gel Electrophoresis (PAGE)

Fresh or frozen pituitaries were homogenized in 25  $\mu\text{l}$  of distilled water and further homogenized after addition of an equal volume of a 1:1 solution of TRIS-Glycine buffer (pH 8.5) and a 40% sucrose solution. The final volume was

approximately 50  $\mu$ l. This homogenate was electrophoresed on a Shandon PAGE apparatus at pH 8.5. Glass tubes with a 4mm inner diameter and 10cm in length were loaded with a 6.5cm running gel (7.5% acrylamide) and a 1.5cm stacking gel (3.5% acrylamide). Electrophoresis was conducted at 4°C to prevent denaturation of the peptides. Samples were layered onto the surface of the large pore gel with a micro-pipette, and a 1 mA current was applied per tube. After five minutes the current was increased to 2.5 mA per tube. This current was maintained until the bromophenol blue indicator band was seen to migrate into the small pore running gel. The current was then increased to 5 mA per tube and electrophoresis was continued until the indicator band had moved approximately 6cm along this gel. On completion of the run, the gels were stained for 24 hours in a solution consisting of 1% fast green in 7% acetic acid (Gorovsky et al., 1970). The relative mobilities (Rf values) were expressed as the ratio of the distance from the origin to the particular protein band and the distance from the origin to the ion front indicated by bromophenol blue. Gels were scanned at 623 nm using a 2.5mm X 0.25mm slit on a Vitatron densitometer model TLD 100 and the amounts of protein were estimated in terms of purified bovine albumin (Sigma Chemical Co.) standards. Beers law, which describes a linear relation between concentration of protein and absorption intensity was verified for 0.8 to 5  $\mu$ g loads per band.

Isolation of Putative Prolactin (PRL) and Growth Hormone (GH)

Several hundred pituitaries were obtained from both fresh and seawater salmon smolts. The rostral pars distalis was removed from approximately 100 and these were pooled and processed separately. About 200 pituitaries were freeze dried and ground to a fine powder. This pituitary powder was homogenized in distilled water and Tris-glycine-sucrose as described previously. The resulting homogenate, made up to 10ml with distilled water, was centrifuged at 5000g for 30 minutes to remove debris and the pellet was then re-extracted in the same way. The supernatants were combined and the final pellet was discarded. The combined supernatants were concentrated by ultrafiltration in an Amicon chamber (Model 10-PA, Amicon Corp.) fitted with an XM 50 membrane which retains components with MW > 50,000. The ultrafiltrate was collected and filtered through a Diaflo PM10 membrane (Amicon Corp.) which retains components with MW > 10,000. These retentates were now presumed to contain all the components with a MW between 10,000 and 50,000 Daltons. The retentates, each of approximately 0.1 ml, were combined and electrophoresed in 50 to 100  $\mu$ l loads per gel. Two gels from each run were stained with fast green then destained electrophoretically. The remaining gels were immediately frozen and stored at  $-25^{\circ}\text{C}$ . Once the presence of desired bands was verified on the stained control gels then the corresponding sections were removed from

the frozen unstained gels and allowed to thaw at 4°C. These gel sections were extracted electrophoretically and the eluates were concentrated. This was accomplished with a disc-electrophoresis apparatus consisting of an upper cathodal and lower anodal buffer reservoirs (Fig. 1-3). The electrodes were 5cm long battery carbons. Two plexiglass discs were located between the two chambers. Each disc has four holes. Dialysis membranes of a pore size selected to retain the desired peptides were placed over the holes in the lower disc and the holes in the top and bottom discs were placed in apposition. The two discs were then fastened together with four plastic screws.

Protein bands were eluted from gels by inserting the appropriate gel segment into the slightly constricted end of a 4cm long glass tube. This end of the tube was then inserted into one of the holes above the dialysis membrane leaving a space, filled with 0.1ml of Tris-glycine buffer, between the membrane and the gel section. The upper end of the glass tube was inserted into the base of the upper reservoir which was then filled with Tris-glycine buffer. The gel section was layered with 0.1ml of .001% aq. bromophenol blue as an indicator dye. The cathode lid was placed on the reservoir and 2 mA of current were applied per tube until the indicator dye migrated through the gel section. Peptides were concentrated by eluting several gel sections containing the same protein band into the same 0.1ml of Tris-glycine buffer. The cathodal buffer was renewed after.

Figure 1

Components of disc gel fraction extraction apparatus. A, anode buffer reservoir lid and electrode; B, anodal buffer reservoir; C, disc gel tube; D, two discs with interposed dialysis membrane; E, cathodal buffer reservoir.

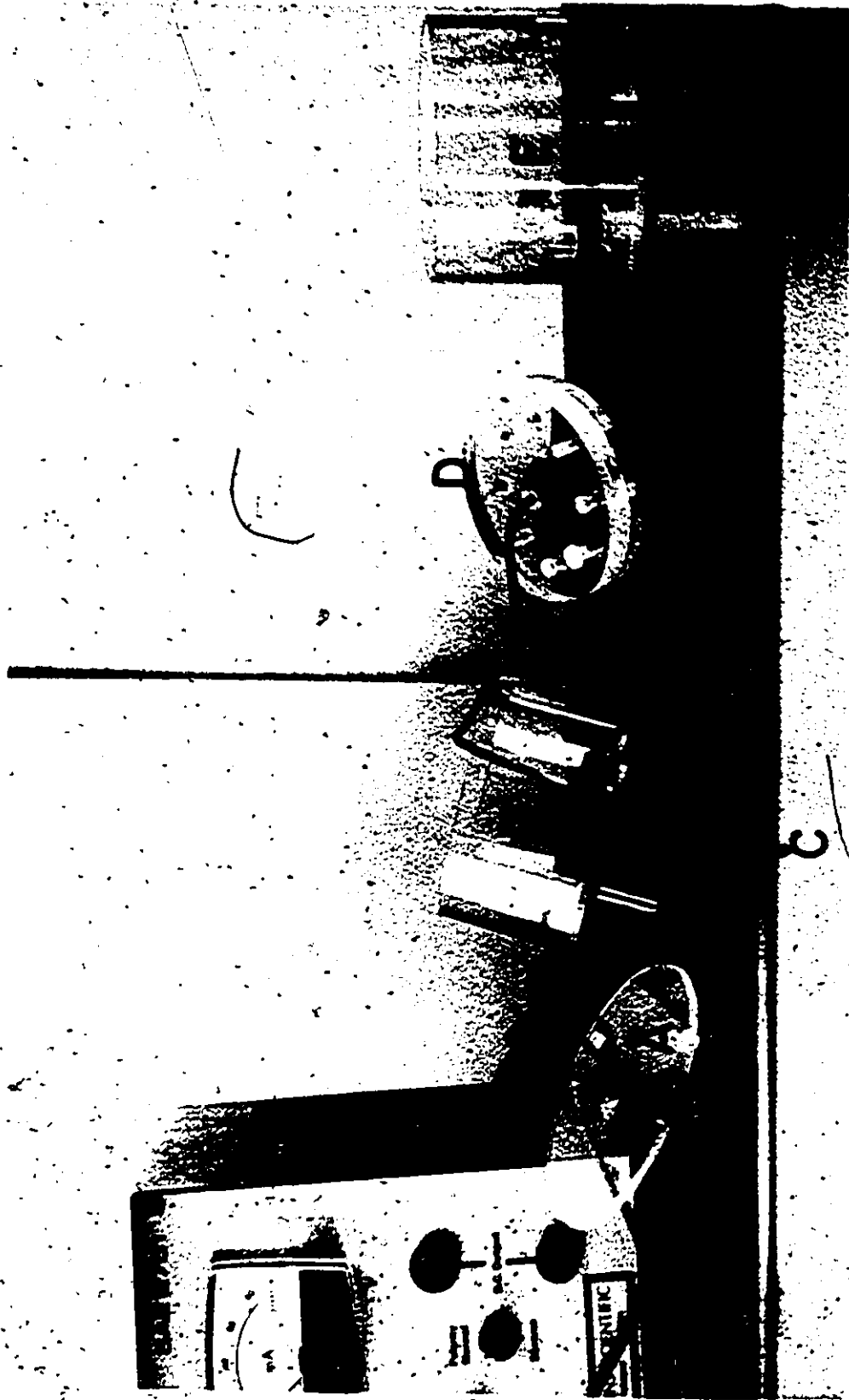
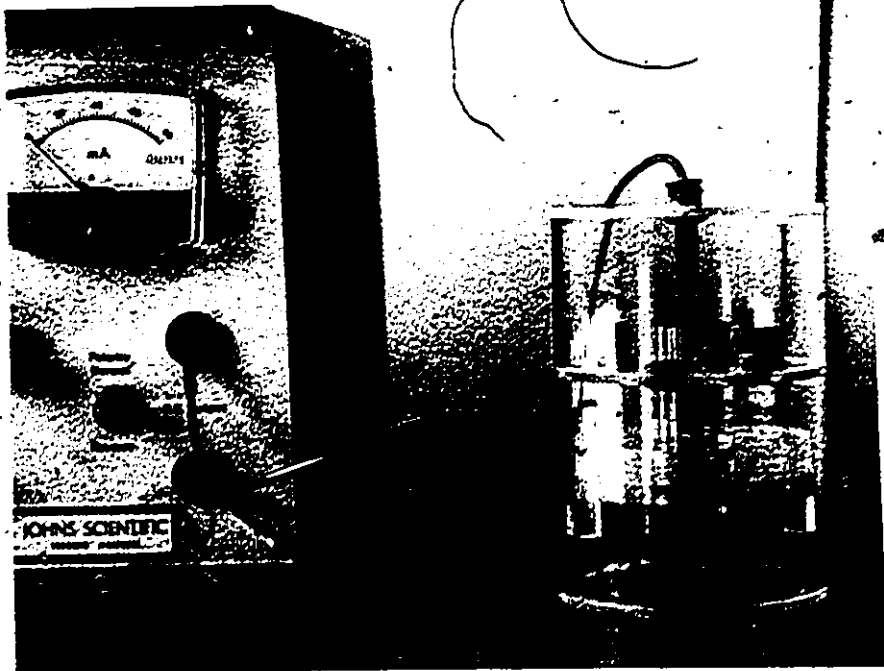
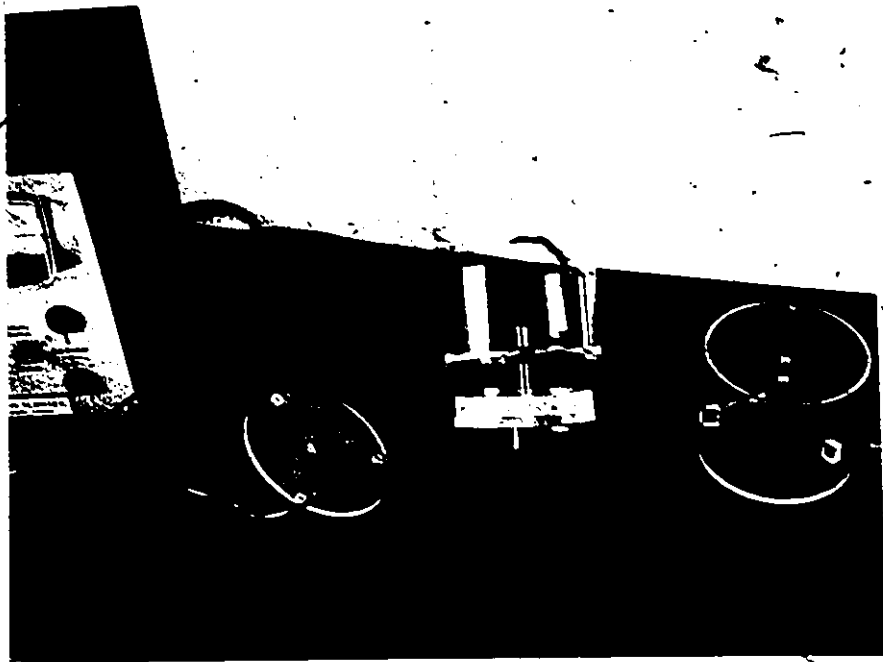


Figure 2

Assembly steps of disc gel extraction apparatus. Upper end of glass tube containing gel to be extracted is inserted in anodal buffer reservoir. Lower end is inserted into upper one of two discs (Fig. 1, D) after dialysis membrane is secured between discs.

Figure 3

Assembled disc gel extraction apparatus.



each extraction run. Prior to removal of the eluate fraction the current polarity was briefly reversed to recover any portion of the peptide that had adhered to the surface of the dialysis membrane. The purity of the extracts was checked by electrophoresis.

All of the bands from some gels were extracted individually for radioimmunoassay of PRL and GH. Each of the individual extracts were contained in 0.1ml of buffer. An additional series of extracts was prepared from gels after electrophoresis of homogenized RPD fragments. Extracts for bioassay of PRL contained only major bands associated with the RPD.

#### Bioassay of Atlantic Salmon Prolactin

The bioassay for PRL was based on the sodium-retaining activity of the hormone in hypophysectomized killifish, Fundulus heteroclitus, following transfer from seawater to fresh water (Pickford et al., 1965, 1966). The fish, approximately two inches in length, were collected in estuaries near the Mt. Desert Island Biological Station, Salisbury Cove, Maine, USA. They were held in seawater (30 ‰ salinity), at 12°C, under a simulated natural photoperiod from October 12th to November 2nd when they were transferred to St. Andrews, N.B. The killifish were initially held under the same conditions for a period of 10 days. The daylight period was then reduced to 8 hours to prevent sexual maturation. Hypophysectomy was performed by the opercular approach.

method of Liversage (1973). The pituitary was exposed by cutting and parting the pharyngeal epithelium and parasphenoid bones as flaps. The gland was then removed by applying moderate suction through a drawn out 50  $\mu$ l-pipette. The bone flaps were carefully replaced to allow proper healing of the incision. Sham operations were performed in an identical manner except that the pituitaries were not removed. Operated and sham-operated animals were dipped in a 0.004% aq. solution of oxytetracycline hydrochloride (Animal formula Terramycin, Pfizer) and returned to seawater for post-operative recovery.

The bioassay was carried out 14 days after surgery. At this time killifish were injected with gel segment eluates containing the major RPD bands in 0.6% NaCl in Tris-glycine buffer (pH 8.3), with whole RPD homogenate in the same vehicle or with just the vehicle. Injections were delivered intraperitoneally via the post-anal hypaxial musculature using 0.25ml tuberculin syringes fitted with 30 gauge needles. Ten control fish, eight sham hypophysectomized and nine hypophysectomized fish were given placebo injections. Two hypophysectomized fish were injected with RPD homogenate, three received eluate from the segment containing the putative prolactin band ( $R_f = 0.47$ ), two were injected with eluate from the section of gel preceding the prolactin band and one was injected with eluate from the remaining portion of the gel. Three hours after the injections one half of each group of placebo injected fish, and all the fish receiving

pituitary extracts, were transferred to fresh water aquaria. After 36 hours the fish were stunned by a blow on the head and blood was collected in microhematocrit tubes lightly coated with ammonium heparin (Sherwood). This was done by sectioning the caudal peduncle and taking blood directly from the caudal artery. Fish were then rapidly killed by overanesthesia in tert-amyl alcohol. The blood was centrifuged at 500g for 10 min and the plasma was stored at  $-25^{\circ}\text{C}$ . Appropriate regions of the brain from the bioassay animals were examined histologically for the presence of pituitary remnants.

Plasma aliquots of 5  $\mu\text{l}$  size were diluted to 7.5ml with distilled water. Duplicate dilutions were made whenever possible. Sodium concentrations were determined by flame emission spectrophotometry on a Perkin Elmer 303 atomic absorption-emission spectrophotometer.

Duncan's new multiple-range test (Steel and Torrie, 1960) was used to test for differences among the experimental means.

#### RIA of Salmon PRL and GH Identities on PAGE

Samples of pituitary homogenate diluted with Tris-glycine buffer and extracts from six gel segments were air-mailed for analysis to a commercial radioassay laboratory (Radioassay Systems Laboratories, Carson, California, USA). The extracts represented the sum total of electrophoresable peptide from three salmon pituitaries (12 mg). Each of these

extracts contained no more than one band with protein concentrations ranging from 10 to 40 ng/ml of the buffer.

Freeze dried extracts of segments covering the entire length of gels were prepared from both whole pituitaries and RPDs. These extracts were transported frozen at  $-15^{\circ}\text{C}$  to the University of Guelph for radioimmunoassay of growth hormone and prolactin. The purpose of these assays was to detect the location of the hormones on the gels. The assays were performed by Dr. B.A. McKeown by the technique of Leatherland and McKeown (1973). This technique involved the use of antipollack (Pollachius-virens) hormone in a solid-phase method. The amounts of putative PRL and GH were electrophoretically estimated to be in the range of 2-4 ng.

#### Vasopressor Activity of PAGE Extracts

Four Sprague-Dawley rats, approximately 300g in weight, were anesthetized with 0.25ml of Nembutal (50mg/ml). A cannula was then inserted into the left carotid artery. This cannula, filled with 0.6% saline and 1 U/ml of heparin, was connected via a pressure transducer to an E & M physiograph in order to record blood pressure. A lead II EKG was also recorded.

Blood pressure and heart rate were allowed to stabilize and a quantity of pituitary homogenate equivalent to 1, 2 or 3 pituitaries and eluates from segments of gels containing electrophoresed salmon pituitaries were tested. Arginine vasopressin

## RESULTS

Polyacrylamide disc gel electrophoresis of whole, frozen or previously frozen Atlantic salmon pituitaries all yielded highly variable results. In many cases no very distinct bands appeared. This made it necessary to pool pituitaries, or their fragments, for each gel load.

The most typical bands from the RPD had Rfs of 0.22, 0.47 and 0.55. The band with an Rf of 0.47 (Fig. 4) was the most common. The band with an Rf of 0.22, though not found as often, was sometimes very intense. A faster moving band could be resolved immediately ahead of the band with Rf = 0.47. However, this could only be accomplished after the indicator dye had migrated at least 4.5 cm into the small pore gel. None of these bands were found in homogenates from other parts of the pituitary.

The surgical isolation of other pituitary regions was difficult because of the neurohypophyseal interdigitations into the proximal pars distalis (PPD) and the pars intermedia (PI). On the other hand, the neurohypophysis does not invade the RPD to any significant degree. Several bands appeared to be related to an area exclusive to the RPD with the most prominent having Rf values of 0.78, 0.84, 0.86, and 0.94. Fractions from the RPD did not give rise to any bands with Rf values > 0.5. Bands with Rf values > 0.74 were found in association with relatively pure PI and neurohypophyseal material. But due to the impossibility of

Figure 4

Polyacrylamide disc.gel of homogenate of whole pituitary gland from Atlantic salmon, Salmo salar, stained with Fast Green. 0, origin; GH, growth hormone; PRL, prolactin; albumin and ion front (If).

← O

← GH

← PRL

← Albumin

← If

separating the PI from the neurohypophysis the origin of these bands could not be established.

Gels of whole pituitary homogenate did not display any bands with strong affinity for either PAS or aldehyde fuchsin. However, weak staining with PAS and aldehyde fuchsin was observed on bands with Rf values of 0.55 and 0.69.

Atlantic salmon serum produced 18 distinct bands after PAGE (Appendix, Table 1) and several of these had Rf values identical or close to those produced by pituitary homogenates. However, no noticeable difference was found between the electrophoretograms of pituitaries flushed with the NaCl-sucrose solution and the patterns produced by fresh whole pituitary homogenates. The histological examination of several of the perfused glands confirmed that blood cells were only found in the adjacent saccus vasculosus and that few, if any, remained within the gland.

The brain tissue electrophoretograms were obscured by intense background streaking but displayed a major band with Rf = 0.69 and a somewhat less intensely staining band with Rf = 0.61. Several other bands were just barely detectable.

#### Extraction of Putative PRL

Several eluates were obtained from segments of gels containing electrophoresed RPD fragments. Electrophoresis of an aliquot of each of the eluates indicated that they

were free of contamination and only the desired bands were present.

The eluates from one of the RPD gel segments contained a composite band with a principal component ( $R_f = 0.47$ ) and a less intense component which migrated on its leading edge. A slight increase in the faster moving component was apparent after handling under alkaline conditions or after freezing.

When the purity of the eluates from gel segments containing the RPD band with an  $R_f$  of 0.22 was checked electrophoretically two bands with variable  $R_f$ 's, close to 0.50, were obtained. The original band ( $R_f = 0.22$ ), however, was seldom present in these eluates.

The band with an  $R_f$  of 0.47 was retained by the Diaflo PM-10 membrane whereas the band with  $R_f = 0.22$  did not appear to be retained at all. Consequently, ultrafiltration provided a means for the preliminary separation of these two RPD components.

#### Prolactin Bioassay

Hypophysectomized killifish transferred to freshwater showed a significant drop in their plasma  $Na^+$  levels relative to either the sham operated or intact group (Table 1). Prior injections of pure eluates of the band with  $R_f = 0.47$  prevented this drop. In fact, the plasma  $Na^+$  levels of this group were higher than all other experimental groups ( $P < 0.01$ ) with the exception of hypophysectomized killifish retained in seawater. Partially purified RPD homogenates also showed

TABLE 1

Effect of PAGE fractions of Salmo salar pituitary rostral pars distalis on the plasma sodium concentration in hypophysectomized seawater adapted Fundulus heteroclitus following transfer to fresh water.

Treatment	N	Plasma Sodium <sup>a</sup> (mEq/liter)
<u>0.6% NaCl I.P.</u>		
FW Control	5	181.7 ± 5.9
SW Control	5	195.7 ± 7.9 <sup>b</sup>
FW Sham operated	4	176.5 ± 10.2
SW Sham operated	4	188.8 ± 11.8
FW Hypophysectomized	5	145.5 ± 9.6 <sup>b</sup>
SW Hypophysectomized	4	205.8 ± 6.8 <sup>b</sup>
<u>PAGE Fractions in 0.6% NaCl I.P.</u>		
FW Hypophysectomized Fraction A (Rf 0.0-0.43) 1 µg protein/g B. Wt	2	158.5 ± 0.6 <sup>b</sup>
FW Hypophysectomized Fraction B (Rf 0.43-0.49) 0.5 µg protein/g B. Wt	3	221.6 ± 12.0 <sup>bc</sup>
FW Hypophysectomized Fraction C (Rf 0.49-1.0) 1 µg protein/g B. Wt	1	155.3
<u>Rostral Pars Distalis extract in 0.6% NaCl I.P.</u>		
3 (pooled)	1	183.9
6 (pooled)	1	170.3

a Values are means ± SE of means.

b Means significantly different from FW control (P < 0.01).

c Means significantly different from FW hypsect (P < 0.01).

Na<sup>+</sup> retaining activity. Injections of eluates from other segments of polyacrylamide gels did not have any significant sodium retaining activity. No significant differences were found among the hematocrits of the different groups.

Histological examination of the floor of the brain of hypophysectomized animals did not reveal any pituitary tissue.

#### RIA of PAGE Eluates

Commercial radioimmunoassay of gel segment eluates using anti-human GH did not indicate any significant reaction with any of the gel eluates. Some reaction was reported with anti-human prolactin in all segments of the gel but this did not vary from the reaction reported with an extract from a blank portion of gel. The reaction appeared to be more specific when antibodies to the pollack hormones were used. Most of the GH activity was reported to be located between Rf 0.33 and 0.40. Prolactin appeared to be present in several segments of the gel but the greatest activity was located between Rf 0.45 and 0.50. Somewhat reduced activity was also detected between Rfs 0.14 and 0.25 and Rfs 0.35 and 0.44.

#### Vasopressor Activity of Gel Eluates

Homogenates of approximately 3 mg whole smolt pituitaries had a rat vasopressor activity equivalent to about 8 I.U. of arginine vasopressin. None of the segment eluates from gels containing whole Atlantic salmon pituitaries

electrophoresed under the alkaline conditions prevalent in this experiment showed any significant vasopressor activity.

Effect of Constant Daylight on Pituitary Electrophoretograms

Transfer of salmon parr, acclimated to 12h daylight periods for three weeks, to a 16h daylight regimen resulted in decreased PAGE detectable PRL and GH levels. However, transfer of salmon from the 12h daylight regimen to an 8h daylight regimen showed only a slight, non significant ( $P > 0.05$ ), decrease in PRL and GH levels. The band with  $R_f = 0.22$  did not show any significant changes.

Effect of a 4h Increase or Decrease in Photoperiod on Pituitary Electrophoretograms

A 4h increase in daylight exposure of salmon parr, acclimated to 12h daylight periods for three weeks, resulted in a significant ( $P < 0.05$ ) decrease in PAGE detectable GH levels (Table 2). A slight non-significant ( $P > 0.05$ ) increase occurred in the PRL levels. Decreasing daylight exposure by 4h resulted in a significant ( $P < 0.05$ ) increase in PRL levels and a non-significant ( $P > 0.05$ ) decrease in GH levels. No significant changes were observed in other electrophoresable pituitary components.

TABLE 2

Effect of increasing or decreasing the daily light exposure by 4h for 18 days on electrophoresable pituitary GH and PRL levels in Atlantic salmon acclimated to 12h daily light periods.

Regimen		Putative Hormone Units <sup>a</sup> (N) <sup>c</sup>	Percentage of Electrophoresable Protein <sup>b</sup>
12h daylight	GH	33.1 ± 5.2 (3)	3.9 ± 0.4
	PRL	13.2 ± 5.1 (3)	1.6 ± 0.6
16h daylight	GH	5.8 ± 0.8 (4)	0.7 ± 0.1
	PRL	19.8 ± 7.6 (4)	4.8 ± 0.8
8h daylight	GH	26.3 ± 1.2 (4)	3.7 ± 0.1
	PRL	35.4 ± 7.7 (4)	4.5 ± 0.4

a Values are means ± SE of mean. Units are arbitrary where the colorimetric density of 1 µg. of albumin stained with fast green is equivalent to 20 units.

b Percentage electrophoresable protein = 
$$\frac{\text{Band protein} \times 100}{\text{Total electrophoresed protein}}$$

c N = pool of 4 pituitaries.

DISCUSSION

The present study indicates that the band with an electrophoretic Rf value of 0.47 is a form of Atlantic salmon prolactin. This band was consistently present, possessed Na-retaining activity in hypophysectomized killifish and cross reacted significantly with anti-pollack prolactin. The band consisted of a slow and a fast moving component. It is not clear whether the RIA detectable PRL activity in eluates from gel segments not containing discrete bands was the result of nonspecific cross-reactions or an indication of further forms of the hormone. The existence of multiple forms of PRL and GH is well established among mammalian species and there is evidence that this multiplicity of bands extends to other tetrapods (Nicoll and Nichols, 1971). Several cases of an apparent conversion between bands have also been reported. Aggregation of  $^{135}\text{I}$  human GH has been described in response to freezing and thawing (Schwartz and Batt, 1973). Human GH has also been found to exhibit a concentration dependent self-association (Squire and Pederson, 1961). In addition, a variety of polymeric forms of PRL have been described in granule extracts from bovine pituitaries (La Bella et al., 1971) and in pure prolactins (Cheever and Lewis, 1969). More direct evidence for this type of transformation was reported after alkaline extraction of pituitaries from the blue shark, Prionace glauca, (Lewis et al., 1972). This procedure re-

sulted in the PRL-like and GH-like protein bands showing fast moving components. Yet all investigations on teleost pituitary electrophoretograms have presented these hormonal principles as single bands (Knight et al., 1970; Chadwick, 1970; Ball and Ingleton, 1973; Clarke, 1973a, b; Hattingh and du Toit, 1973; Farmer et al., 1975). Evidence from the current study suggests that salmon prolactin exists in the form of a two band complex and this can be considered as presumptive evidence for the existence of multiple hormonal components in teleosts.

Some earlier electrophoretic studies on the pituitaries of Poecilia, Tilapia, and Cichlasoma have revealed two distinct bands associated with this gland (Knight et al., 1970; Ball and Ingleton, 1973; Clarke, 1973a, b). As in the present study, the band identified with the rostral pars distalis has been attributed to prolactin. The other major band has generally been assumed to be attributable to growth hormone (knight et al., 1970; Chadwick, 1970; Ball and Ingleton, 1973; Clarke, 1973a, b; Hattingh and du Toit, 1973). The current study suggests that the band with a slightly variable electrophoretic mobility, found between Rf 0.33 and 0.41, was salmon growth hormone. In contrast to the aforementioned studies this band was not always very discrete but it was consistently associated with extracts high in PPD content and was the single band present in the only gel fraction that displayed a significant RIA detectable GH activity.

The identification of PRL and GH as bands with Rf values of 0.45-0.49 and 0.33-0.41, respectively, follows closely the values obtained for other species (Appendix, Table 2). In all the teleosts examined, except for Poecilia, PRL migrates ahead of GH (at or about pH 8.6). This is also true for all tetrapod prolactins with the exception of human PRL (Nicoll and Licht, 1971).

The bands with Rf values greater than 0.76 found in the present study were associated with fragments consisting of pars intermedia material and neurohypophyses and were therefore assumed to originate from one of these regions. The lack of rat vasopressor activity in extracts from the corresponding gel segments suggests that these bands were not vasopressor principles. Preliminary work related to the present study has also revealed that a modification of Wilhelmi's (1968) purification technique for canine GH, based mainly on ammonium sulfate fractionation, selects the bands with Rf values of 0.78, 0.84 and 0.94. It is therefore possible that these bands may have common properties. Unfortunately, sparse information is available about teleost pituitary electrophoretogram components other than prolactin and growth hormone. A fast moving band is found in Clarke's (1973a) study on Tilapia and Cichlasoma but the significance of this is not discussed. Hattingh and du Toit (1973) found that the faster migrating bands of the mudfish, Labeo umbratus, were associated with melanotropic, vasopressor and oxytocic activities. Unfortunately

the Rf values of these bands were not indicated. The neurophysins, a cystine-rich group of proteins of the hypothalamo-neurohypophyseal system associated with oxytocin and vasopressin have also been found to migrate in this area (Moen and Burford, 1973). But it is unlikely that the fast moving bands found in the current study were neurophysins as they did not exhibit the strong affinity for aldehyde fuchsin characteristic of these proteins.

The band with an Rf of 0.69 found in pituitary extracts appeared to be the same as that found at that Rf in serum and brain extracts. It stained lightly with aldehyde fuchsin or the PAS method. This suggests that the band was probably albumin.

It remains indeterminate whether other bands found in the Atlantic salmon electrophoretogram represented hormonal principles. Studies on mammalian pituitaries using a similar PAGE system have indicated that neither ACTH, TSH, nor LH yield discrete bands under these conditions, that is on 7.5% gels and at an alkaline pH.

A number of species such as paddlefish, Polyodon spathula, American catfish, Ictalurus nebulosus, white sturgeon, Acipenser transmontanus, bowfin, Amia calva, and chinook salmon, Oncorhynchus tshawytscha did not show any prominent bands after PAGE (Clarke, 1973a, b). This absence of banding in these species might suggest either a failure of the peptides to migrate as discrete bands under the experimental conditions used or that a minimal amount

of hormone was stored in the pituitary. The likelihood of the latter alternative appears to be suggested by the absence of sodium-retaining activity in the pituitary homogenates of these species.

Much of the difficulty in characterizing and purifying teleost PRL and GH can be attributed to the lack of suitable assays. The pigeon crop sac stimulating assay has never been found to yield positive results with any fish prolactin other than that from the lungfish, Protopterus, (Nicoll and Bern, 1965, 1968). However, Hattingh and du Toit (1972) reported some success using this assay on PRL from the mudfish, Labeo umbratus. Unfortunately, positive results with the pigeon crop stimulating assay might be attributable to inflammation and leucocyte infiltration rather than to a specific hormonal effect (Nicoll and Bern, 1968). An alternative assay which consists of a xanthophore dispersion reaction following the injection of small amounts of prolactin in the goby, Gillichthys mirabilis has been used in several studies (Sage and Bern, 1972). However, a recent study (Farmer et al., 1976) has suggested that the yellowing response characteristic of this assay may not be an effect of prolactin since purified fractions of the hormone were unable to elicit the response.

Prolactin has been shown to be the only pituitary hormone that has sodium-retaining ability in Poecilia (Ensor and Ball, 1968a, b) and in Fundulus (Pickford et al., 1965) and this forms the basis of a reliable bioassay for this

hormone. Bioassays based on the sodium-retaining activity of teleost prolactin have been used successfully by several workers using hypophysectomized Poecilia latipinna (Ensor and Ball, 1968a, b) and intact Tilapia mossambica (Clarke, 1973b; Farmer et al., 1975). The latter assay, though it does avoid the trauma and difficulty of a complicated hypophysectomy, has the severe limitations of less predictable plasma Na-levels, and requires relatively large prolactin doses during priming and testing. This assay also relies on an increase in sodium levels compared to controls. By contrast, in the hypophysectomized Poecilia assay, the absence of endogenous prolactin results in a continuous loss of sodium and death unless prolactin is administered. This assay yields clear differences between the experimental groups.

Of the various possible bioassays for prolactin the hypophysectomized Fundulus assay was considered to be the most practical for the present study. This animal is readily available on the Atlantic coast of north America and can be hypophysectomized with ease by way of the opercular opening. Hypophysectomized Fundulus survive longer in fresh water than do hypophysectomized Poecilia. Further, the decrease in plasma sodium levels is large and predictable (Ensor and Ball, 1968a, b; Griffith, 1974). But in spite of their potential as standard bioassay animals, Fundulus had not previously been used in this type of assay.

The bioassay results from the current study showed that

the band with an Rf value of 0.47 was the only electrophoresable material from Atlantic salmon pituitaries with Na-retaining activity. The response to a relatively small amount of material also suggests that this activity in salmon pituitaries may be quite powerful. This is significant, since the functions of prolactin in salmon, a fish that has yet to be hypophysectomized successfully, remain uncertain (Oliverreau, 1969).

The advantages of PAGE in this type of study are evident. Peptides can be separated and quantified or purified in two steps. Ideal purification would be obtained from distinct bands with a high relative mobility as this helps to eliminate the possibility of background contamination resulting from faster moving bands in the system. Where background is a problem, the fraction of choice could be electrophoresed before the electrophoretic elution is carried out. Further, various histological stains may be applied to control gels to aid in the identification of the bands.

There are, however, several shortcomings to this technique. Disc gel electrophoresis is not an ideal technique for the purification of large amounts of material. Further, the process of synthesis, storage and release of hormones appears to involve changes in both the structure and the activity of the hormones and it is not clear which fraction or portion of the total hormone is being determined. Nicoll (1972) has proposed a model to explain the "depletion", "repletion" and release mechanisms operating in the adeno-

hypophysis. This model, based on studies of GH and PRL, accounts for the existence of at least two forms of the intracellular hormones. One form is a storage form of low molecular weight and the other is an aggregated or bound ("macromolecular") form and shifts are possible between these two forms. Nicoll (1972) has further hypothesized that the "large" form is the presecretory or precursor form of the other. The suggestion is made that RIA detects both forms whereas PAGE and bioassay detect the small or storage form only. But PAGE and bioassay are not always in agreement (Nicoll, 1972). Of all these methods, only PAGE appears to reflect depletion. An obvious inconsistency in Nicoll's model is the failure of bioassay to detect the large or release form. Presumably, then, this form would have to undergo yet a further change since the final circulating form might be expected to be bioassayable.

Another model (Stachura and Frohman, 1974) postulates the existence of four distinct forms called large, big, small and little GH with an exchange between the large and small pools. But in this model the small form is the one which is released.

The above proposed models underline the difficulty in interpreting both qualitative and quantitative assay data based on extracted pituitaries. The described evidence suggests that PAGE reflects only the pre-secretory or storage pool of the hormones. Therefore, it would appear that if this pool is repleted as release occurs, any detectable

increase in the pool would reflect an increase in the pool size rather than an increased rate of secretion.

The current data suggest that photoperiod affects the PAGE detectable levels of PRL and GH. In fish acclimated to 12h daylight periods, GH levels changed more markedly after a 4h increase in light exposure and PRL responded the most to a 4h decrease. In general, more GH was stored under the 8 and 12h light regimens than under the 16h one. Prolactin also showed an increase with the short daylight regimen but the response to reduced light appeared to be much smaller when compared to that shown by GH. Photoperiod effects on these hormones are discussed further in the following section.

SECTION IV

SOME EFFECTS OF PHOTOPERIOD ON PLASMA IONIC LEVELS  
AND GROWTH OF ATLANTIC SALMON

## INTRODUCTION

Pre-adaptation to seawater and lower condition factors are characteristic of smoltification in salmonids (Hoar, 1939; Malikova, 1957; Pinder and Eales, 1969; Saunders and Henderson, 1970; Wagner, 1974a). As this pre-adaptation involves the development of hypoosmoregulatory mechanisms such as an increased specific activity of gill Na-K-ATPase (Wagner, 1974a), it seemed reasonable to expect changes in ionic composition during smoltification. Houston (1960) found that at the time of smoltification, Atlantic salmon showed a sharp decrease in plasma  $Cl^-$  while in fresh water. Moreover, differences in ion levels between parr and smolts have been reported (Fontaine, 1951; Parry, 1960, 1961; Houston and Threadgold, 1963; Fontaine *et al.*, 1969). But neither the changes in plasma ion levels in smolting salmon nor the effect of photoperiod on these parameters appears to have been studied in great detail. Most of the previous studies involving photoperiod manipulation of the parr-smolt transformation have dealt with the ability of the fish to regulate  $Na^+$  and  $Cl^-$  upon transfer to seawater at different times of the year (Koch, 1968; Wagner, 1974b).

Growth appears to play an important role in the pre-adaptation to seawater of salmonids. Salinity tolerance and the ability to regulate ions increase with size (Parry, 1958, 1960; Conte and Wagner, 1965) and this occurs in the absence of elevated gill Na-K-ATPase levels (Wagner, 1974b).

Wagner (1974) suggested that this development indicated the presence of an endogenous cycle in S. gairdneri which was unaffected by a variety of photoperiod regimens including constant daylight and constant darkness. This lack of a photoperiod effect is surprising since photoperiod was previously found to influence growth in Atlantic salmon (Saunders and Henderson, 1970). It appears therefore that the effect of photoperiod on the relationship between growth and ionic regulation deserves further investigation.

The stimulatory effects of long daily light periods in winter on growth and smoltification of Atlantic salmon have been discussed earlier in this thesis. In general, it was established that exposure to longer daylight periods during the winter was associated with an increase in growth, the development of salinity tolerance and a decline in the condition factor.

Based on a study of the effects of daily light exposure on the growth of green sunfish, Lepomis cyanellus, Gross et al. (1965) suggested that growth was more powerfully stimulated by increasing daily light exposure than by constant 16h daylight periods. Pyle (1967) reported that over a 94 week period brook trout, Salvelinus fontinalis, kept under constant daylight grew more than they did under a simulated natural photoperiod or when held in total darkness. No published information exists in the literature on the effects of constant daylight on the growth of Atlantic salmon. This is unfortunate since such a regimen appears

useful for studying the limitations of photoperiod manipulation and the possible existence of endogenous cycles in the physiology of the salmon.

The aims of this study were to observe the effects of constant daylight on growth and smoltification of Atlantic salmon parr and to compare these effects with those obtained under natural and reciprocal photoperiods as were used in an earlier section. Changes in pituitary hormone producing cells were monitored histologically and the hormonal levels were measured by polyacrylamide disc gel electrophoresis. Further, an analysis of plasma  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ , and  $\text{Ca}^{2+}$  was carried out on fish of the different experimental groups.

## MATERIALS AND METHODS

Atlantic salmon parr, aged 1+, of approximately 28cm in length were brought to the St. Andrews Biological Station from the Cobequid Fish Culture Station, Nova Scotia, on December 21, 1973. The fish were placed in freshwater in the same holding facilities described in an earlier section (Section I, Materials and Methods). The fish were divided into three groups of 275 fish each. The water temperature was gradually raised to 10°C as described previously. On December 27th the fish were placed under different experimental regimens consisting of a simulated natural photoperiod, the reciprocal photoperiod described earlier and a constant daylight regimen. Fish were fed Ewos pellet food 4P and 5P (Ewos, AB.) to satiation three times daily.

Morphometric determinations and sampling were carried out every three weeks on all fish. Eighteen fish from each group were killed on the sampling dates by a swift blow on the head. Tails were cut off the first twelve fish and blood from the caudal artery was collected in heparinized (ammonium heparin, Sherwood) centrifuge tubes. The blood samples were immediately centrifuged at 550g for 10 min. The plasma was removed and stored in glass vials at -25°C for ion analysis. The pituitaries from the first six fish from each group were removed and fixed in Bouin's fixative for histological study. The pituitaries from the remaining

twelve fish from each group were removed, blotted with filter paper and quickly frozen at  $-35^{\circ}\text{C}$ . The frozen pituitaries were later freeze dried and stored in a vacuum at  $-35^{\circ}\text{C}$  until analyzed by electrophoresis.

#### Histological Procedures

All tissues were processed as described in Section I. The tissue sections, 4  $\mu\text{m}$  in thickness, were stained by the PAS-OG-hematoxylin method for cellular differentiation and with methyl green-pyronin for RNA demonstration.

#### Plasma Ion Analysis

Plasma  $\text{Cl}^-$  was measured using a Buchler Cotlove automatic chloride titrator (model 4-2000, Buchler Instruments Inc.). Aliquots of plasma were diluted with 0.4% lanthanum chloride for the analysis of Na, K and Ca by flame emission spectrophotometry on a Jarell-Ash (Fisher Scientific) absorption-emission spectrophotometer. Duplicate samples were analyzed where possible.

#### Disc Electrophoretic Quantitation

Freeze dried pituitary samples were pooled in batches of four where possible and were ground to a fine powder. This powder was dissolved and electrophoresed by the methods described in Section III. Electrophoresis was carried out in 8.0cm X 0.4cm gels. All gels were stained with 1% fast green in 7% acetic acid for 16 hours and destained in several changes of 7% acetic acid. Gels which had been stained in

Fast Green tended to change colour from green to blue within a week or two. This was prevented by storing them in individual vials filled with 7% acetic acid in a dark cool place. Scanning densitometry was performed as described earlier. The quantification of protein per band was carried out by scanning densitometry using bovine albumin standards. These standards ranging from 1-2  $\mu\text{g}$  (Bovine albumin, Fraction V, Sigma Chemical Co.) were electrophoresed on individual gels included in every experimental run.

## RESULTS

### Effect of Photoperiod on Growth and Condition Factor

Salmon exposed to constant daylight (LL) grew faster than fish from the reciprocal (RP) or natural photoperiod (NP) regimens (Fig. 1 and 2) and were significantly longer ( $P < 0.01$ ) than both of these groups by early March (Fig. 3). Peak growth rates were recorded in all groups of fish during the interval between January and April and in June. Growth rates of the RP fish decreased during April and all groups showed decreased growth in May (Fig. 1). But by the end of the experiment the NP fish had approximated the RP fish in length. At this time, however, the LL fish remained significantly longer and heavier ( $P < 0.01$ ) than the fish from either of the other groups (Fig. 3 and 4).

Condition factors started to decline following exposure of the fish to longer light periods (Fig. 5). This occurred immediately following exposure in the LL and RP fish. The condition factor of the NP fish did not decline until the daylight period had increased to about 12h. The decline in condition factors coincided with the development of silvering, blackened fin margins and behavioural traits characteristic of smolts such as schooling behaviour and a decrease in aggressive behaviour.



Figure 1

Instantaneous growth rates in length of Atlantic salmon  
under natural, reciprocal and constant daylight photoperiod  
regimens at different times of the year.

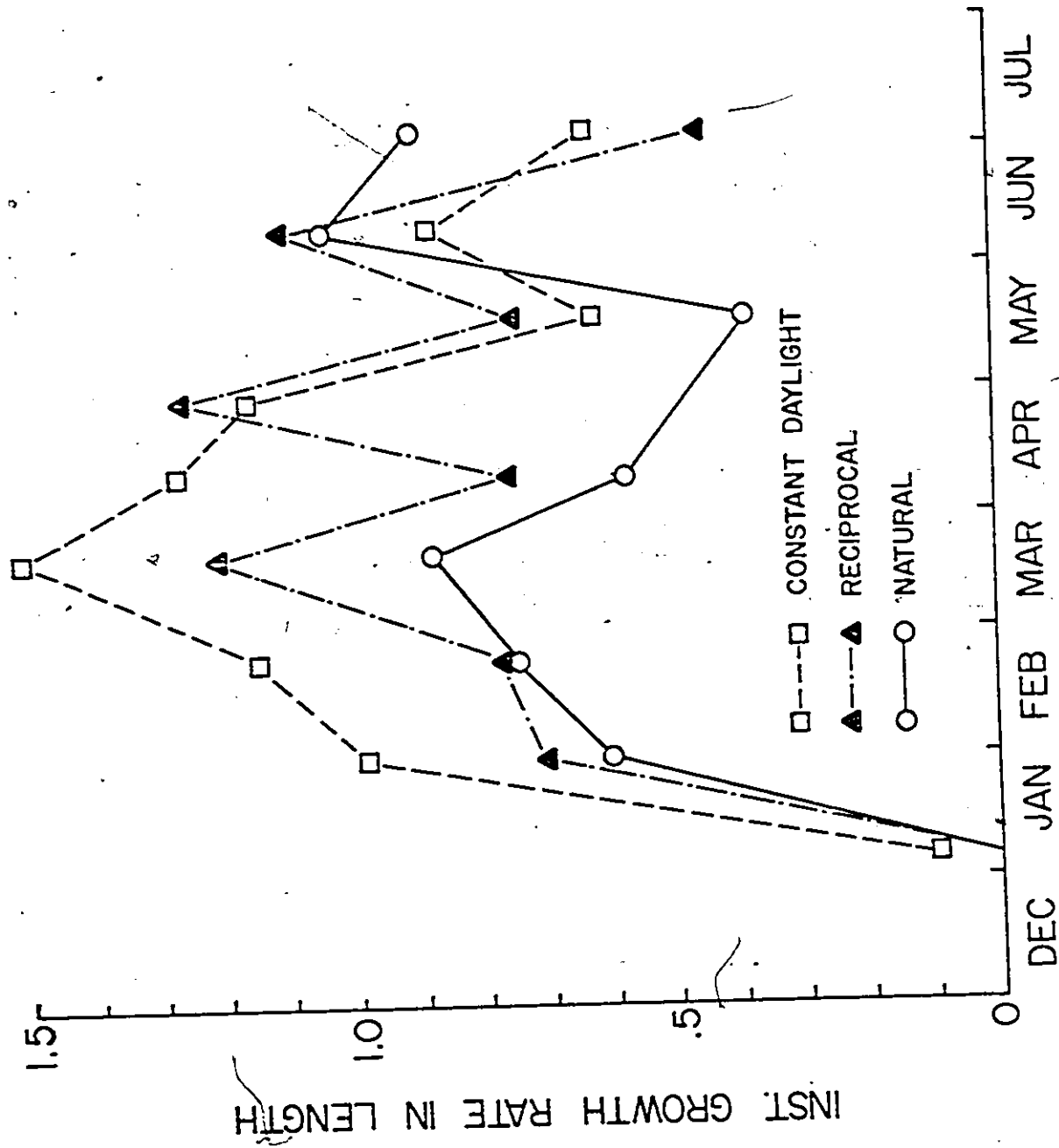
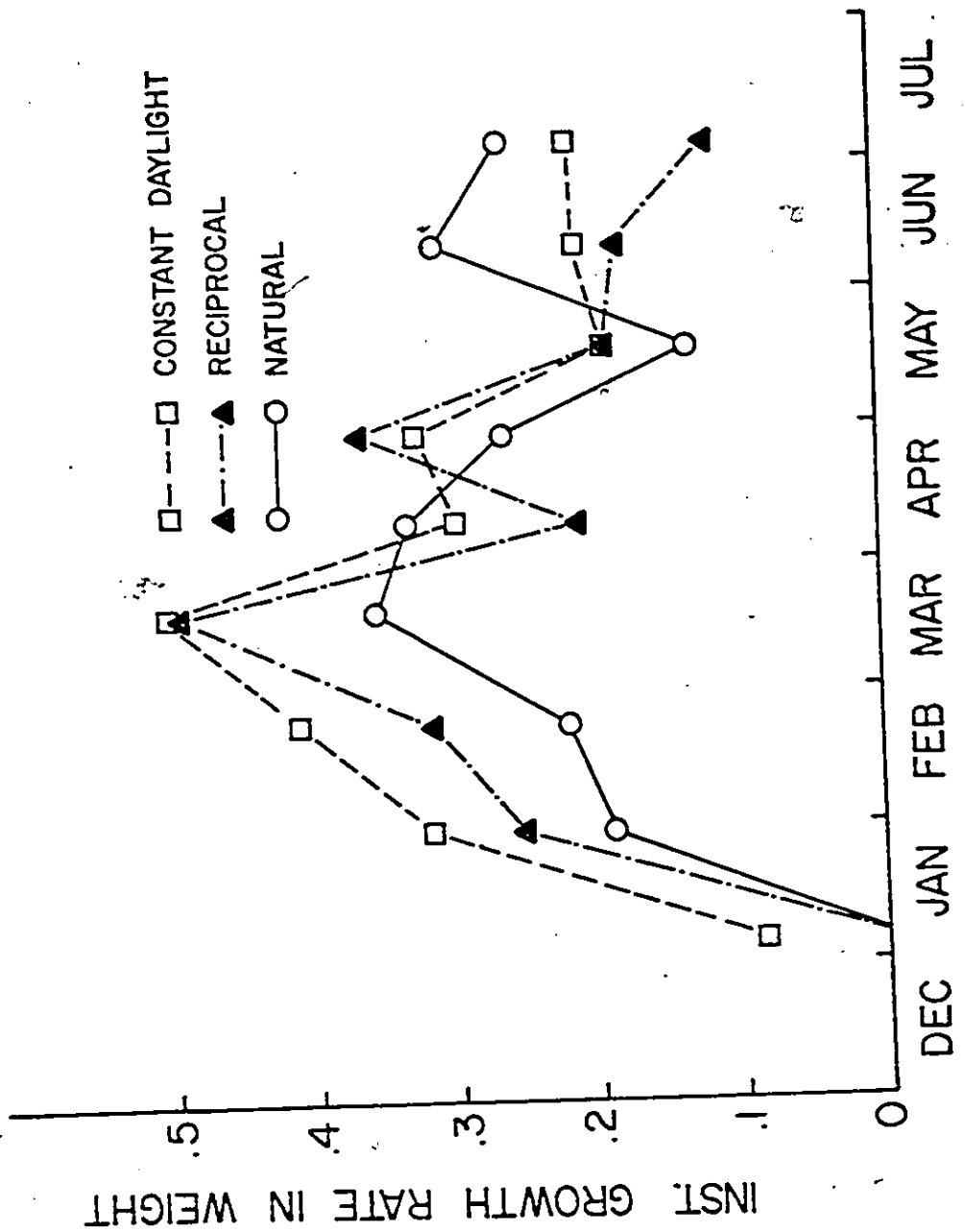


Figure 2

Instantaneous growth rates in weight of Atlantic salmon under natural, reciprocal and constant daylight photoperiod regimens at different times of the year.



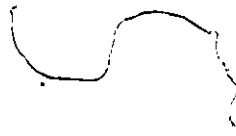


Figure 3

Mean lengths of Atlantic salmon under natural, reciprocal and constant daylight photoperiod regimens at different times of the year (99% confidence intervals for the means are indicated).

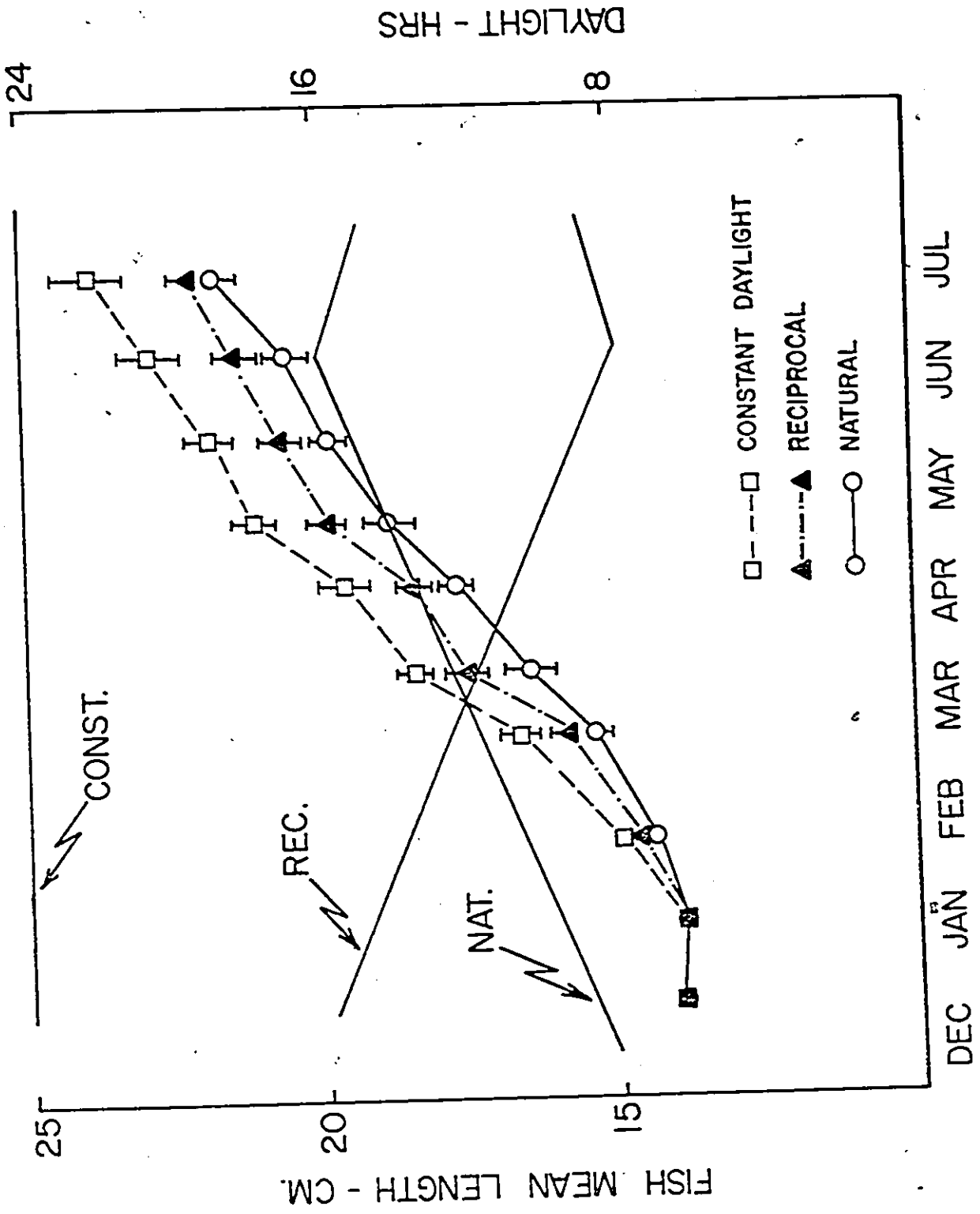
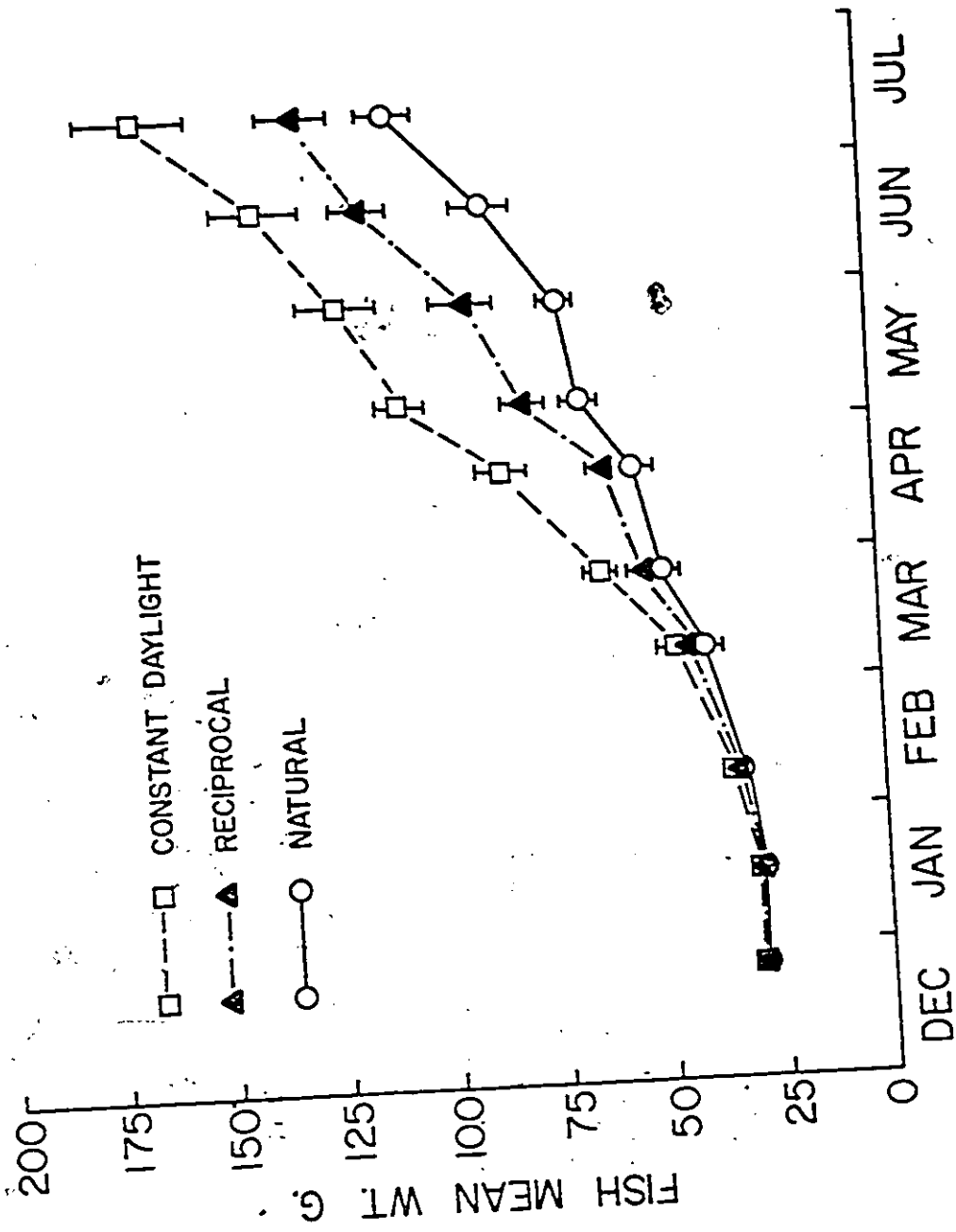


Figure 4

Mean weights of Atlantic salmon under natural, reciprocal and constant daylight photoperiod regimens at different times of the year (99% confidence intervals for the means are indicated).



2

26

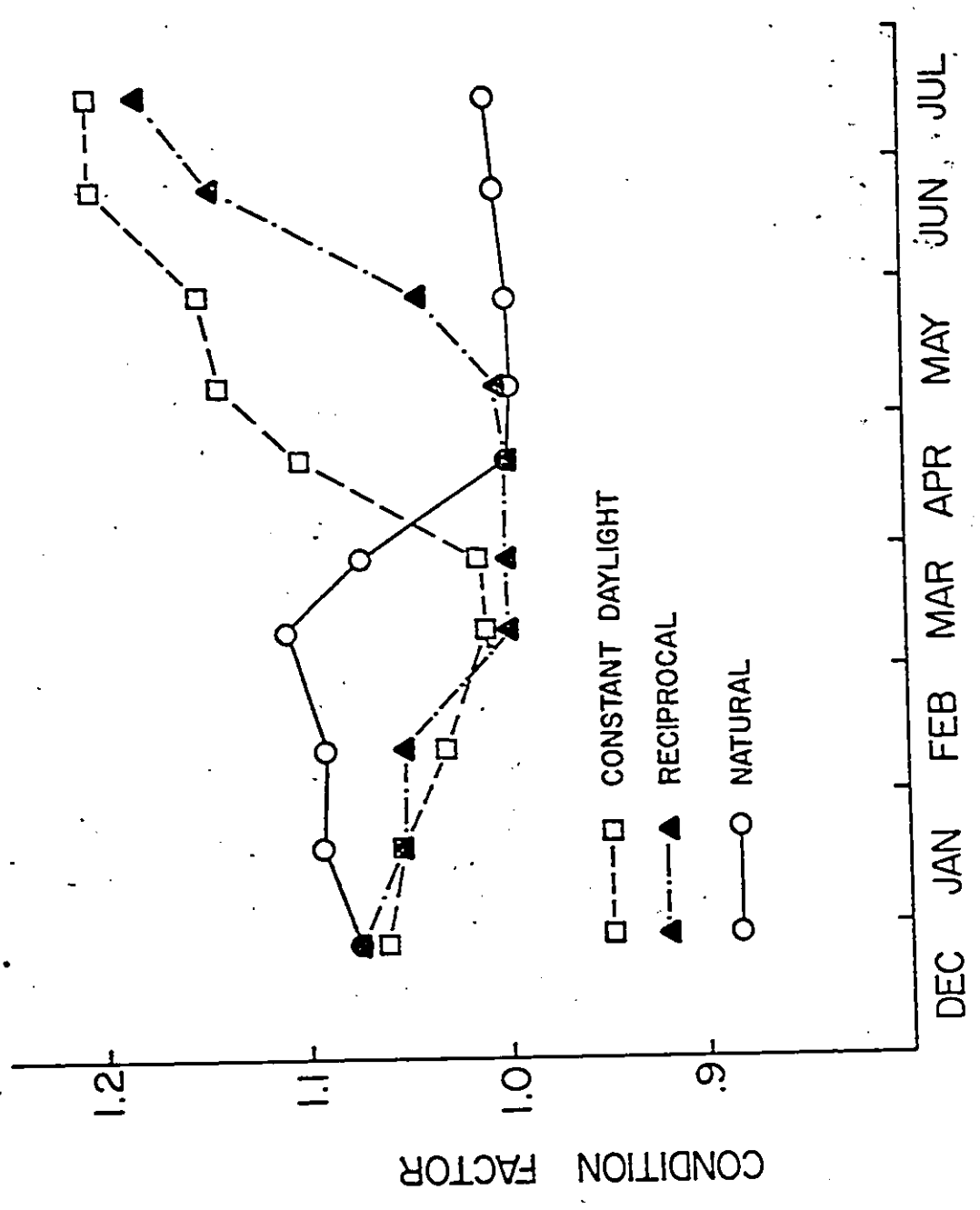
3



Figure 5

Mean condition factor of Atlantic salmon under natural, reciprocal and constant daylight photoperiod regimens at different times of the year.

$$\text{Condition factor} = \frac{100 \cdot W}{L^3}$$



▲

Effects of Photoperiod on Adenohypophyseal Cytology

Fish exposed to 12hr or more of light per day had a noticeable hypertrophy and hyperplasia of their GH cells. These cells also demonstrated an increased pyroninophilia. A moderate stimulation of prolactin, adrenocorticotropic and pars intermedia cells was also evident under the same conditions. Continuous light elicited a more marked activation of all of these cells and particularly of the GH cells (Fig. 6-9). The number of GH cells in the LL group was increased in February, and was about double that found in the NP group at the same time. In some instances the GH cells occupied as much as four-fifths of the adenohypophysis compared to the quarter or third seen in NP animals. Mitotic figures in the pituitaries of LL fish were numerous in all the cell types that were judged to be hyperactive. Several orange-G +ve cells which closely resembled GH cells but were polygonal in shape, instead of the elliptical form characteristic of somatotrops, were found invading the pars intermedia of LL fish in April. At the same time the prolactin cells of these fish exhibited extensive vacuolation (Fig. 9). Three weeks later the activated cells had all become less active and GH cells now occupied only about a third of the adenohypophyseal sectional area.

A slight increase in pyroninophilia was observed in the GH and PRL cells of LL fish in June. This increase was less evident in PRL and GH cells of the RP fish. The activation

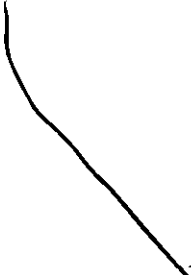
Figure 6

Proximal pars distalis from Atlantic salmon after 6 weeks exposure to continuous illumination starting in mid-December. Note increase in somatotrops (dark grey cells) compared to those in Fig. 7 below. Arrow indicates somatotrop in mitosis; NH, neurohypophysis. PAS-hematoxylin-OG. X320.



Figure 7

Proximal pars distalis from Atlantic salmon after 6 weeks exposure to a reciprocal photoperiod regimen starting in mid-December. NH, neurohypophysis. PAS-hematoxylin-OG. X320.



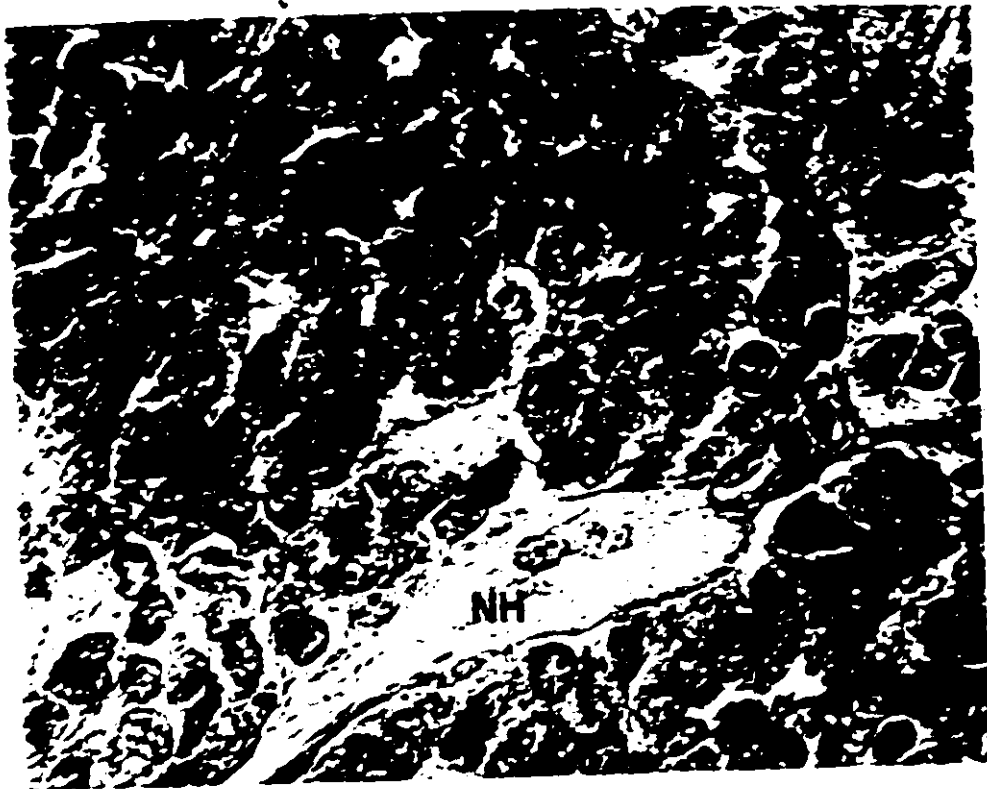
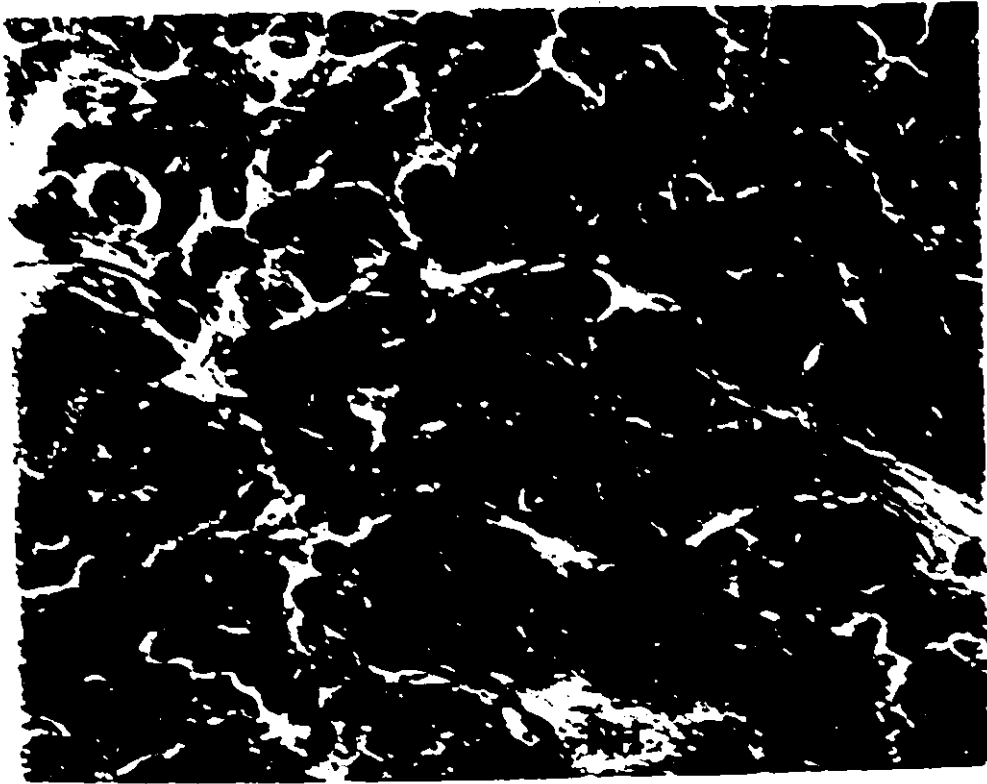


Figure 8

Rostral pars distalis from Atlantic salmon after 9 weeks exposure to continuous illumination. Arrows indicate ACTH cells in mitosis; rbc, red blood cell, NH, neurohypophysis. PAS-hematoxylin-OG. X320.

Figure 9

Follicular region of rostral pars distalis from Atlantic salmon after 4 months exposure to continuous illumination. Arrow indicates mitotic figure. Note vacuolation in prolactin cells. PAS-hematoxylin-OG. X800.



in both groups decreased gradually towards the end of the experiment. No increase in pituitary cell activity was observed in NP fish until May when a moderate stimulation of GH, PRL, ACTH and pars intermedia cells occurred.

With the exception of the GH cells in the proximal pars distalis which maintained sparse but definite orange-ophilic granulation most mitotic cells degranulated completely and were difficult to characterize. Several of the mitotic cells in the rostral pars distalis of LL fish were tentatively identified as thyrotrops on the basis of their location.

During December and January several parr in all groups showed distinct PAS +ve cells in the PPD. Gross anatomical inspection of the abdominal cavity of these animals revealed the presence of mature gonads or the pinkish coloured gonads characteristic of the regressing or reabsorbing sex organs in the salmon. In January, the PAS +ve cell types were less numerous and they disappeared completely by February. At that time, this portion of the PPD region appeared devoid of cells (Fig. 10 and 11). Subsequently, the void appeared to be filled by orange-G+ve cells in the faster growing groups of fish. However, chromophobes predominated in the area until June in fish under the natural photoperiod regimen. The disappearance of the PAS+ve cell type always appeared to precede any noticeable increase in the GH cell population.

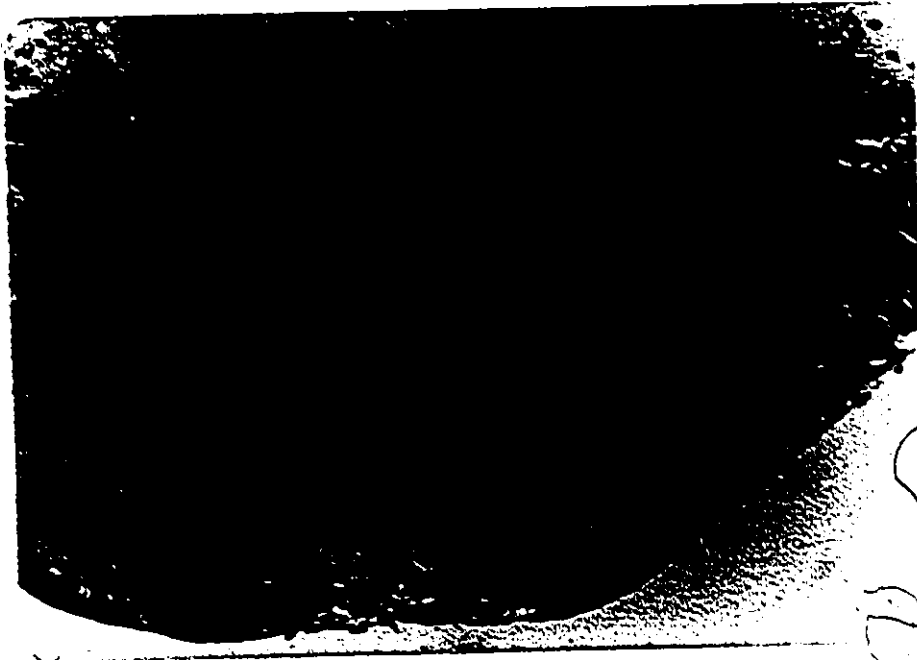
Figure 10

Mid-sagittal section through pituitary of Atlantic salmon parr. Note spaces left by disappearance of PAS+ve gonadotrops in the proximal pars distalis. PAS-hematoxylin-OG.

X125.

Figure 11

Enlarged portion of proximal pars distalis in preceding figure. PAS+ve cells are gonadotrops and Orange-G+ve cells are somatotrops. PAS-hematoxylin-OG.



Effect of Different Photoperiods on Disc-Gel Electrophoresable  
PRL and GH

Pituitaries from rapidly growing fish yielded few definite peaks on their electrophoretograms whereas those from slow growing fish showed erratic but definite banding.

The NP fish yielded definite peaks for GH and PRL between January and March. Faint bands or none were seen thereafter. The LL fish pituitaries only yielded consistent banding after April, when the putative PRL and GH fractions appeared as sizeable, well defined peaks. The disc electrophoretic patterns of pituitaries from RP fish pituitaries was similar to that of the LL ones except that GH banding was much less intense.

Effect of Photoperiod on Blood Electrolytes

Plasma levels of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$  and  $\text{Ca}^{2+}$  all exhibited cycles. These cycles did not appear to be seasonal or to be related to photoperiod (Fig. 12-15). Basic similarities appeared to exist for any one ion in all three experimental groups. But the phases of the cycles of different ions were not synchronous. For example, the  $\text{Ca}^{2+}$  levels of post-smolts rose sharply in June and July but at the same time their  $\text{Cl}^-$  levels decreased. In general, changes in the photoperiod caused only slight shifts in the temporal sequence of the cycles or altered the amplitude of the fluctuations. However, exposure of the fish to constant light virtually abolished the cycling of plasma  $\text{Cl}^-$ .

Figure 12

Plasma sodium concentrations of Atlantic salmon under natural, reciprocal and constant daylight photoperiod regimens, at 10°C, at different times of the year; points are means  $\pm$  SE. (N  $\geq$  7).

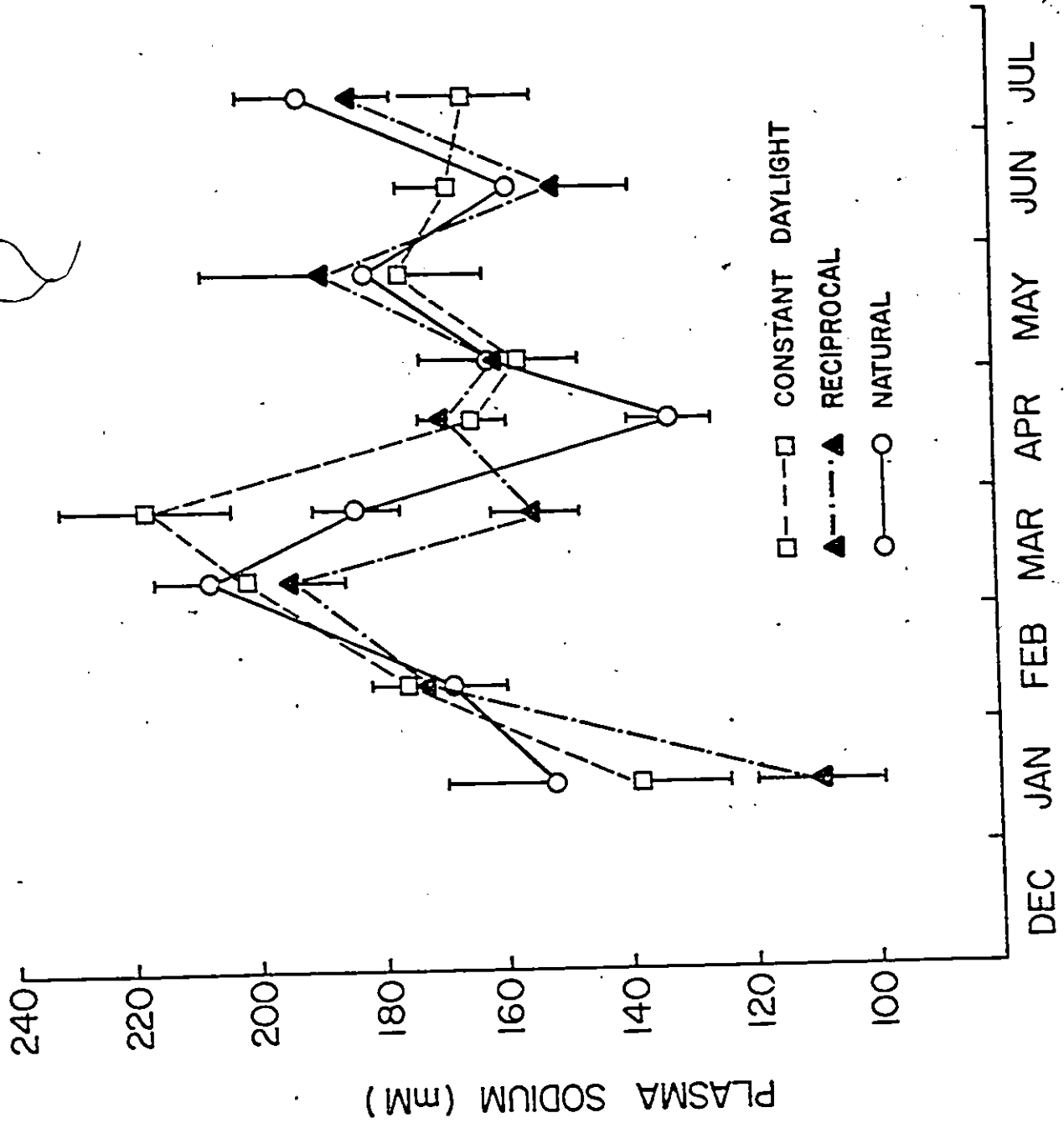




Figure 13

Plasma potassium concentrations of Atlantic salmon under natural, reciprocal and constant daylight photoperiod regimens, at 10°C, at different times of the year; points are means  $\pm$  SE. (N  $\geq$  7).

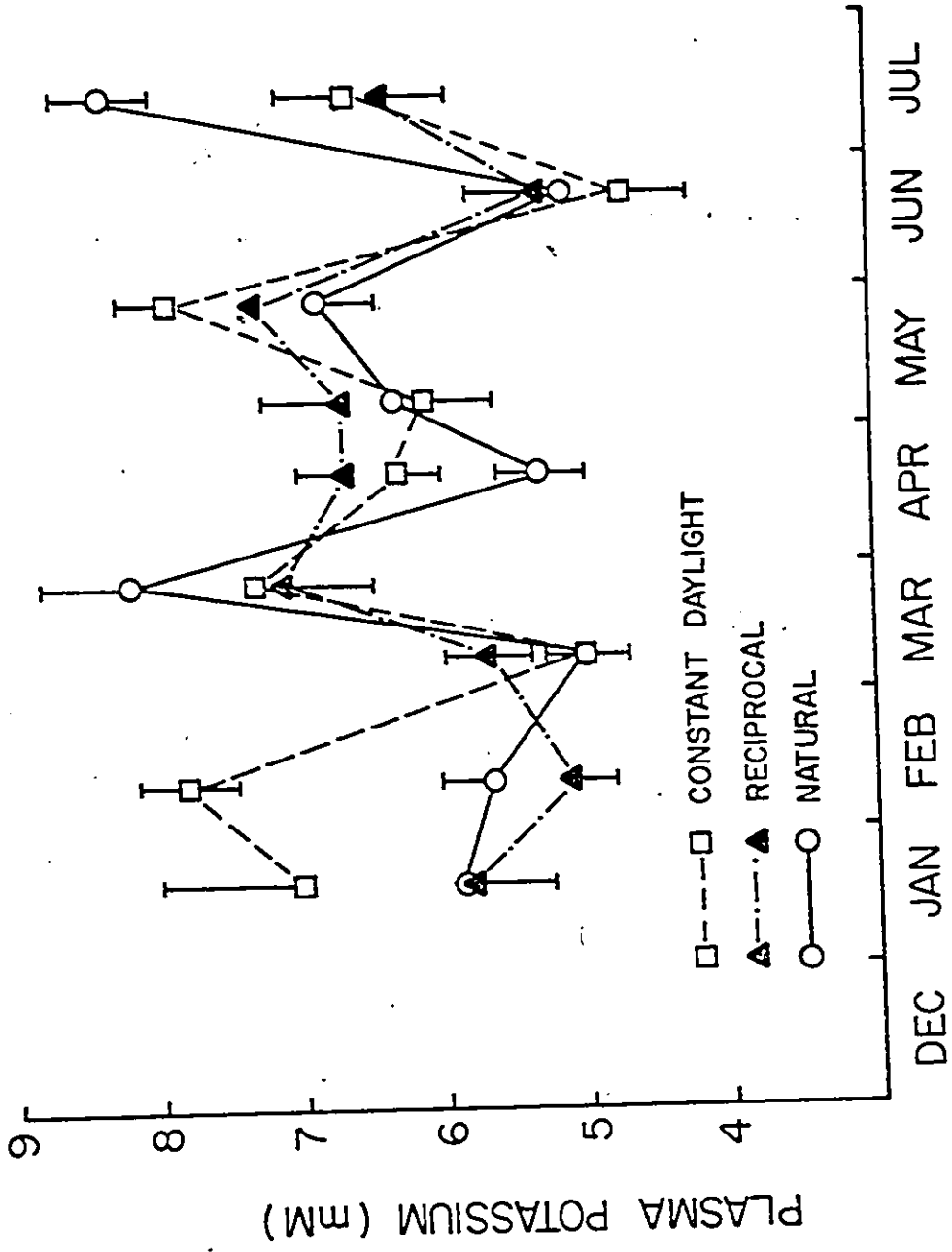


Figure 14

Plasma calcium concentrations of Atlantic salmon, under natural, reciprocal and constant daylight photoperiod regimens, at 10°C, at different times of the year; points are means  $\pm$  SE. (N  $\geq$  7).

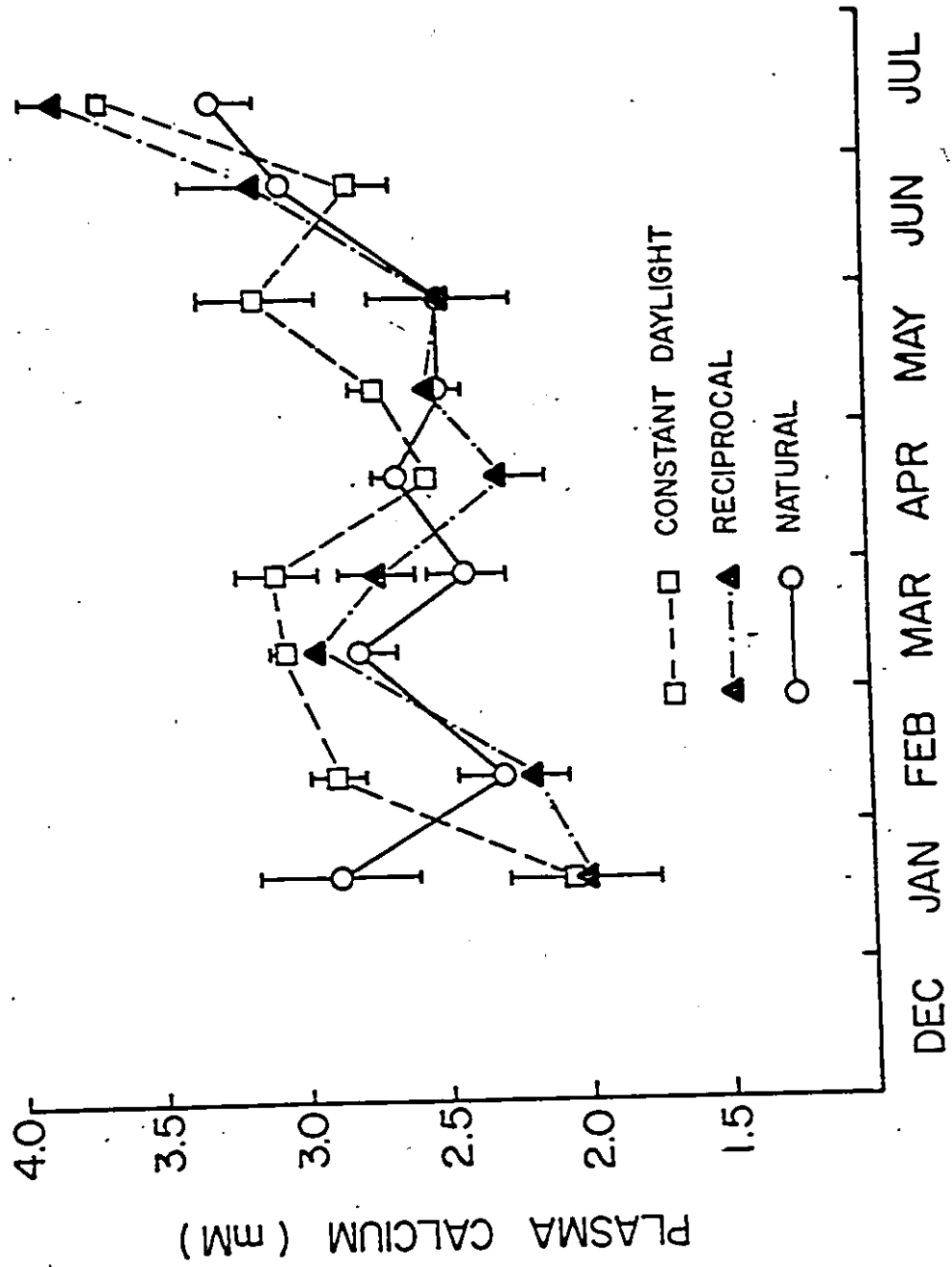
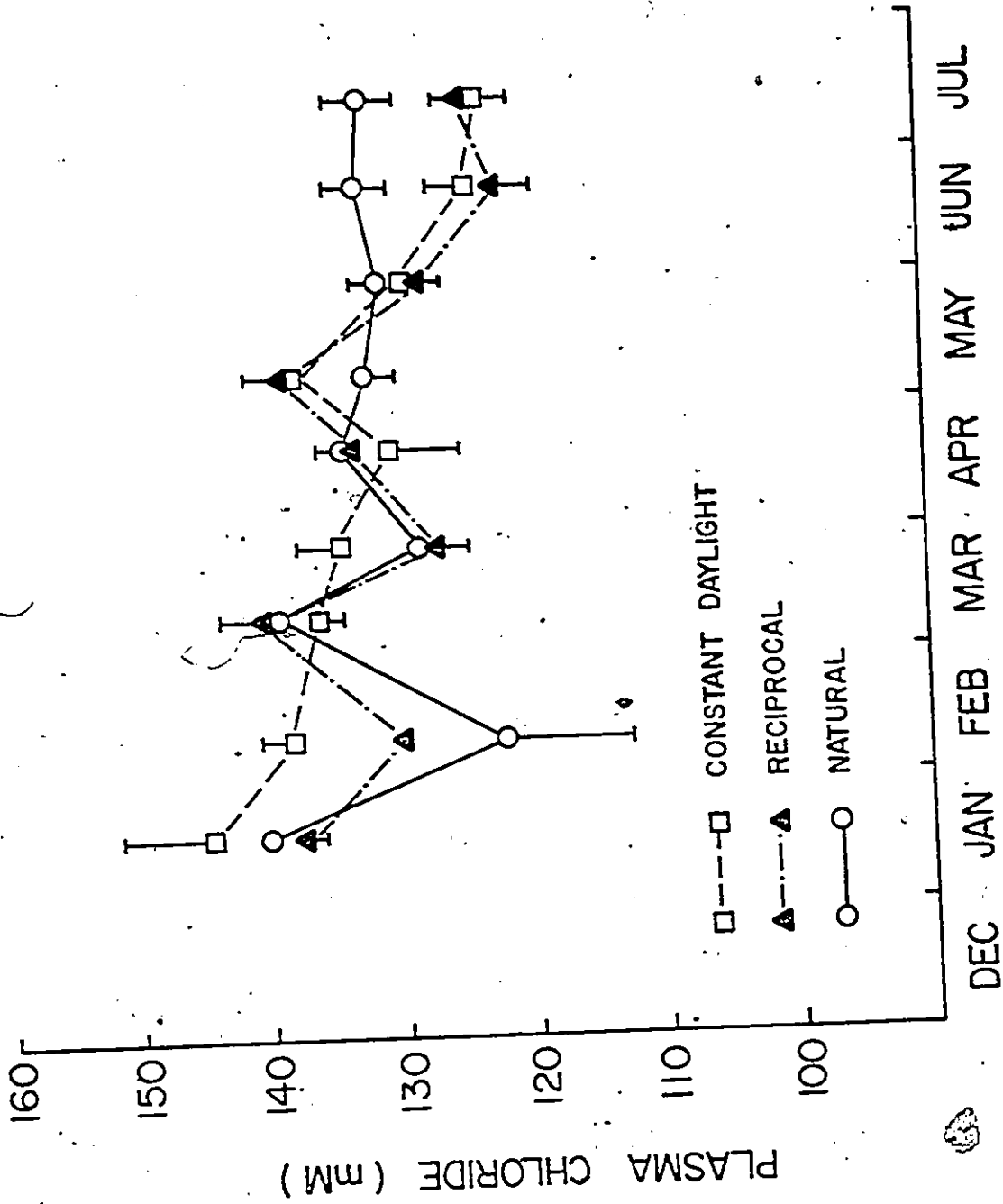


Figure 15

Plasma chloride concentrations of Atlantic salmon under natural, reciprocal and constant daylight photoperiod regimens, at 10°C, at different times of the year; points are means  $\pm$  SE. (N  $\geq$  7).



▲

## DISCUSSION

The results of this study concerning the photoperiodic effects on growth corroborate similar work discussed in section I. In short, longer daylight periods in December stimulated growth and resulted in an earlier smoltification. Concomitantly, various pituitary cell types which can be implicated in the various aspects of the processes of growth and smoltification were stimulated. The photoperiodic effects observed in the RP group appeared maximized in the LL group. Nevertheless, the results suggest that the duration of pituitary activation produced by continuous light is limited and that the magnitude of this activation occurs at the expense of the duration of the effect. Indeed, the subsequent regression of stimulated GH cells occurred more precipitously and somewhat earlier in the LL group than in the RP group.

These data also indicate that a basic biological rhythm exists in the growth patterns of the salmon that cannot be permanently blocked even by the effects of continuous daylight. Signs of such a pattern also appeared in Section I. It was generally found that, although photoperiod influenced growth rates, a number of peaks and decreases in the rates occurred that could not be related to photoperiod. For example, growth rates usually increased in January, March and June whereas a decrease was found in May. Similar observations and a failure to find a primary relation between growth rates of brown trout, Salmo trutta, and environmental variables

were previously reported by Swift (1955).


An extended period of increasing daylight does not seem to be necessary to stimulate growth. In the present study growth was stimulated during constant light conditions in the LL group, and the growth rates of the RP fish exposed to long daylight periods in December continued to increase even as daylight exposure was decreasing. This is in contrast with Wagner's (1974) suggestions that in steelhead trout, Salmo gairdneri, the rate of increase in daylight period length rather than the accumulated daylight hours acted as a cue. However, Wagner's experiment dealt with the photoperiodic manipulation of migration, and growth data were not presented. It is therefore not clear whether the two species respond differently to photoperiod or whether growth and migration are affected in two different ways. That is, migration may respond to an increasing daylight period whereas growth may be more sensitive to the total light received.

The histological observations in the present study are in close agreement with those made in Section I. Light, or shorter dark periods, appeared to stimulate GH, PRL, ACTH and pars intermedia cells. The presence of mitotic figures in the pituitaries of salmon has previously (Olivereau, 1969) been described as an index of activity. Olivereau (1969) termed the occurrence of two or more of the figures per histological section as an index of high activity. Since mitotic

figures were observed infrequently throughout the current work, their abundance in the LL fish pituitaries was deemed indicative of strong pituitary stimulation. In spite of the high apparent activity of cells other than GH cells, the volume of the pituitary occupied by GH cells increased the most. Further, this occurred partly at the expense of other cell types and mainly those of the pars intermedia. However, it is not likely that the cellular turnover rate of the pars intermedia cells was greatly enhanced because no signs of cellular debris were found in the area. This is an obvious inconsistency. An alternative possibility is that not all of the cell types in this region were stimulated. Due to the uncertainty of cell type identity in this area (Section I) this possibility was not investigated in detail.

It is noteworthy that GH cells were not found to be stimulated before the PAS +ve cells found in the PPD, presumed to be gonadotrops, had regressed or disappeared altogether. This finding does not necessarily imply a mutually exclusive situation. Yet, it must be considered in light of reports that sexual maturation coincides with periods of slow growth in various species of teleosts (Atz, 1957).

It is possible that the vacuolation observed in PRL cells of the LL fish which occurred towards the end of their strong activation was related to this stimulation. The significance of the appearance of the larger and more irregularly shaped orange-G+ve cells in the pars intermedia is not apparent. It seems likely that they represented GH producing cells.



Somatotrops in other species have been found to assume irregular shapes as a consequence of increased granulation (Ball and Baker, 1969). This would appear as a possible explanation since GH cells from pituitaries of rapidly growing fish were heavily granulated.

The absence of detectable fractions of PRL and GH from fish pituitaries which appeared highly active on histological examination might indicate a rapid turnover and little storage of these fractions. Whether these fractions represent the active forms of the hormones or short lived precursor forms remains uncertain. Previous results (Section III) suggested that the PRL band with  $R_f = 0.47$  displayed the sodium retaining activity characteristic of the hormone. But the presence of other types of biological activity in the electrophoretic fractions has not yet been studied. It is noteworthy that the histologically active pituitaries from the LL and RP groups in February and March yielded more prominent PRL and GH bands in April, when their histological activity was decreased, than did the equally little stimulated pituitaries from the NP group earlier in the year (Appendix, Table 3). This may be interpreted as an increase in the size of the PAGE detectable pool, in the fast growing fish, without concomitant storage. That is, an increased storage capacity would then be created leading to a larger detectable pool of the hormone when growth was reduced. But this problem is complicated by the fact that fish of slightly different sizes were compared at different times of the year.

It is obvious that many other parameters must be studied before the applications of disc gel electrophoresis may be fully exploited. This technique presents some problems in direct quantitation of pituitary peptides, but it may prove to be a useful tool for studying hormone depletion. In fact, Ball and Ingleton (1973) and Clarke (1973a) reported a decrease in PAGE detectable prolactin of several species of teleosts upon transfer from seawater to fresh water. These reports and the current work support the existence of an inverse relationship between release of the hormone and its content in the pituitary.

The blood analysis data from this study is highly intriguing. The existence of a rhythm in blood levels of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , or  $\text{Cl}^-$  in fresh water salmon has not been described before. However, Wagner (1974a) described seasonal changes in tolerance to seawater in Salmo gairdneri. This species develops salinity tolerance at the time of migration but also has a greater ability to regulate  $\text{Na}^+$  and  $\text{Cl}^-$  at other times of the year. In an earlier study, Conte and Wagner (1965) made similar observations on S. gairdneri. These workers suggested that "two smoltifications" appeared to take place during the first five months of the year. It is not known whether gill Na-K-ATPase develops during this first increase in salinity tolerance. But a study on S. gairdneri by Adams

et al. (1973) would suggest that, at 10°C, gill Na-K-ATPase activity increases early in the year, decreases sharply in May, and rises again thereafter. Unfortunately, this study was reported to have been plagued by disease which also necessitated formalin treatment of the animals.

A sharp decrease in plasma  $\text{Cl}^-$  attributed to an imbalance in osmoregulatory function during smoltification of S. salar has been described by Houston (1960). However, Koch (1968) suggested that the decrease reported by Houston (1960) had been exaggerated by the use of an unusual scale in the presentation of the data, but noted that changes in tissue chloride and water have also been described during smoltification of salmonids. However, Saunders and Henderson (1970) found no change in plasma  $\text{Cl}^-$  of smoltifying Atlantic salmon. Most other studies have only compared ionic levels between the plasma of parr and smolts. Parry (1961) found that during the freshwater phase Atlantic salmon smolts had higher plasma  $\text{Na}^+$  and lower  $\text{Cl}^-$  levels than did the parr. Fontaine et al. (1969) noted a drop in  $\text{Ca}^{2+}$  during smoltification of Atlantic salmon. It is interesting that in the latter study adults showed much higher plasma  $\text{Ca}^{2+}$  levels in April than in December.

Continuous daylight seemed to have a marked effect on all four ions. It is also to be noted that the cycles of the different ions studied were not in phase. For example, in March, the high  $\text{Na}^+$  levels found in all groups coincided with low  $\text{K}^+$  whereas in May and June both  $\text{Na}^+$  and  $\text{K}^+$  were at a peak.

It is tempting to suggest a relation between the drop seen in this study in plasma  $\text{Na}^+$  in NP fish in April with an increase in gill  $\text{Na}^+-\text{K}^+-\text{ATPase}$  reported to occur during smoltification in salmonids (Zaugg and Wagner, 1973). But other work (Kamiya, 1972) suggests that the pump does not activate in fresh-water. Indeed, the activation of a powerful  $\text{Na}^+$  extrusion mechanism in fresh water could lead to a sodium depletion and this might operate so as to stimulate a preference for saline media. Unfortunately, there is little proof for the existence of such a situation. 7

The similarity of the instantaneous growth patterns for weight and those of the ionic cycles might be indicative of growth related changes or a redistribution of body compartment components. In this respect it is probably significant that a decrease in thiocyanate and  $\text{Na}^+$  spaces have been demonstrated during periods of rapid growth of humans and avians (Fellers et al., 1949; Barlow and Manery, 1954; Medway and Kare, 1959). Comparison of ionic and growth cycles in the present study indicate major similarities in phase and period between the two before May but not thereafter. Furthermore, the condition factors do not suggest important changes in the relations of body dimensions that can be correlated with the ion cycles at any time. No consistent behaviour of any of the four ions could be noticed at smoltification when taking the condition factor as an indicator of the transformation. Hoar (1939) found that condition factors of salmon declined sharply at the time of

smoltification. But in the present study neither the beginning of the decline of the condition factors nor their lowest levels appeared to be related to a specific stage of an ionic cycle.

The rise in plasma  $\text{Ca}^{2+}$  levels observed in all groups in June and July and the concomitant decline in  $\text{Cl}^-$  levels were only slightly more pronounced in animals that had smolted before the NP group. This appears to indicate some modifying effect of photoperiod on the cycles of the ions studied.

GENERAL DISCUSSION

Photoperiod has been shown to be effective in controlling the growth rate of various species of teleosts (Gross et al., 1965; Saunders and Henderson, 1970). It has also been shown to exert an effect via the pituitary gland in ionic regulation (Lam, 1965) and to markedly affect the cytology of the pituitary gland (Rasquin and Rosenbloom, 1954). A similar action of light has been proposed in the regulation of the parr-smolt transformation which involves changes in osmoregulatory function and the process of growth (Hoar, 1965; Saunders and Henderson, 1970).

Photoperiod was found to affect growth in Salmo salar smolts (Saunders and Henderson, 1970), and to accelerate smoltification, as judged by a downstream movement of the fish, in Salmo gairdneri (Wagner, 1974) and, in this study, to influence growth and smoltification of Salmo salar parr. In the present study, the actions of photoperiod were paralleled at the cytological level by an activation of the GH cells as light periods became longer and growth rates increased. A lesser, but nevertheless noticeable activation of PRL, ACTH and pars intermedia cells indicated that the pituitary might mediate other photoperiod controlled parameters of smoltification in addition to growth.

The current study indicates that growth is probably under the principal influence of growth hormone since the rapid growth phase which occurs under long daylight periods could be mimicked by exogenous porcine GH administration.

It is, however, difficult to interpret increases in growth rates which occurred during summer months in fish held under short daylight periods and whose GH cells did not appear active on histological inspection. It is possible that other factors were responsible for these results. For example, the thyroid appears to possess an endogenous cycle in salmonids with one peak early in the year and a larger peak in summer (Eales, 1965). Thyroid hormone has growth promoting activity in mammals (Raben, 1973) and in fishes (Pickford, 1957). Moreover, since increases in growth rates in Swift's (1955) study and in the present one occurred at times of the year when thyroid activity might also be expected to increase, part of the seasonal growth pattern could be attributable to the action of thyroid hormone.

The occurrence of a significant stimulation of GH cells at the onset of smoltification and the promotion of growth by GH therapy in the current study might be indicative of important roles for GH in the metamorphosis. Moreover, the effects of GH therapy were elicited under conditions that were not consistent with high levels of production of any of the pituitary hormones (Section II). Therefore, if the presence of important quantities of contaminants in the GH preparation used in this experiment can be ruled out, the results would suggest that a variety of effects previously attributed to other salmon hormones might actually be elicited by porcine GH. Briefly, porcine GH therapy elicited pigmentary responses and promoted salinity tolerance. These actions

obviously warrant further investigation.

It also remains to be established whether the induction of growth and salinity tolerance by photoperiod manipulation or by hormone therapy is possible at times of the year other than those at which the present studies were conducted. Certainly, the work by Saunders and Henderson (1970) suggests that growth manipulation by photoperiod in salmon is also possible in March. However, it must be considered that maximum growth stimulation resulting from artificial long light periods will probably be obtained when the natural growth rate is low. This situation was found to occur more than once a year (Section IV).

It is probably significant that links between growth and the acquisition of salinity tolerance have been reported in the literature. Elson (1957) suggested that the attainment of 10cm in length by the fish may be necessary for smoltification to occur. However, genetic factors may underlie this qualification. Observations concerning growth regulation are of even more importance in the light of findings that faster growing salmon migrate earlier as smolts than slow growing ones (Pyefinch, 1955; Parry, 1958). From these studies the rate of growth would appear to be more important in smoltification than the attainment of a specific size.

Houston (1961) stressed the importance of the changes in gill area to body surface ratio that accompany growth. He argued that a decrease in this ratio might favour survival

in seawater. Further, Johnson (1973) speculated that GH may increase the differentiation rate of chloride cells. If this were demonstrated one might also surmise the existence of a parallel increase in gill Na-K-ATPase activity. However, the current study did not indicate size as being of primary importance. Indeed the timing of smoltification was more dependent on the type of photoperiod regimen than on the size of the fish.

The state of activation of prolactin cells has previously been shown to be seasonal, and stimulated by long daylight period regimens, in the stickleback, Gasterosteus aculeatus (Lam and Hoar, 1967; Lam and Leatherland, 1969). On the basis of prolactin cell activation during long daylight periods, the present investigation suggests that a similar situation may exist in the Atlantic salmon.

The current work also indicates the presence of sodium retaining activity in Atlantic salmon prolactin and this is in agreement with previous reports of this activity in the prolactin from other species (Ball, 1969). It also reinforces the hypothesis that prolactin may be a fresh water hormone in salmonids (Oliverreau, 1969). If this is the case, then the increased production of prolactin prior to and at the time of the catadromous migration, as demonstrated by the current study, becomes difficult to explain. But possible roles for prolactin in maintaining the activity of Na-K-ATPase at a minimum in fresh water (Kamiya, 1972) and in fat

mobilization (de Vlaming et al., 1974) have been suggested in other species. These roles would appear as important in the physiology of the smolting salmon as they would constitute means for preventing or lessening the osmoregulatory and energetic challenges inherent in the catadromous migration. The possibility that salmonid prolactins might share such functions is underscored by the findings that prolactin cells of eels (Hanke et al., 1969) and salmonids (Olivereau, 1969) become inhibited a few days after transfer from fresh to seawater. Similar findings have been made using in vitro experiments on salmonid prolactin cells (Ingleton et al., 1973). Thus, migration itself might result in at least a temporary reduction in prolactin secretion.

The present study suggests that a decrease in pituitary activity might occur following smoltification after the salmon has migrated seaward and as a result of shortening daylight periods. Since no work is available on this stage of the Atlantic salmon's life cycle it is not known if such a decrease might actually occur. But growth patterns of salmon at sea (Allen et al., 1972) appear to be similar to those found in fresh water. Thus, the effects of photoperiod may be similar in both media. However, the effect of seawater on pituitary hormones other than prolactin is not known. The need for further work in this area is underscored by observations that the transfer of


salmon from fresh water to seawater results in an increased utilization of GH (B.A. McKeown cited in Chester Jones, 1974).

The use of histology as a tool for assessing pituitary function in teleosts is well established (Ball, 1969) and for prolactin cells it has been found to agree with results obtained by various assays (Ball and Ingleton, 1973; Ingleton et al., 1973). Furthermore, responses to changes in environmental salinity are readily detectable in prolactin cells at the light microscopic level (Mattheij et al., 1971; Schreibman et al., 1973). It is possible that the appearance of the pituitary gland may vary somewhat within a few hours, as a result of circadian rhythms or otherwise (Leatherland, personal communication). However, well defined changes such as a pronounced hypertrophy, hyperplasia or the presence of mitotic figures, as were found in the current study, are usually considered to be signs of increased cellular activity (Olivereau, 1969) and are less likely to vary over short periods of time (Olivereau, personal communication). Less certainty can be attached to the interpretation of the degree of granulation present in a particular cell type as a measure of hormonal release. This is illustrated by the lack of degranulation found in the present study in GH cells of rapidly growing fish subjected to long daylight periods. This appeared to contradict the finding that these same fish showed little or no PAGE detectable GH. If it is assumed that granulation reflects hormone storage (Ball, 1969) this would

imply that the granules visible in GH cells of Atlantic salmon contain a form of the hormone which is not detectable by PAGE, possibly a precursor form.

The findings in the current study suggest that disc gel electrophoresis might be a potential tool for measuring depletion, or depletion and release, of hormones in Atlantic salmon. This corroborates earlier findings in other species (Ball and Ingleton, 1973; Clarke, 1973a). The indication is that the pituitary content of PAGE detectable PRL and GH might be inversely related to their secretion rates. These findings are in agreement with the present work. The present work also suggests that, although specific radioimmunoassays are not yet available for quantitating pituitary hormones in salmon, the technique can be used in the qualitative assessment of partially purified pituitary hormones. The possibility of marked cross reactions with more than one pituitary hormone cannot, however, be neglected.

Relkin's (1972a, b) findings in rats suggest that the pineal gland is involved in the reception and possibly in the mediation of photoperiod effects on prolactin and growth hormone. The pineal, a conspicuous structure in salmon, is particularly well adapted as a possible light receptor (Fenwick, 1970a). Further, it is possible that the hormone melatonin which has been demonstrated in fish pineals (Fenwick, 1970b) may have a messenger role in a "light-pituitary" axis. In this context it is interesting



that, in rats, an increased pineal function in the dark might be responsible for inhibiting secretion of GH releasing factor with a subsequent decrease in GH production and release (Belkin, 1972a). Thus, if a similar pathway is demonstrated in salmon, the decrease in the dark period rather than the increase in the light period would be responsible for an increased GH production.

Photoperiod might serve as a cue to regulate and coordinate all the individuals of any species. Hoar (1965) suggested that some salmonid species undergo physiological and behavioural cycles, and that each year these cycles preadapt them to seawater. These cycles are probably endogenous as constant illumination (Hoar, 1965) or constant darkness (Fontaine, 1965) do not abolish them. Evidence has since accumulated in favour of such an hypothesis. A circannual cycle in condition factors of Atlantic salmon is indicated by data from Pinder and Eales (1969). Smoltification appears to interrupt or terminate this rhythm. Rhythms are also suggested by data on blood serum levels of cholesterol and lipid-phosphorus of brown trout (McCartney, 1966, 1967) and in the activity of the salmonid thyroid gland (Eales, 1965). The present study suggests the presence of cycles in growth and blood electrolyte levels in Atlantic salmon. The endogenous nature of these cycles is suggested by the persistence of the basic patterns of most of these cycles under continuous light. Unfortunately, a complete analytical comparison of the cycles cannot be

made as the study only spanned part of a year.

The information available at the present time supports, to a large extent, Baggerman's (1960) interpretation of the events involved in the catadromous migration of salmonids. This hypothesis proposes the existence of external "priming" factors that prepare the animal to respond to external "releasing" factors which trigger migratory behaviour. Furthermore, this hypothesis also accommodates the existence of possible endogenous cycles. The acceptance of such a scheme would still rest on a fuller understanding of the precise role of the different environmental cues (zeitgeber) involved in it. The difficulty in assessing migration makes it difficult to understand the true impact of releasing factors. Clearly, photoperiod meets the qualifications of a priming factor as regards salmonids.

There is little evidence for a role of temperature as a "priming" factor with the exception of that found for the salmonid thyroid which is affected by temperature and photoperiod (Eales, 1965). What evidence has accumulated suggests that temperature is preponderantly a "releasing" factor (Baggerman, 1962). However, it is likely that temperature and photoperiod may both play some role as "primers" and "releasers". Differences in the growth response of hypophysectomized F. heteroclitus to standard doses of somatotropin at different temperatures (Pickford, 1953) suggests that temperature acts as a modulator of

effects of the pituitary hormones. In this sense photo-period might be regarded as having a "central" effect in contrast to a "peripheral" one for temperature.

Under the present state of the art, a model for smoltification must remain somewhat theoretical but there is now enough information available to indicate the necessity to review the significance of the parr-smolt transformation. This rather vague term describes a number of events (Fontaine, 1960) which are thought to be part of an extensive metamorphosis at one point in the life cycle of salmonids. This is contradicted by observations that salmon that fail to migrate will "desmoltify" (Malikova, 1957) and will once again smoltify next year. Further, changes involved in the metamorphosis have been experimentally induced independent of one another. In the current study, for example, salinity tolerance was induced by a GH treatment while parr marks remained visible. This substantiates field observations (Saunders, personal communication) that migrant salmon, particularly in the early part of the season, often display parr marks. Therefore, it is possible that silvering, and perhaps other traditional indicators of smoltification are not obligatory components of this transformation. Migration, which follows smoltification has been interpreted as a definite indication of the completeness of the transformation (Wagner, 1974). But migration is clearly a more difficult event to assess accurately and is often confused with mere escape behaviour (Hoar, 1968).

In re-evaluating the initial hypothesis of a photoperiod-pituitary axis in the mediation of the parr-smolt transformation of Atlantic salmon it is found that, within certain limitations, the evidence presented here strongly suggests the presence of such an axis. Photoperiod does influence the timing of smoltification in this species. Moreover, it is apparent that the pituitary is involved in the mediation of the effects, many of which can be attributed to specific hormones produced by this gland. Further, growth hormone is capable of accelerating growth rates during a slow growth phase imposed by short daylight periods. However, the seasonal periodicity of physiological function in Atlantic salmon imposes limitations to its manipulation by photoperiod.

SUMMARY

1. Atlantic salmon exposed to long daylight periods grew faster than those under short daylight.
2. Atlantic salmon under long photoperiods had more active pituitaries with the GH, PRL, ACTH, and Pars intermedia cells all showing signs of increased activity. The GH cells were judged to be activated the most.
3. Long daylight photoperiod regimens resulted in blackening of fin margins. This effect was seasonal and coincided with activation of pars intermedia cells.
4. Total muscle lipid declined under long daylight regimens.
5. Porcine growth hormone mimicked effects of long daylight exposure in Atlantic salmon held under short daylight periods. The hormone promoted growth and smoltification as indicated by increased growth rates, sharply lower condition factors, darkened fin margins, smolt-like behaviour and increased salinity tolerance.
6. Plasma levels of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Cl}^-$  described cycles that were not primarily related to the prevailing environmental conditions. Photoperiod did, however, modify the period and frequency of these cycles.
7. Atlantic salmon growth hormone and prolactin were identified as the PAGE bands with Rf's 0.39 and 0.47 respectively.

8. Atlantic salmon prolactin may exist in two forms.
9. The concentration of the growth hormone and prolactin bands on PAGE were inversely related to the apparent histological activation of the corresponding pituitary cells.
10. Atlantic salmon prolactin showed strong Na-retaining activity in hypophysectomized killifish.
11. Radioimmunoassay using anti-pollack GH or anti-pollack PRL detected Atlantic salmon growth hormone and prolactin. But anti-human GH and anti-human PRL did not cross react specifically with the salmon hormones.
12. Smoltification appeared to consist of a number of independent as well as interdependent changes.
13. The effect of photoperiod manipulation is limited to a temporary modification of some endogenous cycles.

SYNOPSIS

Even though limitations are imposed by apparent endogenous rhythms, it is now indicated that photoperiod manipulation can accelerate the growth and smoltification of Atlantic salmon. This potential may be useful in salmonid aquaculture. More rapid growth and an earlier release of the fish into seawater appear as economically desirable goals. A shortened fresh water stage is desirable as the retention of salmon in fresh water is a costly undertaking when compared to sea-ranching or allowing the fish to feed in the wild at sea and harvesting returning fish. Naturally, the receptivity of the fish to environmental manipulation, including photoperiod, temperature and salinity, probably depends greatly on the period of the year at which this is attempted and such manipulation does create certain problems. An advance in the timing of the metamorphosis such as that elicited in the current study would oblige an earlier transfer, or release, of the fish to seawater. This would clearly be hazardous in nature where environmental conditions will likely be hostile to the fish at that time of the year. The optimum environmental conditions for this transfer have yet to be studied.

Major advances in the study of photoperiodic control of endocrine processes will likely be made in the near future. Already it appears that some type of photoperiod control exists over pituitary function in mammals (Relkin,

1972a, b; Schams and Reinhardt, 1974; Wurtman, 1975). It is probable, therefore, that the study of light-endocrine relationships in teleosts will remain attractive for the comparative physiologist.

APPENDIX

TABLE 1

Relative mobilities (Rf's) of disc gel electrophoretic bands from pituitary extracts, serum, and brain tissue of Atlantic salmon.

---

<u>Serum</u>		<u>Pituitary</u>	<u>Brain</u>
0.03	0.29*	0.05	0.16
0.06	0.30*	0.10	0.20
0.08	0.34*	0.22*	0.35
0.10	0.41*	0.33	0.44
0.13*	0.45*	0.39*	0.50
0.14*	0.52*	0.41	0.60*
0.19	0.61	0.47*	0.69*
0.21	0.69*	0.49	
0.27*	0.77	0.55	
		0.61*	
		0.75	
		0.78*	
		0.84*	
		0.86	
		0.94*	

---

\* Major component of electrophoretogram.

TABLE 2

Disc-gel electrophoretic mobilities (Rf's) of fish growth hormones and prolactins at an alkaline pH.<sup>a</sup>

<u>Species</u>	<u>GH</u>	<u>PRL</u>	<u>Reference</u>
<u>Pleuronectes flesus</u>	--	0.80	Chadwick (1970)
<u>Anguilla anguilla</u>	0.38	0.46	Knight et al. (1970)
<u>Prionace glauca</u>	0.25	0.41	Lewis et al. (1972)
<u>Poecilia latipinna</u>	0.31	0.16	Ball and Ingleton (1973)
<u>Tilapia mossambica</u>	--	0.47	Clarke (1973a)
<u>Cichlasoma labiatum</u>	--	0.42	Clarke (1973a)
<u>Labeo umbratus</u>	(0.39) <sup>b</sup>	(0.45) <sup>b</sup>	Hattingh and du Toit (1973)
<u>Salmo salar</u>	0.39	0.47	Present study

a Davis (1964)

b Values estimated from data.

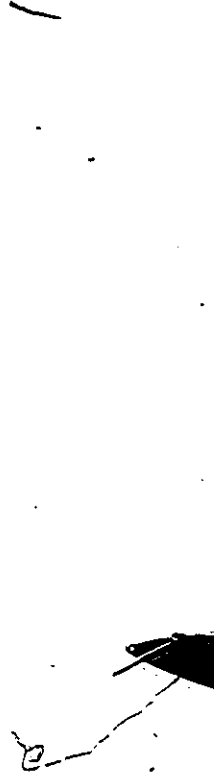


TABLE 3  
 Effects of Photoperiod on Disc-Gel Electrophoresable Pituitary Prolactin  
 (PRL) and Growth Hormone (GH) in Atlantic Salmon<sup>a</sup>

	Continuous Illumination		Natural Photoperiod		Reciprocal Photoperiod	
	PRL	GH	PRL	GH	PRL	GH
Dec. 27	24.0±2.5	6.0±1.0	20.0±4.5	7.0±0.7	21.5±4.2	6.0±0.8
Jan. 17	TR <sup>b</sup>	TR	15.0±6.0	5.5±1.6	-	TR
Feb. 8	TR	-	18.0±8.9	6.0±1.0	-	-
Mar. 6	-	TR	TR	TR	-	TR
Mar. 26	-	-	TR	TR	20.0±6.0	6.5±0.3
Apr. 18	30.2±2.0	8.0±0.2	-	-	23.0±0.6	7.0±0.2
May 5	28.8±3.8	8.0±0.2	TR	TR	20.0±2.0	8.5±0.4
May 29	33.1±3.0	8.5±0.5	-	-	32.0±2.3	9.0±0.3
June 18	37.4±2.8	8.5±0.5	-	-	30.0±2.4	8.5±0.2
Jul. 11	32.0±3.0	7.5±0.3	-	TR	34.0±3.0	8.5±2.0

<sup>a</sup> Values are means of arbitrary units ± SE. N = 4 pools of 3 pituitaries each in all cases.

<sup>b</sup> Trace.

LITERATURE CITED

- Abbott, F.S. 1973. Endocrine regulation of pigmentation in fish. *Am. Zool.* 13: 885-894.
- Adams, B.L., W.S. Zaugg and L.R. McLain. 1973. Temperature effect on parr-smolt transformation in steelhead trout (Salmo gairdneri) as measured by gill sodium-potassium stimulated adenosine triphosphatase. *Comp. Biochem. Physiol.* 44A: 1333-1339.
- Aler, G. 1971. The study of prolactin in the pituitary gland of the Atlantic eel (Anguilla anguilla) and the Atlantic salmon (Salmo salar) by immunofluorescence technique. *Acta Zool.* 52: 145-156.
- Allen, K.R., R.L. Saunders and P.F. Elson. 1972. Marine growth of Atlantic salmon (Salmo salar) in the north-west Atlantic. *J. Fish. Res. Board Can.* 29: 1373-1380.
- Anderson, R.O. 1959. The influence of season and temperature on growth of the bluegill Lepomis macrochirus (Rafinesque). Ph.D. Thesis. University of Michigan.
- Atz, J.W. 1957. The relation of the pituitary to reproduction in fishes. In: *The physiology of the pituitary gland of fishes.* Edited by G.E. Pickford and J.W. Atz. New York Zoological Society. New York, pp. 84-99.
- Baggerman, B. 1960. Salinity preference, thyroid activity and the seaward migration of four species of Pacific salmon (Oncorhynchus). *J. Fish. Res. Board Can.* 17: 296-322.
- Baggerman, B. 1962. Some endocrine aspects of fish migration. *Gen. Comp. Endocrinol. Suppl.* 1: 188-205.

- Baker, B.I. 1963. Effect of adaptation to black and white backgrounds on the teleost pituitary. *Nature* 198: 404.
- Baker, B.I. 1967. Factors affecting in vitro thyrotropic activity in teleosts. *Gen. Comp. Endocrinol.* 9: 430.
- Ball, J.N. 1961. On the food of the brown trout of Llyn Tegid. *Proc. Zool. Soc. (Lond.)* 137: 599-622.
- Ball, J.N. 1969a. Prolactin and osmoregulation in teleost fishes: a review. *Gen. Comp. Endocrinol. Suppl.* 2: 10-25.
- Ball, J.N. 1969b. Prolactin (fish prolactin or paralactin) and growth hormone. In *Fish physiology*. Vol. II. The endocrine system. Edited by W.S. Hoar and D.J. Randall. Academic Press, New York. pp. 207-240.
- Ball, J.N. and B.I. Baker. 1969. The pituitary gland: anatomy and histophysiology. In *Fish physiology*. Vol. II. The endocrine system. Edited by W.S. Hoar and D.J. Randall. Academic Press, New York. pp. 1-119.
- Ball, J.N. and D.M. Ensor. 1967. Specific action of prolactin on plasma sodium levels in hypophysectomized Poecilia latipinna (Teleostei). *Gen. Comp. Endocrinol.* 8: 432-440.
- Ball, J.N., and J.W. Jones. 1960. On the growth of the brown trout of Llyn Tegid. *Proc. Zool. Soc. (Lond.)* 134: 1-41.
- Ball, J.N., and P.M. Ingleton. 1973. Adaptive variations in prolactin secretion in relation to external salinity in the teleost Poecilia latipinna. *Gen. Comp. Endocrinol.* 20: 312-325.

- Barlow, J.S., and J.F. Manery. 1954. The changes in electrolytes, particularly chloride, which accompany growth in chick muscle. *J. Cell. Comp. Physiol.* 43: 165-191.
- Beckman, L. 1943. Annulus formation on the scales of certain Michigan game fishes. *Papers Mich. Acad. Sci.* 28: 281-312.
- Ben-David, M., and A. Chrambach. 1973. Electrophoretic identity of biologically active pituitary and serum prolactins in the lactating and perphenazine-treated rat. *Acta Endocrinol.* 72: 654-662.
- Bilton, H.T., and G.L. Robins. 1971. Effects of starvation, feeding, and light period on circulus formation on scales of young sockeye salmon (*Oncorhynchus nerka*). *J. Fish. Res. Board Can.* 28: 1749-1755.
- Bjorklund, R.G. 1958. The biological function of the thyroid and the effect of length of day on growth and maturation of goldfish *Carassius auratus* Linn. Ph.D. Thesis. University of Michigan, 171 p.
- Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37: 911-917.
- Brown, M.E. 1946. The growth of brown trout (*Salmo trutta*, L.) II. The growth of two year old trout at a constant temperature of 11.5°C. *J. Exp. Biol.* 22: 130-144.
- Brown, M.E. 1957. Experimental studies on growth. In

- Physiology of fishes. Vol. I. Edited by M.E. Brown.  
Academic Press, Inc., New York. pp. 361-400.
- Chadwick, A. 1970. Pigeon crop sac-stimulating activities  
of the flounder (Pleuronectes flesus). J. Endocrinol.  
47: 463-469.
- Chartier-Baraduc, M.M. 1959. Influence de l'hormone soma-  
totrope sur les teneurs en eau et en électrolytes du  
plasma et du muscle de la truite arc-en-ciel (Salmo  
gairdnerii). C.R. Acad. Sci. Paris 153: 1757-1761.
- Cheever, E.V. and U.J. Lewis. 1969. Estimation of the  
molecular weights of the multiple components of growth  
hormone and prolactin. Endocrinology 85: 465-473.
- Cheever, E.V., B.K. Seavey and U.J. Lewis. 1969. Prolactin  
of normal and dwarf mice. Endocrinology, 85: 698-703.
- Chester Jones, I., J.N. Ball, I.W. Henderson, T. Sandor, and  
B.I. Baker. 1974. Endocrinology of fishes. In Chemical  
Zoology. Vol. 8. Deuterostomians, cyclostomes and fishes.  
Edited by M. Florin and B.T. Scheer. Academic Press.  
New York. pp. 524-578.
- Clarke, W.C. 1973a. Disc-electrophoretic identification of  
prolactin in the cichlid teleosts Tilapia and Cichla-  
soma and densitometric measurement of its concentration  
in Tilapia pituitaries during salinity transfer experi-  
ments. Can. J. Zool. 51: 687-695.
- Clarke, W.C. 1973b. Sodium-retaining bioassay of prolactin  
in the intact teleost Tilapia mossambica acclimated to  
seawater. Gen. Comp. Endocrinol. 21: 498-512.

- Conte, F.P., and H.H. Wagner. 1965. Development of osmotic and ionic regulation in juvenile steelhead trout Salmo gairdneri. Comp. Biochem. Physiol. 14: 603-620.
- Davis, B.J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. 121: 404-427.
- Deason, H.J., and R. Hile. 1947. Age and growth of the kiyi, Leucocichthys kiyi, Koelz, in lake Michigan. Trans. Am. Fish. Soc. 74: 88-142.
- de Vlaming, V.L., M. Sage, C.B. Charlton and B. Tiegs. 1974. The effects of melatonin on lipid deposition in cyprinodontid fishes and on pituitary prolactin activity in Fundulus similis. J. Comp. Physiol. 94: 309-319.
- Donaldson, E.M. 1973. Reproductive endocrinology of fishes. Am. Zool. 13: 909-927.
- Donaldson, E.M., F. Yamazaki and W.C. Clarke. 1968. Effect of hypophysectomy on plasma osmolarity in goldfish and its reversal by ovine prolactin and a preparation of salmon pituitary "prolactin". J. Fish. Res. Board Can. 25: 1497-1500.
- Eales, J.G. 1965. Factors influencing seasonal changes in thyroid activity in juvenile steelhead trout, Salmo gairdneri. Can. J. Zool. 43: 719-729.
- Eisler, R. 1957. The influence of light on the early growth of chinook salmon. Growth, 21: 197-203.
- Elson, P.F. 1957. The importance of size in the change from parr to smolt in Atlantic salmon. Can. Fish. Cult. 21: 1-6.

Ensor, D.M., and J.N. Ball. 1968a. A bioassay for fish prolactin (paralactin). Gen. Comp. Endocrinol. 11: 104-110.

Ensor, D.M., and J.N. Ball. 1968b. Prolactin and fresh water sodium fluxes in Poecilia latipinna (Teleostei). J. Endocrinol. 41: xvi.

Farmer, S.W., W.C. Clarke, H. Papkoff, R.S. Nishioka, H.A. Bern, and C.H. Li. 1975. Studies on the purification and properties of teleost prolactin. Life Sci. 16: 149-158.

Fellers, F.X., H.L. Barnett, K. Hare, and H. McNamara. 1949. Changes in thiocyanate and sodium<sup>24</sup> spaces during growth. Pediatrics, 3: 622-629.

Fenwick, J.C. 1970a. Effects of pinealectomy and bilateral enucleation on the phototactic response and on the conditioned response to light of the goldfish, Carassius auratus. Can. J. Zool. 48: 175-182.

Fenwick, J.C. 1970b. Demonstration and effect of melatonin in fish. Gen. Comp. Endocrinol. 14: 86-97.

Finney, D.J. 1952. Probit Analysis. 2nd ed. Cambridge Univ. Press. London and New York.

Follenius, E. 1963. Ultrastructure des types cellulaires de l'hypophyse de quelques poissons téléostéens. Arch. Anat. Microscop. Morphol. Exp. 52: 429-468.

Follenius, E. 1965. Bases structurales et ultrastructurales des corrélations hypothalamo-hypophysaires chez quelques espèces de poissons téléostéens. Ann. Sci. Nat. Zool. 7: 1-150.

- Fontaine, M. 1951. Sur la diminution de la teneur en chlore du muscle des jeunes saumons (smolts) lors de la migration. C.R. Acad. Sci. Paris, 232: 2477.
- Fontaine, M. 1965. Quelques aspects des mécanismes physiologiques des phénomènes migratoires. In La distribution temporelle des activités animales et humaines. Edited by R. Chauvin. VIème session d'études de l'union internationale des sciences biologiques. Masson et Cie (Paris).
- Fontaine, M., M.M. Chartier-Baraduc, J. Deville, E. Lopez and M. Poncet. 1969. Sur les variations de la calcémie observées chez Salmo salar L. à diverses étapes de son cycle vital. Leur déterminisme endocrinien et leur intervention éventuelle sur le comportement. C.R. Acad. Sci. Paris, 268: 1958-1961.
- Fontaine, M., and M. Olivereau. 1949. L'hypophyse du saumon (Salmo salar, L.) à diverses étapes de sa migration. C.R. Acad. Sci. Paris, 228: 772-774.
- Forrest, J.N., A.D. Cohen, D.A. Schon, and F.H. Epstein. 1973. Na transport and Na-K-ATPase in gills during adaptation to seawater: effects of cortisol. Am. J. Physiol. 224: 709-713.
- Gerking, S.D. 1966. Annual growth cycle, growth potential and growth compensation in the bluegill sunfish in Northern Indiana lakes. J. Fish. Res. Board Can. 23: 1923-1956.
- Gorovsky, M.A., K. Carlson and J.L. Rosenbaum. 1970. Simple method for quantitative densitometry of polyacrylamide

- gels using fast green. Anal. Biochem. 35: 359-370.
- Griffith, R.W. 1974. Pituitary control of adaptation to fresh water in the teleost genus Fundulus. Biol. Bull. 146: 357-376.
- Gross, W.L., E.W. Roelófs, and P.O. Fromm. 1965. Influence of photoperiod on growth of green sunfish, Lepomis cyanellus. J. Fish. Res. Board Can. 22: 1379-1386.
- Hanke, W., K. Bergerhoff and D.K.O. Chan. 1969. Histological and histochemical changes of organs associated with adjustment to the environment. Gen. Comp. Endocrinol. 2: 331-341.
- Hattingh, J., and P.J. du Toit. 1973. Partial separation of the pituitary proteins of Labeo umbratus; Smith (mudfish). J. Fish. Biol. 5: 41-47.
- Hoar, W.S. 1939. The weight-length relationship of the Atlantic salmon. J. Fish. Res. Board Can. 4: 441-460.
- Hoar, W.S. 1958. The evolution of migratory behaviour among juvenile salmon of the genus Oncorhynchus. J. Fish. Res. Board Can. 15: 391-428.
- Hoar, W.S. 1963. The endocrine regulation of migratory behaviour in anadromous teleosts. Proc. XVI Internat. Congr. Zool., 3: 14-20.
- Hoar, W.S. 1965. The endocrine system as a chemical link between the organism and its environment. Trans. R. Soc. Can. Ser. IV. Vol. III, Sect. III: 175-200.
- Hoar, W.S. 1966. Hormonal activities of the pars distalis in cyclostomes, fish and amphibia. In "The pituitary

- gland". Vol. 1. Edited by G.W. Harris and B.T. Donovan. Butterworth, London and Washington, D.C. pp. 242-294.
- Hoar, W.S. 1968. Comments on the contribution of physiological and behavioural studies to the ecology of salmon in streams. In H.R. MacMillan lectures in fisheries. Symposium on salmon and trout in streams. Edited by T.G. Northcote. Institute of fisheries. Univ. British Columbia. pp. 177-180.
- Hodges, D.R., and W.H. McShan. 1970. Electrophoretic separation of hormones associated with secretory granules from rat anterior pituitary glands. *Acta Endocrinol.* 63: 378-384.
- Holmes, R.L., and J.N. Ball. 1974. The pituitary gland, a comparative account. Cambridge Press, London.
- Houston, A.H. 1960. Variations in the plasma level of chloride in hatchery reared yearling Atlantic salmon during parr-smolt transformation and following transfer into sea water. *Nature (Lond.)*, 185: 632-633.
- Houston, A.H. 1961. Influence of size upon the adaptation of steelhead trout (Salmo gairdneri) and chum salmon (Oncorhynchus keta) to sea water. *J. Fish. Res. Board Can.* 18: 401-415.
- Houston, A.H., and L. Threadgold. 1963. Body fluid regulation in smolting Atlantic salmon. *J. Fish. Res. Board Can.* 20: 1355-1369.
- Ingleton, P.M., B.I. Baker and J.N. Ball. 1973. Secretion of

- prolactin and growth hormone by teleost pituitaries  
in vitro. I. Effect of sodium concentration and  
osmotic pressure during short-term incubations. J.  
Comp. Physiol. 87: 317-328.
- Johnson, D.W. 1973. Endocrine control of hydromineral  
balance in teleosts. Am. Zool. 13: 799-818.
- Jones, A.E., J.N. Fischer, U.J. Lewis and W.P. VanderLaan.  
1965. Electrophoretic comparison of pituitary glands  
from male and female rats. Endocrinology, 76: 578-583.
- Kamiya, M. 1972. Hormonal effect on Na-K-ATPase activity in  
the gill of Japanese eel, Anguilla japonica, with special  
reference to seawater adaptation. Endocrinol. Japon.  
19: 489-493.
- Knight, P.J., P.M. Ingleton, J.N. Ball and M.P. Hancock. 1970.  
Separation and identification of eel prolactin by disc  
electrophoresis. J. Endocrinol. 48: xxix-xxxii.
- Knowles, F., and L. Vollrath. 1966. Neurosecretory innerva-  
tions of the pituitary of the eels Anguilla and Conger.  
I. The structure and ultrastructure of the neuro-  
intermediate lobe under normal and experimental conditions.  
Phil. Trans. R. Soc. (Lond.) B250: 311-327.
- Koch, H.J.A. 1968. Migration. In Perspectives in endocri-  
nology. Hormones in the lives of lower vertebrates.  
Edited by E.J.W. Barrington and C.B. Jørgensen. Academic  
Press. New York. pp. 305-349.
- Kragt, C.L., and J. Meites. 1966. Separation of rat anterior

- pituitary hormones by polyacrylamide gel electrophoresis. Proc. Soc. Exp. Biol. Med. 121: 805-808.
- Kurnick, N.B. 1955. Pyronin Y in the methyl-green-pyronin histological stain. Stain Technol. 30: 213-230.
- LaBella, F., M. Krass, W. Fritz, S. Vivian, S. Shin and G. Queen. 1971. Isolation of cytoplasmic granules containing growth hormone and prolactin from bovine pituitary. Endocrinology 89: 1094-1102.
- Lam, T.J., and W.S. Hoar. 1967. Seasonal effects of prolactin on freshwater osmoregulation of the marine form (trachurus) of the stickleback Gasterosteus aculeatus. Can. J. Zool. 45: 509-516.
- Lam, T.J., and J.F. Leatherland. 1969. Effect of prolactin on freshwater survival of the marine form (trachurus) of the threespine stickleback, Gasterosteus aculeatus, in the early winter. Gen. Comp. Endocrinol. 12: 385-394.
- Landgrebe, F.W. 1941. The role of the pituitary and the thyroid in the development of teleosts. J. Exp. Biol. 18: 162-169.
- Leatherland, J.F., and B.A. McKeown. 1973. Circadian rhythm in the plasma levels of prolactin in goldfish, Carassius auratus L. J. Interdiscipl. Cycle Res. 4: 137-143.
- Le Cren, E.D. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (Perca fluviatilis). J. Anim. Ecol. 20: 201-219.

Lewis, U.J., R.N.P. Singh, B.K. Seavey, R. Lasker, and G.E. Pickford. 1972. Growth hormone- and prolactin-like proteins of the blue shark (Prionace glauca). Fish. Bull. 70: 933-939.

Liversage, R.A. 1973. Hypophysectomy and pectoral fin regeneration in adult Fundulus heteroclitus (killifish). Can. J. Zool. 51: 1047-1054.

Lovern, J.A. 1934. Fat metabolism in fishes. V. The fat in the salmon in its young freshwater stages. Biochem. J. 28: 1961-1963.

Malikova, E.M. 1957. Bioshimicheskaja otsenka molodi lososia pri perekhode v sostoianie, blizkoe k pokatnomu, i pri zadershke serebriank v presnoi vode. (Biochemical analysis of young salmon at the time of their transformation to a condition close to the smolt stage, and during their retention in freshwater). (Fish. Res. Board Transl. Ser. 232) Tr. Latv. Otd. Vniro 2: 241-245.

Mattheijj, J.A.M., M.W.J. Stroband, and F.J. Kingma. 1971. The cell types in the adenohypophysis of the Cichlid fish Cichlasoma biocellatum Regan, with special attention to its osmoregulatory role. Z. Zellforsch. Mikroskop. Anat. 118: 113-126.

McCartney, F.H. 1967. Monthly variations of the serum total cholesterol and serum total lipid-phosphorus of mature brown trout. Cortland Hatchery Report No. 35 for 1966. Fish. Res. Bull. N.Y. Cons. Dept. 30: 42-45.

- McCartney, T.H. 1966. Monthly variations of the serum total cholesterol of mature brown trout. Cortland Hatchery Report No. 34 for 1965. Fish. Res. Bull. 29: 72-75.
- McKeown, B.A., and J.F. Leatherland. 1973. Fine structure of the adenohypophysis in immature sockeye salmon, Oncorhynchus nerka. Z. Zellforsch. Mikroskop. Anat. 140: 459-472.
- McKeown, B.A., and A.P. Van Overbeeke. 1971. Immunohistochemical identification of pituitary hormone producing cells in the sockeye salmon (Oncorhynchus nerka, Walbaum). Z. Zellforsch. Mikroskop. Anat. 112: 350-362.
- Medway, W., and M. Kare. 1959. Thiocyanate space in growing domestic fowl. Am. J. Physiol. 196: 873-875.
- Moens, L., and G.D. Burford. 1973. A simple method for detection of neurosecretory products after polyacrylamide gel electrophoresis. Anal. Biochem. 51: 466-469.
- Nicoll, C.S. 1972. Some observations and speculations on the mechanism of "depletion", "repletion", and release of adenohypophyseal hormones. Gen. Comp. Endocrinol. Suppl. 2: 86-96.
- Nicoll, C.S., and H.A. Bern. 1965. Pigeon crop-stimulating activity (prolactin) in the adenohypophysis of lungfish and tetrapods. Endocrinology, 76: 156-160.
- Nicoll, C.S., and H.A. Bern. 1968. Further analysis of the occurrence of pigeon crop sac-stimulating activity (prolactin) in the vertebrate adenohypophysis. Gen. Comp. Endocrinol. 11: 5-20.

Nicoll, C.S., and P. Licht. 1971. Evolutionary biology of prolactins and somatotropins. II. Electrophoretic comparison of tetrapod somatotropins. Gen. Comp. Endocrinol. 17: 490-507.

Nicoll, C.S., and C.W. Nichols Jr. 1971. Evolutionary biology of prolactins and somatotropins. 1. Electrophoretic comparison of tetrapod prolactins. Gen. Comp. Endocrinol. 17: 300-310.

Oide, H. 1971. Annual variation of renal function in freshwater Japanese eel (Anguilla japonica). Zool. Mag. 80: 58-60.

Olivereau, M. 1954. Hypophyse et gland thyroïde chez les poissons. Etude histophysiologique de quelques corrélati-  
ons endocriniennes, en particulier chez Salmo salar L. Ann. Inst. Oceanog. Monaco 29: 95-296.

Olivereau, M. 1962. Action de la thyroxine sur l'hypothalamus, l'hypophyse et la glande thyroïde de l'anguille. Anat. Anz. 109: 468-478.

Olivereau, M. 1963. Cytophysiologie du lobe distal de l'hypophyse des Agnathes et des poissons, à l'exclusion de celle concernant la fonction gonadotrope. In Cytologie de l'adenohypophyse. Edited by J. Benoit and C. Da Lage. C.N.R.S. (Paris) pp. 315-329.

Olivereau, M. 1969. Functional cytology of prolactin-secreting cells. Gen. Comp. Endocrinol. Suppl. 2: 32-41.

Olivereau, M. 1972. Action d'un rapport de cortisol sur la cytologie de l'hypophyse chez l'anguille. Acta Zool. 53: 179-194.

Oliverreau, M., and J.N. Ball. 1963. Fonction corticotrope et cytologie hypophysaire chez deux Téléostéens:

Mollinesia latipinna Le Sueur et Anguilla anguilla.

C.R. Acad. Sci. Paris 256: 3766-3769.

Oliverreau, M., G. La Roche and A.N. Woodall. 1964. Modifications cytologiques de l'hypophyse de la truite à la suite d'une carence en iode et d'une radiothyroïdectomie. Ann. Endocrinol. (Paris). 25: 481-490.

Parry, G. 1958. Size and osmoregulation in salmonid fishes. Nature (Lond.), 181: 1218-1219.

Parry, G. 1960. The development of salinity tolerance in the salmon, Salmo salar (L.) and some related species. J. Exp. Biol. 37: 425-434.

Parry, G. 1961. Osmotic and ionic changes in blood and muscle of migrating salmonids. J. Exp. Biol. 38: 411-427.

Pearse, A.G.E. 1961. Histochemistry, theoretical and applied. 2nd ed. Churchill, London.

Pickford, G.E. 1953. The response of hypophysectomized male Fundulus to injections of purified beef growth hormone. Bull. Bingham Oceanogr. Coll. 14: 46-68.

Pickford, G.E. 1953b. A study of the hypophysectomized male killifish, Fundulus heteroclitus (Linn.). Bull. Bingham Oceanogr. Coll. 14: 5-41.

Pickford, G.E. 1954. The response of hypophysectomized male killifish to purified fish growth hormone as compared with the response to purified beef growth hormone. Endocrinology. 55: 274-287.

- Pickford, G.E. 1957. The growth hormone. In The physiology of the pituitary gland of fishes. Edited by G.E. Pickford and J.W. Atz. New York Zoological Society. New York. pp. 84-99.
- Pickford, G.E., E.E. Robertson and W.H. Sawyer. 1965. Hypophysectomy, replacement therapy, and the tolerance of the euryhaline killifish Fundulus heteroclitus to dilute media. Gen. Comp. Endocrinol. 5: 160-180.
- Pickford, G.E. and E.F. Thompson. 1948. The effect of purified mammalian growth hormone on the killifish, Fundulus heteroclitus. (Linn).. J. Exp. Zool. 109: 367-383.
- Pickford, G.E., P.K.T. Pang and W.H. Sawyer. 1966. Prolactin and serum osmolality of the hypophysectomized killifish, Fundulus heteroclitus, in fresh water. Nature (Lond.), 209: 1040-1041.
- Pinder, L.J. and J.G. Eales. 1969. Seasonal buoyancy changes in Atlantic salmon Salmo salar parr and smolt. J. Fish. Res. Board Can. 26: 2093-2100.
- Pyefinch, K.A. 1955. A review of the literature on the biology of the Atlantic salmon (Salmo salar, Linn). Sci. Invest. Freshwater Fish. Scotland, 9: 1-24.
- Pyle, E.A. 1969. The effect of constant light or constant darkness on the growth and sexual maturity of brook trout. Cortland Hatchery Report No. 36 for 1967. Fish. Res. Bull. N.Y. Cons. Dept. 31: 13-19.

- Raben, M.S. 1973. Growth hormone. Physiology: hormonal effects. In Methods in investigative and diagnostic endocrinology. Vol. 2A. Peptide hormones. Edited by S.A. Berson and R.S. Yalow. North-Holland Publishing Co., Amsterdam. pp. 261-267.
- Rasquin, P. and L. Rosenbloom. 1954. Endocrine imbalance and tissue hyperplasia in teleosts maintained in darkness. Bull. Am. Mus. Nat. Hist. 104: 359-426.
- Relkin, R. 1972a. Effects of pinealectomy, constant light and darkness on growth hormone levels in the pituitary and plasma of the rat. J. Endocrinol. 53: 289-293.
- Relkin, R. 1972b. Effects of variations in environmental lighting on pituitary and plasma prolactin levels in the rat. Neuroendocrinology, 9: 278-284.
- Robertson, O.H. 1949. Production of silvery smolt stage in rainbow trout by intramuscular injection of mammalian thyroid extract and thyrotropic hormone. J. Exp. Zool. 110: 337-355.
- Russell, J.A. 1955. Effects of growth hormone on the metabolism of amino acids. In "The hypophyseal growth hormone, nature and actions". Edited by R.W. Smith, O.H. Gaebler and C.H. Long. McGraw Hill, New York. pp. 213-224.
- Sage, M. and H.A. Bern. 1971. Cytophysiology of the teleost pituitary. Int. Rev. Cytol. 31: 339-376.
- Sage, M. and H.A. Bern. 1972. Assay of prolactin in vertebrate pituitaries by its dispersion of xanthophore pigment in the teleost, Gillichthys mirabilis. J. Exp. Zool. 180: 169-174.

- Saunders, R.L. and E.B. Henderson. 1970. Influence of photoperiod on smolt development and growth of Atlantic salmon (Salmo salar). J. Fish. Res. Board Can. 27: 1295-1311.
- Schreibman, M.P., J.F. Leatherland and B.A. McKeown. 1973. Functional morphology of the teleost pituitary gland. Am. Zool. 13: 719-742.
- Schwartz, P.L., and M. Batt. 1973. The aggregation of  $^{125}\text{I}$  human growth hormone in response to freezing and thawing. Endocrinology, 92: 1795-1798.
- Smith, D.C.W. 1956. The role of the endocrine organs in the salinity tolerance of trout. In The comparative endocrinology of vertebrates. Part II. The hormonal control of water and salt electrolyte metabolism in vertebrates. Mem. Soc. Endocrinol. 5: 83-101.
- Squire, P.G. and K.O. Pederson. 1961. The sedimentation behaviour of human pituitary growth hormone. J. Am. Chem. Soc. 83: 476-481.
- Stachura, M.E., and L.A. Frohman. 1974. "Large growth hormone": ribonucleic acid-associated precursor of other growth hormone forms in rat pituitary. Endocrinology 94: 701-712.
- Steel, R.G.D., and J.H. Torrie. 1960. Principles and procedures of statistics with special reference to the biological sciences. McGraw-Hill, New York.
- Swift, D.R. 1954. Influence of mammalian growth hormone on rate of growth of fish. Nature (Lond.) 173: 1096.

- Swift, D.R. 1955. Seasonal variations in the growth rate, thyroid gland activity, and food reserves of brown trout (Salmo trutta Linn.). J. Exp. Biol. 32: 751-764.
- Swift, D.R. 1961. The annual growth rate cycle in brown trout (Salmo trutta Linn.) and its cause. J. Exp. Biol. 38: 595-604.
- Swift, D.R. and G.E. Pickford. 1962. Seasonal variations in the growth hormone content of pituitary glands of the perch Perca fluviatilis. L. Am. Zool. 2: 451.
- Wagner, H.H. 1974a. Photoperiod and temperature regulation of smolting in steelhead trout (Salmo gairdneri). Can. J. Zool. 52: 219-234.
- Wagner, H.H. 1974b. Seawater adaptation independent of photoperiod in steelhead trout (Salmo gairdneri). Can. J. Zool. 52: 805-812.
- Wilhemí, A.E. 1968. Canine growth hormone. Yale J. Biol. Med. 41: 199-207.
- Woodman, A.S. 1939. The pituitary gland of the Atlantic salmon. J. Morphol. 65: 411-435.
- Wurtman, R.J. 1975. The effects of light on man and other mammals. Ann. Rev. Physiol. 37: 467-483.
- Yadley, R.A., D. Rodbard and A. Chrambach. 1973. Isohormones of human growth hormone. III. Isolation by preparative polyacrylamide gel electrophoresis and characterization. Endocrinology, 93: 866-873.

Zaugg, W.S. and H.H. Wagner. 1973. Gill ATPase activity related to parr-smolt transformation and migration in steelhead trout (Salmo gairdneri): Influence of photoperiod and temperature. Comp. Biochem. Physiol. 45: 955-965.



12