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HISTOLOGY AND PROTEIN PATTERNS OF THE PITUITARY OF GOLDFISH, CARASSIUS AURATUS, IN RELATION TO FOOD SUPPLY

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HISTOLOGY AND PROTEIN PATTERNS OF THE PITUITARY OF GOLDFISH, CARASSIUS AURATUS, IN RELATION TO FOOD SUPPLY

APPROVED BY

Howard A. Clemens

John M. Augenfeld

Arnold Braver

S.H. Wender

DISSERTATION COMMITTEE

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CHAPTER I

INTRODUCTION

Fresh water fishes are adapted so that the timing of events in the reproductive cycle is adjusted by certain factors in the external environment. The effect of the environment on the fish gonad is, however, indirect. The pituitary responds initially (via the central nervous system) to certain alterations in the external environment and changes in pituitary target organs are a consequence of secretory changes in the pituitary (Gerbil'skii, 1951). Thus a major role of the pituitary is that of an intermediary between the external and internal environments (Pickford and Atz, 1957). Ample evidence has accumulated to show that pituitary secretions regulate the reproductive processes of teleosts (Dodd, 1955; Hoar, 1955).

Most investigations dealing with the effects of environmental manipulation on fish reproduction have been made with reference to changes in the gonad. Several

investigators have studied the response of the gonad to controlled light (Ogneff, 1911; Bullough, 1939, 1959; Baggerman, 1957; and Harrington, 1959) and temperature (Turner, 1919; Craig-Bennet, 1931; Burger, 1939; Merriman and Schedl, 1941; and Kawamura and Otsuka, 1950). However, study of the corresponding condition of the pituitary has been largely neglected and, as a result, the response of the pituitary to specific environmental factors is poorly understood. Thus a key point in the series of causally related events that lead from the environment to the act of reproduction is not adequately explained. The increasing use of fish as a source of protein has recently emphasized the need to understand and control the reproductive processes of fishes. Yet, progress in gaining some measure of control over fish reproduction awaits a clearer understanding of the factors regulating pituitary activity. Further studies correlating changes in the gonad, pituitary, and specific environmental factors are indicated.

Under natural conditions, the environmental factor most readily regulated, on a large scale, is food. Yet food and its influences on fish reproduction have received less attention than other environmental factors (Fontaine and Fontaine, 1962). The response of the gonad to lack of food has been recently reported (Reed, 1965) but the corresponding condition of the pituitary was not investigated.

The morphology and histology of the teleostean

pituitary gland have been extensively studied (Pickford and Atz, 1957), and several authors have described seasonal changes in pituitary histology (Scruggs, 1951; Beach, 1959; Sundararaj, 1960; Sokol, 1961; Sathyanesan, 1963; Barr, 1963a; and Lagios, 1965). However, changes in pituitary protein contents have not been studied seasonally or under controlled environmental conditions.

The objectives of this study were to describe and compare changes in pituitary histology and protein contents associated with dietary controlled gonadal regression and recovery from regression of male goldfish (Carassius auratus) and, if possible, to relate those changes to food supply. A study of fish from a natural environment was included in order to compare the pituitary and gonad conditions in the natural and experimental environments.

CHAPTER II

METHODS

This study was conducted at the University of Oklahoma Fisheries Research Center. The fish used were taken from stock maintained on a year-round basis in the Fisheries Research Center ponds. This provided the advantage of using homogeneous groups of fishes with the same history.

The investigation included a study of four groups of male goldfish. Three groups were kept under controlled conditions in the laboratory. These were: (1) fish placed on a regression diet and (2) their controls, which were placed on full feed; and (3) regressed fish placed on full feed. The fourth group was maintained in a natural (pond) environment. Fishes of uniform body weight were selected for each group.

Fish kept in the laboratory were placed in 100 gallon troughs supplied with well-aerated, filtered water, circulated at a rate of 5 gal per minute. Temperature was maintained at 24 $\pm$ 2° C and the fish received natural light. A commercial fish food (Clark's New Age Complete Trout Grower Crumbles) was given at a controlled level.

Regression

The fish used in the regression study were adult males, transferred from the ponds to the laboratory in October, 1964. These were divided into two groups: 225 experimental fish and 100 control fish.

The experimentals were fed at a regression level of one per cent of the initial body weight, five days a week for a period of thirty-two weeks. The daily feed was distributed during at least three and often as many as six feeding periods. Food was evenly distributed in the troughs to counteract the effect of aggressiveness. Samples were taken at two week intervals according to the subsequently described sampling technique.

Control

The controls for this experiment were fed at a level of four per cent of the initial body weight per day, five days a week throughout the experiment. Treatment of the control and experimental fish was otherwise identical. Control samples were taken monthly.

Recovery from Regression

One hundred male goldfish with nutritionally regressed testes were placed under an experimental regimen identical to the one described above except that they were fed at a recovery level of four per cent of the initial body weight per day, five days a week. Samples were taken monthly over a period of twenty-eight weeks.

Nature

In October, 1964, a population of nine thousand (estimated on a weight basis) six-month old male and female goldfish was established in a one-tenth acre pond. Commencing December, 1964, samples of the males of this population were taken at two week intervals for a period of one year. The natural diet was supplemented by a mixture of cottonseed meal and bran fed at a level of three per cent of the body weight, three times weekly.

Sampling Technique

Samples of both laboratory and pond fish were taken as follows: Twelve fish were taken for each sample. From six of these, body and gonad weights were recorded and the pituitaries preserved for histological examination. The remaining six provided pituitaries for protein determination.

The sample fish were weighed intact, then quickly dissected to remove the pituitaries and testes. The testes were weighed to the nearest milligram and their relative weight expressed as a Gonosomatic Index (GSI), defined as (testes weight/body weight) x 100.

To remove the pituitary, the roof of the skull was split sagittally, pulled apart, and the brain lifted out. The pituitary usually remained attached to the brain and was easily removed. The pituitaries used for protein determination were quickly frozen after dissection and stored at -30° C.

Histology

The pituitaries collected for histological purposes were fixed in Baker's ten per cent calcium-formalin solution (Baker, 1958) and imbedded in paraffin. Sagittal sections were cut at six to eight micra, mounted serially, and stained using a modified Mallory-Heidenhain technique (Cason, 1950).

The terminology used for the lobes and regions of the pituitary is that suggested by Pickford and Atz (1957). The total basophiles (cyanophiles) and acidophiles which form the meso-adenohypophysis were counted and the differential cell count used in a quantitative analysis of cell types. A micrometer was used to determine the total area of the pituitary and the areas of the pro-, meso-, and meta-adenohypophysis. A mediosagittal section of the pituitary was used for cell counts and area determinations. The medial section of the pituitary was identified with maximum width of the stalk of the neurohypophysis.

Protein Determinations

Pituitary protein patterns were determined by the technique of disc electrophoresis, essentially as described by Ornstein (1964) and Davis (1964). Electrophoresis was performed in columns of polyacrylamide gel consisting of three sections: (1) a large pore anti-convection gel into which the protein sample is introduced; (2) a large pore spacer gel in which the constituents of the sample are

electrophoretically separated and concentrated into thin, contiguous zones; and (3) a small pore gel in which the sample is separated by both electrophoresis and molecular sieving. The gel was placed in glass tubes 65 mm in length and 6 mm inside diameter.

The six sample pituitaries were pooled and homogenized with unpolymerized gel. After centrifuging, half of the sample was added to each of two gel tubes. Thus each gel tube contained an amount of protein equivalent to three pituitaries. Electrophoresis was run at five milliamps per tube for approximately one hour. Electrophoresis was stopped when the tracking dye (Bromophenol Blue) had migrated 30 mm into the small pore gel. The proteins in the gels were simultaneously fixed and stained in 7.5 per cent aqueous acetic acid containing Amido-Schwartz dye. Removal of unbound dye was accomplished electrophoretically. The electrophoretic patterns were analyzed quantitatively using a CANALCO Model E Microdensitometer. This instrument provides a densitometric trace of the gel column. Total protein was expressed as the total area under the tracing and separate bands were quantitated on the basis of their optical densities.

CHAPTER III

RESULTS

Regression

GSI and Body Weight

The GSI of fish placed on a regression diet decreased from 2.3 to 0.1 in eight months (Fig. 1). During the first two months of the experiment (until December 21) the GSI decreased from 2.3 to 0.6. After December 21 the GSI increased over a ten week period to a value of 1.9. This was followed by a final fourteen week period during which the GSI decreased regularly to a minimum value of 0.1. At this GSI, the gonad is reduced to a thin string of tissue. Histologically, it is composed primarily of spermatogonia, spermatocytes, and fibrous connective tissue (Reed, 1965).

Body weights are shown in Fig. 3. There was no statistically significant difference (at the five per cent level) between the original and final body weights of fish kept on a regression diet. Mortality during the experiment was seven per cent. The mortality was not as high as anticipated and twelve experimental fish were left after all control fish were used. These supplied protein and GSI data

for two additional experimental samples.

Histology

The balance between the two cell types of the meso-adenohypophysis is shown as a basophile/acidophile (B/A) ratio in Fig. 1. Fig. 2 shows the average total cell counts.

The B/A ratio showed a sequence of changes similar to those seen in GSI. The original and final values were 2.1 and 0.7, respectively. Cell counts show that the inversion of the B/A ratio was due to both basophile decrease and acidophile increase.

Total mediosagittal pituitary area is shown in Fig. 2. The areas of the pro-, meso-, and meta-adenohypophysis are given in Table I.

The mediosagittal area decreased during the latter half of the regression experiment. The areas of the separate regions of the adenohypophysis show that the total area decrease was largely due to a decrease in the meta-adenohypophysial area. The area of the pro-adenohypophysis tended to decrease during the experiment while the meso-adenohypophysial area showed little change.

Protein

Disc electrophoresis resolved the extractable pituitary proteins into seventeen fractions, i.e. following electrophoresis seventeen separate bands were detectable in the separation gel. These bands were numbered in order

starting with the band showing the greatest distance of migration. The R_f values of the separate bands are given in Table II. The resulting patterns represent the pooled protein of six sample pituitaries. Protein patterns of duplicate gels were identical.

Total pituitary protein is shown in Fig. 3. The total pituitary protein showed a series of changes. Protein increased for eight weeks (until the December 21 sample) then decreased for eight weeks (to the February 15 sample). This was followed by a six week period of increasing protein (to the March 29 sample) and a final ten week period of decreasing protein.

Changes in the optical density of the individual protein bands are shown in Fig. 4. Bands 1, 2, 3, 4, 6, 10, 11, 12, and 15 showed an overall decrease; bands 5, 13, and 17 showed an overall increase; and band 14 showed no overall change. Bands 7, 8, 9, and 16 did not appear in all samples. Most bands followed a similar pattern of change and the band changes tended to parallel the changes in total protein.

Control

GSI and Body Weight

The GSI of the controls followed a pattern of change similar to that seen in nature at the same time of year (Fig. 1). The GSI decreased until the December 21 sample then increased regularly, stabilizing around 4.5 in April

and May.

The controls showed a decrease in body weight until the December 21 sample (Fig. 3). During the remainder of the experiment, body weight showed an increase. The regression experiment was much longer than anticipated and all the control fish originally selected on the basis of uniformity were used prior to the termination of the experiment. This necessitated taking several large fish, not originally intended for use, for the final control sample. The large final control body weight average should, therefore, be disregarded in a consideration of body weight changes.

Histology

The B/A ratio decreased from 2.1 to 1.45 during the first two months of the experiment, i.e. until the December 21 sample (Fig. 1). During the remainder of the experiment the B/A ratio increased from 1.45 to 3.1. Cell counts show that the B/A ratio increase resulted from a great increase of basophiles and little change in acidophiles (Fig. 2).

The mediosagittal area changed irregularly throughout the experimental period with no recognizable trend (Fig. 2). The changes in total area were mainly due to changes in the meta-adenohypophysial area (Table I). The area of the meso-adenohypophysis increased during the experiment while the pro-adenohypophysial area remained relatively constant.

Protein

Total protein in the control pituitaries increased for eight weeks (until the December 21 sample) then decreased until the February 15 sample (Fig. 3). During the remaining twelve weeks of the experiment, protein increased.

The pattern of change was similar in all the individual protein bands (Fig. 5). The time and direction of change was the same as with total protein. Seventeen bands appeared in all samples.

Recovery

GSI and Body Weight

The GSI of regressed fish placed on full feed increased from 0.7 to 3.6 over an experimental period of seven months (Fig. 1). The initial GSI (0.7) showed little change for two months (until December 21). From the December 21 sample to the end of the experiment (May 10) the GSI increased from 0.6 to a final value of 3.6.

Body weight also showed little change until after the second sample (Fig. 3). During the remainder of the experiment, the body weight increased from 20 gm to a final sample weight of 36 gm.

Histology

During recovery from regression, the B/A ratio showed little change until after the December 21 sample (Fig. 1). During the remainder of the experiment, the B/A

ratio increased from 0.5 to a final value of 2.0. The inversion in the B/A ratio was due to both an increase of basophiles and a decrease of acidophiles (Fig. 2).

The mediosagittal area increased during the first three months of the experiment and showed little change thereafter (Fig. 2). The total area increase was largely due to an increase in meta-adenohypophysial area (Table I). The areas of the pro- and meso-adenohypophysis showed little change during the experiment.

Protein

Total pituitary protein of regressed fish placed on full feed decreased slightly during the first three months (until January 18) then increased during the remaining four months of the experiment (Fig. 3). Several degrees of change appeared among the individual protein bands (Fig. 6). Bands 1, 2, 3, 4, 6, 7, 8, 9, 11, 14, 15, and 16 showed an overall increase; bands 10, 12, 13, and 17 showed an overall decrease; and band 5 showed no overall change. The same pattern of change was not followed by all bands but most bands tended to parallel total protein. Seventeen bands appeared in all samples.

Nature

GSI and Body Weight

In nature the seasonal changes in GSI indicate the annual reproductive cycle (Fig. 1). The most striking change

is the precipitous drop during the spring spawning period. A post-spawning interval of gonadal quiescence is followed by a short period of rapid development in the fall. Further gonadal development is arrested during the winter and a pre-spawning GSI increase commences in February. The maximum GSI (6.3) was just prior to spawning with the minimum value (1.2) during August and September.

Average body weights show that the immature fish grew rapidly during the winter months and body weight continued to increase until spawning (Fig. 3). Spawning was followed by reduced body weights during May and June. A further decrease in body weight occurred during July and August. In September the weights returned to the post-spawning level.

Histology

In nature, changes in the B/A ratio did not parallel changes in GSI. Peaks in the B/A ratio were in February and September (Fig. 1). Total cell counts show that the cyclic changes in the B/A ratio were primarily due to cyclic changes in the acidophile number (Fig. 2). The basophile count showed greater short term fluctuation than the acidophile count but remained at the same general level above the acidophiles. Throughout most of the year, the basophile count showed a cycle of alternating increase and decrease.

Total mediosagittal area increased during January and February then showed little change during spring and

early summer (Fig. 2). During July, August, and September the total area was reduced; in October and November it returned to the same level as in the spring. The summer reduction in total area was primarily due to a decreased meta-adenohypophysial area (Table I).

The area of the pro-adenohypophysis showed little change throughout the year. Since the basophile count remained at the same level during the year, changes in the meso-adenohypophysial area tended to follow changes in the acidophile count.

Protein

In nature, the yearly maximum for total protein was in December (Fig. 3). After December, protein decreased to April. Following April, protein increased until June, then decreased to the yearly minimum in September.

The degree and direction of fluctuation varied among individual protein bands (Fig. 7). Bands 1, 2, 3, 6, 8, 9, 10, 11, and 12 were present throughout the year; bands 7, 13, and 17 failed to appear during some parts of the year; bands 4, 5, and 14 rarely appeared; and bands 15 and 16 were not present at any time during the year. Most regularly appearing bands tended to follow a pattern of change somewhat similar to total protein.

CHAPTER IV

DISCUSSION

A comparison of the experimental and control pituitaries at the end of the regression experiment shows the overall effect of food deprivation on the pituitary. The pituitaries of regressed fish showed an inversion of the B/A ratio due to increased acidophiles and decreased basophiles. The experimental pituitaries showed decreased total protein and a decrease in most separate protein bands. The mediosagittal pituitary area was reduced in regressed fish.

During regression, however, the direction of change in the pituitary and gonad reversed twice. This sequence of changes cannot be readily explained on the basis of food alone; the influence of an additional factor is suggested. Changes in the pituitary and gonad, before and after winter solstice, follow a similar pattern in both laboratory and pond fish. The coincidence of change in photoperiod and physiology suggests the influence of light on the pituitary at this time of year. While correlation does not prove a causal relationship, the degree of coincidence implies that one exists.

If increasing photoperiod stimulated the gonads through the pituitary, the effect was temporary and apparently overcome by lack of food within two and one-half months. Lack of food could result in depressed activity of the pituitary, a refractory gonad, or both. Reed (1965) reported results suggesting that the pituitary of regressed fish is depleted of gonadotropin.

GSI is taken as a measure of the functional maturity of the gonad (Pickford and Atz, 1957). If the gonad is responsive, changes in GSI thus presumably reflect quantitative and/or qualitative changes in the gonadotropin(s) released by the pituitary. During the regression experiment, the influence of inadequate food in the experimentals is implicated in the accelerated decrease in GSI in the experimentals prior to December 21, the retarded increase after December 21, and in the final regression.

During the first part of the regression experiment, total pituitary protein appeared to be independent of diet; protein increased in both the experimentals and controls until the December 21 sample, then decreased. The coordinated change in photoperiod and pituitary protein again suggests the influence of light on pituitary physiology. Full restitution from the post-solstice protein decrease, however, appeared to require adequate food.

The sequence of changes in the pituitary and gonad suggest that the regression experiment may be divided into

two phases, the first phase dominated by the effects of changing photoperiod on pituitary activity and the second phase dominated by the effects of food deprivation.

Changes in pituitary protein were not clearly related to changes in pituitary histology; neither total protein nor any separate protein band followed changes in a cell type. Therefore, if secretory products (hormones) are among the separate fractions of pituitary protein, the functional significance of the histological changes seems questionable. It is, of course, possible that the pituitary protein contents do not reflect the functional condition of the pituitary. Turnover rate may be a more important consideration than the quantity present at any one time. The quantity of hormone present may result from a balance between synthesis and secretion. If both increased or decreased together, no change in the quantity present would result.

In the experimentals and controls, individual band changes tended to parallel each other, i.e. the proportions of the constituent protein fractions remained approximately the same. Again, turnover rate of separate pituitary proteins may be the major consideration.

Among individuals, the overall shape and size of the pituitary are quite variable and the mediosagittal area divided by body weight yields a range of figures. It is, of course, possible that gland dimensions change laterally

without changing vertically. No frontal sections of the pituitary were made. The decrease in pituitary area during the latter stages of regression may result from an actual decrease of the parenchyma of the gland. At this stage, "clusters" of nuclei with very little cytoplasm are often visible along the margins of the neurohypophysis.

The results with regressed fish placed on full feed show that adequate food can reverse the effects of food restriction in both the pituitary and the gonad. The lack of response until after December 21 and the results of the regression experiment suggest that while food is required for hormone synthesis, secretion may be, at least in part, under the influence of light.

The tendency for acidophile increase and basophile decrease (and vice versa) to be concomitant during regression and recovery from regression suggests the possibility of the interchangeability of these cell types. Although little information directly concerning this question is available, it is interesting that Bretschneider and DeWit (1947) described a cycle in the meso-adenohypophysial cells of the bitterling (Rhodeus amarus) in which a basophilic phase alternated with an acidophilic phase.

Since there are more pituitary hormones than cell types, a cell type must be capable of producing more than one hormone or there are separate types of basophiles and acidophiles. Numerous investigators have associated

specific hormones with basophiles or acidophiles (Pickford and Atz, 1957) and two types of basophiles, identified as thyrotrophs and gonadotrophs, have been reported by Atz (1953), Olivereau and Herlaut (1954), and Barrington and Matty (1955). Total cell counts may, therefore, reflect the sum of several separate cell populations each with its characteristic cycles, functional significance, etc. Unfortunately, the distinction between thyrotrophs and gonadotrophs could not be made using Halmi's method (Halmi, 1952). In the interpretation of histological data of the pituitary, one is thus beset by the lack of knowledge concerning the cellular components of the pituitary and an accurate interpretation of changes in pituitary histology awaits further knowledge of pituitary cytology and histochemical differentiation of cell types.

It is difficult to assign a functional significance to the changes in area of the regions of the adenohypophysis. They may reflect only normal variability. Some of the results, however, seem worthy of comment. The pro-adenohypophysis remained relatively constant in all groups, i.e. the area of this region did not change with body weight or environmental conditions. Unfortunately, the functional significance of this area remains uncertain for most species. From observations on one fish, Ball (1965) suggested that the pro-adenohypophysis may be a source of prolactin-like hormone. Olivereau and Ball (1965) considered the

pro-adenohypophysis a probable source of both prolactin and corticotropin. In the controls, changes in the meso-adenohypophysial area result mainly from basophile changes. Since the basophile and acidophile counts tended to change in opposite directions during regression and recovery from regression, the meso-adenohypophysial area in these groups remained at the same general level. The region with the most variable area was the meta-adenohypophysis. Changes in this region were a major cause of changes in total mediosagittal pituitary area. The only recognized function of this region is the production of a chromatophore dispersing hormone.

In these experiments, changes in GSI appear more closely related to the B/A ratio than to either acidophiles or basophiles alone. In nature, the beginning of the fall and early spring periods of gonadal development were marked by peaks in the B/A ratio. Under laboratory conditions, an increase in GSI was accompanied by an increase in the B/A ratio. Thus gonadal development or its initiation was associated with a predominantly basophilic meso-adenohypophysis. Similar results have been reported by several investigators (Scruggs, 1951; Beach, 1959; Sundararaj, 1960; Sokol, 1961; Barr, 1963a; Olivereau, 1963; Sathyanesan, 1963; and Lagios, 1965). However, the B/A ratio changes result primarily from acidophile changes in nature, basophile changes in the controls, and both acidophile and basophile changes during regression and recovery from

regression. GSI changes do not, therefore clearly relate to independent changes of either total acidophiles or basophiles. If the B/A ratio is of physiological significance, a balance of factors may be implied as critical for gonadal stimulation. It is interesting that in nature the two peaks in the B/A ratio precede periods of increasing GSI. Barr (1963b) found that in the plaice (Pleuronectes platessa L.) the critical, gonadotropin-dependent phase of spermatogenesis is the initiation of meiotic division.

The cyclic changes in the testes of seasonally spawning fishes is well known (Hoar, 1955). The study of pond fish was included for the purpose of comparing the pituitary and gonad conditions in the experimental and natural environments.

According to the work of Fry (1947) on the standard metabolic rate of goldfish, the metabolism of the pond fish would increase twelve fold from the minimum winter to maximum summer temperature. Food consumption may not vary to this extent and body fat stores are not large. If food intake can not keep pace with metabolic rate increase, then a regression condition may exist during the summer period of high temperature.

Seasonal changes in pond water temperature are given in Table III. In 1965, unusually hot weather persisted until mid September. The mid September increase in GSI and a sudden drop in temperature corresponded. In past years

both temperature decrease and GSI increase have been earlier.

Thus temperature and food supply may both be factors determining the amount of metabolic material available for synthetic processes in the pituitary and gonad. The yearly maximum temperature in July was accompanied by a decreasing GSI, decreasing pituitary proteins, reduced body weight, the yearly minimum B/A ratio, and a decreased mediosagittal pituitary area, i.e. changes comparable to those associated with regression. The decrease of pituitary protein during regression or in nature may reflect a depletion of hormones, precursors, and enzymatic materials. It seems relevant that Houston and Fenwick (1965) reported reduced plasma proteins in goldfish acclimated at 30° C.

In these experiments, total basophile count was not obviously related to light or temperature. It is perhaps significant that body weight increase was associated with an increase of basophiles in the laboratory fish and in pond fish during the period of rapid growth (December 1964 to February 1965). The rather regularly alternating sequence of basophile increase and decrease in nature suggests a possible cyclic process in this cell type.

Although these experiments were not designed to study the influence of light or temperature, the results have required a consideration of these factors. In nature, several factors may be operating singly or in combination at different times of the year. Further studies of separate

factors under controlled conditions are needed.

The interrelationships among changes in protein bands, physiology, and environmental conditions invite much speculation. However, the true physiological significance of band changes awaits the identification of the individual bands.

CHAPTER V

SUMMARY

1. This study describes and compares changes in pituitary histology and protein contents associated with dietary controlled gonadal regression and recovery from regression of goldfish (Carassius auratus). A one year study of fish from a natural environment is included to compare the pituitary and gonad conditions in the natural and experimental environments. Pituitary proteins were analyzed by disc electrophoresis. Cell counts and regional areas were determined from mediosagittal pituitary sections.

2. Pituitaries of regressed fish showed decreased total protein and a decrease in most separate fractions of pituitary protein.

3. Pituitary protein and histological changes, however, did not appear related.

4. Gonadal regression was accompanied by an inversion of the basophile/acidophile ratio of the meso-adenohypophysis, due to increased acidophiles and decreased basophiles.

5. Opposite and concomitant changes in acidophile

and basophile numbers during regression and recovery from regression suggest the interchangeability of these cell types.

6. Total mediosagittal pituitary area decreased during the last stages of regression largely due to a decrease in the meta-adenohypophysial area.

7. Adequate food reversed the effects of restricted diet in both the pituitary and gonad within seven months.

8. The gonosomatic index was more closely related to the basophile/acidophile ratio than to either basophiles or acidophiles alone.

9. In nature, the yearly maximum temperature was accompanied by a decreasing gonosomatic index, decreasing pituitary proteins, reduced body weight, the yearly minimum in the basophile/acidophile ratio, and a reduced mediosagittal pituitary area.

10. Changes in the pituitary and gonad of fish in nature during the summer are comparable to those associated with regression.

11. The results of this study suggest that a true understanding of the physiological significance of changes in pituitary histology and proteins will require further knowledge of pituitary cytology, the histochemical differentiation of cell types, and the identification of the separate fractions of pituitary protein.

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TABLE I

MEDIOSAGITTAL AREA OF ADENOHYPOPHYSIAL REGIONS

Average Area ($\times 10^4 = \mu^2$)[a]

Date	Regression			Control		
	Pro-[b]	Meso-[b]	Meta-[b]	Pro-	Meso-	Meta-
1964 Oct. 26	11.3	25.4	69.0	11.3	25.8	69.0
Nov. 9	10.2	22.7	72.5			
Nov. 23	13.5	27.5	51.3	10.7	32.8	70.6
Dec. 7	10.2	29.0	69.0			
Dec. 21	10.2	22.0	58.4	11.0	17.0	54.6
1965 Jan. 4	9.3	20.0	68.5			
Jan. 18	11.3	20.5	72.0	11.3	22.7	73.0
Feb. 1	8.5	22.7	73.0			
Feb. 15	8.5	17.7	69.5	11.0	34.8	95.4
Mar. 1	10.0	25.5	67.5			
Mar. 15	8.8	20.0	69.3	9.5	27.0	76.1
Mar. 29	8.8	24.7	57.8			
Apr. 12	8.5	20.2	53.5	10.2	31.8	49.3
Apr. 26	7.0	21.7	51.7			
May 10	6.0	21.7	53.0	10.5	47.4	96.7

[a]Sample size is 6 for all columns.

[b]Indicates Pro-, Meso-, and Meta-adenohypophysis.

TABLE I--Continued

Date	Nature			Recovery		
	Pro-	Meso-	Meta-	Pro-	Meso-	Meta-
1964 Oct. 26				8.5	18.2	42.0
Nov. 23				9.0	22.2	61.5
Dec. 7	10.2	18.0	53.5			
Dec. 21	7.5	18.0	42.7	7.5	19.2	43.8
1965						
Jan. 18	11.8	26.2	63.0	10.0	29.0	85.0
Feb. 1	8.2	26.8	50.7			
Feb. 15	9.3	29.8	24.3	10.7	20.8	69.0
Mar. 1	13.3	28.0	59.3			
Mar. 15	15.0	30.8	58.8	7.2	22.0	81.5
Mar. 29	16.3	29.2	70.0			
Apr. 12	13.7	40.8	50.5	6.5	23.2	70.0
Apr. 26	11.0	28.2	53.0			
May 10	10.2	33.2	57.0	7.7	27.8	61.5
May 24	12.5	30.2	55.5			
June 7	11.0	36.5	59.0			
June 18	9.0	32.5	44.2			
July 2	7.0	21.2	51.8			
July 19	10.0	28.2	48.0			
Aug. 2	9.0	29.2	42.7			
Aug. 16	10.5	25.5	54.0			
Aug. 30	10.0	30.0	46.3			
Sept. 13	8.2	24.0	52.2			
Sept. 27	11.3	27.3	43.0			
Oct. 11	11.8	27.8	59.8			
Oct. 25	13.7	33.2	74.0			
Nov. 8	11.0	27.0	53.0			
Nov. 22	14.7	34.2	61.0			

TABLE II

R_f VALUES OF PITUITARY PROTEIN BANDS

Band No.	R_f	Band No.	R_f	Band No.	R_f
1	.88	7	.57	13	.36
2	.81	8	.54	14	.27
3	.75	9	.51	15	.22
4	.70	10	.46	16	.16
5	.65	11	.42	17	.02
6	.62	12	.39		

TABLE III

AVERAGE POND WATER TEMPERATURES (°C)

	Date	Temperature		Date	Temperature
1964	Dec. 1-15	4.7	1965	June 1-15	26.8
	Dec. 16-31	5.7		June 16-30	28.3
1965	Jan. 1-15	6.9		July 1-15	31.4
	Jan. 16-31	4.6		July 16-31	31.0
	Feb. 1-15	4.6		Aug. 1-15	28.4
	Feb. 16-28	8.0		Aug. 16-31	29.5
	Mar. 1-15	6.7		Sept. 1-15	29.0
	Mar. 16-31	7.0		Sept. 16-30	21.5
	Apr. 1-15	21.3		Oct. 1-15	20.4
	Apr. 16-30	19.9		Oct. 16-31	17.9
	May 1-15	23.2		Nov. 1-15	16.8
	May 16-31	24.4		Nov. 16-30	14.1

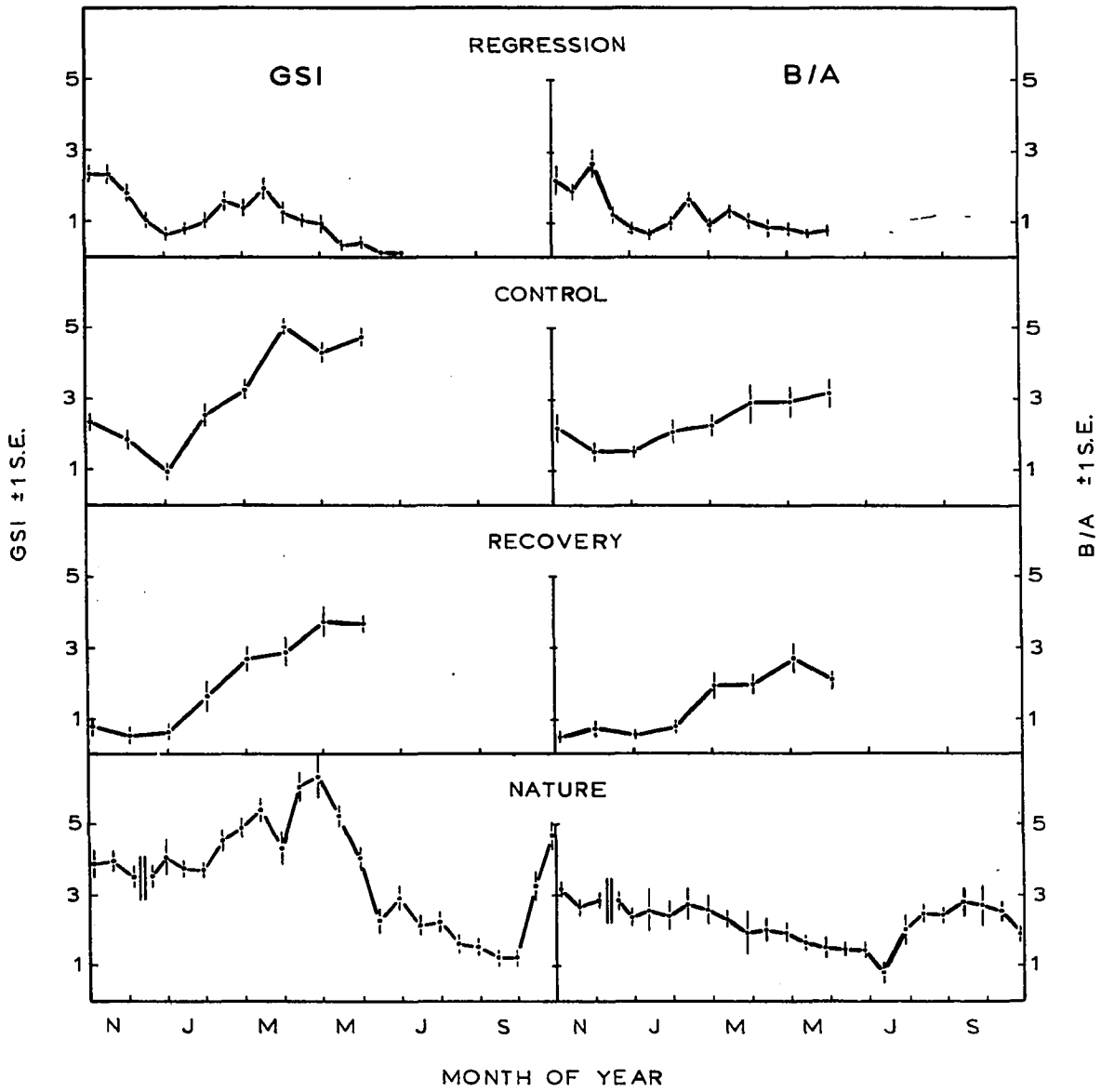

Fig. 1.--Gonosomatic indices and basophile/acidophile ratios

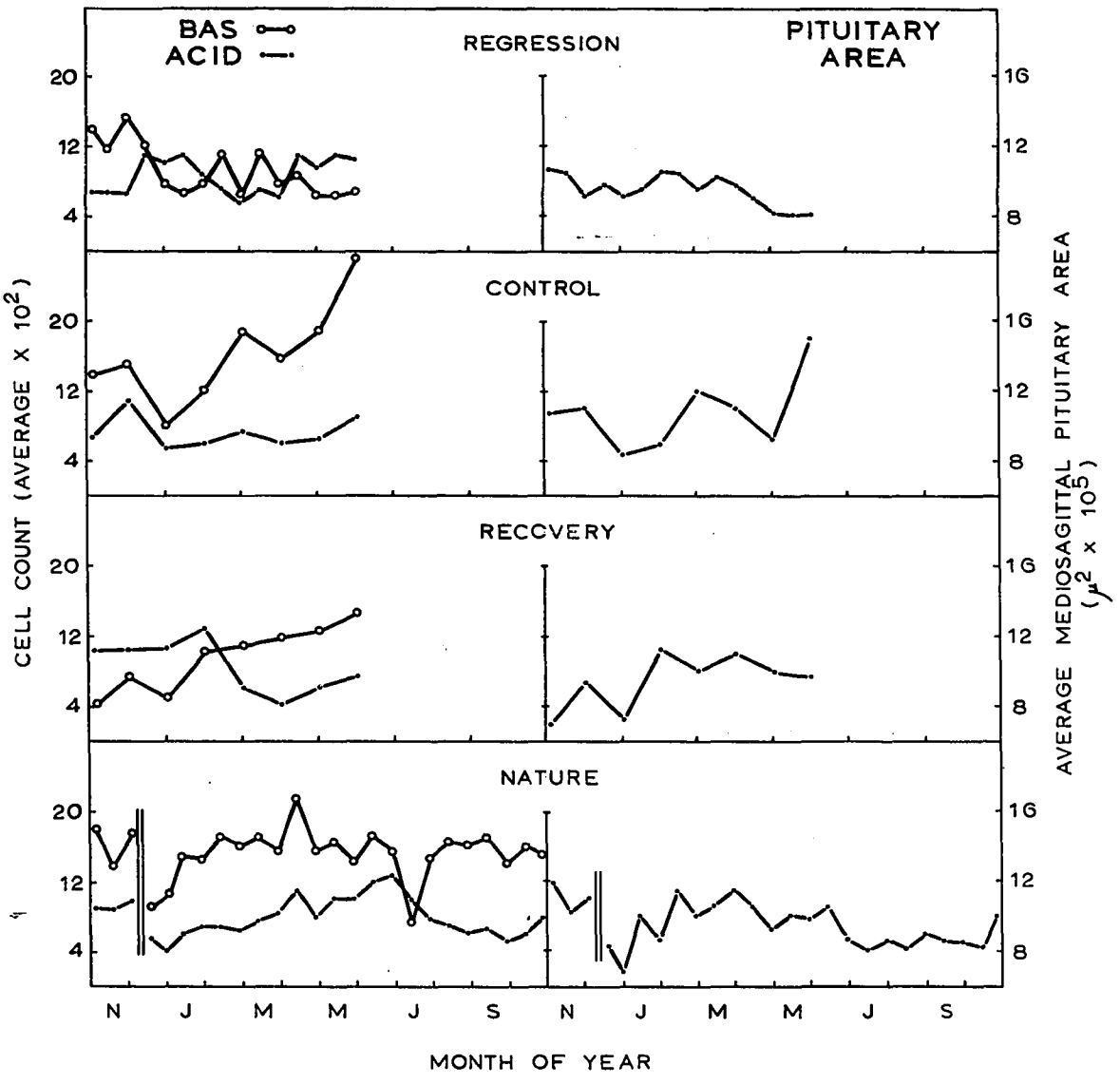

Fig. 2.--Basophile, acidophile counts and mediosagittal pituitary areas

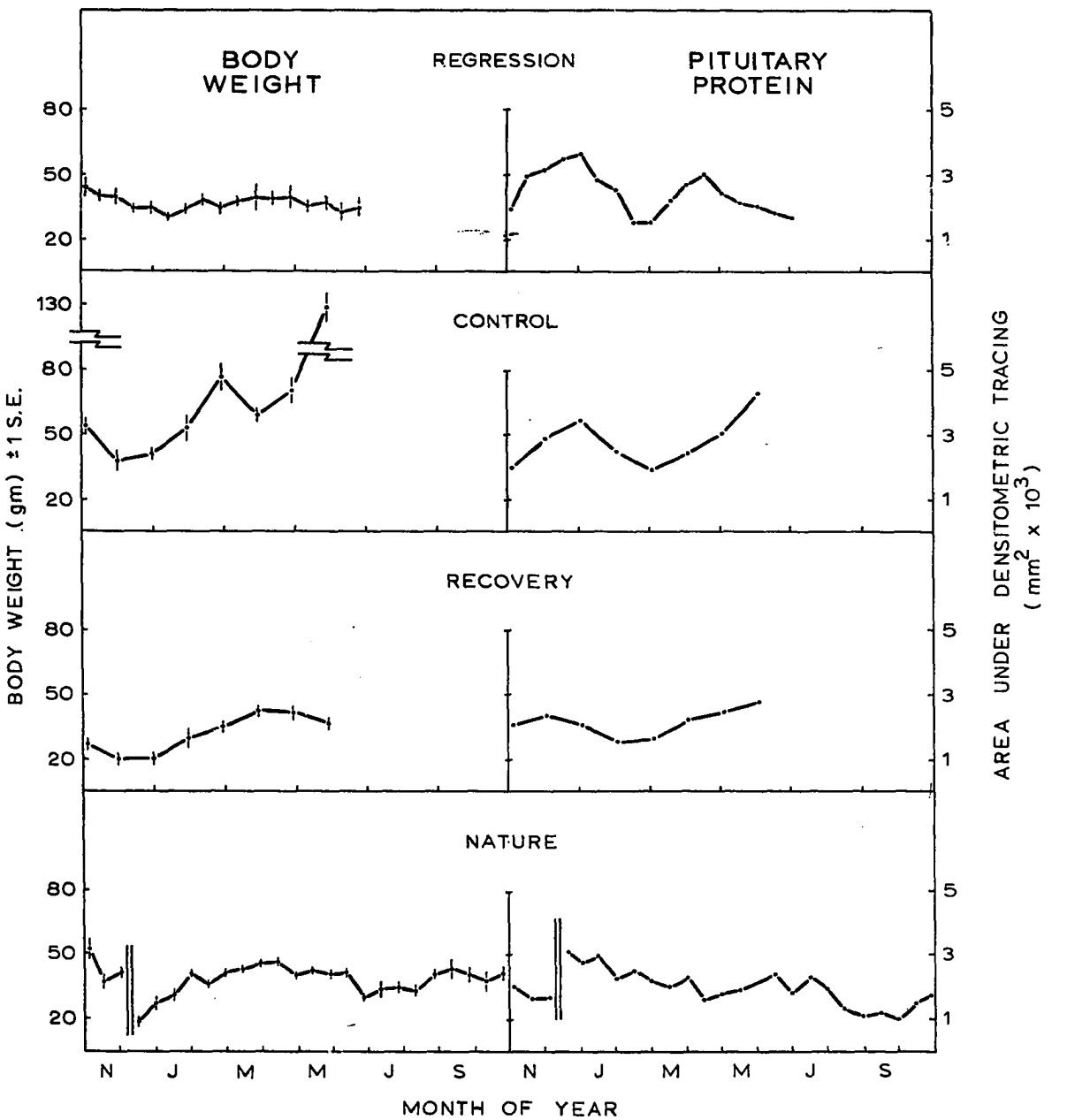

Fig. 3.--Body weights and total pituitary protein

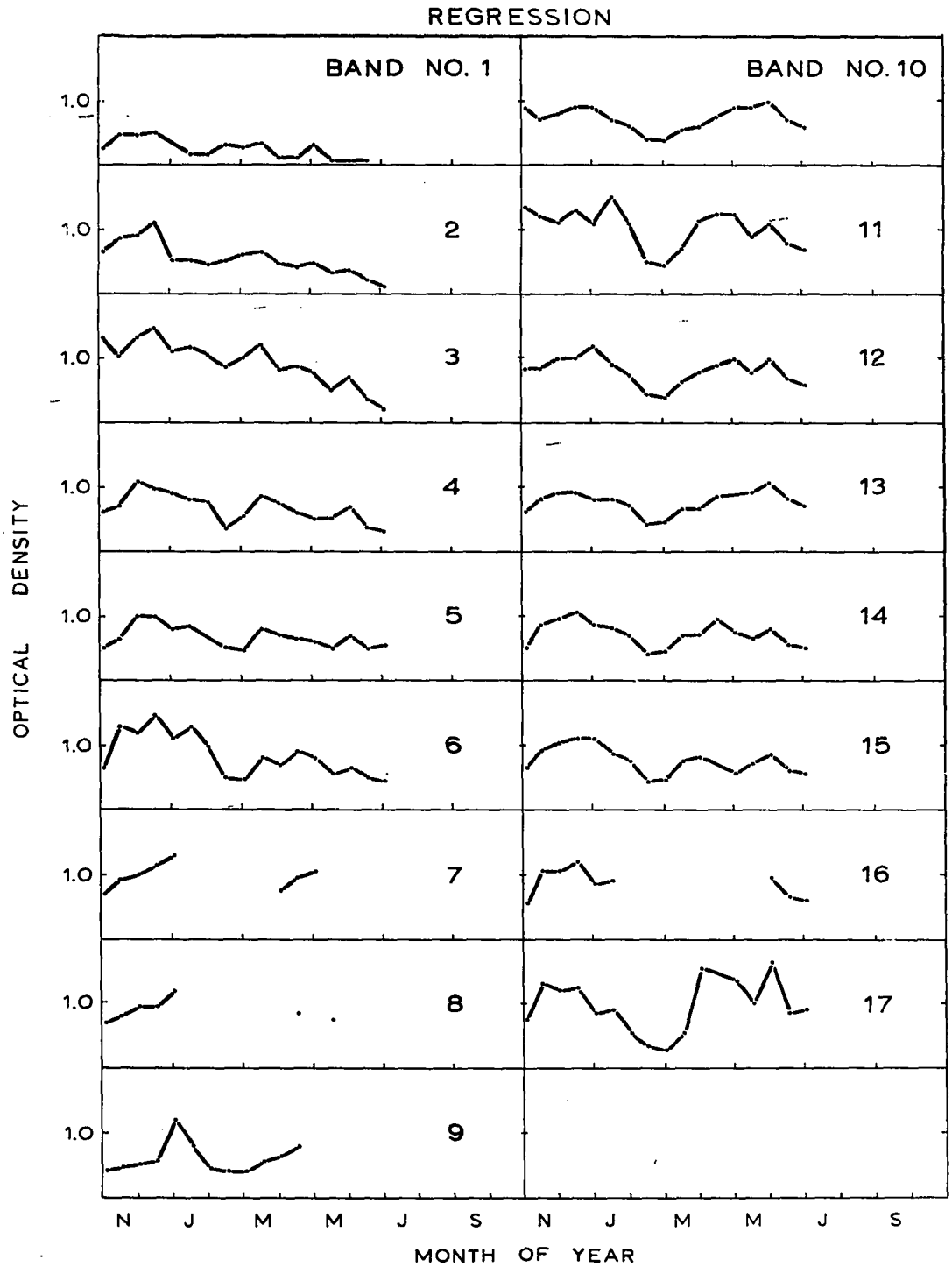

Fig. 4.--Regression--pituitary protein bands

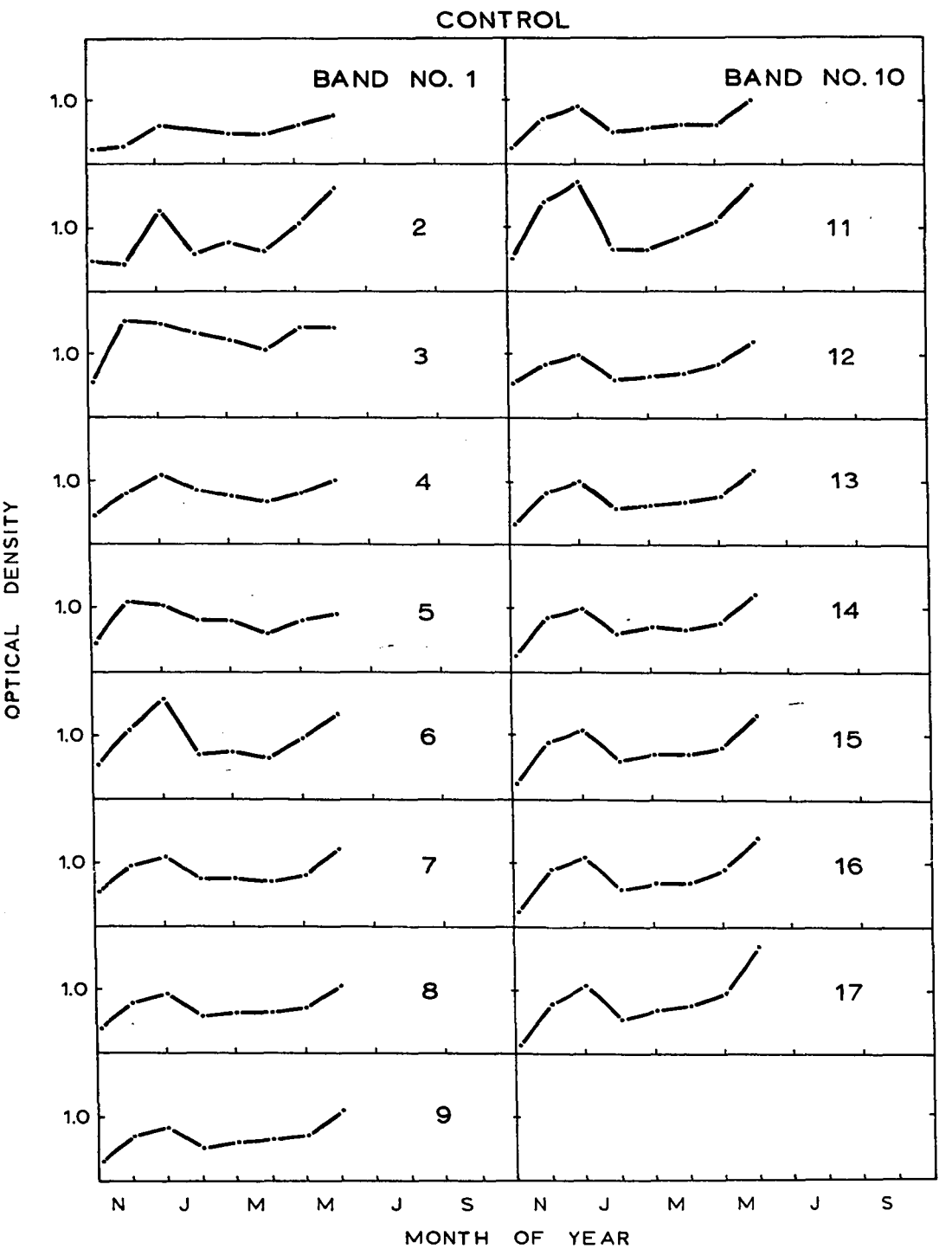

Fig. 5.--Control--pituitary protein bands

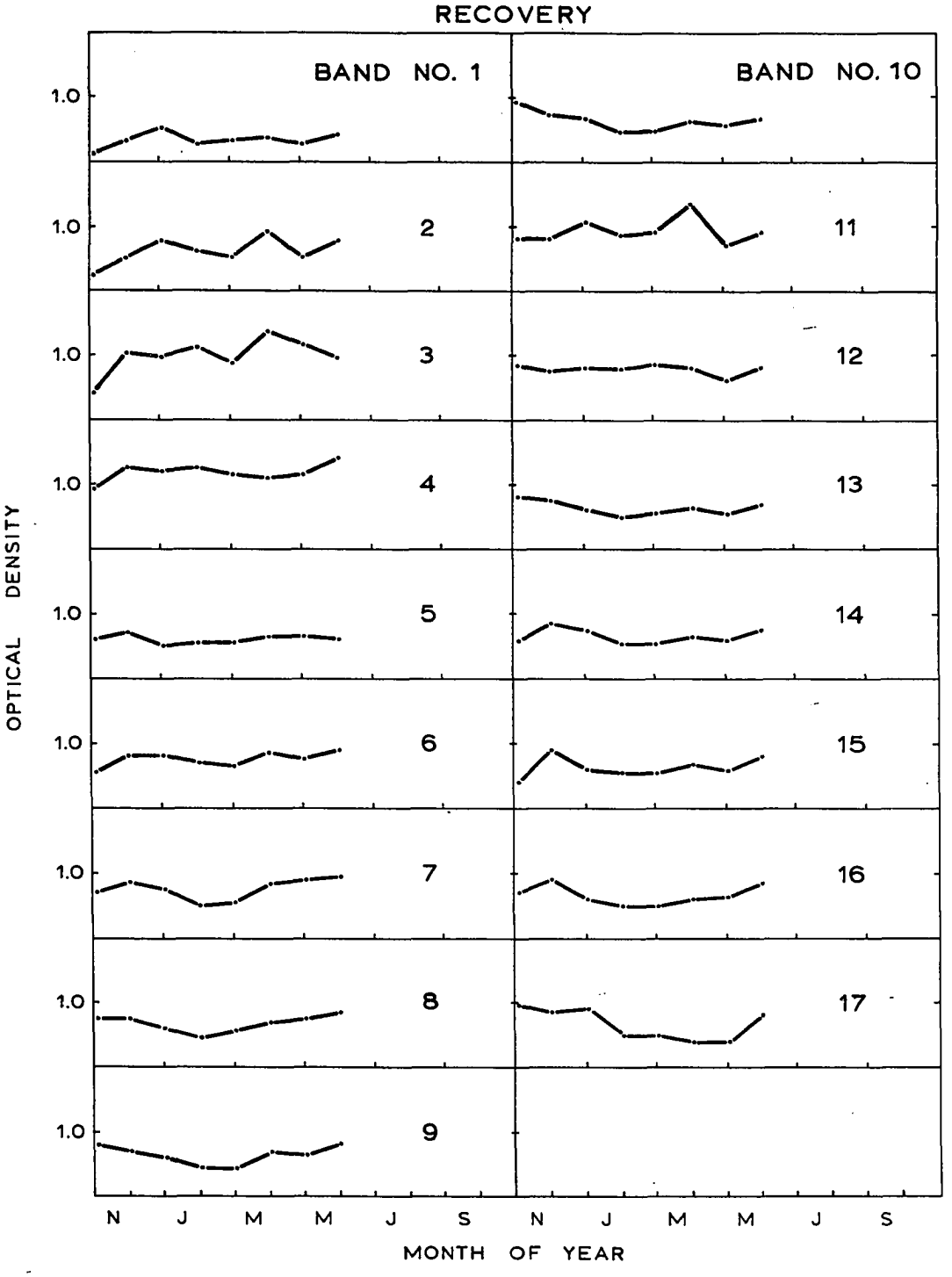

Fig. 6.--Recovery--pituitary protein bands

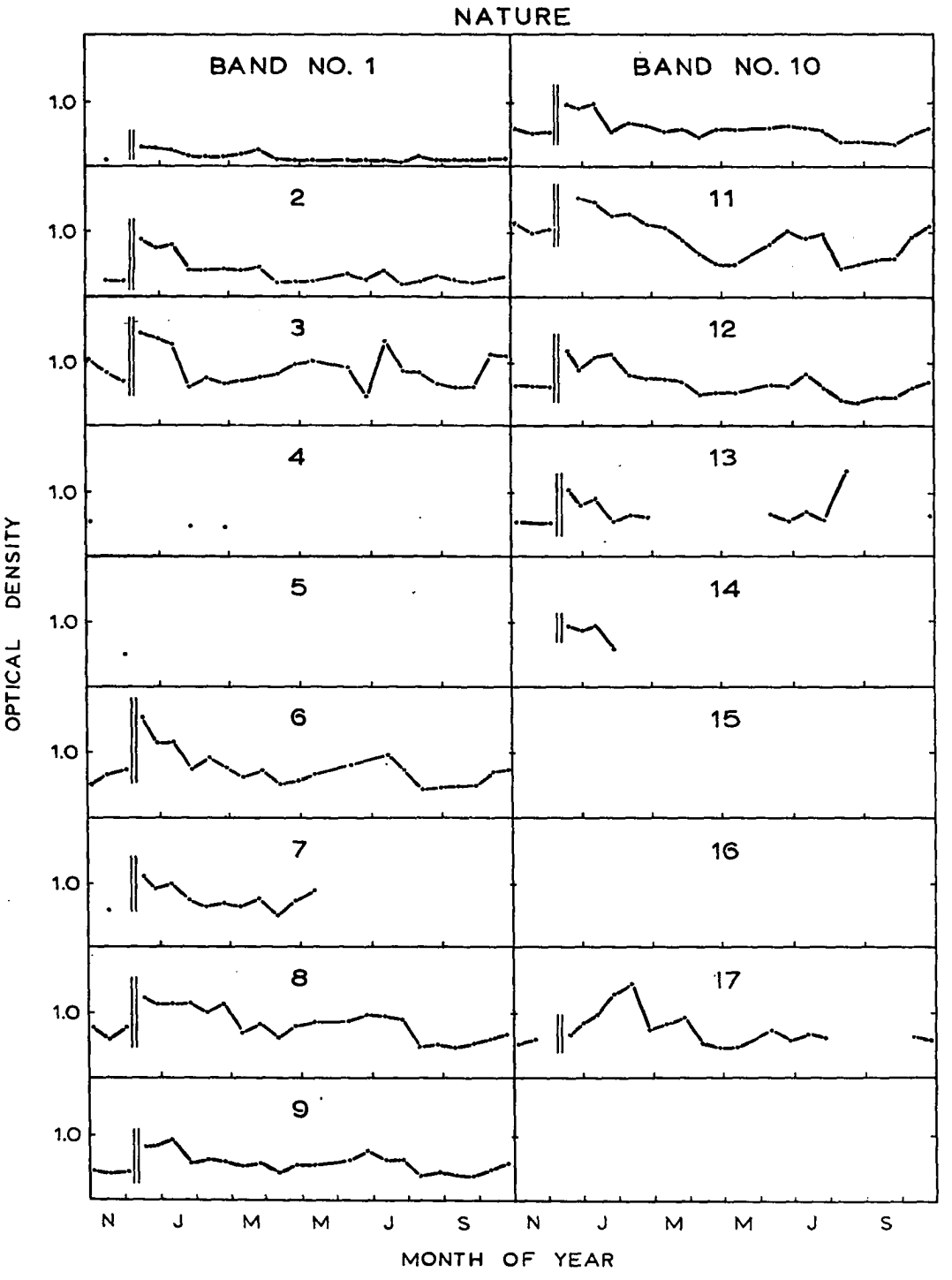

Fig. 7.--Nature--pituitary protein bands