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SEASONAL VARIATION IN PROTEIN CONTENT OF BLUEGILL PITUITARY GLANDS [1]

By Percy W. Laarman

Abstract

Seasonal variations in the protein content of pituitary glands of the bluegill, Lepomis macrochirus Rafinesque were measured. Collections were made from March to October 1969, inclusive. The acetone-dried pituitary glands represented 14.5% of the wet weight. Seventy-six per cent of the annual fish growth occurred between 1 May and 1 August. Per cent protein in the acetone-dried glands increased from 25% in early spring to 56% in August. The pituitary gland was large and active before growth and reproduction began, and became smaller with a higher percentage protein content, after growth and spawning were completed. Care should be taken in evaluating experiments in which fish metabolism is a factor since the response of the fish may vary significantly during different times of the year.

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Introduction

The purpose of this study was to measure the seasonal variation in protein content of pituitary glands of the bluegill, Lepomis macrochirus Rafinesque, and to determine the relationship of this variation to the seasonal growth pattern. This report makes no attempt to delve into the intricate physiological functions of the pituitary gland, but rather presents data that may be of interest to fishery biologists who are working in the area of fish metabolism.

The physiology of the pituitary gland of fishes has been summarized by Pickford and Atz (1957). More recent work on the pituitary gland has been summarized by Hoar and Randall (1969). These two references are indispensable to anyone interested in the histophysiology of the pituitary gland of fishes. Swift and Pickford (1965) found (by bioassay with Fundulus heteroclitus) a seasonal change in the growth-promoting activity of the pituitary glands of perch, Perca fluviatilis. They concluded that the seasonal growth rate pattern must have resulted primarily from different concentrations of growth hormone in the pituitary glands.

Methods

In September 1968, about 300 yearling bluegills were placed in a one-half-acre pond located at our research station

in Saline, Michigan. These fish were progeny of bluegills collected from Sugarloaf Lake, Washtenaw County.

Collections were made by angling or seining on the first of each month from March to October 1969, inclusive. Total lengths and weights were recorded and scale samples and pituitary glands were taken from eight fish during each collection. Whole pituitary glands were removed by cutting off the head and removing the top of the cranium just above the eyes. With a forceps the brain was lifted out, exposing the pituitary gland. Sometimes the gland remained attached to the ventral part of the brain, and sometimes it remained in the floor of the brain case. The glands were picked out with a forceps and immediately placed in vials containing chilled acetone and stored in a freezer. After 24 hours, the acetone was decanted and replaced with fresh chilled acetone. At the end of 36 hours, the glands were dried on filter paper and weighed collectively.

Total protein concentration of the glands was determined by the method of Lowry et al. (1951). Each pituitary was allowed to dissolve in 1 ml of 1 N NaOH. Five ml of a reagent consisting of 0.5 ml of Na Tartrate, 0.5 ml $CuSO_4$ and 50 ml of Na_2CO_3 was added to 0.5 ml of the NaOH solution containing the dissolved pituitary gland. The solutions were mixed and allowed to stand for 10 minutes. After that, 0.5 ml of a 1:1 dilution of phenol reagent with distilled water was added rapidly and with a thorough mixing. Tubes were allowed to stand for 30 minutes and then optical density values were read on

a Beckman DU spectrophotometer at 750 mμ. Lyophilized human serum was used as a reference to establish a standard curve which gave the relationship between quantity of protein and optical density values.

Seasonal fish growth was determined from scales by the back-calculation method.

Results

All the fish that were sampled were sexually mature and belonged to age-group II. Thus, all the fish had completed two growing seasons, and growth in the third season had not started when sampling began. Linear growth is probably a better criterion for measuring fish growth than weight increase, because of changes that occur in gonad weights during the reproductive season.

Pickford and Atz (1957) reviewed the literature concerning weight loss in acetone-dried pituitaries and found that the defatted dry weight represented 13 to 17% of the wet weight of pituitaries from four species of teleosts. These belonged to three different orders. Two of the species were freshwater, one anadromous, and one marine. Clemens and Sneed (1962) found the acetone-dried pituitary weights of 26 female goldfish were 24% of the wet weight. Weight loss due to acetone drying was determined from a separate sample of 16 pituitary glands collected in May. The acetone-dried weight represented 14.5% of the wet weight of bluegill pituitary glands.

Since the proteins measured in the pituitary gland are different from the proteins (human serum) used to establish a standard curve, the color values may be different. Therefore, interest is not in the absolute protein values, but rather in the seasonal trend that was observed. The per cent protein per acetone-dried pituitary was determined by dividing the average quantity of protein in milligrams by the average pituitary weight in milligrams times 100. This per cent protein was then plotted against the time of year as shown in Figure 1. Three distinct periods are evident in the per cent protein content of the pituitary gland. March, April and May collections represent period one; June and July, period two; and August, September and October, period three. During the first period, in early spring, the protein content is about 25%. This has increased to about 38% in the second period and by the first of August to about 56%. The second period represents an increase in the magnitude of 50% over the first period. The third period average is about 119% greater than the first period during early spring.

Back calculations of bluegill growth from the scale samples showed that 76% of the year's growth occurred between the May and August collections, and that 92% of the total season's growth was completed by 1 August. It appears that the per cent protein in the pituitary gland is at a much higher level after completion of most growth and reproductive activities.

The size of the pituitary gland also underwent a seasonal change. To compensate for changes in fish size in relation to pituitary size, an index was determined by dividing the average pituitary weight in milligrams by the average fish length in millimeters times 100 as given in Table 1. The highest indices were obtained in the June and July collections. This is also the period when physiological processes expressed in growth and reproduction are highest. Clemens and Sneed (1962) found a coefficient of correlation of 0.887 between male channel catfish length and pituitary weight, and for females it was 0.817. My data indicate that the time of year of collection would be a factor to consider in determining the relationship between pituitary gland weights and lengths of bluegills.

The data presented suggest the following hypothesis. Early in the spring, before spawning and growth occur, the pituitary gland is active in the secretion of protein hormones, but little is actually stored in the gland. During peak spawning and growth activity, the pituitary gland is large and contains an intermediate quantity of protein. When spawning and growth are almost completed, the pituitary is less active and small in size, but contains a relatively high percentage of protein. In other words, the gland goes into a resting stage.

The pituitary gland, sometimes called the master gland of the endocrine system, is involved in an intricate way in regulating

physiological processes through secretion of various hormones. If the seasonal variation in the protein content of the pituitary gland, as noted in this report, is typical of all temperate-zone fishes, then great care must be taken in evaluating experiments in which fish metabolism is a factor. The response of experimental fish might be significantly different during two different times of the year, even under otherwise controlled conditions.

Acknowledgments

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Percy W. Laarman

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Table 1.--Seasonal changes in pituitary protein content, pituitary size and fish growth for the bluegill in 1969

Month	Protein per pituitary (mg)	Pituitary weight (mg)	Total length of fish (mm)	Index of pituitary weight to fish length x 100	Current year's growth (mm)
March	0.0601	0.2556	141	0.181	-
April	0.0790	0.2881	158	0.182	-
May	0.0537	0.2250	137	0.164	10
June	0.1196	0.3125	160	0.191	16
July	0.1304	0.3500	176	0.199	29
Aug.	0.1381	0.2500	182	0.138	58
Sept.	0.1427	0.2500	194	0.129	63
Oct.	0.1453	0.2500	196	0.128	63

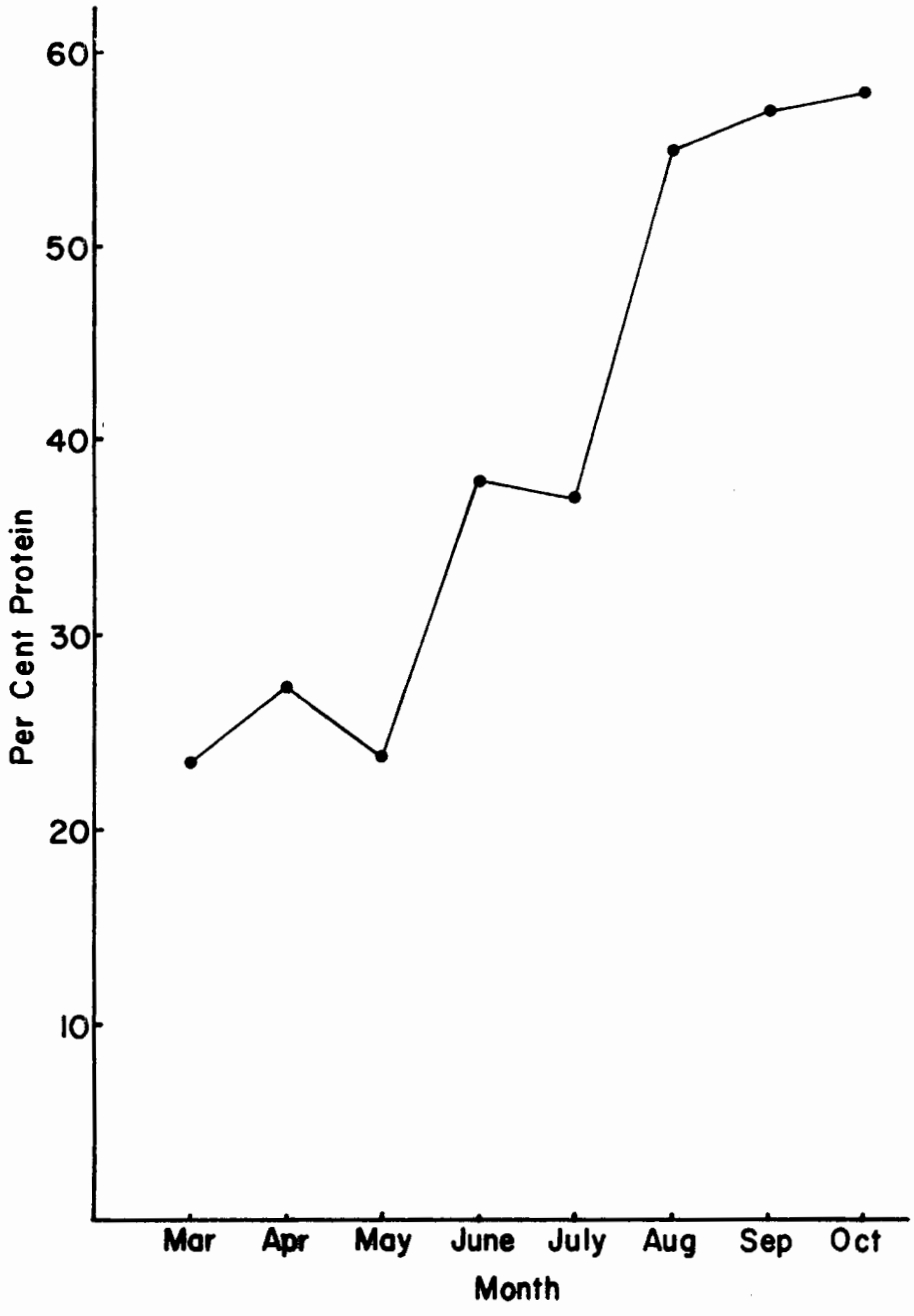

Figure 1. --Seasonal change of per cent protein found in the pituitary gland of bluegills.