



12-1983

The Effects of Photoperiod on the Prolactin Cells of Notropis Cornutus (Mitchell)

Matthew Kevin Hettinger

Follow this and additional works at: https://scholarworks.wmich.edu/masters_theses



Part of the Aquaculture and Fisheries Commons, and the Biology Commons

Recommended Citation

Hettinger, Matthew Kevin, "The Effects of Photoperiod on the Prolactin Cells of Notropis Cornutus (Mitchell)" (1983). *Master's Theses*. 1595.

https://scholarworks.wmich.edu/masters_theses/1595

This Masters Thesis-Open Access is brought to you for free and open access by the Graduate College at ScholarWorks at WMU. It has been accepted for inclusion in Master's Theses by an authorized administrator of ScholarWorks at WMU. For more information, please contact wmu-scholarworks@wmich.edu.



THE EFFECTS OF PHOTOPERIOD ON THE
PROLACTIN CELLS OF NOTROPIS CORNUTUS
(MITCHELL)

by

Matthew Kevin Hettinger

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Master of Arts
Department of Biology

Western Michigan University
Kalamazoo, Michigan
December 1983

THE EFFECTS OF PHOTOPERIOD ON THE
PROLACTIN CELLS OF NOTROPIS CORNUTUS
(MITCHELL).

Matthew Kevin Hettinger, M.A.

Western Michigan University, 1983

Three groups of the common shiner (Notropis cornutus, Mitchell) were adapted to three different photoperiods. These photoperiods were 16L:8D (summer), 12L:12D (controls), and 8L:16D (winter). The prolactin cells were studied histologically and morphologically with respect to staining characteristics, cell and nuclear morphology, nuclear size, and cellular density. The prolactin cells of those fish under the long photoperiod exhibited greater cellular activity than the controls as evidenced by an increase in nuclear size, cellular density, and by becoming more circular. The prolactin cells of those fish under the short photoperiod exhibited the same characteristics as those in the long photoperiod. The staining characteristics did not vary between photoperiods. The results indicate that prolactin synthesis and secretion may be under seasonal control.

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation for the advice, direction, and encouragement given to me by Dr. Richard Pippen. I wish to thank Drs. Dubien and McIntire for their assistance with the statistics and histology respectively, and for putting up with some unusual circumstances. I also wish to thank my brother, Doug, for aiding me in preparing some of the slides, and Cathy Stump for typing this manuscript. And, of course, I wish to thank my parents for their encouragement and guidance.

Matthew Kevin Hettinger

1322425

HETTINGER, MATTHEW KEVIN

THE EFFECTS OF PHOTOPERIOD ON THE PROLACTIN CELLS OF NOTROPIS
CORNUTUS (MITCHELL)

WESTERN MICHIGAN UNIVERSITY

M.S. 1983

University
Microfilms
International 300 N. Zeeb Road, Ann Arbor, MI 48106

Copyright 1983

by

HETTINGER, MATTHEW KEVIN

All Rights Reserved

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy.
Problems encountered with this document have been identified here with a check mark ✓.

1. Glossy photographs or pages ✓
2. Colored illustrations, paper or print ✓
3. Photographs with dark background _____
4. Illustrations are poor copy _____
5. Pages with black marks, not original copy _____
6. Print shows through as there is text on both sides of page _____
7. Indistinct, broken or small print on several pages ✓
8. Print exceeds margin requirements _____
9. Tightly bound copy with print lost in spine _____
10. Computer printout pages with indistinct print _____
11. Page(s) _____ lacking when material received, and not available from school or author.
12. Page(s) _____ seem to be missing in numbering only as text follows.
13. Two pages numbered _____. Text follows.
14. Curling and wrinkled pages _____
15. Other _____

University
Microfilms
International

TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENTS	ii
LIST OF FIGURES	v
LIST OF TABLES	vi
 CHAPTER	
I. INTRODUCTION	1
II. LITERATURE SURVEY	3
Physiological Effects of Prolactin in Fish	3
Responses related to osmoregulation	3
Non-osmoregulation Responses	4
The Prolactin Cell	7
Regulation of Prolactin Cell Activity	9
Regulation by ambient osmolarity	9
Regulation by light	11
Hypothalamic Control of Prolactin Cell Activity.	14
III. METHODS AND MATERIALS	17
Collection of Fish	17
Photoperiod Chambers.	17
Fish C.N.S. Anatomy	18
Histological Techniques	21
Fixation procedures	21
Preparation for staining	21
Staining procedures	23

CHAPTER

	Measurements and Techniques	24
	Qualitative analysis	24
	Quantitative analysis	24
	Statistical analysis	26
IV.	RESULTS	30
	Statistical Results	31
V.	DISCUSSION.	40
	Cell Density and Nuclear Size	40
	Qualitative Morphology and Staining Properties.	43
	Hypothalamic Control of Photoperiod Response	44
	Tissue Responses to Photoperiod via Prolactin	45
VI.	SUMMARY	47
VIII.	BIBLIOGRAPHY	55

LIST OF FIGURES

FIGURES	PAGE
1. Ventral View of the Head After Removing the Lower Jaw and Showing Relative Location of the Pituitary in Relation to Skeletal Parts.	19
2. Sagittal View of the Pituitary in Relation to the Brain Case and Different Structures of the Brain.	20
3. Method of Measuring Nuclear Area.	27
4. Method of Measuring Cell Density.	28
5. Prolactin Cells Showing Chord Arrangement.	32
6. Prolactin Cells Under a Light Regime of 16L:8D.	33
7. Prolactin Cells Under a Light Regime of 8L:16D.	34
8. Prolactin Cells Under a Light Regime of 12L:12D.	35
9. Relative Shape of Cell and Nucleus and Relative Nuclear Areas for the Three Photoperiods.	36

LIST OF TABLES

TABLES	PAGE
1. Nuclear Area and Cell Density of Prolactin Cells (μm^2) Under the Influence of Photoperiod.	37
2. Analysis of Variance of Nuclear Areas.	37
3. Analysis of Variance of Cell Densities.	38
4. Least Significant Difference Analysis of Nuclear Areas.	38
5. Least Significant Difference Analysis of Cell Densities.	39
6. Nuclear Area of Prolactin Cells-16L:8D. Appendix	50
7. Nuclear Area of Prolactin Cells-12L:12D. Appendix	51
8. Nuclear Area of Prolactin Cells-8L:16D. Appendix	52
9. Cell Densities of Prolactin Cells. Appendix	54

CHAPTER I

INTRODUCTION

Prolactin is a hormone found in the pituitary gland of vertebrates. It is of physiological interest because of the variety of physiological processes it regulates. In teleosts (fish) prolactin has been reported to regulate ion movement across the gills, pigmentation, skin mucous secretion, seminal vesicle growth and secretion, fin fanning and nest building behavior, renal excretion, thyroid stimulation, fat deposition, resistance to thermal stress, and is an antagonist to toxic effects of estrogen (Bern 1967).

It is the purpose of this investigation to study and describe the morphological and histological characteristics of the prolactin cells of a fish, Notropis cornutus, as influenced by artificial photoperiods. Some investigators of various vertebrates found that seasonal changes did influence the secretion of prolactin (Applington 1942; Hartmann 1944; Lam 1967; Lam and Hoar 1967; Lam and Leatherland 1969; deVlaming 1973). Serum prolactin levels and morphological changes in the prolactin cells were found to correlate. Photoperiod effects on prolactin cell cytology has been done only to one species of fish: Gasterosteus aculeatus. Leatherland (1970) studied the marine form (trachus). The prolactin cells appeared minimally active in early winter collected in the sea, and maximally active in fish collected in freshwater in the spring. Benjamin (1974) studied the freshwater form (leuries). The prolactin cells showed significant increases in the number of secretory granules, the

number of free ribosomes, the relative volume of rough endoplasmic reticulum (RER), and the relative volume of the rostral pars distalis from winter to spring.

The prolactin cells of teleosts are found in the rostral portion of the pars distalis and forms one-third to one-half of the pituitary gland. The cells take a variety of stains including the Massons' stain used in this study (Ball and Baker 1969).

CHAPTER II

LITERATURE SURVEY

Physiological Effects of Prolactin in Fish

Responses Related to Osmoregulation

Fish, whether they are migratory or not, are subject to seasonal changes in ambient osmolarity. To remain active, or even to survive, fish must adapt to these seasonal changes. Thus those organs responsible for these adaptations undergo rhythms. All of these organs have been shown to be responsive to prolactin cell activity (Ensor 1978).

The interest in prolactin as an osmoregulatory hormone originally stems from the work of Burdon (1956), who showed that the euryhaline killifish Fundulus heteroclitus, exhibited osmotic failure in freshwater after being hypophysectomized. Pickford and Phillips (1957) then showed that injections of ovine prolactin permitted the survival of hypophysectomized fish. It is now known that prolactin prevents osmotic failure in a number of teleost species: Poecilia latipinna (Ball and Olivereau 1964), Xiphoporous maculatus (Schreibman and Kallman 1966), Tilapia mossambica (Dharmamba et al. 1968), Oryzias latipes (Utida et al. 1971), Ictalurus onelas (Chidamberum et al. 1972), Mugil cephalis (Sage 1973), and Salmo trutta (Oduleye 1976).

The target organs of prolactin concerned with osmoregulation

include gill and skin epithelium, the gut, the kidneys, and the bladder (Ensor 1978). The primary effect of prolactin on these organs is to retain sodium. Prolactin minimizes sodium loss across the gills by increasing active uptake by the ionocytes in the gill lamellae. Prolactin also minimizes sodium loss by producing a mucous sheath surrounding the gill, and by reducing blood flow through the lamellae. Both of these mechanisms reduce passive efflux (Ensor 1978). Prolactin stimulates mucous production by integumentary mucous cells (Blum and Fielder 1964; Schreibman and Kallman 1965; Ogawa and Johnson 1967). In the kidney, prolactin reduces the urine-to-plasma ratio (Lahlou and Sawyer 1967; Lam 1972), increases the glomerular filtration rate by expanding the glomerular tuft and increasing urine production (Leatherland and Lam 1969), and increased reabsorption of prolactin caused a decrease in the permeability of the bladder to water which was correlated with an increase in the rate of sodium absorption. The gut seems to reduce water flux and decrease the chloride transport in response to prolactin (Utida 1972).

Non-osmoregulatory Responses

Various nonosmoregulatory tissues and metabolic processes have been shown to be responsive to prolactin. Among these are reproductive structures, the thyroid, fat deposits and parental behavior (Ensor 1978). These tissues and metabolic processes seem to undergo seasonal rhythms similar to tissues responsible for osmoregulation.

Reproductive Structures

The reproductive structures studied thus far in fish are the sem-

inal vessicles and the ovaries. During the prespawning period of Heteropneustus fossilis, prolactin inhibits vitellogenesis and induces follicular atresia. During the spawning period prolactin induces ovarian regression (Sundararaj and Kishavanath 1975). In male fish the seminal vesicles regress with hypophysectomy (Tavolga 1955; Sundararaj and Goswami 1965; deVlaming and Sundararaj 1972). After hypophysectomy, prolactin injections prevent seminal vesicle regression (deVlaming and Sundararaj 1972). Prolactin probably stimulates the growth of seminal vesicles by potentiating the effects of testosterone since it has no effect on the seminal vesicles by itself (Sundararaj and Noyzer 1969; deVlaming and Sundararaj 1972).

Parental Behavior

A well documented effect of prolactin on parental behavior is the promotion of skin sloughing which produces discus milk in the chichlid Symphysodon discus (Blum and Fielder 1965). The epidermal cells of this fish undergo division and then are shed with mucus which forms a milky substance for the young to use as food. Another well documented effect of prolactin is its participation in parental care in the male sea horse Hippocampus (Ball and Baker 1969; Ensor 1978). It seems that prolactin proliferates the marsupial epithelium and stimulates the synthesis and secretion of an epithelial protease. This protease digests proteins derived from the yolk of eggs that were arrested during early development and are present in the marsupial fluid. The resulting amino acids are then absorbed by the embryo.

Prolactin may also play a role in inhibiting aggressive and sexual behavior during the parental period. In chichlids, Blum and Fielder

(1965) found that prolactin inhibits aggressive behavior by suppressing androgen synthesis or blocking gonadotrophin release. Bleick (1975) found that prolactin causes the regression of the nuchal hump, a protrubence on the forehead of the male and female during the mating season, in the chichlid Cichlasoma citriovellus. Bleick proposed that the main role of prolactin is to prevent the development of mating characteristics during the parental period, which is associated with the regression of the hump.

Fin-fanning is another parental behavior attributed to prolactin (Blum and Fielder 1965; Blum 1974). Prolactin causes the pectoral fins to move in a fanning motion to move water across the nest in chichlids.

The Thyroid

It has been shown that ovine prolactin induces an increase in the activity of the thyrotrophs, which was associated with an increase in thyroid activity in eels (Oliverieu 1966). This effect was not observed in hypophysectomized eels. Oliverieu concluded that prolactin may act as a thyroid stimulating hormone-releasing factor exerting its effects at the hypothalmo-hypophysial level.

Fat Metabolism

Prolactin has been shown to affect fat metabolism for a number of fish (Lee and Meier 1967; Merhle and Fleming 1970; Joseph and Meier 1971; deVlaming, Sage and Charlton 1973; deVlaming and Pardo 1974; Mckeown, Leatherland and John 1975). The seasonal fluctuations in fat metabolism seems to be related to the seasonal fluctuations in

serum prolactin (Lee and Meier 1967; Meier 1969).

The mechanism by which photoperiod, prolactin, and fat metabolism are related has not been worked out yet. However, there is speculation that the circadian rhythm of serum prolactin and fat metabolism may be involved. These rhythms fluctuate seasonally, and it is thought that it is these phase shifts that give the observed seasonal responses (Lee and Meier 1967; Spieler, Meier and Loesch 1976). This mechanism is thought to be involved at the hypothalamo-hypophysial level. (Spieler, Meier and Loesch 1976).

Another possible mechanism involves the pineal. In 1973 and 1974, deVlaming and co-workers reported that melatonin effects both prolactin levels and fat metabolism. It is known that melatonin decreases pituitary prolactin, but beyond that the mechanism is not clear (deVlaming et al. 1973).

The Prolactin Cell

The prolactin cell is essentially the same throughout all vertebrate classes. They have similar morphologies, cytologies, and staining characteristics (Holmes and Ball 1974). One main difference between the teleost prolactin cells and the cells of other vertebrates is that they are located in a distinct region apart from other cell types (Schreibman, Leatherland and Mckeown 1973; Holmes and Ball 1974).

The prolactin cells of fish lie in the anterior portion of the rostral pars distalis and may form one-third to one-half of the pituitary. They extend ventrally and laterally for various distances around the proximal pars distalis. The secretory granules stain red with erythrosin in Alizarin Blue Tetrachrome, Cleveland-Wolfe stain,

and Herlants Tetrachrome (Kracier, Herlant and Duclos 1966; Ball and Baker 1969). They also stain red with azocarmine or acid fucsin in Azana stain, Mallory's stain, and Masson's Trichrome (Ball and Baker 1969). They are negative to Periodic Acid Shift, Aldehyde Fucsin, and Aniline Blue (Ball and Baker 1969).

The teleost prolactin cell, like other cells, contains a nucleus, endoplasmic reticulum, Golgi apparatus, mitochondria, and secretory granules. Depending on the species, the prolactin cells can be in one or two forms. They can be columnar, have apical cilia, have secretory granules concentrated at the basal pole of the cell, and are arranged in follicles. However, they may be circular evenly granulated, have a kidney-shaped nucleus, have Golgi appearing as one or more clear tubes shaped in a U or C, and not arranged in follicles (Ball and Baker 1969; Holmes and Ball 1974). The secretory granules are membrane-bound structures ranging in size from 1200 to 3500 μ A, depending on the species and the environmental conditions (Schreibman, Leatherland and McKeown 1973; Holmes and Ball 1974). These granules are released by exocytosis under the appropriate physiological conditions (Weiss 1965; Leatherland 1970; Holmes and Ball 1974; Benjamin 1978).

It was first suspected that the prolactin cells contained prolactin when hypophysectomized fish failed to survive in freshwater, when the prolactin cells appeared more active in freshwater than seawater, and when prolactin injections allowed the survival of hypophysectomized fish (Ball and Baker 1969; Holmes and Ball 1974). It was shown by bioassay that there is approximately double the prolactin activity in pituitary glands taken from fish adapted to

freshwater when compared to the pituitary glands of fish adapted to seawater (Ball and Baker 1969).

It was confirmed that the prolactin cells contain prolactin by the fluorescent antibody technique. Fluorescent antibody to ovine prolactin locates specifically on the granules of the prolactin cells. This has been done with Fundulus, Onchoryncus, Cirrhosoma, Cerassics, Levcisus, Anguilla, Salmo, and Clupea (Emmart, Pickford and Wilhelmi 1966; Emmart 1969; Holmes and Ball 1974). Emmart and Mossakowski (1967) used cultured cells of Fundulus heteroclitus with this technique.

Environmental Regulation of Prolactin Cell Activity

Changes in Ambient Osmolarity

Within the past fifteen years numerous studies have been done to delineate the relationship between ambient osmolarity and prolactin cell activity. Changes in ambient osmolarity, whether due to changing sodium concentrations or other ions, causes changes in the activity of the prolactin cell. The prolactin cells appear more active in freshwater than in seawater. This is seen from evidence on the changes in cellular size and shape, cell density and the relative volume taken up by the prolactin cells in the pituitary, nuclear size and shape, activity of cytoplasmic organelles and staining characteristics of the cytoplasm, and the number and size of the secretory granules (Dharmamba and Nishioka 1968; Ball and Baker 1969; Olivereau and Ball 1973; Holmes and Ball 1974; Nagahama et al. 1975; Bonga 1978; Benjamin 1978).

Cellular Size and Shape

The size of the prolactin cell increases and the shape becomes spherical as fish move from saltwater to freshwater. Bonga (1978), in his work with G. aculeatus (trachurus), has shown that fish adapting to freshwater had approximately twice the cellular volume than fish adapting to saltwater. This increase in size has also been shown for the prolactin cells of T. mossambica (Dharmamba and Nishioka 1968). The prolactin cells of G. aculeatus (trachurus) also shows circular shapes when sampled from freshwater as compared to those sampled in seawater habitats (Leatherland 1970).

Cell Density and Relative Volume

The cell density decreases, and the relative volume in the pituitary taken up by the prolactin cells increases when fish move from seawater to freshwater. In 1978, Benjamin transferred Pungitius pungitius from freshwater to seawater for periods of 10 hours, 3, 6, 9, and 21 days, and found that the cell density decreases. The prolactin cells of M. cephalis and P. latipinna show a similar pattern. The cell densities of these fish are two to three times greater in seawater than in freshwater. Along with the decrease in cell density, the relative volume taken up by the prolactin cells increases approximately two to three times (Ball and Pickford 1964; Blanc-Livni and Abraham 1970; Ball and Ingleton 1973).

Nuclear Shape and Size

The nucleus of the prolactin cells of M. cephalus appears three times larger in freshwater than in seawater (Abraham 1971). This increase

in size has also been shown for P. pungitius and P. latipinna (Ball and Ingleton 1973; Benjamin 1978). Nuclear shape has been reported to be round and oval with occasional evaginations for fish adapted to freshwater, whereas the nucleus appears kidney shaped in fish adapted to saltwater (Ball and Ingleton 1973; Benjamin 1978).

Cytoplasmic Organelles and Staining Characteristics

The Golgi apparatus and the endoplasmic reticulum seem to change with the activity of the cell. The Golgi is small and indistinct in seawater but large in freshwater, and may be seen to be shaped in the form of a U or C (Dharmamba and Nishioka 1967; Ball and Baker 1969; Ball and Ingleton 1973). The endoplasmic reticulum can be seen as a cap or halo surrounding the nucleus in active cells if stained appropriately (Ball and Baker 1969). It appears that the prolactin cells of fish in freshwater take stains more intensely than those cells of fish in saltwater. P. pungitius stained heavily with Orange G in freshwater, and became erythromophobic with longer periods of exposure to saltwater (Benjamin 1978).

Number and Size of Secretory Granules

Both the number and size of secretory granules increase with decreasing salinity. This has been shown for T. mossambica (Dharmamba and Nishioka 1968), M. cephalus (Abraham 1971), P. latipinna (Ball and Ingleton 1973), and X. maculatus (Weiss 1965).

Regulation by Light

Circadian Rhythms

The circadian rhythm of prolactin cell activity has been demonstrated in birds, rats, man, amphibians, and reptiles (Ensor 1978). In teleosts these rhythms have been demonstrated by fluctuations in serum and pituitary prolactin concentrations, and by fluctuations in prolactin cell morphology (Leatherland and Mckeown 1973; Sage and deVlaming 1973; Leatherland, Mckeown and John 1974; Battan and Ball 1976; Mckeown and Peter 1976; Spieler, Meier and Loesch 1976). Peaks in serum prolactin levels during the day differ among species. The peak serum prolactin level occurs between 03.00 and 06.00 hr for Onchorhynchus nerka (Leatherland and Mckeown 1973; Leatherland, Mckeown and John 1974), at approximately 16.00 hr for Fundulus grandis (Spieler, Meier and Noeske 1979), at 09.00 and 24.00 hr for C. auratus in freshwater, and at 06.00 and 24.00 hr in saltwater (Mckeown and Peter 1976). Fundulis similis releases prolactin just after the onset of light (Sage and deVlaming 1973). M. cephalus also exhibits a peak in serum prolactin after the onset of light which leads to daily fluctuations during the course of the year (Spieler, Meier and Loesch 1976). During the Fall a peak is reached between 04.00 and 06.00 hr, and between 16.00 and 18.00 hr in the Winter. Thus it seems that circadian rhythms fluctuate seasonally.

The prolactin cell also undergoes circadian morphological changes. In P. latipinna nucleoli numbers were greater during midday than the morning. Golgi also occupied a greater volume at midday (Battan and Ball 1976). So far no studies have been done to see if the circadian peaks of cell activity, as represented by morphological structures, vary seasonally.

Circannual Rhythms

Along with the work of Spieler, Meier and Loesch (1976), circannual rhythms seem to be present in other teleosts as well as vertebrates in general (Meier, Burns and Dusseau 1969; Butter 1973; Pelletier 1973; Forbes et al. 1974; Quadri and Spies 1976; Ensor 1978). One of the earliest studies done on the correlation between photoperiod and prolactin cell morphology was done by Hartmann (1944) with the garter snake. Hartmann described the prolactin-containing carminophilic cell (Holmes and Ball 1974) as having coarse, dense, red granules with round or oval nuclei. The nuclei also stained red. The shape of the cell was oval or columnar. These cells decreased in number during the Spring and increased during the Summer. They reach a maximum in July and the decrease to winter levels in the fall. Hartmann also noted that the size of the cells fluctuate seasonally reaching a maximum in July although no quantitative measurements were given.

In the teleosts, only two studies have been done to relate photoperiod with prolactin cell morphology. Leatherland (1970) studied G. aculeatus (trachurus) since previous evidence presented by Lam and Hoar (1967), and Lam and Leatherland (1969) indicated that the marine stickleback does not synthesize and/or release prolactin in the winter when placed in freshwater. Leatherland found in freshwater juveniles of G. aculeatus (trachurus) that the prolactin cells were spherical with many densely packed granules that stain with erythrosin and Orange G. The eccentric nucleus was irregular in shape. The cytoplasm was divided into two regions: a region for the storage of granules, and a zone with Golgi bodies next to the

nucleus. In juveniles collected at sea, the granules were smaller and less erythrosinophilic, but just as well granulated.

Adult sticklebacks collected in late winter had prolactin cells that were more ellipsoid and more erythrosinophilic than early winter fish. The cellular diameter was significantly larger. The storage zone also appeared larger. In spring fish the trend of increasing cellular diameter and erythrosinophilic stainability continued. These characteristics were maximal in freshwater spring fish. After spawning, the prolactin cells appeared similar to those found in winter fish.

In general, the number of cells per unit area (cell density) was found to be inversely proportional to the diameter of the cell. However, the prolactin cells of fish collected in seawater during the spring exhibited increasing cellular diameter and density.

Benjamin (1974) compared the morphological characteristics of the prolactin cells of G. aculeatus (leurius) collected in the spring to the morphological characteristics of prolactin cells in winter fish and found that there was no significant difference in staining properties or cell size. The size of the Golgi did not change significantly either, but there were significant differences at the electron microscope level. It was concluded from these observations that the prolactin cell is more active in the spring.

Hypothalamic Control of Prolactin Cell Activity

Hypothalamic control of prolactin cell activity was first reported by Ball (1965). It was shown that transplants of the pitu-

pituitary gland allowed fish to survive in freshwater, and that the prolactin cells were still functional while other cell types were not. This has been confirmed in a wide range of teleosts including C. auratus (Leatherland 1972; Peter 1972; Leatherland and Ensor 1973), Gillichthys mirabilis (Zambrano 1971), M. cephalus (Abraham 1971), and Ictalurus melas (Chidambaren, Myer and Hasler 1972). Leatherland and Ensor (1974), in their studies with C. auratus, injected hypothalamic extracts from fish maintained in distilled water and seawater and found that the extracts from the fish in distilled water had a higher concentration of a prolactin inhibiting factor. They hypothesize that osmotic regulation of prolactin release may operate through the hypothalamus rather than acting directly on the prolactin cells.

It has been shown that the prolactin cells of P. latipinna are synaptically innervated by type B (aminergic) nerve fibers arising from the hypothalamus (Battan and Ball 1976; Ingleton, Battan and Ball 1977). It has also been shown that destruction of these fibers by L-hydroxydopamine causes an increase in the ability of fish to survive in freshwater (Zambrano et al. 1974). This suggested that dopamine may be the inhibitor. Oliverieu (1975) found that injections of L-dopa inhibited prolactin release in Anguilla anguilla. The same results were found with Gillichthys (Magahama et al. 1975). Dopamine has been shown to be the inhibitor in both in vivo and in vitro studies. Wigham and his co-workers (1975) found that in in vitro studies with isolated pituitary glands and various agonists and antagonists, and with dopamine alone and in combination with dimethylphenylethylamine (a specific dopamine antagonist) shows dopamine to be the prolactin inhibiting factor. Wigham and Ball (1976) confirmed

the in vitro work with P. latipinna by showing that dopamine inhibited the synthesis and release of prolactin.

Wigham and Ball (1976) also found that when the aminergic innervation of the prolactin cells was destroyed, there was a rise in plasma osmotic pressure along with an increase in plasma prolactin. Ingleton (1973) found that prolactin release was stimulated by low plasma osmotic pressure. Thus it would seem that osmotic regulation may not be through the aminergic system since it is thought that low osmotic pressure stimulated hypothalamic response (Ensor 1978). Catecholaminergic control may then act independently of osmotic influences and may explain the existence of circadian and circannual rhythms in prolactin cell activity.

CHAPTER III

METHODS AND MATERIALS

Collection of Fish

N. cornutus were collected on 15 Feb. 1980 from an inlet off of Morrow Lake located in Kalamazoo County, Michigan. Using a sein, the fish were trapped and placed in buckets filled with stream water and transported to the lab within 15 minutes. Other buckets were filled with stream water to serve as a source of replenishment water in the aquaria. The temperature of the water was maintained at 2° C.

Photoperiod Chambers

Fifteen fish, that ranged in length between 9cm and 15cm (adults), were placed in each of three black-bottomed 10 gallon aquaria. This number was established by previous experiments to determine the number of fish that can survive in this size aquarium. Inlet water was maintained in each aquarium at 22cm from the bottom by the extra water obtained at the stream. The filters used were Penn Plax hi-low filters which contained super activated carbon (Aquarium Pharmaceutical Inc.) and 100% polyester aquarium filter floss (Flexi-Mat Corp.). These filters served to prevent the build-up of gaseous and solid wastes, and also served as a secondary air supply. The filters were powered by Hush 1000 power paks which were connected to the filters by plastic hoses. The primary air supply came from the lab bench. Plastic hoses connected the outlets on the bench to air stones inside the aquaria.

Each aquarium was placed in a Sherer Gillett Gro-Lab chamber preset to a temperature of 2°C and a light intensity of 5400 foot candles. This prevented thermal stress that the fish would have been exposed to when adjusting to higher temperatures. The light intensity was measured using a Gossen Lux meter (Panlus Electronic). The source of light was Sylvania cool white fluorescent bulbs (relative irradiance 520nm-620nm) and G.E. Plant Gro and Show incandescent bulbs (relative irradiance, 762nm). The photoperiod for each chamber was set at 12L:12D for a period of one week with the start of the photoperiod 08.00 h. This allowed the fish to acclimate to lab conditions. After one week one chamber was set at 12L:12D, and another was set at 8L:16D. The third chamber was left at 12L:12D and served as the control. All light cycles started at 08.00 h. at which time the fish were fed Guppy Fare (Wardlap). The fish were maintained on these photoperiods for 60 days. The extra water was placed in one of the chambers to maintain the temperature at 2°C.

Fish Central Nervous System Anatomy

The pituitary gland lies on the ventral side of the brain, and is connected to the hypothalamus by the infundibular stalk. The gland lies in a hollow cartilagenous tube. The roof of the tube surrounds the infundibular stalk, and the floor is the base of the brain case.

After decapitation, the lower jaw and any excess gill was removed along with a layer of connective tissue posterior to the palate.

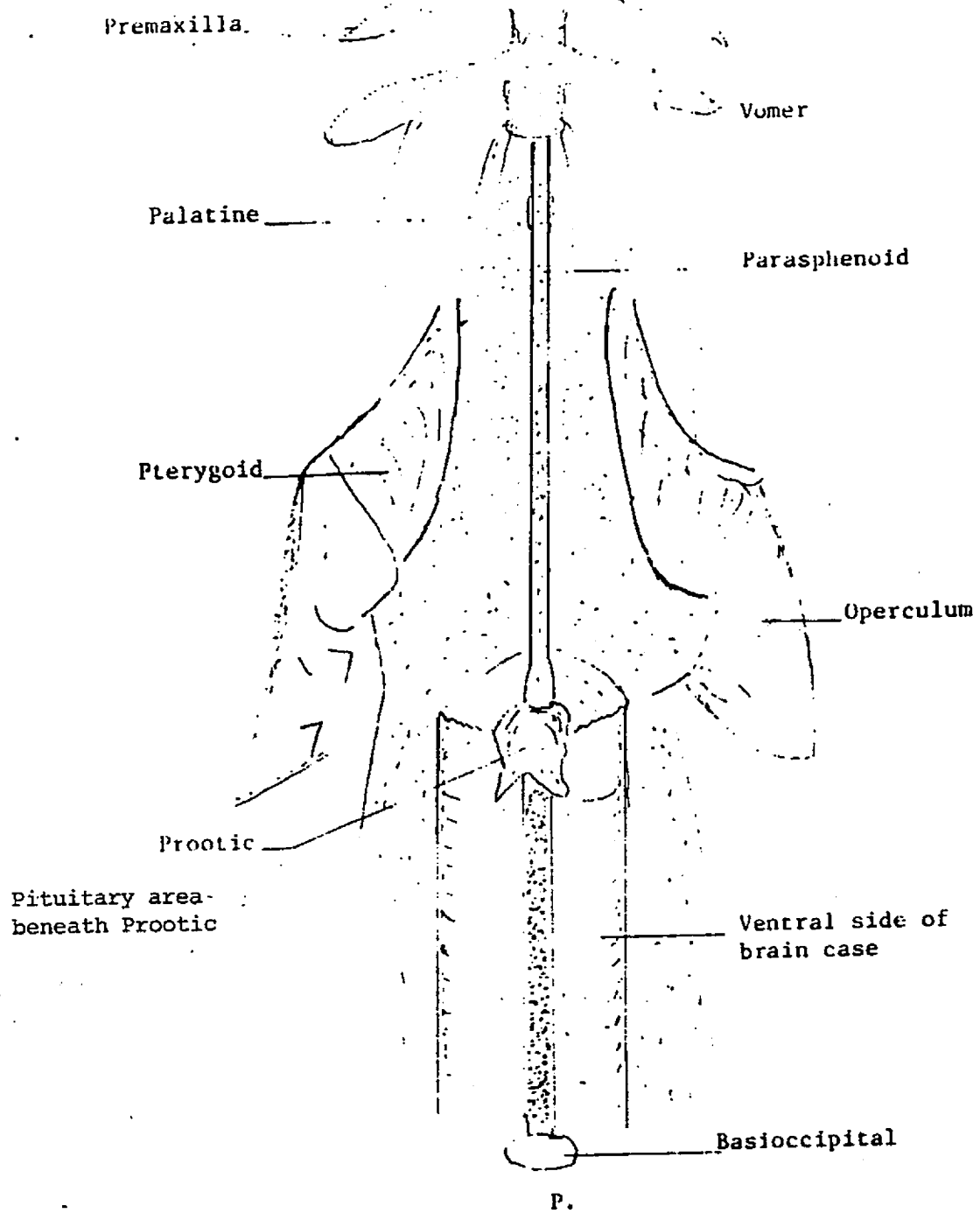


Figure 1. Ventral view of the head after removing the lower jaw and showing relative location of pituitary in relation to skeletal parts.

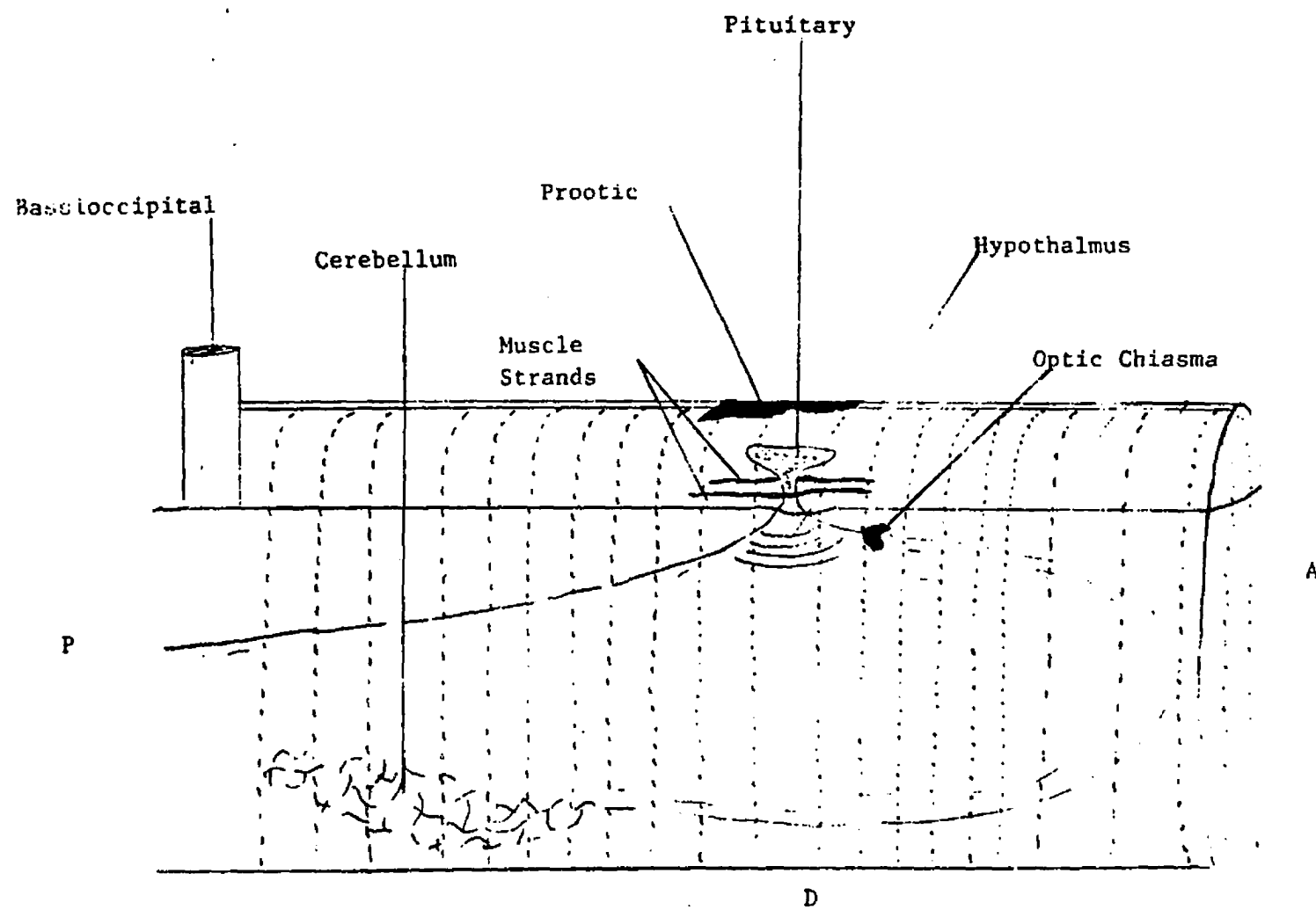


Figure 2. Sagittal view of the pituitary showing its relation to different structures of the brain.

The pterygoids were then visible. The pterygoids were removed under a dissecting scope along with the palate, exposing the parasphenoid and the base of the brain case (Fig. 1). Tissue was then scraped off the case and the anterior portion of the head was snipped off to free the parasphenoid. This allowed the bottom of the case to be lifted off by raising the parasphenoid so the pituitary would not be pulled with it. Two sets of muscle fibers were seen running alongside the pituitary. The entire pituitary was removed along with excess cartilage, after removing the muscle strands, and placed in fixative. The dissection was completed in one minute. All dissections were done between 10.00 and 12.00 h.

Histological Techniques

Fixation Procedures

Three fixatives were tried with various staining techniques. These fixatives were Bouin's solution, Bouin-Hollande Sublimate, and Helley's fixative. After evaluation of the stains with these fixatives, it was decided that Bouin's solution would be used. Pituitaries from each photoperiod were placed in this solution for 24 hours (Humason 1979).

Preparation for Staining

Dehydration

After fixing, tissues were placed in 70% ethanol for washing and initial dehydration for two hours. Dehydration consisted of passing the tissues through a series of ethanol baths: 70%, 80%, 95%, 100% and 100%.

The tissues remained in each bath for two hours. The tissues were then infiltrated with xylene and Paraplast by passing them through the following baths: ethanol:xylene, 3:1; xylene:ethanol, 3:1; xylene; xylene:Paraplast, 3:1; ; Paraplast:xylene, 3:1; Paraplast; and Paraplast. All Paraplast baths were held at 59°C. The tissues remained in each bath for two hours.

Embedding and Sectioning To embed the tissues, melted para-

To embed the tissues, melted paraplast was poured into metal molds that had previously been sprayed with mold release (Tissue Tak). Plastic holders were put in place prior to pouring the paraplast. The tissues were then placed and oriented in the paraplast with a dissecting needle that had been heated in a bunsen burner. The heated needle kept the paraplast melted to aid in orientation. Tissues were oriented so sections would be cut transversely, sagittally, or frontally. After the paraplast hardened, it was released from the metal mold, the excess paraplast was trimmed away with a razor blade, and the entire pituitary was sectioned in 4um increments using a Spencer "820" microtome (American Optical). Using soft-hair water-color brushes, ribbons of seven sections were transferred to a water bath (Lipshaw) kept at 45°C. Three strings of seven sections were placed on each slide. The slides had previously been washed with Alconox, had tissue adhesive applied to them (Tissue Tak), and placed on a slide warmer (Chicago Surgeon and Electrical Co.). The ribbons were placed on the slides by passing the slides underneath the ribbons floating in the water bath. The slides were then placed on the slide warmer for at least 30 minutes before rehydration.

Rehydration Rehydration consisted of passing the slides

Rehydration consisted of passing the slides through the following baths: xylene, xylene, 100% ethanol, 100% ethanol, 95% ethanol, 70% ethanol, and tap water. The slides were left in each bath for five minutes.

Staining Procedures

Of the staining techniques tried, it was found that the Hemotoxylin and Eosin stain, and the Aldehyde Fucsin stain gave poor results. Massons' Trichrome was chosen over the other two on the basis of being more consistent, and more convenient. The procedure for Massons' Trichrome is as follows (Humason 1979):

- 1) Place slide in iron alum for one-half hour.
- 2) Wash in running water for five minutes.
- 3) Differentiate in saturated aqueous picric acid.
- 4) Wash in running water for ten minutes.
- 5) Stain in acid fucsin for five minutes.
- 6) Rinse in distilled water until excess stain is removed.
- 7) Stain in ponceau de xyloidine for five minutes.
- 8) Rinse in tap water.
- 9) Differentiate in phosphomolybdic acid for five minutes.
- 10) Transfer to fast green for two minutes.
- 11) Differentiate in acidified water.

Good differentiation of the acidophils was obtained. Prolactin cells stained brick-red while the growth hormone cells stained yellow-orange to pink. The basophils stained various shades

of blue. After completion of the staining procedure, the tissues were dehydrated by passing the slides through the following baths: 70% ethanol, 95% ethanol, 95% ethanol, 100% ethanol, xylene, and xylene. The slides were left in each bath for five minutes. Permunt was used to coverslip the slides.

Measurements and Techniques

Ten fish were exposed to each photoperiod, however, the number of pituitary glands used for each photoperiod was ten, nine, and seven for the photoperiods 16L:8D, 8L:16D, and 12L:12D, respectively. This was due to the loss of some glands during tissue preparation. All sections of all photoperiods were examined at a magnification of 1600x with a Ziess Photomicroscope under the same illumination conditions. This allowed a comparison of nuclear and cellular shape, and staining characteristics between photoperiods under similar lighting conditions. These parameters were compared within each section, within each gland, and between each photoperiod. These parameters were compared between photoperiods by observing sections of a slide from one photoperiod which was then followed by sections from slides of the second and third photoperiod. This procedure was followed until all slides have been examined.

Quantitative Analysis

The quantitative description of the prolactin cells included nuclear area and cell density (the number of cells per unit area). The nuclear area was obtained by selecting a slide with sections of a particular gland and selecting a particular section on that slide, both at random. Ten nuclei were randomly measured on this

section. This was done for each pituitary gland until 100 nuclei were measured for each photoperiod. Additional sections were used from previous slides if there were not enough glands to obtain the measurement of 100 nuclei. The nuclear area was calculated from the measurements of the major and minor axis of the nuclear diameter, and from the equation $A = r^2$. The axis were measured with micrometer at a magnification of 1600x (Fig. 3). Each division on the micrometer was found to be equal to .90909um. The radius was found by averaging the major and minor axis measurements and dividing by two. This value was then used to find the area with $A = r^2$.

The cell density was obtained by using the same sections that were used to measure the nuclear area. Two 10um^2 areas in each section were selected with the micrometer at 1600x (Fig. 4). Each division on the micrometer was one-tenth of a millimeter. The number of nuclei contained in these two areas was averaged and divided by ten to obtain the number of cells per squared micrometer in each section. A cell was counted to be within the area if more than half of its nucleus was contained within the boundry. One section per gland was used to obtain 20 cell densities for each photoperiod. Additional sections were used if 20 measurements could not be made for a given photoperiod. The average cell density for each photoperiod was calculated by averaging the cell densities from each gland for that photoperiod.

Statistical Analysis

Both data sets (nuclear area and cell density) were subjected to a one-way analysis of variance (ANOVA) using the F distribution to determine if there was a significant difference between the means of any two of the three photoperiods. Both data sets were also subjected to a least significant difference analysis (LSD), using the t distribution, to determine which means were significantly different and to place them in rank order. Both analysis were carried out by the statistics lab of Western Michigan University. All calculations were performed at a significance level of 5%.

The Minitab program calculated the degrees of freedom, the sum of squares, and the mean square for the photoperiod, the experimental error between photoperiods, and the sampling error within photoperiods. The test statistic F was calculated by $MSPP / MSEE$, where MSPP is the mean square of the photoperiods and MSEE is the mean square of the experimental error. These values were compared to a value derived from the F distribution table. The degrees of freedom for the photoperiods were found with the equation $n-1$; where n is the number of variables (i.e. $n=3$ for three photoperiods). The degrees of freedom for the experimental error were calculated from the difference between the degrees of freedom for the fish (25, where $n=26$) and the degrees of freedom for the photoperiods. if $F \leq F_{.05} = 3.42$, then it can be concluded that the difference between the means for at least two of the photoperiods is not due to experimental error, but is due to the effects of photoperiod.

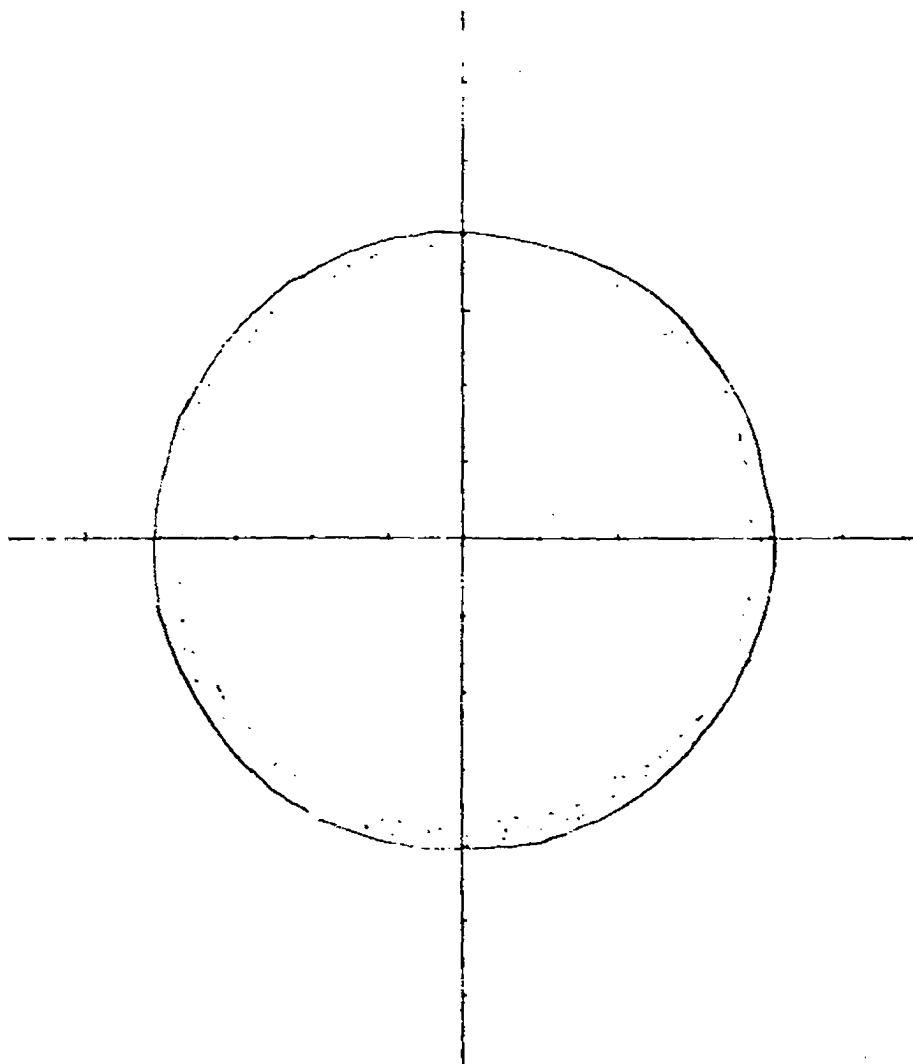


Figure 3. Measurement of Nuclear Area (1600x). The micrometer was placed on the major axis and the minor axis. The number of divisions were recorded for each axis and averaged to get the radius. This converted to μm and used to find area by $A = \pi r^2$ 1 Div. = .90909 μm

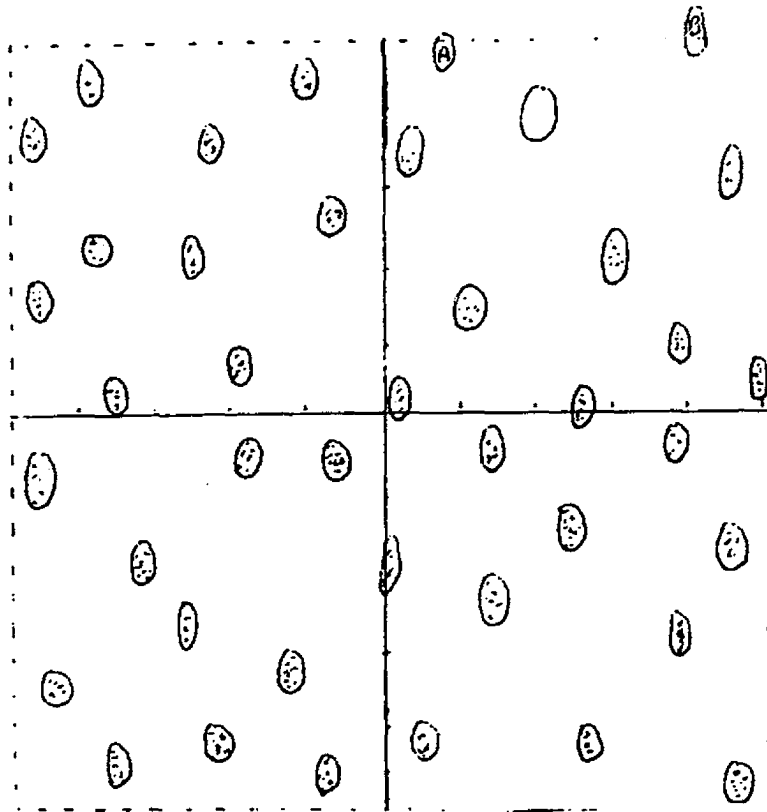


Figure 4. Measurement of Cell Density (1600x). The micrometer was placed vertically on the field of vision and then moved 90° . This formed a vertical and horizontal axis from which a 10mm^2 area was outlined. The number of nuclei (the number of cells) within this area was then counted. If more than one-half of the nucleus was inside the area (nucleus A) the cell was counted. If more than one-half was on the outside (nucleus B) it was not counted. $1\text{cm}=1\text{mm}$.

For the LSD analysis, the differences between the means for all combinations of photoperiods were calculated and compared to the values 0.08549 and 0.19116 for the nuclear area and cell density data respectively. These values were calculated from the equation $LSD (.05) = t_{23} \sqrt{2MSEE/20}$, where $t=2.069$ which was obtained from the t distribution table. If the calculated difference of the means between any two photoperiods is greater than the value calculated for that data set, then there is a difference due to daylength, and not due to experimental error, for those two photoperiods. This allowed the means to be placed in rank order by ordering the means from smallest to largest for those photoperiods that show significant differences.

CHAPTER IV

RESULTS

The shape of the prolactin cell was round to oval in all photoperiods. There was a tendency towards round with the long daylength. The same results were true for nuclear shape. The nuclei did not have any major evaginations. Golgi and other cytoplasmic organelles did not stain. There appeared to be a uniform distribution of cellular size within the pituitary; i.e. there were no longer cells located peripherally or centrally. Occasionally cells were seen to be arranged in chords in all photoperiods. The cells were located at the anterior end and took up approximately one-third to one-half of the pituitary gland.

The majority of prolactin cells of those fish exposed to the long daylength appeared round. Some cells appeared oblong. This was true for the nucleus also. The nucleus took up approximately two-thirds of the cell. Both the cellular and nuclear membranes appeared intact with little or no evaginations. The mean nuclear area for these cells was $67.28 \pm 3.09 \mu\text{m}^2$, and the mean cell density was $5.34 \pm .23$ cells/unit area (Table 1).

The cells appeared brick-red with some variations in intensity being noted for individual cells, and between cells in different pituitary glands. The nucleus also appeared red and there was black heterochromatin concentrated around the periphery of the nucleus in some cells, while being evenly distributed in others. The cytoplasmic organelles did not stain; hence their location and distribution

could not be determined. At times the cells were seen to be arranged in chords. Approximately seven cells were found to be arranged in a fascicular pattern to delineate a chord (Figs. 5 and 6).

The prolactin cells of those fish exposed to the other two photoperiods (8L:12D, 12L:12D), were similar to the above except for nuclear and cellular shape, nuclear area, and cellular density. The cells, and the nuclei of the cells, of those fish exposed to the short photoperiod were more ellipsoid than those of the long photoperiod. Some cells did maintain a circular geometry. The nuclear area of these cells is $60.13^{+3.55}\mu\text{m}^2$, and the cell density is $4.23^{+.22}$ cell/unit area (Table 1). The cells, and the nuclei of the cells, of the fish exposed to the intermediate photoperiod exhibited the greatest ellipsoidal geometry. The nuclear area and cell density of these cells are $49.66^{+4.27}\mu\text{m}^2$, and $3.77^{+.16}$ cells/unit area respectively (Table 1).

Statistical Results

The one-way analysis of variance shows significant differences between the means of at least two of the photoperiods for both nuclear area and cell density (Tables 1 and 2). The F value for the nuclear analysis is 75.88, which is greater than 3.42 found in the tables. The F value for the cell density analysis is 162.66, which is also greater than 3.42.

The least significant difference analysis shows that the nuclear areas and cell densities of all photoperiods are significantly different from each other. The order from smallest to largest for both data

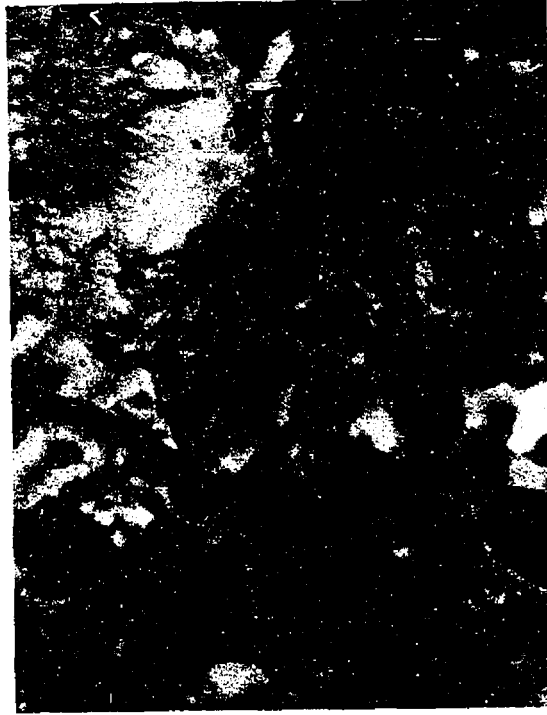


Figure 5. Prolactin cells arranged in cords (1000x, 16L:8D). This arrangement was occasionally seen with all photoperiods.

1



Figure 6. Prolactin cells under a light regimen of 16L:8D (Massons' Trichrome, 1000x). The nuclear and cellular shapes are relatively circular and a relatively high cell density is shown.



Figure 7. Prolactin cells under a light regimen of 8L:16D (Massons' Trichrome, 1000x). These cells show nuclear and cellular shapes intermediate to those shown for cells exposed to 12L:12D and 16L:8D. Cell density is also intermediate.



Figure 8. Prolactin cells under a light regimen of 12L:12D (Masson's Trichrome, 1000X). The majority of the cells show nuclear and cellular shapes that are ellipsoid. The cell density is relatively low.

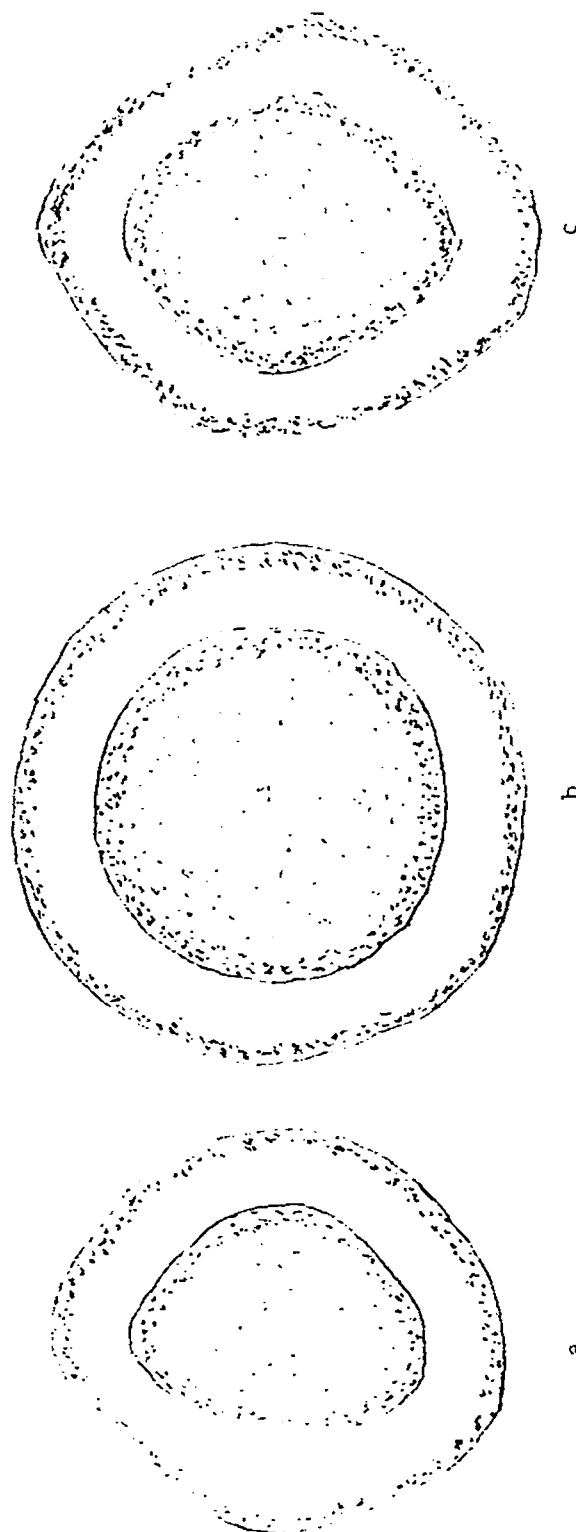


Figure 9. Relative shape of cell and nucleus and relative nuclear areas for the three photoperiods. $a=12L:12D$, $b=16L:8D$, $c=8L:16D$. $5mm = 1\mu m$

sets is 12L:12D, 8L:16D and 16L:8D (Tables 3 and 4). All calculated differences for both data sets were greater than the values of the LSD (.05) test, which was 2.95 for the nuclear area, and .19115 for cell density.

Table 1

Nuclear Area and Cell Density of Prolactin Cells (μm^2)
Under the Influence of Photoperiod

Photoperiod	Fish	Nuclear Area (AVE ⁺ -S.D.)	Cell Density (AVE ⁺ -S.D.)
8L:16D	9	60.13-3.55	4.22-0.22
12L:12D	7	49.66-4.27	3.77-0.16
16L: 8D	10	67.28-3.89	5.33-0.23

Table 2

ANOVA TABLE FOR NUCLEAR AREA

Source	df	SS	MS
Total	299	58393.75	
Fish	25	18028.91	
Photoperiod (PP)	2	15689.01	7844.50
Experimental Error (EE)	23	2339.90	101.74
Within Fish (Sampling Error)	274	40364.84	147.32

Note: df=degrees of freedom, SS=sum of squares, MS=mean square
.05

$F_{\text{test}} = \text{MSPP}/\text{MSEE} = 77.10$. $f_{\text{test}} f_{2,23} = 3.42$, at least two of the photoperiods differ with respect to nuclear area.

Table 3
ANOVA Table For Cell Densities

Source	df	SS	MS
Total	59	33.096	
Fish	25	29.7593	
Photoperiod (PP)	2	27.7690	13.8845
Experimental Error (EE)	23	1.9633	0.08536
Within Fish (Sampling Error)	34	3.3367	0.09814

Note: $f_{\text{test}} = 162.66$. Since $F_{\text{test}} f_{2,23} = 3.42$, at least two of the photoperiods differ with respect to cell density.

Table 4
LSD Analysis For Nuclear Area

	16L:8D(67.28)	8L:16D(60.13)	12L:12D(49.66)
12L:12D(49.66)	17.61	10.48	
8L:16D(60.13)	7.13		
16L:8D(67.28)			

Note: Since all values are greater than $LSD(.05) = t_{.025}^{23} 2MSEE/100 = 2.95$, all means are significantly different and may be placed in rank order from largest to smallest as follows: 16L:8D 8L:16D 12L:12D.

Table 5
LSD Analysis For Cell Density

	16L:8D(5.34)	8L:16D(4.23)	12L:12D(3.77)
12L:12D(3.77)	1.62	0.52	
8L:16D(4.23)	1.11		
16L:8D(5.34)			

23

Note: Since all values are greater than $LSD(.05) = t_{.025}^{23} \sqrt{2MSEE/20} = .19115$,
all means are significantly different from each other and may be placed
in rank order from largest to smallest as follows: 16L:8D 8L:16D 12L:12D.

CHAPTER V

DISCUSSION

Cell Density and Nuclear Size

Differences in cell density and nuclear size between photoperiods indicate that seasonal variations in photoperiod may influence prolactin cell activity. The significant increase in cell density and nuclear area of the prolactin cells of fish subjected to the long photoperiod (16L:8D), as compared to those on the intermediate photoperiod (12L:12D), suggests that the prolactin cells are more active during periods of greater daylength.

The increase in nuclear area correlating with increasing daylength has not been previously reported. The cellular area must have increased also. An increase in cellular area has previously been reported for the migratory form of G. aculeatus (Leatherland 1970). Benjamin (1974) found no change in cellular area in the freshwater form. However, Benjamin did note significant increases in RER, the number of immature secretory granules, and the number of free ribosomes in spring fish when compared to winter fish. Benjamin attributed these increases to greater cellular activity.

The increase in cell density in response to increasing daylength has also been reported for G. aculeatus form trachurus (Leatherland 1970). Benjamins' (1974) findings that the increase in the relative

proportion of the pituitary taken up by the prolactin cells in the spring as compared to the winter, along with the fact that cellular area did not increase, suggests that cell density increases for the freshwater form also. An increase in density usually means the cells have undergone mitosis. No mitosis was observed; however, active mitosis probably ceased before the fish were sacrificed.

In Leatherland's study (1970) the correlation between cell density and photoperiod ceased to exist, while the correlation between cellular area and photoperiod continued, when fish migrated to freshwater. Cell density and cellular area were inversely correlated at this time. Leatherland concluded that cell activity is regulated by two factors: photoperiod and ambient salinity. Leatherland hypothesises that photoperiod controls hormone synthesis which causes an increase in cellular size and number, whereas ambient salinity controls hormone secretion which decreases cell density as fish move to freshwater. Based on E.M. results, Benjamin (1974) concluded that an increase in photoperiod stimulates an increase in hormone secretion as well as hormone synthesis, and suggested that the mechanism of hormone release is different for freshwater fish. However, Benjamin collected fish in their natural environment. These fish were undoubtedly subjected to seasonal changes in ambient osmolarity just as frequently migratory forms although not to such a great extent. These osmolarity changes could be responsible for hormone secretion in freshwater fish as well as migratory fish which might be reflected by cellular density (Oreg

and Chadwick 1975). The quantitative results reported here strengthens the hypothesis that increases in photoperiod increases hormone synthesis, but sheds no light on the mechanism of hormone secretion.

It was expected that the nuclear area and cell density of the fish subjected to the short photoperiod (8L:16D) would be smaller, and thus less active, than the prolactin cells of those fish on the intermediate photoperiod. The fact that this was not the case may be explained by possible seasonal fluctuations in circadian rhythms of prolactin cell activity (Meier, Burns and Dusseau 1969; Spieler, Meier and Loesch 1975; Battan and Ball 1976; Ball and Grier 1976; McKeown and Peter 1976). Thus at the time N. cornutus was sacrificed (10.00 - 12.00hr), the prolactin cell activity may have reached a maximum for the short photoperiod, and a minimum for the intermediate photoperiod.

Another explanation may be that the fish subjected to the short photoperiod were under greater stress. It was observed during the experiment that these fish were more agitated, which may be due to stress, than the fish subjected to other photoperiods. Stress has been found to alter serum prolactin levels, which indicates greater cellular activity (Dunn, Arimura and Scheving 1972).

It appears that the nuclear area of the prolactin cells of N. cornutus is the largest that has been reported for fish. Excluding the cells of N. cornutus, nuclear areas range from $2.9\mu\text{m}^2$ to $43\mu\text{m}^2$ (Abraham 1971; Ball and Ingleton 1973; Benjamin 1974, 1978; Bonga 1978; Olivereau 1978; Marshall 1979). The cell density appears to be the

smallest to be reported. Cell densities reported in other studies range from 17 cells/unit area to 79 cells/unit area (Abraham 1971; Ball and Ingleton 1973; Benjamin 1978; Marshall 1979).

Qualitative Morphology and Staining Properties

Differences in nuclear and cellular shape also indicate that seasonal variations in photoperiod influence prolactin cell activity. The circular shapes of both the nuclei and cells of those fish exposed to the long photoperiod, as compared to the nuclei of those fish on the short photoperiod, suggests that the prolactin cells are more active during periods of greater daylength. Similar results have been shown for the migratory form of G. aculeatus (Leatherland 1970).

Since cytological organelles did not stain, it could not be determined if they fluctuate with photoperiod. Golgi usually appear as U or C shaped tubules in active cells (Ball and Baker 1969; Holmes and Ball 1974). Golgi would thus be expected to be observed in cells subjected to the long photoperiod. Endoplasmic reticulum would also be observed in cells subjected to the long photoperiod (Ball and Baker 1969; Holmes and Ball 1974). The significance of the distribution of heterochromatin around the periphery of the nucleus is not known and has not been previously reported.

The apparent uniformity of cellular size throughout the rostral pars distalis is not usual. The prolactin cells that are located next to blood vessels are usually larger, and are localized either centrally or peripherally. In general, the prolactin cells follow

the morphological pattern found in more advanced fish such a Poecilia, Fundulis, and cyprinodonts (Ball and Baker 1969; Holmes and Ball 1974).

Staining characteristics did not change with photoperiod. Benjamin (1974) reported similar results for G. aculeatus (leurio). However, Leatherland (1970) found an increase in stainability with increasing daylength. The intensity of the stain indicates the amount of hormone present (Ball and Baker 1969). The lack of changing intensity is interpreted to mean that photoperiod may regulate hormone secretion as well as hormone synthesis. One would expect greater stainability of cells subjected to the long photoperiod when compared to cells subjected to the control photoperiod if there were no hormone secretion. It seems then that the mechanism for hormone secretion from the prolactin cells of freshwater fish is different from those cells found in migratory forms (Benjamin 1974).

It was also observed under the dissecting scope that the rostral half of the pituitary gland was dense white and opaque, whereas the distal end appeared translucent. This observation is similar to others involving Fundulus and Poecilia where the rostral half contained prolactin cells (Ball and Baker 1969).

Hypothalamic Control of Photoperiod Responses

Morphological studies with dopamine and/or dopamine antagonists show similar results to those reported here with photoperiod (Ensor 1978). Type B Fibers (aminergic), found in the hypothalamus, innervate the prolactin cells and synthesize and secrete dopamine, which in turn inhibits the synthesis and secretion of prolactin (Nagahama et al. 1975;

Olivereau 1975; Wigham et al. 1975; Battan and Ball 1976; Wigham and Ball 1976; Ingleton, Battan and Ball 1977). Thus, secretion of dopamine inhibits prolactin cell activity as evidenced by decreasing nuclear and cellular area, cell density, and stainability.

The synthesis and secretion of dopamine is thought to be regulated by an endogenous clock in the hypothalamus, which may be entrained by photoperiod (Axelrod 1974; Ensor 1978). Therefore seasonal changes in prolactin cell activity, as evidenced by the present study, may be regulated by the entrainment of an endogenous clock of dopamine synthesis and secretion in the hypothalamus. Photoperiod must act as an inhibitor to dopamine if this hypothesis is to work.

Tissue Responses to Photoperiod via Prolactin Cell Activity

N. cornutus may have circannual changes in blood glucose, free fatty acids fat deposition, reproductive structures, and the kidney although no studies in these areas have been performed on this fish. All of these seasonal fluctuations have been attributed to photoperiod and/or prolactin in other animals (Applington 1942; Lee and Meier 1967; Pauley and Scheving 1967; Lam and Leatherland 1968; Ball and Baker 1969; Joseph and Meier 1971; Leatherland, Mckeown and John 1973; Bartke, Croft and Dalterio 1975; Bangalor, Sundararaj and Keshavanath 1976). Thus, there is a correlation between seasonal prolactin cell activity, as evidenced by changing morphological and cytological characteristics, and seasonal changes in other tissues and metabolic process (Applington 1942; Hartmann 1944; Ball and Baker 1969).

Serum levels of blood glucose and free fatty acids exhibit circadian rhythms which vary seasonally (Joseph and Meier 1971; Leatherland, Mckeown and John 1973). This would tend to support the hypothesis that it is possible for the prolactin cells of N. cornutus undergo circadian rhythms that vary seasonally, and may support the explanation for the intermediate values found for the prolactin cells subjected to the short photoperiod in this study. This would also tend to support the hypothesis of photoperiod entraining an endogenous clock in the hypothalamus that regulates prolactin cell activity.

CHAPTER VI

SUMMARY

The prolactin cells, and the heterochromation of their vessicular nuclei, appeared brick-red for all photoperiods with no significant difference in intensity between photoperiods. Heterochromatin appeared either concentrated around the nuclear membrane, or evenly distributed throughout the nucleus for all photoperiods. The cellular and nuclear membranes remained intact with little or no evaginations throughout the photoperiods. Cytoplasmic organelles did not stain in any photoperiod. Both nuclear and cellular shape ranged from ellipsoidal (12L:12D) to circular (16L:8D). The nuclear areas were $67.29^{+3.89}\mu\text{m}^2$ (16L:8D), $60.13^{+3.55}\mu\text{m}^2$ (8L:16D), and $49.66^{+4.27}\mu\text{m}^2$ (12L:12D). The cell densities were 5.34 cells/unit area (16L:8D), 4.23 cells/unit area (8L:16D), and 3.77 cells/unit area (12L:12D).

Cells were found to be grouped in chords in all photoperiods

It was concluded from these results that photoperiod may influence prolactin cell activity as evidenced by changes in nuclear and cellular shape, nuclear area, and cell density. Increases in photoperiod may change nuclear and cellular shape from ellipsoidal to circular, increases nuclear area, and cell density. These results strengthen the hypothesis that photoperiod regulates hormone synthesis. The lack of a significant difference in the intensity of staining between photoperiods suggested that hormone secretion may also be regulated by photoperiod. The greater activity of the prolactin cells of fish

exposed to the short photoperiod, when compared to the control photoperiod (12L:12D), was explained by a possible circadian rhythm which may vary seasonally.

APPENDIX

Table 6

Nuclear Area of Prolactin Cells (μm^2)-16L:8D

Cell#	Pit#									
	1	2	3	4	5	6	7	8	9	10
1	78.53	78.53	78.53	64.91	52.58	93.47	64.91	52.58	78.53	64.91
2	52.58	52.58	64.91	64.91	64.91	78.53	78.53	52.58	64.91	52.58
3	64.91	93.47	52.58	93.47	78.53	93.47	52.58	78.53	64.91	52.58
4	64.91	93.47	64.91	78.53	64.91	52.58	64.91	64.91	52.58	64.91
5	64.91	64.91	52.58	64.91	64.91	64.91	64.91	78.53	78.53	78.53
6	78.53	93.47	52.58	64.91	52.58	78.53	52.58	64.91	52.58	64.91
7	78.53	78.53	78.53	52.58	52.58	64.91	52.58	64.91	78.53	64.91
8	52.58	64.91	52.58	64.91	78.53	64.91	78.53	52.58	52.58	78.53
9	93.47	64.91	64.91	64.91	93.47	52.58	64.91	78.53	64.91	52.58
10	64.91	64.91	78.53	52.58	78.53	78.53	64.91	52.58	64.91	64.91
AVE	64.40	74.98	64.06	66.66	68.15	72.25	63.94	64.06	64.91	63.94

The average nuclear area of prolactin cells exposed to a light regime of 16L:8D is 67.28 ± 3.89 . N=10

TABLE 7

Nuclear Area of Prolactin Cells (μm^2)-12L:12D.

Cell#	Pit#	1	2	3	4	5	6	7
1		64.91	31.80	78.53	52.58	41.53	64.91	41.53
2		52.58	64.91	41.53	41.53	41.53	52.58	64.91
3		52.58	52.58	52.58	52.58	31.80	52.58	64.91
4		41.53	52.58	78.53	41.53	52.58	31.80	52.58
5		78.53	64.91	31.00	52.58	41.53	78.53	52.58
6		41.53	31.80	41.53	52.58	31.80	41.53	64.91
7		52.58	41.53	52.58	52.58	41.53	23.36	52.58
8		41.53	52.58	41.53	41.53	52.58	52.58	64.91
9		52.58	64.91	41.53	41.53	41.53	52.58	41.53
10		52.58	52.58	41.53	64.91	64.91	52.58	52.58
11		64.91	31.80	64.91	52.58	52.58	52.58	41.53
12		52.58	52.58	64.91	52.58	64.91	23.36	31.80
13		52.58	41.53	52.58	31.80	64.91	41.53	52.58
14		41.53	64.91	31.80	31.80	52.58	41.53	52.58
15		52.58	52.58					
AVE		53.00	50.23	57.84	47.33	48.30	47.28	56.00

Average nuclear area for prolactin cells exposed to a light regime of 12L:12D is $49.66 \pm 4.27 \mu\text{m}^2$. N=7

TABLE 8

Nuclear Area of Prolactin Cells (μm^2)-8L:16D

Cell#	Pit#	1	2	3	4	5	6	7	8	9
1		41.53	64.91	52.58	78.53	52.58	31.80	64.91	52.58	31.80
2		52.58	78.53	64.91	41.53	52.58	64.91	64.91	52.58	52.58
3		52.58	52.58	64.91	64.91	31.80	41.53	78.53	64.91	78.53
4		64.91	78.53	52.58	52.58	64.91	52.58	52.58	64.91	64.91
5		52.58	64.91	78.53	64.91	64.91	78.53	64.91	64.91	52.58
6		64.91	64.91	52.58	64.91	64.91	64.91	52.58	52.58	78.53
7		78.53	52.58	64.91	52.58	52.58	78.53	52.58	64.91	64.91
8		64.91	64.91	52.58	64.91	64.91	52.58	52.58	78.53	52.58
9		52.58	52.58	64.91	52.58	52.58	41.53	64.91	52.58	64.91
10		41.53	64.91	52.58	52.58	78.53	64.91	52.58	64.91	52.58
11		64.91	64.91	78.53	78.53	64.91	78.53	52.58	64.91	64.91
12										64.91
AVE		51.52	64.02	61.79	60.78	58.58	59.11	57.52	61.65	60.30

The average nuclear area of prolactin cells exposed to a light regime of 8L:16D is 60.13 ± 3.55 . N=9

Continuation of Table 8

8L:16D										
Area#	Pit #	1	2	3	4	5	6	7	8	9
1		4.1	3.9	4.0	4.1	4.0	4.8	4.7	3.9	4.3
2		4.3	4.2	4.5	4.4	4.0	4.4	4.3	4.1	4.1
3									4.3	4.0
AVE		4.20	4.05	4.25	4.25	4.00	4.60	4.60	4.10	4.13

Note: The mean cell densities for the photoperiods 12L:12D, 16L:8D, and 8L:16D are 3.705 cells/mm², 5.330 cells/mm², and 4.220 cells/mm² at 1600x.

TABLE 9

		Cell Densities (Cells/mm ²)-Grid Count at 1600x																		
		12L:12D									16L:8D									
	Pit#	1	2	3	4	5	6	7			1	2	3	4	5	6	7	8	9	10
Area#		4.4	3.1	4.0	3.9	3.5	3.3	3.7			5.6	5.0	5.3	5.0	5.3	5.6	5.3	5.4	5.5	5.1
1		4.4	3.1	4.0	3.9	3.5	3.3	3.7			5.6	5.0	5.3	5.0	5.3	5.6	5.3	5.4	5.5	5.1
2		3.2	4.2	3.6	4.1	4.0	3.7	3.1			5.8	5.7	5.2	4.8	5.2	5.5	5.2	5.1	5.7	5.4
3		3.8	3.2	3.7		3.6	3.9	41.												
AVE		3.80	3.50	3.77	4.00	3.70	3.67	3.97			5.70	5.35	5.25	4.90	5.25	5.55	5.25	5.25	5.60	5.25

BIBLIOGRAPHY

- Abraham, M. 1971. The ultrastructure of the cell types and of the neurosecretory innervation in the pituitary of Mugil cephalis L. from freshwater, the sea, and a hypersaline lagoon. 1. The rostral pars distalis. Gen. Comp. Endocrinol. 17:334-350.
- _____, and Blanc-Livni, N. 1969. The ultrastructure of prolactin cells in the pituitary of Mugil cephalus. Gen. Comp. Endocrinol. 13:489 (ABSTR).
- Alder, G., Bage, G., and Fernholm, B. 1971. On the existence of prolactin in cyclostomes. Gen. Comp. Endocrinol. 16:498-503.
- Applington, H.W. 1942. Correlative cyclical changes in the hypophysis and gonads of Necturus maculosus rafinesque. Anat. Rec. 70:201-242.
- Axelrod, J. 1974. The pineal gland: A neurochemical transducer. Science 184:1341-48.
- Ball, J.N. 1965a. A regenerated pituitary remnant in a hypophysectomized killifish Fundulus heteroclitus: further evidence for the cellular source of the teleostean prolactin-like hormone. Gen. Comp. Endocrinol. 5:181-5.
- _____. 1965b. Partial hypophysectomy in the teleost Poecilia; separate identities of teleostean growth hormone and teleostean prolactin-like hormone. Gen. Comp. Endocrinol. 5:654-61.
- _____. 1965c. Effects of autotransplantation of different parts of the pituitary gland on freshwater survival in the teleost Poecilia latipinna. J. Endocrinol. 33:v-vi.
- _____, and Baker, B.I. 1969. The pituitary gland. In Fish Physiology, ed. W.S. Hoar and D. S. Randall. New York and London: Academic Press.
- _____, and Ingleton, P.M. 1973. Adaptive variations in prolactin secretion in relation to external salinity in the teleost Poecilia latipinna. Gen. Comp. Endocrinol. 20:312-25.
- _____, and Olivereau, M. 1964. Role de la prolactin dans la survie en eau douce de Poecilia latipinna hypophysectomisee et arguments in favor de sa synthesis par les cellules crythrosinopiles de l'hypophyse des teleosteans. C.r. Acad. Sci., Paris. 259:1443.

- _____, and _____. 1965. Pituitary and autotransplants and freshwater adaptation in the teleost Poecilia latipinna. Amer. Zool. 5:167A.
- _____, and Pickford 1964. Pituitary Cytology and freshwater adaptation in the killifish Fundulus heteroclitus. Anat. Rec. 148:358.
- Bartke, A., Croft, B.T., and Dalreria, S. 1975. Prolactin restores plasma testosterone levels and stimulates testicular growth in hamsters exposed to short day-length. Endocrinology. 97:1901-04.
- Battan, T.F.C., Ball, J.N., and Grick, T.M. 1976. Circadian changes in prolactin cell activity in the pituitary of the teleost Poecilia latipinna in freshwater. Cell Tissue Res. 165:267-280.
- Benjamin, M. 1974. Seasonal changes in the prolactin cell of the pituitary gland of the freshwater stickleback Gasterosteus aculeatus, form leuries. Cell Tissue Res. 152:93-102.
- _____. 1978. Cytological changes in prolactin, ACTH, and growth hormone cells of the pituitary gland of Pungitius pungitius L. in response to increased environmental salinities. Gen. Comp. Endocrinol. 36:48-58.
- Bern, H.A. 1967. Hormones and the endocrine glands of fishes. Science 158:455-62.
- Bhattacharyya, G.K., and Johnson, R.A. 1977. Statistical Concepts and Methods. New York: John Wiley and Sons.
- Blanc-Livni, N., and Abraham, M. 1970. The influence of environmental salinity on the prolactin-and gonadotropin-secreting regions in the pituitary of Mugil (teleostei). Gen. Comp. Endocrinol. 14:184-97.
- Bleick, C.R. 1975. Hormonal control of the nuchal hump in the cichlid fish Cichlasoma. Gen. Comp. Endocrinol. 26:192-98.
- Blum, B. 1974. Prolactin and behavior in fish. ACTH Endocr. (Copah). 151:172.
- _____, and Fielder, K. 1964. Hormonal control of reproductive behavior in some cichlid fish. Gen. Comp. Endocrinol. 5:186-96.
- Bonga, S.E.W. 1978. The effects of changes in external sodium, calcium, and magnesium concentrations on prolactin cells, skin, and plasma electrolytes of Gasterosteus aculeatus. Gen. Comp. Endocrinol. 34:265-75.

- Bourne, R.A., and Tucker, H.A. 1975. Serum prolactin and LH responses to photoperiod in bull calves. Endocrinology. 97: 473-475.
- Burdon, G.E. 1956. The failure of hypophysectomized Fundulus heteroclitus to survive in freshwater. Biol. Bull. 110: 8-29.
- Buttle, H.L. 1974. Seasonal variation of prolactin in plasma of male goats. J. Reprod. Fert. 37:95-9.
- Chadwick, A. 1966. Prolactin-like activity in the pituitary gland of fishes and amphibians. J. Endocrinol. 35:75-81.
- Chan, D.K.O., Jones, C.I., and Mosley, W. 1968. Pituitary and adrenocortical factors in the control of water and electrolyte composition of the freshwater european eel. J. Endocrinol. 42:91-8.
- Chidambaram, S., Meyer, R.K., and Hasler, A.D. 1972. Effects of hypophysectomy, prolactin autografts, prolactin, temperature, and salinity of the medium on the survival and natremia in the bullhead, Ictalurus melas. Comp. Biochem. Physiol. 43A:443-57.
- Clark, R.H., and Baker B.L. 1963. Circadian periodicity in the concentration of prolactin in the rat hypothesis. Science 141: 375-76.
- Cleveland, R., and Wolfe, J.M. 1932. A differential stain for the anterior lobe of the hypophysis. Anat. Rec. 51:409-13.
- Cook, H., and van Overbeeks, A.P. 1969. Ultrastructure of the eta cells in the pituitary gland of adult migratory sockeye salmon (Oncorhynchus nerka). Can. J. Zool. 47:937-41.
- Dharamamba, M., 1970. Studies of the effects of hypophysectomy and prolactin on plasma osmoregularity and plasma sodium in Tilapia mossambica. Gen. Comp. Endocrinol. 14:256-69.
- _____, and Nishioka, R.S. 1968. Response to "prolactin-secreting" cells of Tilapia mossambica to environmental salinity. Gen. Comp. Endocrinol. 10:409-20.
- Donaldson, E.M., and McBride, I.R. 1967. The effects of hypophysectomy in the rainbow trout Salmo gairdnerii with special reference to the pituitary-interrenal axis. Gen. Comp. Endocrinol. 9:93-101.
- Dunn, J.D., et al. 1972. Effect of stress on circadian periodicity in serum LH and prolactin concentrations. Endocrinology. 90: 29-33.

- Eddy, S., and Underhill, J.C. eds. 1978. How to know the freshwater fishes. Dubuque: Wm. C. Brown Company Publishers.
- Emmart, E.W. 1969. The localization of endogenous "prolactin" in the pituitary gland of the goldfish, Carassius auratus Linnaeus. Gen. Comp. Endocrinol. 12:519-25.
- _____, and Mossakowski, M.J. 1967. The localization of prolactin in cultured cells of the rostral pars distalis of the pituitary Fundulus heteroclitus (Linnaeus). Gen. Comp. Endocrinol. 9: 391-400.
- _____, Pickford, G.E., and Wilhelmi, A.E. 1966. Localization of prolactin within the pituitary of a cyprinodont fish, Fundulus heteroclitus (Linnaeus), by specific fluorescent antiovine prolactin globin. Gen. Comp. Endocrinol. 7:571-83.
- Ensor, D.M. 1978. Comparative endocrinology of prolactin. London: Chapman and Hall.
- Forbes, J.M., et al. 1974. The effect of daylength and level of feeding on serum prolactin in growing lambs. J. Endocrinol. 64:549-54.
- Forbes, S.A., and Richardson, R.E. 1908. The fishes of Illinois. Danville: Illinois Printing Company.
- Giunta, C., et al. 1973. Biochemical data on some organs from normal or prolactin-treated Xenopus laevis (Daudin) adults reared in fresh and in saltwater. Gen. Comp. Endocrinol. 20:107-111.
- Hartmann, J.F. 1944. Seasonal cytological changes in the anterior hypophysis of the garter snake. Am. J. Anat. 75:121-49.
- Hoar, W.S., and Lam, T.J. 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. 12:385-94.
- Holmes, R.L., and Ball, J.N. 1974. The pituitary gland. Cambridge: Cambridge University Press.
- Humason, G.L. 1979. Animal tissue techniques. San Francisco: W.H. Freeman and Company.
- Ingleton, P.M., Battan, T., and Ball, J.N. 1977. Observations on the catecholaminergic innervation of the prolactin cells in the teleost Poecilia latipinna. J. Endocrinol. 73:9P.
- Johnson, D.W. 1972. Time course of response of starry flounder (Platichthys stellatus) urinary bladder to prolactin and to salinity transfer. Gen. Comp. Endocrinol. 24:373-80.

- Joseph, M.M., and Meier, A.H. 1971. Daily variations in the fattening response to prolactin in Fundulus grandis held on different photoperiods. J. Exp. Zool. 178:59-62.
- Kizer, S.S., et al. 1975. The nyctohemeral rhythm of plasma prolactin: effects of ganglionectomy, pinealectomy, constant light, constant darkness or 6-OH-dopamine administration. Endocrinology. 96:12:3-40.
- Kraicer, J., Herlant, M., Duclos, P. 1967. Changes in adenohipophysial cytology and nucleic acid content in the rat 32 days after bilateral adrenalectomy and the chronic injection of corticoid. Can. J. Physiol. Pharm. 45:948-55.
- Lahlou, B., and Sawyer, W.H. 1969. Electrolyte balance in the hypophysectomized goldfish Carassius auratus. Gen. Comp. Endocrinol. 12:370-77.
- Lam, T.J. 1968. Effect of prolactin on plasma electrolytes of the early-winter marine threespine stickleback, Gasterosteus aculeatus, form trachurus, following transfer from sea to freshwater. Can. J. Zool. 46:1095-97.
- _____. 1972. Prolactin and hydromineral regulation in fishes. Gen. Comp. Endocrinol. Suppl 3:328-38.
- _____, and Hoar, W.S. 1967. Seasonal effects of prolactin on freshwater osmoregulation of the marine form (trachurus) of the stickleback Gasterosteus aculeatus. Can. J. Zool. 45:509-16.
- _____, Leatherland, J.F. 1969a. Effect of prolactin on freshwater survival of the marine form (trachurus) to the threespine stickleback Gasterosteus aculeatus, in the early winter. Gen. Comp. Endocrinol. 12:385-87.
- _____, _____. 1969b. Effect of prolactin on the glomerulus of the threespine stickleback Gasterosteus aculeatus L., form trachurus, after transfer from seawater to freshwater during late autumn and early winter. Can. J. Zool. 47:247-50.
- Leatherland, J.F. 1970. Seasonal variation in the structure and ultrastructure of the pituitary of the marine form (trachurus) of the threespine stickleback, Gasterosteus aculeatus L. I. Rostral pars distalis. Z. Zellforsch. 104:301-17.
- _____. 1972. Histophysiology and innervation of the pituitary gland of the goldfish Carassius auratus L. A light and electron microscope study. Can. J. Zool. 50:835-44.

- _____, and Ensor, D.M. 1973. Activity of the autotransplanted pituitary glands in the goldfish Carassius auratus L. maintained in different ambient salinities. Can. J. Zool. 51:225-35.
- _____. 1974. Effects of hypothalamic extracts on prolactin secretion in the goldfish, Carassius auratus. Comp. Biochem. Physiol. 47A:419-26.
- _____, and Lam, T.J. 1969. Effect of prolactin on the density of the mucous cells on the gill filaments of the marine form (trachurus) of the threespined stickleback, Gasterosteus aculeatus. Can. J. Zool. 47:7878-92.
- _____, and McKeown, B.A. 1973. Circadian rhythm in the plasma levels of prolactin in goldfish, Carassius auratus L. J. Interdiscipl. Cycle Res. 4:137-43.
- _____. 1974. Effect of ambient salinity on prolactin and growth hormone secretion and on hydromineral regulation in Kokanee salmon smolts (Oncorhynchus nerka). J. Comp. Physiol. 89:215-26.
- _____, _____, and John, T.M. 1974. Circadian rhythm of plasma prolactin, growth hormone, glucose and free fatty acid in juvenile Kokanee salmon, Oncorhynchus nerka. Comp. Biochem. Physiol. 47A:821-28.
- Lee, R.W., and Meier, A.H. 1967. Diurnal variations of the fattening response to prolactin in the golden topminnow, Funulus chrysotus. J. Exp. Zool. 166:307-16.
- Lodi, G., Biciotti, G.L., and Sacerdote, M. 1978. Osmoregulatory activity of prolactin in the skin of the crested newt. Gen. Comp. Endocrinol. 36:7-15.
- MacLeod, R.M., and Abad, A. 1968. On the control of prolactin and growth hormone synthesis in rat glands. Endocrinology. 83: 799-806.
- Marshall, W.S. 1979. Effects of salinity acclimation, prolactin growth hormone, and cortisol on the mucous cells of Leptocottus armatus (Teleostei Cottidae). Gen. Comp. Endocrinol. 37: 358-68.
- McKeown, D., and van Overbeeke, B. 1969. Immunohistochemical localization of prolactin-secreting cells of adult migratory salmon (Onchorhynchus nerka). J. Fish Res. Bd. Canada
- _____, and Peter, R.E. 1976. The effects of photoperiod and temperature on the release of prolactin from the pituitary gland of the goldfish, Carassius auratus L. Can. J. Zool. 34:1960-68.

- Meier, A.H. 1969. Diurnal variations in metabolic responses to prolactin in lower vertebrates. Gen. Comp. Endocrinol. Suppl. 2:55-62.
- _____, Burns, J.T., and Dusseau, S.E. 1969. Seasonal variations in the diurnal rhythm of pituitary content in the white-throated sparrow, Zonotrichia albicollis. Gen. Comp. Endocrinol. 12:282-89.
- Nagahama, Y., et al. 1975. Control of prolactin secretion in teleosts with special reference to Gillichthys mirabilis and Tilapia mossambica. Gen. Comp. Endocrinol. 25:166-88.
- Narasimhan, P.V., and Sundararaj, B.I. 1971. Circadian variation in carbohydrate parameters in two teleosts, Notropis notopterus (Pallas) and Colisa fasciata (Bloch and Schneider). Comp. Biochem. Physiol. 39A:89-99.
- Nikolaitsky, G.V. 1963. The ecology of fish. New York: Academic Press.
- Oduleye, S.O. 1976. The effects of calcium on water balance of the brown trout (Salmo trutta). J. Exp. Biol. 63:343-56.
- Ogawa, M., and Johanson, P.H. 1967. A note on the effect of hypophysectomy on the mucus on the goldfish Carassius auratus. Can. J. Zool. 45:885-86.
- Olivereau, M. 1966. Modifications of the "prolactin cells" in seawater eels. Am. Zool. 6:598.
- _____. 1975. Dopamine, prolactin control and osmoregulation in eels. Gen. Comp. Endocrinol. 26:550-61.
- _____. 1978a. Effect of primozide on the cytology of the eel pituitary. I. Prolactin-secreting cells. Cell Tissue Res. 189:219-30.
- _____. 1978b. Effect of parachlorophenylalanine, a brain serotonin depletor, on the prolactin cells of the eel pituitary. Cell Tissue Res. 191:93-99.
- _____, and Ball, J.N. 1970. Pituitary influences osmoregulation in teleosts. Mem. Soc. Endocrinol. 18:57-85.
- Pauly, J.E., and Scheving, L.E. 1967. Circadian rhythms in blood glucose and the effect of different lighting schedules, hypophysectomy, adrenal medullectomy and starvation. Am. J. Anat. 120:627-36.

- Pelletier, J. 1973. Evidence for photoperiodic control of prolactin release in rams. J. Reprod. Fert. 35:143-47.
- Peter, R.E. 1972. Feedback effects of thyroxine in goldfish Carassius auratus with an autotransplanted pituitary. Neuroendocrinology. 10:273-81.
- Peterson, R.R., and Weiss, J. 1955. Staining of adenohypophysis with acid and basic dyes. Endocrinology. 57:96-108.
- Pickford, G.E., and Atz, J.W. 1957. The physiology of the pituitary gland of fishes. New York: Zoological Society.
- _____, and Phillips, J.G. 1959. Prolactin, a factor in promoting survival of hypophysectomized billfish in fresh-water. Science. 130:454-55.
- _____, Robertson, G.E., and Sawyer, W.H. 1965. Hypophysectomy, replacement therapy, and the tolerance of the euryhaline killifish, Fundulus heteroclitus, to hypotonic media. Gen. Comp. Endocrinol. 5:160-80.
- Quadri, S.K., and Spies, H.G. 1976. Cyclic and diurnal patterns of serum prolactin in the chesus monkey. Biol. Reprod. 14: 495-501.
- Relkin, R. 1972. Effects of variation in environmental lighting on pituitary and plasma prolactin levels in the rat. Neuroendocrinology. 9:278-84.
- _____, Adachi, M., and Kahan, S.A. 1972. Effects of pinealectomy and constant light and darkness on prolactin levels in the pituitary and plasma and on pituitary ultrastructure of the rat. J. Endocrinol. 54:263-68.
- Richards, G.E., et al. 1980. Regulation of prolactin and growth hormone secretion. Neuroendocrinology. 30:139-43.
- Sage, M. 1968. Responses to osmotic stimuli of Xiphophorus prolactin cells in organ culture. Gen. Comp. Endocrinol. 10:70-74.
- _____. 1973. The relationship between the pituitary content of prolactin and blood sodium levels in mullet (Mugil cephalus) transferred from seawater to freshwater. Contr. Mar. Sci. 17: 163-67.
- _____, and deVlaming V.L. 1973. A diurnal rhythm in hormone effectiveness and in pituitary content in Fundulus similis. Tex. Rep. Biol. Med. 31:101-2.

- Schreibman, M.P., and Kallman, K.D. 1966. Endocrine control of freshwater tolerance in teleosts. Gen. Comp. Endocrinol. 6:144-55.
- _____, Leatherland, J.F., and McKeown, B.A. 1973. Functional morphology of teleost pituitary gland. Am. Zool. 13:719-42.
- Spieler, R.E., Meier, A.H., and Loesch, H.C. 1976. Seasonal variations in circadian levels of serum prolactin in striped mullet, Mugil cephalis. Gen. Comp. Endocrinol. 29:156-60.
- _____, _____, and Noeske, T.A. 1979. Ambient salinity and the daily variation of circulating prolactin in the gulf killifish Fundulus grandis. J. Interdiscipl. Cycle Res. 10: 233-38.
- Steel, R.G.D., and Torrie, J.H. 1960. Principles and Procedures of Statistics. New York: McGraw-Hill Book Co. Inc.
- Sundararaj, B.I., and Goswami, S.V. 1966. Effects of mammalian hypophysical hormones, placental gonadotropins, gonadal hormones and adrenal corticosteroids on ovulation and spawning in hypophysectomized catfish, Heteropneustes fossilis Bloch. Gen. Comp. Endocrinol. Suppl. 2:69-80.
- _____, and Keshavanath, P. 1976. Effects of melatonin and prolactin treatment on the hypophysical-ovarian system in the catfish, Heteropneustes fossilis (Bloch). Gen. Comp. Endocrinol. 29:84-96.
- _____, and Nayyer, S.K. 1969. Effect of prolactin on the "seminal vesicles" and the neural regulation of prolactin secretion in the catfish Heteropneustes fossilis Bloch. Gen. Comp. Endocrinol. Suppl. 2:69-80.
- Swift, D.R., and Pickford, G.E. 1965. Seasonal variation in the hormone content of the pituitary gland of the perch, Perca fluriatilis. L. Gen. Comp. Endocrinol. 5:354-65.
- Tavolga, W.N. 1955. Effects of gonadectomy and hypophysectomy on pre-spawning behavior in males of the gobiid fish Bothygobius soporator. Physiol. Zool. 28:218-31.
- Tindal, J.S. 1974. Hypothalamic control of secretion and release of prolactin. J. Reprod. Fert. 39:437-61.
- Utida, S., et al. 1971. Effect of prolactin on survival and plasma sodium levels in hypophysectomized medaka Oryzias latipes. Gen. Comp. Endocrinol. 16:566-73.
- _____, et al. 1972. Hormonal control of the intestine and urinary bladder in the teleost osmoregulation. Gen. Comp. Endocrinol. Suppl. 3:317-27.

- deVlaming, V.L., 1973. The effects of temperature and photoperiod on reproductive cycling in the estuarine gobiid fish, Gillichthys mirabilis. U.S. Natl. Mar. Fish Serv. Fish Bull. 70:1137-52.
- _____, and Pardo, R.J. 1974. In vitro effects of prolactin on liver lipid metabolism in the teleost fish Notemigonus chrysolaucus. Am. Zool. 14:230 (ABSTR).
- _____, Sage, M., and Charlton C.D. 1973. The effects of melatonin and prolactin on body lipids in teleosts and amphibians and an effect of melatonin on pituitary prolactin in a teleost. Am. Zool. (ABSTR).
- _____, and Sundararaj, B.I. 1972. Endocrine influences on seminal vesicles in the estuarine gobiid fish, Gillichthys mirabilis. Biol. Bull. 142:243-50.
- _____, et al. 1974. The effects of melatonin on lipid deposition in cyprinodontid fishes and on pituitary prolactin activity in Fundulus similis. J. Comp. Physiol. 94:309-20.
- Weiss, M. 1965. The release of pituitary secretion in the platyfish, Xiphophorus maculatus (Guenther). Z. Zellforsch. Mikrosk. Anat. 68:783-94.
- Wighma, T., and Ball, J.N. 1976. In vivo evidence for catecholaminergic inhibition of prolactin secretion in teleost Poecilia latipinna. J. Comp. Physiol. 110:135-43.
- _____, _____. 1976. In vivo evidence for catecholaminergic inhibition of prolactin secretion in the teleost Poecilia latipinna. J. Comp. Physiol. B. Metab. Transp. Funct. 110:135-43.
- _____, _____, and Ingleton, P.M. 1976. Secretion of prolactin and growth hormone by teleost pituitaries in vitro. III. Effect of dopamine on hormone release in Poecilia latipinna. J. Comp. Physiol. 104:87-96.
- _____, Nishioka, R.S., and Bern, H.A. 1977. Factors affecting in vitro activity of prolactin cells in the euryhaline teleost Sarotherodon mossambicus (Tilapia mossambica). Gen. Comp. Endocrinol. 32:120-31.
- Wurtman, R.J. 1975. The effects of light on human body. In Hormones and Reproductive Behavior. Readings from Scientific American 1979. San Francisco: W.H. Freeman and Company.

Zambrano, D. 1971. The nucleus lateralis tuberalis system of the gobiid fish Gillichthys mirabilis. III. Functional modifications of the neurons and the gonadotrophic cells. Gen. Comp. Endocrinol. 17:164-82.

_____, et al. 1974. Influence of G-hydroxydopamine on the hypothalamic control of prolactin and ACTH secretion in the teleost fish, Tilapia mossambica. Neuroendocrinology. 13: 284-98.