THE EFFECT OF PHOTOPERIOD AND THYROID ACTIVITY ON GROWTH OF THE GREEN SUNFISH *LEPOMIS CYANELLUS* (RAFINESQUE)

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ABSTRACT

THE EFFECT OF PHOTOPERIOD AND THYROID ACTIVITY ON GROWTH OF THE GREEN SUNFISH *LEPOMIS CYANELLUS* (RAFINESQUE)

by Willard Louis Gross

The effect of photoperiod was evaluated in four independent experiments, three of which were performed in conjunction with the effect of thyroid activity. The fish were held in light-tight aquaria at constant temperatures under four photoperiods (8-hour constant, 16-hour constant, variable increasing photoperiod 8 to 16 hours, and variable decreasing photoperiod 16 to 8 hours). Increment in weight and total length, rate of food consumption, and efficiency of food conversion were determined. Results of the first four experiments demonstrated that photoperiod does have an effect upon growth in both length and weight of the green sunfish. Generally, a greater growth occurred in fish held at longer photoperiods. Significant differences in growth in terms of weight gain occurred in two experiments. Growth in weight was better associated with photoperiod than was total length.

Significant correlations between the rate of food consumption and growth at given photoperiods indicate that photoperiod mediates its effect through increased appetite of the fish. Food conversion was generally most efficient under an increasing photoperiod and least efficient under a decreasing photoperiod.

Results of the four experiments demonstrated that varying photoperiod has a greater effect upon growth than a constant photoperiod. An increasing photoperiod (8 to 16 hours) stimulated growth above that for a constant 16-hour photoperiod and a decreasing photoperiod (16 to 8 hour) depressed growth below that of a constant 8-hour photoperiod.

Differences in growth of fish given injections of artificial thyroxine (hyperthyroid), radioiodine (hypothyroid), and saline solution (control) demonstrated an effect of thyroid activity on growth in length and weight. Hyperthyroid fish attained the greatest growth and hypothyroid fish the least. The differences between the thyroidal groups were not statistically significant, but consistent results were obtained in the experiments. Thyroid activity had no effect on food consumption, but an increased efficiency of food conversion was generally associated with greater thyroid activity.

Using the rate of loss of radioiodine from the head region as an index of thyroid activity, an effect of photoperiod on thyroid activity was demonstrated. Greater thyroid activity was associated with short photoperiods and low thyroid activity with long photoperiods.

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By

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INTRODUCTION

The objective of this investigation was to obtain a more precise understanding of the effect of photoperiodicity and thyroid activity on growth of fish. The experimental animal was the green sunfish *Lepomis cyanellus* Rafinesque.

Seasonal Growth Patterns in Fish

Growth in length and weight of fish is continuous throughout their life, the rate of growth declining with age. However, this growth occurs at a seasonal rate on an annual basis in many fish. Seasonal growth patterns have been found in yellow perch (Langford and Martini, 1941), river carpsucker (Bucholz, 1957), warmouth (Larimore, 1957), white-sucker (Spoor, 1938), bluegill (Anderson, 1959; Beckman, 1943; Sprugel, 1954), smallmouth bass (Brown, 1960), rock bass (Brown, 1960), brook trout (Cooper, 1953; McFadden, 1961), and green sunfish (Hubbs and Cooper, 1935). The period of maximum growth varies slightly but, in general, growth is greatest from April to July, declines slowly during August and September, drops rapidly the latter part of September and October, and almost ceases during the period November to March. Swift (1955;1961) found a slightly different annual growth cycle in 3-year-old and yearling brown trout in England. The fish had a maximum growth rate in spring (May to June) and usually a smaller increase in growth rate in autumn (September and October) with a low growth rate during winter and mid-summer. These studies indicate that growth bears a definite relationship to the seasons.

Factors Influencing Growth

The regular variation in the growth patterns of different species is probably due to rhythmic changes in environmental factors. Several environmental factors listed by Brown (1957) as affecting growth are temperature, light, chemical factors, space factors and availability of food. Chemical and space factors vary throughout the year, but these changes are usually very minor and would not account for the repeated annual growth cycle each year. Availability of food can influence growth if the supply of food becomes critical at times. However, this situation does not affect most fish with great regularity under natural conditions. The two factors which appear primarily responsible are temperature and light (photoperiod) or an interaction of these two factors. Seasonal temperature changes are nearly in phase with changes in photoperiod in temperate regions making it difficult to evaluate these effects individually under natural conditions.

Effect of Temperature on Growth

Since fish are poikilothermous animals, one would expect that temperature would affect their growth. Literature referring to the effect of temperature on growth of poikilotherms is reviewed by Pickford and Atz (1957). There appears to be an optimal temperature for growth above or below which there is a decline in growth. An exception is the brown trout for which Brown (1946b) and Swift (1955) found two optimal temperatures. From his studies on the effect of temperature on the seasonal growth rate of the bluegill, Anderson (1959) concluded that temperature was the primary factor influencing seasonal growth. Swift (1959; 1961) also concluded that temperature was the primary factor influencing the seasonal growth rate of brown trout. Temperature is believed to affect the growth of fish by influencing food consumption and metabolism, especially respiration and digestion.

Temperature may not be the only factor affecting the seasonal growth of fish. If the theory of an optimal temperature were true, one would expect an increased growth rate in fall when the optimal temperature is again reached. This does occur in brown trout (Swift, 1961) but is not generally true for other species. In a laboratory study under constant temperature and variable photoperiod corresponding to different seasons of the year, Anderson (1959) found a variation in the potential growth of the bluegill which was independent of temperature. The greatest increments in length at each temperature coincided with a photoperiod comparable to that for the period of May 30 to June 28 for Michigan. Although he had no direct evidence, Anderson suggested that this difference involved an endocrine mechanism.

Effect of Photoperiod on Growth

The effect of photoperiod on the growth pattern of fish has not been adequately studied. Much of the difficulty lies in the similarity in variations of temperature and day-length. Most studies have involved only the effect of constant photoperiod on growth. Brown (1946a) found that in brown trout kept at 11.5° C., under standard conditions, the average specific growth rates were significantly lower with 12 or 18 hours of standard light than with 6 hours of light per day. Bjorklund (1958) studied the effect of photoperiod on growth of goldfish. There was no significant difference between the growth of fish held in darkness, constant 10-hour day, and on what he termed a constant 16-hour day which began with a 11 3/4-hour day decreased to a 10-hour day over a period of 11 days and then increased in 15 minute increments over a period of 23 days to a 16-hour day where it remained constant for a period of 38 days. The greatest increments in length and weight occurred in fish held in total darkness. Growth was inversely related to photoperiod which

Bjorklund felt was due to differences in the activity of the fish. Eisler (1957), in studies of the influence of light on early growth of chinook salmon, found a significantly greater growth in fish reared at different light intensities than those reared in the dark. Anderson (1959) performed an experiment on the effects of photoperiod using two groups of four fish each; the first group was held on a constant 15-hour photoperiod and the other group on a constant 10-hour photoperiod for a period of six weeks. No significant differences in gain of length or weight were found; although, the average values were slightly greater for the short day group. Swift (1955) suggested that the seasonal growth rate might be better correlated with photoperiod than temperature; however, he later found no relationship between photoperiod and the seasonal growth rate of brown trout. In these studies, the effect of a varying photoperiod upon growth was not considered. Swift (1961) states that it is important to try to distinguish between the effects of day to day changes in photoperiod length and the effect of the length of the photoperiod when it is constant from day to day. All other studies of photoperiod have involved its effect upon maturation of gonads or temperature resistance in fish.

Endogenous Cycles of Growth in Fish

One investigator suggests that an endogenous seasonal growth pattern may exist. Brown (1946a) maintained brown trout for more than a year in a constant environment (11.5° C., constant 12-hour photoperiod) and found a cycle of seasonal growth and maturation of the gonads at the same time of year as under natural conditions. The seasonal growth rate decreased to a minimum in October and November, rose to a maximum in February, fell gradually throughout the summer to August, and then decreased markedly. This pattern is somewhat similar to that found by Swift (1955). Further studies by Swift (1961) have revealed a slightly different seasonal pattern. He discusses the work of Brown and concludes that an endogenous cycle does not exist.

Role of the Thyroid Gland and Its Relation to Growth of Fish

Despite the large amount of research on the thyroid of poikiotherms, the role of the thyroid gland in fish is still not clearly understood (Gorbman, 1959). The literature has been reviewed by Gorbman (1959), Lynn and Wachowski (1951), and more fully by Pickford and Atz (1957). Thyroid activity or thyroid hormone in fish has been associated with sexual development, osmoregulation, calcium and phosphorus metabolism, fin regeneration, development of epidermis and subcutaneous tissue, and transformations such as the smolt transformation in salmonids and metamorphosis in the lamprey.

The control of maturation by the thyroid and the synergistic action between thyroid hormone and growth hormone is well-known in mammals. Several investigators have attempted to show a similar role in fish. Much controversy exists in the literature as to whether the thyroid exerts a calorigenic effect. Investigations of thyroidal function in fish have involved a variety of methods such as injection or immersion in water containing anti-thyroid drugs, injection or feeding of thyroxine or thyroid powders, radiothyroidectomy, or simply a comparison of groups in different environments. It appears that the contradictory results obtained may in part be due to the variation in methods. Recent investigations not reviewed by Pickford and Atz afford evidence of an effect of the thyroid on growth. Barrington, Barron and Piggins (1961) noted an increased growth of rainbow trout given thyroxine and thyroid powder. Three independent experiments by Bjorklund (1958) gave conflicting results. One experiment involving injections of thiourea and of thyroxine retarded growth in both instances. In two other experiments, transitory increases in length and weight were observed in fish injected with thyroxine and also in fish injected with triiodothyronine. Bjorklund felt that the negative results of his first experiment were the result of use of pharmaceutical doses and concluded that the thyroid had a calorigenic effect. Hoar (1952) suggested that the iodine content of the water may limit growth and reproduction in populations of fish. He cites, as an example, the alewife, a marine species, which has become landlocked in the Great Lakes. The freshwater fish is only a little more than one-half the size of the marine relative; the thyroid is extremely hyperplastic or occasionally atrophic compared with the thyroid of the marine relative.

Seasonal fluctuations in thyroid activity have been studied by several investigators. Seasonal variations have been found in the minnow Phoxinus (Barrington and Matty, 1954; Fortune, 1955), brown trout (Swift 1955; 1959), salmon parr (Hoar, 1939) and the killifish Fundulus (Berg, Gorbman and Kobayashi, 1959). Periods of peak activity seem to vary with species. In the above investigations, the results were correlated with spawning activity. Seasonal fluctuation in thyroid activity has not been extensively studied with respect to growth. Swift (1955) found that the peak activity of the thyroid corresponded to maximum activity of trout. He suggested that the increased locomotor activity of the fish led to an increase in the amount of metabolites needed for maintenance which reduced the amount available for growth thereby reducing the growth rate. In the investigations cited above, the peak activity of the thyroid, or one of the peaks where two peaks were observed, occurred in spring. This is the usual period of maturation of gonads in fish which are predominently spring spawners. However, this is also the period of rapid growth for many species. The maximum thyroid activity observed in yearling trout (immature) cannot be accounted for by maturation of the gonads. These results plus those cited previously dealing with the influence of thyroid hormone on growth indicate a possible effect of the thyroid gland on the seasonal growth pattern.

Effect of Photoperiod on Thyroid Activity

Another aspect of thyroid physiology involves factors affecting thyroid activity. Leloup and Fontaine (1959) state that among the numerous ecological and ethologic factors able to influence iodine metabolism in lower vertebrates are genital maturity, nutrition, environmental concentration of iodine, salinity, season, photoperiod, amphibiosis and desiccation. These factors have been investigated to varying extents. The effect of photoperiod has not been extensively investigated. Several investigations have demonstrated an effect of light on thyroid activity, although the results at times are contradictory. Buser and Blanc (1949) as quoted by Pickford and Atz (1957) found no effect of continuous illumination on thyroids of *Ameiurus nebulosus*. Rasquin (1949) found that the thyroid of *Astanax mexicanus* undergoes hypertrophy when the fish are kept in total darkness. Further studies on this species by Rasquin and Rosenbloom (1954) demonstrated that the hypertrophied thyroids of fish exposed to darkness could be restored to normal by returning the fish to light. More recently, Robertson (1958), as quoted by Hoar (1959), found a slightly greater uptake of radioiodine in goldfish maintained on a short-day basis (8 hours of light) compared with those maintained on a long day (16 hours of light). Baggerman (1959), in studies of the migration of juvenile coho salmon, found that day-length influenced the time of induction of the migration disposition. Fish held under an 8-hour day retained a preference for freshwater which was associated with a low level of thyroid activity since thiourea treated fish responded similarly. Fish held under a 16-hour day exhibited an earlier change in preference for salt water than fish held under an 8-hour photoperiod. This change in preference was associated with a high level of thyroid activity since thyroxine-treated fish responded similarly. In studies of the seasonal production of thyroxine by the thyroid, Berg *et al.* (1959) suggest photoperiod as a possible mechanism of thyroid control. The pattern of production appeared directly related to photoperiod although this effect was not tested separately. These results lead the investigator to believe that a possible interaction may exist between photoperiod and thyroid activity which has an effect upon growth.

Summary

A review of literature indicates that the effects on growth of photoperiod and thyroid activity or their interrelationship are unsettled problems. Few of the past studies involved an extensive investigation of the effect of photoperiod, and no studies involved a measurement of the effect of a varying photoperiod. An analysis of the role of the thyroid gland frequently involved the use of anti-thyroid drugs which are known to have toxic side effects. Some reported effects of the thyroid on growth most probably represent the response to pharmaceutical rather than physiological doses.

In this study, an effort was made to hold constant such variables as temperature, availability of food, and space factors in order to determine more accurately the effect of photoperiod and thyroid activity on growth of fish. This study represents the results of four separate experiments. Each of the first three experiments involved a combined study of the effect of photoperiod and thyroid activity. The fourth experiment involved the use of only "normal" fish to further assess the effect of photoperiod. In conjunction with the second experiment, a study was made of the thyroid activity of the fish using the rate of loss of radioiodine from the head region as an index of thyroid activity.

METHODOLOGY

Experimental Fish

The experimental animal used throughout the study was the green sunfish *Lepomis cyanellus* Raf. This species was selected because it is readily accessible, well adapted to aquarium life, and is believed to have a seasonal growth pattern. A seasonal growth pattern was indicated by the work of Hubbs and Cooper (1935) who showed the presence of a spawning mark on the scales of the green sunfish which was much nearer the following winter annulus than the preceding one.

The fish were obtained from two sources. Fish for the first experiment were obtained from Burke Lake in the Rose Lake Experiment Station, Clinton County, Michigan. Fish for all the other experiments were obtained from the private ponds of Dr. Peter Tack, Clinton County, Michigan.

Fish ranged from 2 to 4 years in age, as determined by scale reading. Both mature and immature fish were used. The size range varied slightly in each experiment depending upon the size of fish captured prior to the experiment. The size range and the mean total length and weight of the fish in each experiment are given in Table 1.

In each of the first three experiments, 120 fish were used. Four photoperiods were included in this study. There were 10 fish in each thyroidal condition (hyperthyroid, hypothyroid, and control) under each of the photoperiods. The fourth experiment involved only 60 fish, 15 fish under each photoperiod (5 in each tank). All fish were distributed on a random basis.

Table 1. The size range and mean total length and weight of the fish at the beginning of each of the experiments.

Expt.	Total Length (mm.) Range	Mean	Total Weight (gm.) Range	Mean
1	68 - 110	89.1	5.2 - 23.4	13.5
2	82 - 110	96.4	7.6 - 25.3	16.6
3	81 - 98	89.1	7.5 - 16.6	11.6
4	89 - 103	89.8	9.0 - 20.4	12.8

Aquaria and Accessories

Four light-tight aquaria of approximately 100-gallon capacity were used in the study. Each aquarium had a separate filtering apparatus using glass wool and activated charcoal and having a filtration rate of 60 gallons per hour. Each aquarium represented one of the four photoperiods used in the study. Each was subdivided, by screen dividers, into three compartments (designated hereafter as tanks) approximately 15 by 21 by 12 inches. The water supply was tap water from the wells of Michigan State University.

Control of Photoperiod

The four photoperiods used in this study were assigned to the aquaria on a random basis. The photoperiods given below were used in the first three experiments: (1) a constant 8-hour photoperiod throughout the experiment; (2) a constant 16-hour photoperiod throughout the experiment; (3) a variable photoperiod increasing from 8 to 16 hours; and (4) a variable photoperiod decreasing from 16 hours to 8 hours. An experiment lasted six weeks. In the aquaria having variable photoperiod, changes in day-length were made at the rate of 1 hour over a 5-day period. This was accomplished by changing the day-length 15 minutes on the first and second day, making no change the third day, and then changing the day-length 15 minutes again on the fourth and fifth day. The lights were controlled by 24-hour timers.

The fourth experiment differed slightly from the first three. A regular six-week experiment was performed using the above photoperiods; at the end of the six weeks, the experiment was continued for another three weeks holding the variable photoperiods constant at the day-length that they finished the first six week period. This was done so that a comparison could be made between the growth of fish held at the two constant photoperiods throughout the experiment and the growth of fish at similar photoperiods but having a prior history of a varying photoperiod.

The light source in the first experiment consisted of two 25-watt frosted bulbs placed at each end of the aquarium. This did not provide an even distribution of light to all tanks and also caused a heating problem. Therefore, a three-foot fluorescent fixture, with a 30-watt cool white tube, was installed overhead in the hood covering each aquarium. The fixture was covered with translucent polyethylene. This resulted in a more even distribution of light in all tanks of the aquarium and was used in all other experiments.

Control of Temperature

The temperature of the aquaria was maintained as nearly constant as possible by adjustment with cool or warm water in the daily cleaning routine. The temperatures in the aquaria varied closely with the temperature in the laboratory. As a result, temperatures at which the four experiments were run differed slightly depending upon the season of the year. The median temperature for each experiment and the maximum deviation between aquaria is given in Table 2.

Table 2. Dates, median temperature and maximum deviation in temperature between aquaria for each of the experiments.

Expt.	Dates	Median Temp.	Maximum Deviation Between Aquaria
1	8/24/60 - 10/4/60	$78^{\circ} \pm 4^{\circ}$ F.[1]	2.0° F.
2	7/13/61 - 8/22/61	$77^{\circ} \pm 4^{\circ}$ F.	1.5° F.
3	9/23/61 - 11/3/61	$77^{\circ} \pm 3^{\circ}$ F.	2.0° F.
4	2/10/62 - 4/12/62	$74^{\circ} \pm 2^{\circ}$ F.	1.0° F.

[1]Variation from median during experiment.

Heaters were installed in the aquaria for the third and fourth experiments to maintain the water temperatures above the laboratory temperature and comparable to the previous experiments.

Temperature variations between the aquaria were infrequent, small and never directional;one aquarium was never consistently higher than another in any of the experiments.

Thyroidal Conditions

There were three thyroidal conditions used in the experiment, each of which was assigned to one of the tanks of an aquarium on a random basis.

An attempt was made to produce a hypothyroid condition by radiothyroidectomy of fish with carrier-free radioiodine (I^{131}). Fish in the first experiment received a single intraperitoneal injection of 100 microcuries (volume .05 cc.). Fish in the second experiment also received 100 microcuries given in two injections, of 50 microcuries each, into the peritoneal cavity, one week apart. In the third experiment, the fish were given a single intraperitoneal injection of 200 microcuries.

Thyroid follicles were present in the lower jaw of fish of the second experiment. Therefore, the dosage was increased in the third experiment. Thyroid follicles were also found in fish of the third experiment. Using histological criteria, no difference could be demonstrated between the control and thyroidectomized fish in both the second and third experiments. No thyroid follicles were found in fish from the first experiment, but there is some question whether complete thyroidectomy had been obtained in view of the findings of the second and third experiments.

An evaluation of the hypothyroid condition was made on the fish at the end of the second experiment using the rate of loss of radioiodine from the head region as an index of thyroid activity. The results of the study demonstrated an inhibition of thyroid activity in the radioiodine injected fish compared with the controls. Statistical analysis showed a significant difference. Therefore, although follicles were present, a "hypothyroid" condition was evident in all experiments.

Fish in the first experiment were injected with radioiodine one week prior to use in the experiment. The fish in all other experiments were injected three weeks prior to the start of the experiment to allow for utilization of reserve stores of thyroxine in the tissue. Simpson, Asling and Evans (1950) found 20 days is required for utilization of reserve stores of thyroxine in the rat.

A hyperthyroid condition was produced by injection of Na-L-thyroxine solution. The solution was prepared by dissolving Na-L-thyroxine in sodium hydroxide; it was then neutralized with hydrochloric acid and diluted with distilled water to a concentration of 100 micrograms of Na-L-thyroxine/.05cc. The dosage given to the fish was 100 micrograms L-thyroxine/fish/week. This dosage is based on a study by Hoffert and Fromm (1959) in which two-year-old rainbow trout held at 13° C. were found to have a thyroxine secretion rate of .303 micrograms L-thyroxine/100gms./day. It was desired to give the fish as much thyroxine as possible while still maintaining a physiological dose. It was felt that 100 micrograms of L-thyroxine per week would constitute a factor several times the secretion rate and still constitute a physiological dose. All injections had a volume of .05 cc. and were made intraperitoneally. This dosage was used in the three experiments in which the effect of the thyroid was evaluated.

In order to overcome bias due to weekly handling of hyperthyroid fish, the control fish and hypothyroid fish received intraperitoneal injections of isotonic saline solution each week at the same time the hyperthyroid fish were injected. The injection was .05 cc. of 0.6% saline solution. All injections were made with a 0.25 cc. syringe fitted with a 22 gauge needle.

Pre-experimental Acclimation and Conditioning

The fish were captured 4 to 8 weeks prior to an experiment and were therefore completely adapted to aquarium life and the feeding of artificial food.

All fish were treated for parasites and disease in one or more of the following ways: formalin treatment 1:4000 solution for approximately 45 minutes; saline treatment 3.0% solution for approximately five minutes; terracycline treatment 0.02% solution for 24 hours. These treatments were given shortly after the fish were collected.

The acclimation of the fish to a given photoperiod and constant temperature conditions, in the experimental aquaria, varied in the different experiments. Fish in the first experiment were held at a photoperiod of 12 hours of light per day for a period of three weeks prior to the start of the experiment. It was later felt that it would be more advantageous to acclimate the fish to the photoperiod that they would begin an experiment. Therefore, in subsequent experiments fish in the constant 8-hour aquarium and variable increasing photoperiod aquarium were held at a 8-hour day-length; the constant 16-hour aquarium and variable decreasing photoperiod aquarium were held at a 16-hour day-length. Fish were acclimated under these conditions for 3 weeks, 1 week and 4 weeks prior to the second, third, and fourth experiments, respectively.

An attempt was also made to acclimate the fish to receiving injections prior to the start of an experiment, except for the first experiment. The fish were given their respective injections each week during the three week acclimation period in the second experiment. In the third experiment, all fish received their respective injection twice before the start of the experiment. This also allowed the establishment of a hyperthyroid condition before the start of an experiment.

Physical Measurements

Measurements were made of the total length, standard length, and weight of each fish. Measurements of total length and standard length were made to the nearest millimeter on a standard measuring board. Weight was measured to the nearest 0.1 gram on a triple-beam balance after the fish had been blotted with absorbant paper to remove excess water. Prior to taking measurements, all fish were anesthetized with tricaine methano sulfonate (M.S.222). All fish were given a three-minute treatment in 3% saline solution after measurements were taken and the fish revived.

Measurements were made at the start of the experiment, end of the third week, and at the end of the sixth week. In the case of the fourth experiment, measurements were also taken at the end of the ninth week.

Growth in this study was considered as any increment (gain), in total length or weight. The fish in the first three experiments were not marked and the data are therefore expressed as the average increment in total length and weight per fish in a tank. In the fourth experiment, the fish were marked so that growth of individual fish could be determined. The percent increase in growth over the initial length and weight was also determined to remove any influence of average size of the fish in a tank.

Food and Feeding Routine

Two types of food were used in this study, a commercially prepared, dry pelleted food and frozen beef liver. Fish in the first experiment were fed the pellets. There was a high incidence of disease and a high mortality in the experiment. Dr. Allison (personal communication), fish pathologist for the state of Michigan, felt that part of the problem may have been dietary and recommended the use of beef liver. Frozen beef liver was fed in the second and third experiments. In these two experiments, occasional periods of cessation of growth were observed which could be correlated with periods of clouding of the aquarium water. It was felt that the clouding of the water may have resulted from the liver. The feeding of pellets and liver to fish in two other aquaria in the laboratory resulted in periodic clouding of the aquarium fed the beef liver. Under the circumstances, it was decided to feed pellets once more in the fourth experiment.

Fish were fed daily allowing 1/2 hour to 1 hour for feeding. They were given as much as they would consume. The amount of excess food was determined, the food and waste removed by siphon, and the tanks filled with fresh water.

The method of determining the amount of excess food varied with the two types of food. In the first experiment, the number of pellets remaining on the bottom of the tank was recorded. The average number of pellets in one gram of food was determined and the weight of the excess food determined by dividing the number of pellets counted as waste by the average number of pellets in a gram of food. In the fourth experiment, the number and size of the pellets remaining in the tank were noted and an equal number of pellets of nearly equal size were weighed on an electric balance. When the fish were fed beef liver, the excess food was removed, blotted, and weighed to the nearest 0.1 gram on a triple-beam balance.

Food consumption was recorded by providing a jar of food for each tank of fish. The jar of food was weighed at the start of the experiment, third week, sixth week, and also the ninth week in the case of the fourth experiment. The difference in the weight of a jar between two periods minus the weight of the excess food represented the amount of food consumed by a tank for that period.

The rate of food consumption is expressed as a percentage of the mean total body weight of a tank of fish per unit of time, and is calculated by the formula:

$$\underset{\text{(\% body weight)}}{\text{R.F.C.}} = \frac{\text{Food consumed by a tank of fish (gms.)}}{\text{Ave. total body weight of tank (gms.)}} \times 100$$

Inasmuch as the food consumed by individual fish was not known, body weight had to be based upon the total body weight of all fish in a tank. The average total weight of the fish in a tank, in a given period, is considered to be the mean of the total weight at the beginning and end of each period of measurement. Measurements were made on a 3-week and 6-week basis. For purposes of evaluation, the entire tank of fish is considered a single "organism." The value represents the rate of food consumption for a particular tank.

The efficiency of food conversion was also determined on a per-tank basis by the following formula:

$$\text{Eff. of Food Conversion (\%)} = \frac{\text{Total increment in weight of tank (gms.)}}{\text{Food consumed by tank (gms.)}} \times 100$$

The value obtained expresses the percent efficiency with which food is utilized for growth by a particular tank.

Histology

The branchialregions of the lower jaw of radiothyroidectomized fish and control fish were sectioned to determine the degree of thyroidectomy. The tissue was fixed in Dietrich's fix for 24 hours, embedded in paraffin, sectioned at 6 to 15 microns, and stained using the standard hematoxin-eosin technique.

Mortality and Disease

Mortality occurred in all four experiments. The highest incidence of mortality and disease occurred in the first experiment during which 12 fish died (10% mortality). Mortality in the second experiment was limited to 3 fish (2.5% mortality). The third experiment had a mortality of 8 fish (6.6% mortality). In the fourth experiment, only 1 fish died (1.6% mortality) in the nine-week period.

Mortality in the first and third experiments was due to a type of fin rot which attacked the caudal and pectoral fins. Death usually ensued after the fin was completely eroded away. Attacks on the weakened fish by other fish at times hastened death. Mortality in the second and fourth experiment could not be explained by disease. Those of the second experiment may have been the result of handling or faulty injecting. In the fourth experiment, the mortality appeared due to attacks by other fish. Other minor infections occurred but these were not believed to have caused any mortality.

The only treatment applied during an experiment was in the first experiment. The fish were fed pellets containing the antibiotic sulfamerazine. This did not appear to be effective and was discontinued.

All fish which died during the course of an experiment were entirely eliminated from the experiment for purposes of analysis of growth. The weight measurements of these fish were used, during the period they survived, for the purpose of determining the rate of food consumption and efficiency of food conversion. Fish observed to be in poor condition, at the time measurements were taken, were eliminated from an experiment.

Determination of Thyroid Activity

The rate of loss of radioiodine from the head region of fish, after injection of a tracer dose of radioiodine, was taken as an index of thyroid activity. The method is similar to that employed by Swift (1955). Measurements of thyroid activity were made before and after the second experiment.

Counts were detected with a sodium-iodide crystal scintillation tube (Nuclear Instrument Corp. Model DS-1), and counts were recorded by use of a count rate meter (Nuclear-Chicago Model 1620) and an Esterline Angus Strip-Recorder. The Esterline strip-recorder was not employed for counting at the end of the experiment. All fish were marked with numbered flutter tags so that individual fish could be recognized and individual records maintained.

Measurements of radioactivity were made *in vivo* using a flow type apparatus with the fish held in a glass tube narrowed at one end for positioning of the fish. The fish were oriented upstream with the flow of water entering at the narrow end of the tube. The tube was then placed in position next to the scintillation tube which lay horizontally on a table. The region of the fish which was counted comprised the head region from about the eye down and posterior to the edge of the opercle. The scintillation tube had a collimated head with an opening one inch in diameter. The region which was examined therefore varied slightly with the size of the fish.

The radioactivity of the fish is expressed in terms of percent injected dose. This is determined by dividing the activity of the fish, in counts per minute (c.p.m.), by the activity of a standard in c.p.m. after both values have been corrected for background. This procedure corrects for isotope decay, as well as standardizes the geometry if the standards are counted in the same position as the sample. The activity of the standard is determined from the average of two standards which are counted several times during the counting period. The effect of the glass tube in which the fish were held is negligible.

The radioactivity, in percent injected dose, determined each day was plotted against time (days after injection) on a semilog scale. The regression coefficient (slope of the line), fitted by the method of least squares, was taken as the index of thyroid activity. A larger negative regression coefficient indicates a more active gland. This holds true if time is allowed for the loss of extrathyroidal iodine.

Prior to the start of the second experiment, a group of four normal fish were acclimated to each of the two initial photoperiods, constant 8-hour and constant 16-hour. The fish were placed under the photoperiod on June 8, 1961, acclimated for 12 days after which they received an intraperitoneal injection of 10 microcuries of radioiodine. Counting was initiated 8 days after injection and continued for 8 consecutive days. One fish died between the time of injection and when counting was initiated. The two standards for this period were prepared by placing 1/5 of the injected dose in each glass planchet. A casein solution was added to bind the iodine and prevent volatilization. The glass planchets were then dried under a heat lamp. Counts of the two standards were made at the beginning and end of the counting period each day.

The thyroid activity was determined for the control and hypothyroid fish, under each of the four photoperiods, at the end of the second experiment. This study involved 73 fish; 36 fish were controls and 37 were hypothyroid fish. The fish were given a tracer dose of 10 microcuries of radioiodine, intraperitoneally, on August 23, 1961. Counting was started 10 days after injection and continued each day for 8 days except for the 6th day. One fish of the group died 6 days after the start of counting.

The two standards for this period were prepared with the same amount of radioiodine that was injected into the fish. Other than this, the standards were prepared in the same manner as at the start of the experiment. Counts were taken of the standards at the start, middle, and end of each counting period.

RESULTS AND DISCUSSION

Effect of Photoperiod

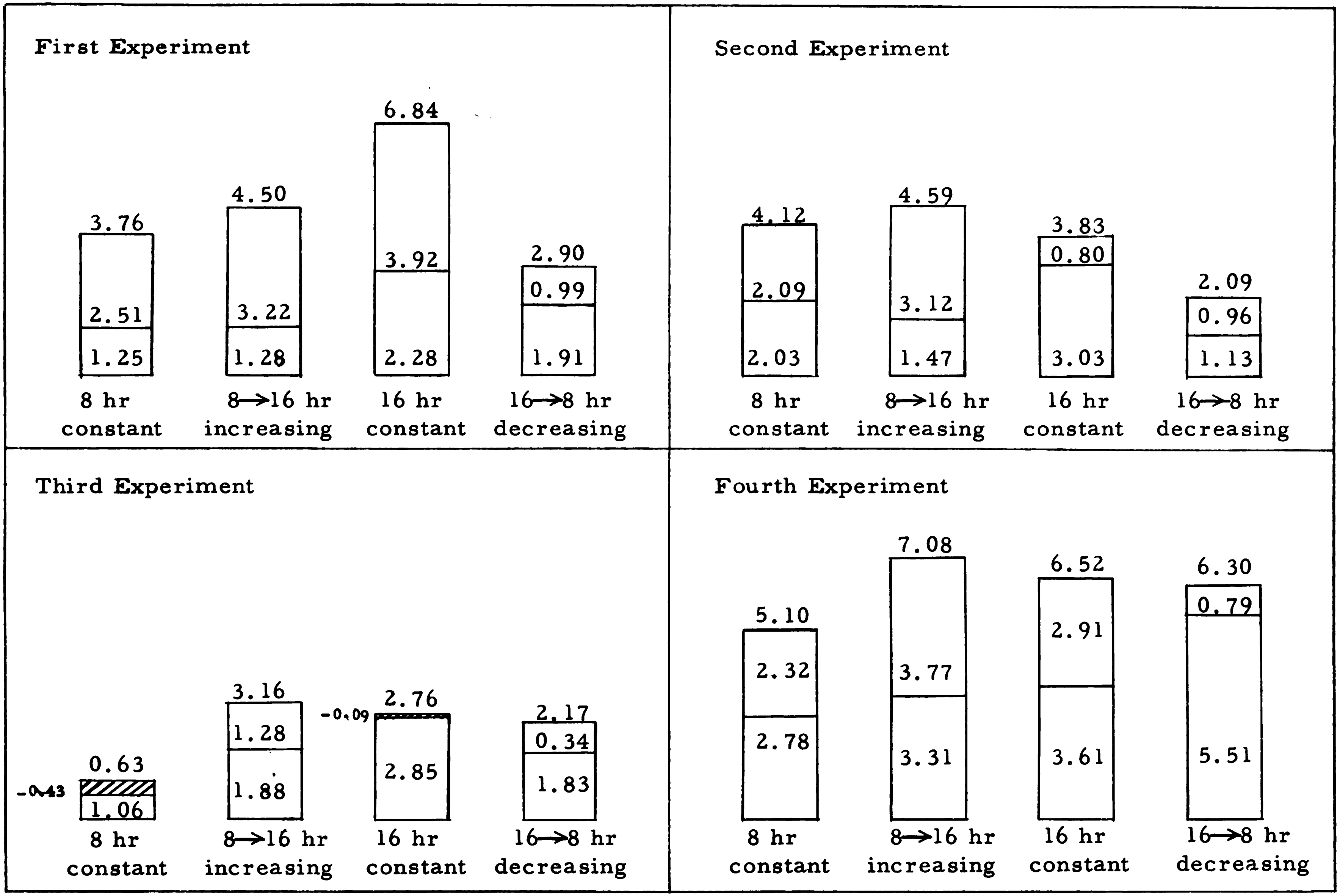
The effect of photoperiod on growth in length and weight, food consumption, and efficiency of food conversion was determined. The data were analyzed from three points of view: 1) differences between the four photoperiods over an entire experiment, 2) comparison of the first and second three-week periods of the two varying photoperiods, 3) a comparison of the two constant photoperiods. In the first three experiments, the value for each photoperiod was determined by combining the three thyroidal conditions under each photoperiod.

Results of Increment in Weight

The average increment in weight per fish under each photoperiod is given in Figure 1. The data for the average increment in weight per fish in each tank for the four experiments is presented in Appendix A. Results of the second and third experiments must be interpreted with caution, as these two experiments experienced periods of inhibited growth which was believed due to the feeding of liver as discussed previously. Those photoperiods particularly affected in the second experiment were the first three week period of the decreasing photoperiod and the second three week period of the 16-hour photoperiod. Those affected in the third experiment were the second three-week period of both the constant photoperiods. Periodic clouding of the water occurred in all aquaria during the third experiment rendering the results somewhat questionable.

A comparison of the results of the four photoperiod groups for all experiments shows that the fish in the increasing photoperiod had the

Figure 1. Average increment in weight (grams) of a fish under each photoperiod of the four experiments. The lower portion of each bar and the value enclosed represents the gain in weight the first three weeks; the upper portion and the value enclosed represents the gain in weight the second three weeks. The values at the top of each bar represent the gain in weight over the six week period. Hatched areas and values to the left of the bar represent losses incurred the second three weeks.


First Experiment
3.76
2.51
1.25
8 hr constant
4.50
3.22
1.28
8→16 hr increasing
6.84
3.92
2.28
16 hr constant
2.90
0.99
1.91
16→8 hr decreasing
Second Experiment
4.12
2.09
2.03
8 hr constant
4.59
3.12
1.47
8→16 hr increasing
3.83
0.80
3.03
16 hr constant
2.09
0.96
1.13
16→8 hr decreasing
Third Experiment
0.63
-0.43
1.06
8 hr constant
3.16
1.28
1.88
8→16 hr increasing
-0.09
2.76
2.85
16 hr constant
2.17
0.34
1.83
16→8 hr decreasing
Fourth Experiment
5.10
2.32
2.78
8 hr constant
7.08
3.77
3.31
8→16 hr increasing
6.52
2.91
3.61
16 hr constant
6.30
0.79
5.51
16→8 hr decreasing

greatest growth in weight in all but the first experiment in which those in the 16-hour photoperiod had the greatest growth. A two-way analysis of variance of the effect of photoperiod and thyroid activity on the mean increment in weight was performed on the first three experiments to determine if any of the differences were statistically significant. In the fourth experiment, a one-way analysis of variance was performed using the individual increments of each fish. These results are given in Table 3. A Tukey Multiple Range Test was applied to the first and third

Table 3. Results of a statistical analysis of the differences in the mean increment in weight of a fish over the entire experiment.

Expt.	F ratio	df	Significance
1	11.17	(3, 6)	1%
2	1.84	(3, 6)	no significant difference
3	5.14	(3, 6)	5%
4	0.58	(3, 55)	no significant difference

experiments to determine which photoperiods were significantly different. In the first experiment, the 16-hour photoperiod was significantly different from the decreasing photoperiod at the 1% level and significantly different from the 8-hour and increasing photoperiods at the 5% level. In the third experiment, 16-hour, increasing photoperiod and decreasing photoperiod were all significantly different from the 8-hour photoperiod at the 5% level.

The effect of photoperiod may also be evaluated by a comparison of the gain in weight between the first and second three week periods, of the varying photoperiods (see Figure 1). Under the increasing photoperiod, there was a greater gain the second three weeks (12 to 16 hours light) than the first three weeks (8 to 12 hours light) in three of the four experiments. This did not occur in the third experiment for reasons stated previously. Similarly, there was a decrease in the weight gain the second three weeks (12 to 8 hr. light) compared with the first three weeks (16 to 12 hr. light) of the decreasing photoperiod group. These differences are especially significant since in the first experiment there was an increased growth the second three weeks for all photoperiods except the decreasing photoperiod, and in the second and fourth experiments there was a decrease in increment the second three weeks in all photoperiods except the increasing photoperiod. The differences in the average increment in weight between the first and second three-week periods of the varying photoperiods, in the four experiments, were tested by the Student's "t" test for matched observations. There was no significant difference between the two 3-week periods for either the increasing or decreasing photoperiod.

A comparison was also made of the average increment in weight of a fish between the two constant photoperiods. Over an entire experiment, the gain in weight was greater for fish in the 16-hour than the 8-hour photoperiod in three of the four experiments. This did not occur in the second experiment; however, here growth was greatly inhibited the second three week period of the 16-hour photoperiod whereas the 8-hour photoperiod appeared to be unaffected.

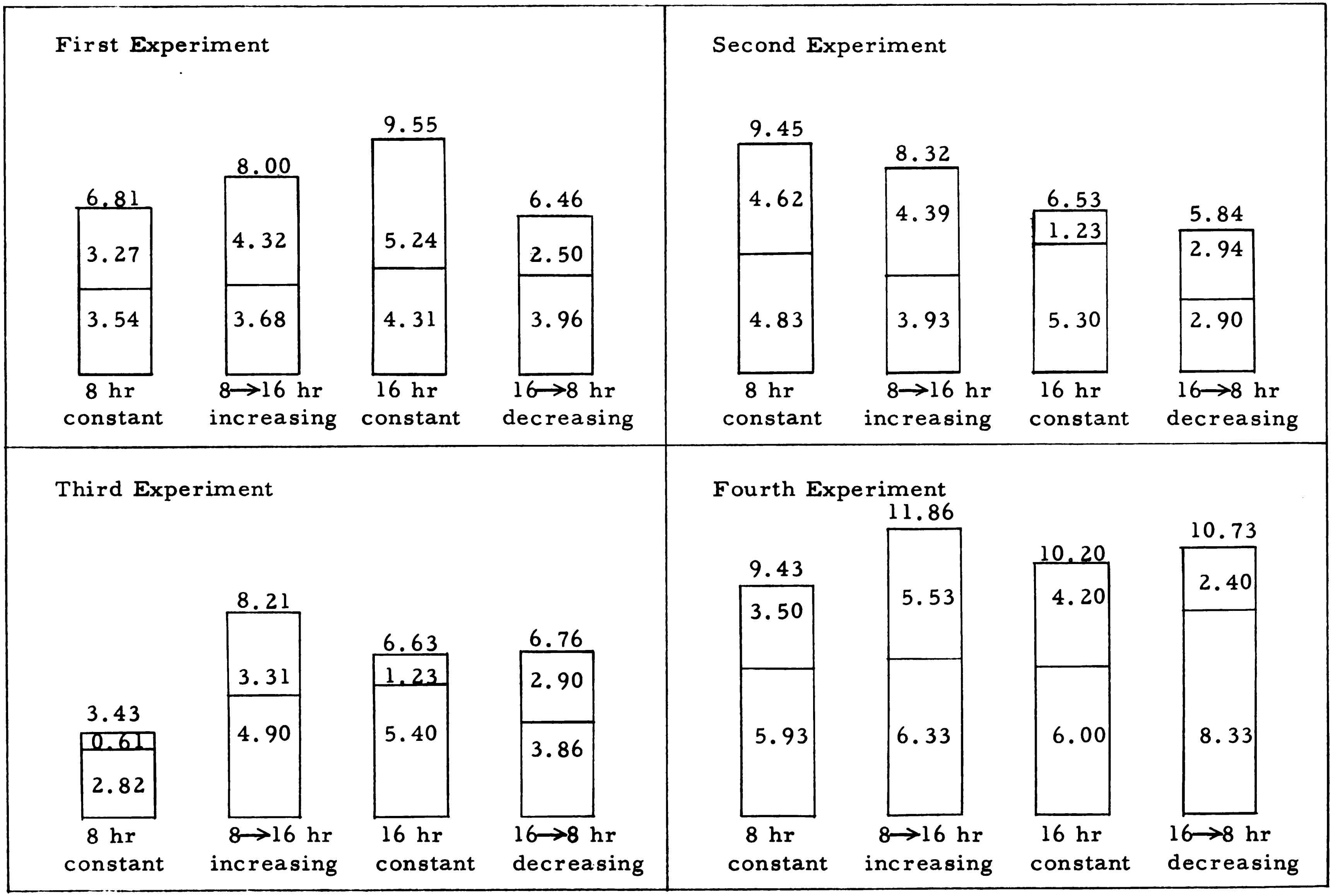
Since all fish of the fourth experiment were marked, it was possible to make a better statistical analysis of the effects of photoperiod. The results of a one-way analysis of variance for the individual increments of the fish under each photoperiod has been presented in Table 3. To determine if there was a significant difference in the growth of fish in different tanks under the same photoperiod, a hierarchical analysis of variance (Simpson, Roe, and Lewontin, 1960) was performed.

Equal replications are necessary for the analysis; since one fish was lost in the 8-hour photoperiod, one fish from each tank under each photoperiod was randomly omitted. There was no significant difference in increment in weight between the different photoperiods or between the tanks under each photoperiod. Using the individual increments of the marked fish, differences in increment between the two 3-week periods of the varying photoperiods were also analyzed by the "t" test for matched observations. There was no significant difference, at the 5% level, between the two 3-week periods of increasing photoperiod group. Under a decreasing photoperiod, the increment in weight between the two 3-week periods was significant at the 1% level ($t = 5.68 > t_{.995} = 2.977$ df = 14).

Results of Increment in Total Length

The average increment in total length of the fish under each photoperiod is given in Figure 2. The average increment in total length of a fish in each tank is presented in Appendix A. The differences in length increment between photoperiods, over an entire experiment, conform to a degree with differences in weight increment. The greatest gain in length occurred in the same photoperiod as the greatest gain in weight in all but the second experiment in which the 8-hour photoperiod group had the greatest gain in length. The photoperiods have the same order of rank for length and weight in each experiment except for the third and fourth experiments in which there was a slightly greater gain in length by fish under a decreasing photoperiod than under 16-hour photoperiod. However, measurements were made only to the nearest millimeter and the slight differences may be due to experimental error. The inhibition of growth in weight that occurred in the second and third experiments also occurred with respect to length; therefore the results of the second and third experiments are again somewhat questionable. Generally, differences in gain in length of fish between the photoperiods of an

Figure 2. Average increment in total length (millimeters) of a fish under each photoperiod of the four experiments. The lower portion of the bar and the enclosed value represent the gain in length the first three weeks; the upper portion and the enclosed value represent the gain in length the second three weeks. The values at the top of the bar represent the gain in length over the six week period.


First Experiment
6.81
3.27
3.54
8 hr constant
8.00
4.32
3.68
8→16 hr increasing
9.55
5.24
4.31
16 hr constant
6.46
2.50
3.96
16→8 hr decreasing
Second Experiment
9.45
4.62
4.83
8 hr constant
8.32
4.39
3.93
8→16 hr increasing
6.53
1.23
5.30
16 hr constant
5.84
2.94
2.90
16→8 hr decreasing
Third Experiment
3.43
0.61
2.82
8 hr constant
8.21
3.31
4.90
8→16 hr increasing
6.63
1.23
5.40
16 hr constant
6.76
2.90
3.86
16→8 hr decreasing
Fourth Experiment
9.43
3.50
5.93
8 hr constant
11.86
5.53
6.33
8→16 hr increasing
10.20
4.20
6.00
16 hr constant
10.73
2.40
8.33
16→8 hr decreasing

experiment are not as great as gains in weight.

A two-way analysis of variance was performed in the first three experiments to determine if differences in increment in length were significantly different. The analysis showed that a significant difference occurred only in the third experiment (5% level). A one-way analysis of variance of the fourth experiment demonstrated no significant difference.

A comparison of increment in length between the two 3-week periods of the varying photoperiods demonstrated a greater gain with longer photoperiods although the results were not as consistent as for increase in weight. Under an increasing photoperiod, the average length increment of a fish was greater the second three weeks than the first three weeks in two of the four experiments. There also was a decrease in gain in length the second three weeks compared with the first three weeks for fish under a decreasing photoperiod in three of the four experiments. In the second experiment, there was basically no change. The mean increment in total length of a fish under each photoperiod for the four experiments was analyzed by the "t" test for matched observations. There was no significant difference between the two 3-week periods under either of the varying photoperiods.

A comparison of the fish under 8-hour photoperiod and the 16-hour photoperiod showed a tendency for greater gains in length under a 16-hour than an 8-hour photoperiod. The second experiment is again the exception.

A statistical analysis of increment in total length was also made for the first six weeks of the fourth experiment. A one-way analysis of variance was performed based on increments in length of the individual fish. To determine if differences among the different tanks of fish existed, a hierarchial analysis of variance was also performed. There was no significant difference between photoperiods. The effect of tanks was also negligible.

The difference in growth of fish between the two 3-week periods of increasing photoperiod was not significant when analyzed by the "t" test. However, the difference in growth between the two 3-week periods of the decreasing photoperiod group was significant at the 1% level ($t = 7.57 > t_{.995} = 2.977$, df = 14).

Results of Growth Expressed as a Percentage

Since the fish in each experiment were distributed on a random basis, slight differences in the average initial total length and weight of fish occurred in each tank. Anderson (1959) found that there was a tendency for larger fish to have greater growth increments. A comparison of the growth of marked fish of the fourth experiment showed this to be generally true. Size hierarchies existed in almost all tanks in each experiment, and this factor had a profound influence on the growth of individual fish. The growth of fish in length and weight was therefore calculated as a percent gain of the mean initial total length or weight for each of the three-week periods and over a six-week period. Increment in weight calculated on this basis is given in Figure 3 and for increment in total length in Figure 4. The results are nearly the same as observed for actual increments in length and weight. The only noticeable differences occurred in the fourth experiment. The percent growth in weight for the 16-hour photoperiod group was very nearly the same as for increasing photoperiod group, and the percent growth of the decreasing photoperiod group is less than it appears to be in terms of actual increment in weight. Overall, the growth is so similar, that the effects of the average initial length and weight of the fish in a tank was believed negligible and that the actual increments accurately describe the growth of the fish.

Figure 3. Growth in weight of a fish expressed as the percent gain of the average initial weight of a fish under each photoperiod. The lower portion of the bar and the enclosed value represents the percent gain the first three weeks; the upper portion and the enclosed value represents the percent gain the second three weeks. The values at the top of each bar represent the percent gain over the six week period. Hatched areas and values to the left of the bar represent losses incurred the second three weeks.

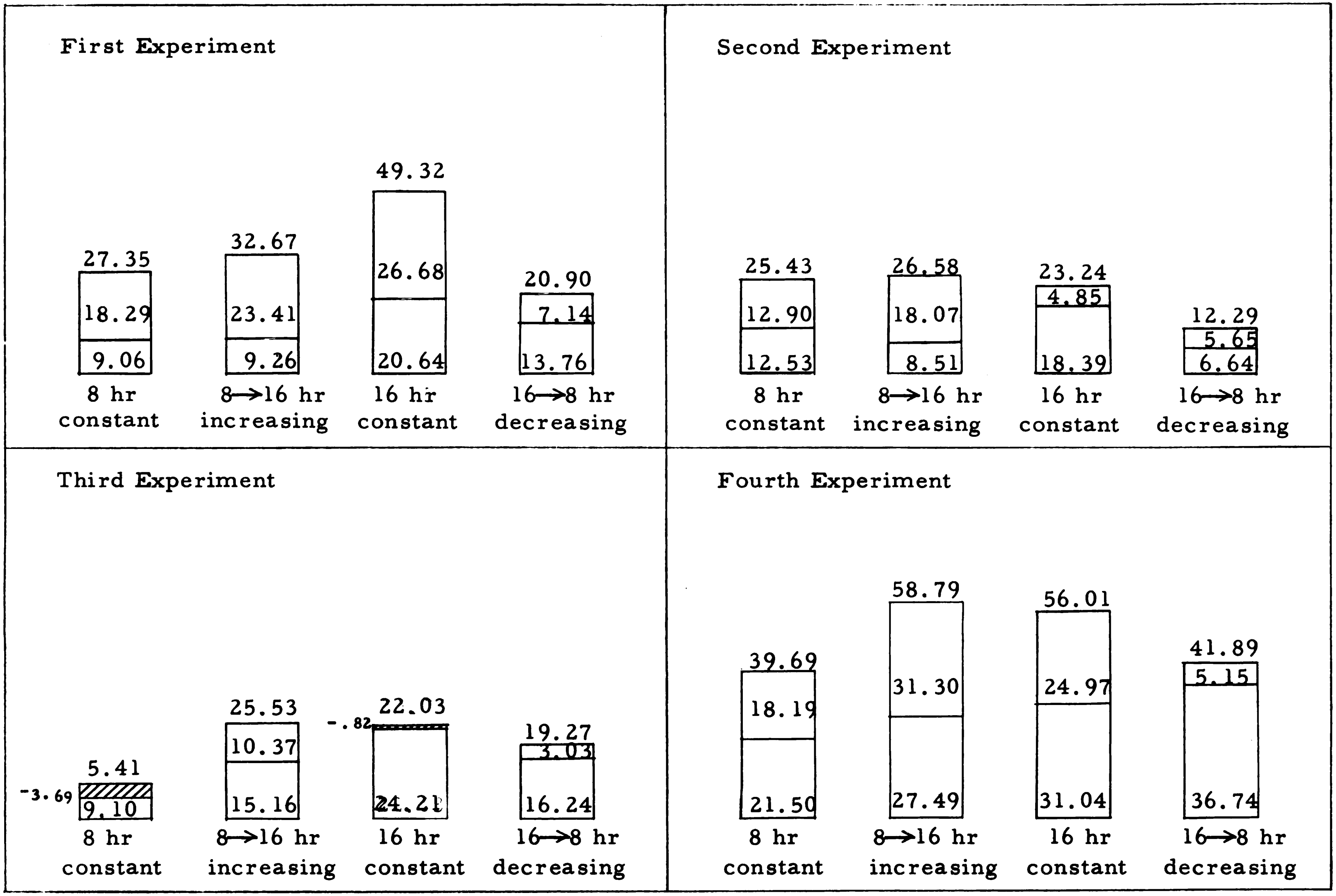
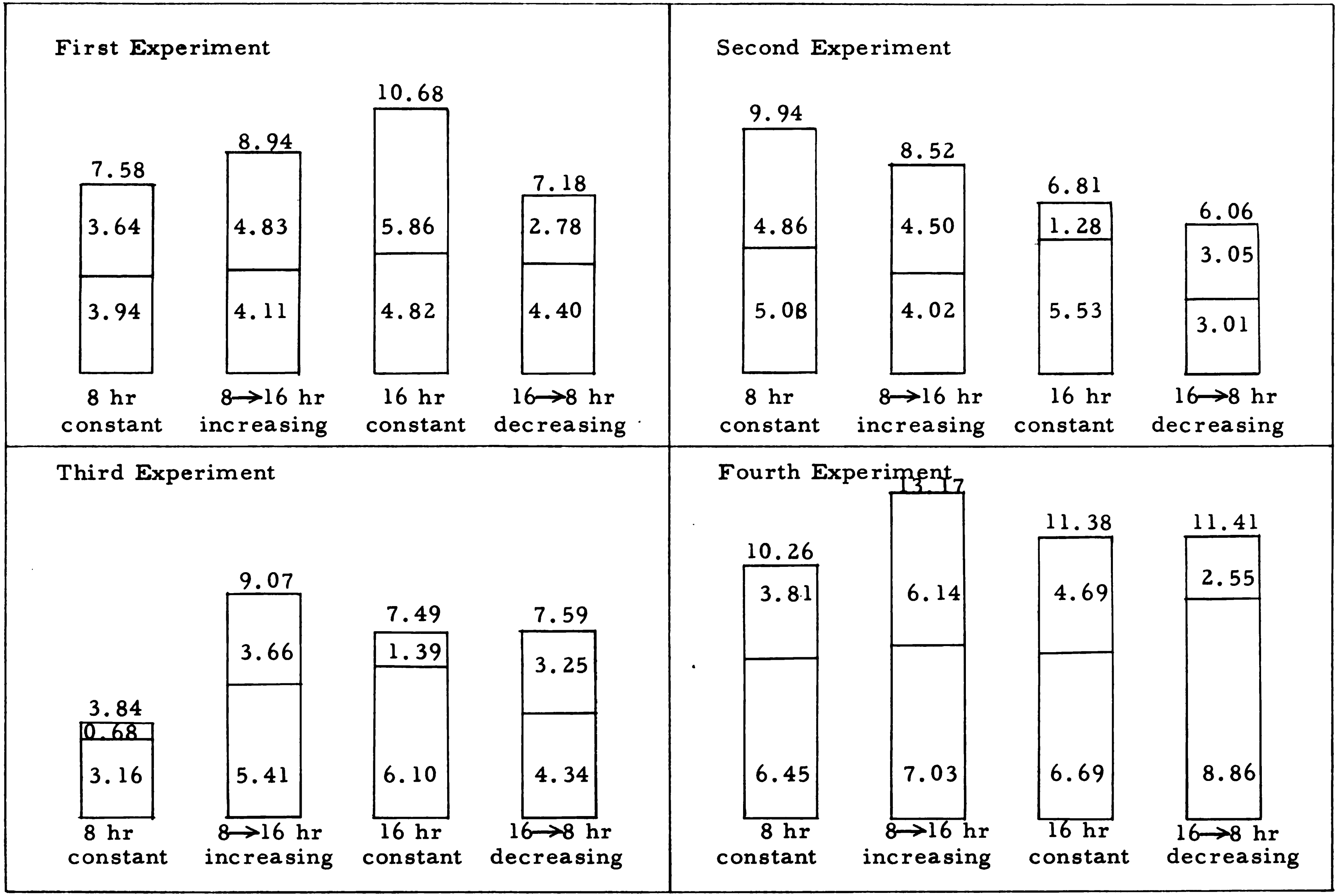

First Experiment
27.35
18.29
9.06
8 hr constant
32.67
23.41
9.26
8→16 hr increasing
49.32
26.68
20.64
16 hr constant
20.90
7.14
13.76
16→8 hr decreasing
Second Experiment
25.43
12.90
12.53
8 hr constant
26.58
18.07
8.51
8→16 hr increasing
23.24
4.85
18.39
16 hr constant
12.29
5.65
6.64
16→8 hr decreasing
Third Experiment
5.41
-3.69
9.10
8 hr constant
25.53
10.37
15.16
8→16 hr increasing
22.03
-.82
16 hr constant
19.27
3.03
16.24
16→8 hr decreasing
Fourth Experiment
39.69
18.19
21.50
8 hr constant
58.79
31.30
27.49
8→16 hr increasing
56.01
24.97
31.04
16 hr constant
41.89
5.15
36.74
16→8 hr decreasing

Figure 4. Growth in total length of a fish expressed as a percent gain of the average initial total length of a fish under each photoperiod. The lower portion of the bar and the enclosed value represents the percent gains the first three weeks; the upper portion and the enclosed value represents the percent gain the second three weeks. The values at the top of each bar represent the percent gain over the six weeks.


First Experiment
7.58
3.64
3.94
8 hr
constant
8.94
4.83
4.11
8→16 hr
increasing
10.68
5.86
4.82
16 hr
constant
7.18
2.78
4.40
16→8 hr
decreasing
Second Experiment
9.94
4.86
5.08
8 hr
constant
8.52
4.50
4.02
8→16 hr
increasing
6.81
1.28
5.53
16 hr
constant
6.06
3.05
3.01
16→8 hr
decreasing
Third Experiment
3.84
0.68
3.16
8 hr
constant
9.07
3.66
5.41
8→16 hr
increasing
7.49
1.39
6.10
16 hr
constant
7.59
3.25
4.34
16→8 hr
decreasing
Fourth Experiment
10.26
3.81
6.45
8 hr
constant
13.17
6.14
7.03
8→16 hr
increasing
11.38
4.69
6.69
16 hr
constant
11.41
2.55
8.86
16→8 hr
decreasing

Effect of Photoperiod on Growth in Length and Weight

The results of the four experiments certainly suggest that photoperiod has an influence on growth in length and weight of green sunfish. Greater growth is associated with longer photoperiods and increasing photoperiods at constant temperature. The results are not statistically significant for all four experiments; however, the consistent results of growth, particularly in weight, under each photoperiod in each of the four experiments and the consistent differences between the two 3-week periods of the varying photoperiods certainly afford strong evidence of an effect of photoperiod. The lack of statistical significance is probably due in part to the large variance in the growth of the fish due to the size hierarchies established in the tanks and the use of various sized fish.

Increment in weight appears to be more closely correlated with photoperiod than increment in total length. Differences in increment in weight between the two 3-week periods of the varying photoperiods were correlated with changes in photoperiod with only one exception. In length increment, there were three exceptions to this correlation. It appears that growth in length is less dependent upon photoperiod than is weight.

The findings of this study are in agreement with the results of Eisler (1957) and Tryon (1942) both of which used very small fish in their study. Eisler (1957) studied the growth of chinook salmon fingerlings in darkness and under various constant light intensities for 12 weeks. There was a significant difference in growth in length of fish reared at different light intensities compared with fish reared in the dark; the fish in the light attained a much greater growth in length. Increase in weight was also greater (57%) for fish reared in light. No significant differences occurred between the different light intensities. Tryon (1942) compared the growth of cutthroat fingerlings reared in hatchery troughs which were covered or exposed to natural light conditions for a period of 50 days.

A significant increase in growth (1% level) occurred in the fish reared in open troughs.

Other investigations described in the literature in which older, mature fish were used have shown either no relationship or an inverse relationship between growth and photoperiod. The work of Bjorklund (1958) on goldfish and Brown (1946a) on brown trout, which were discussed previously, showed an inverse relationship. Anderson (1959) in studies of the bluegill found no relationship, although the mean gain in weight and length was slightly greater for the short day period (10-hour light). Swift (1961) studied the specific growth rate in length of yearling brown trout under natural conditions in which temperature and photoperiod were recorded at monthly intervals, and also under constant environmental conditions with the same fish being exposed for a period of four weeks to a 4-, 8-, and 12-hour light period. In the study under natural conditions, Swift (op. cit.) felt that a closer relationship existed between the annual seasonal growth cycle of length and the annual temperature cycle than with the cycle of photoperiod. He remarked that under the constant environmental conditions (data not given) specific growth rate in length showed no consistent response to photoperiod.

To this investigator's knowledge, the present study is the first in which a positive correlation between growth and photoperiod has been found for older fish (2- to 4- year-old fish). It would be of interest to determine whether there is any variation in the response of different sized fish to photoperiod.

The reasons for the dissimilarity in the results reported in this study and those reported in the literature are unknown. Since each involved different species, one might feel that various species respond differently. This does not appear to be the case when comparing Anderson's (1959) work on the bluegill and the present study of the green sunfish. The bluegill and green sunfish are closely related species attaining approximately the same growth, and occupying the same niche in nature. The conclusions of Anderson (op. cit.) are based on a limited number of fish (3 under each photoperiod) and over a limited period of time (30 days). A larger sample of fish held for a longer period of time may have yielded different results. There may be a lag period before the effects of photoperiod are apparent. This was not particularly true for the present study, but a period of 8 weeks elasped before an effect was apparent in the work of Eisler (1957). Brown (1946a) held trout for periods of two and five months to obtain significant differences. The study by Bjorklund (1958) may have been influenced by the limited food supply given the fish (approximately 1.7% of body weight daily). Under the circumstances, differences in activity of the fish would have a much greater influence on their growth rate than on that of fish given an unlimited food supply.

Effect of Varying Photoperiod on Growth

An attempt was made to determine whether a varying photoperiod has a greater effect upon growth than a constant photoperiod. As previously stated, Swift (1961) felt that a study was necessary to distinguish between the effect of day to day changes in day-length and the effect of day-length when it is constant from day to day. That a varying photoperiod has a greater influence on growth is apparent in this study. In the second, third and fourth experiments, fish exposed to an increasing photoperiod attained the greatest increment in weight. The influence of varying photoperiod is also apparent in Table 4 which shows the percent gains or losses the second three weeks over the first three weeks ($\frac{\text{wt. gain second 3 weeks}}{\text{wt. gain first 3 weeks}}$ x 100). The percent gains in weight were greatest the second three weeks for fish under an increasing photoperiod. In the third experiment in which there was a reduction in growth rate the second three weeks under all photoperiods, the reduction was least for the fish under increasing photoperiod. For fish held under a

Table 4. Percent gains or losses in length and weight the second three weeks compared to the first three weeks for all photoperiods of each experiment.

Expt.	8 hr. percent	8-16 hr. percent	16 hr. percent	16-8 hr. percent
Weight				
1	+100.80	+151.56	+21.57	-58.40
2	-4.34	+112.24	-73.59	-15.04
3	-140.56	-12.04	-103.15	-55.73
4	-16.55	+13.90	-16.62	-85.66
Total Length				
1	-7.62	+17.39	+21.58	-36.87
2	-4.35	+11.70	-76.79	+1.38
3	-78.37	-32.45	-77.22	-24.87
4	-40.98	-12.64	-30.00	-71.19

decreasing photoperiod, there was a general reduction in weight gain the second three weeks (indicated by a percent loss in Table 4). In the first and fourth experiments, the reduction in weight (percent loss) was greater under the decreasing photoperiod than any other photoperiod. These differences in the percent gain or loss between the two 3-week periods were not as apparent for total length; however, as mentioned previously, growth in total length is possibly somewhat independent of the effect of photoperiod. Another point suggesting a greater influence of the varying photoperiods is shown in the study of the effect of the prior history of photoperiod on growth which will be discussed in the next section.

In general, data from all four experiments suggest that decreasing photoperiod has a depressing effect upon growth, but the possibility of a stimulating effect of increasing photoperiod is not well-shown. The first experiment indicates that 16-hour photoperiod acts as the greatest stimulant to growth. The fourth experiment suggests that increasing photoperiod stimulates growth. Fish in the 16-hour photoperiod generally had the greatest growth the first three weeks (Figures 1 and 2). If growth had occurred during the last half of the second and third experiments at a rate comparable to that occurring in the first half, the greatest growth may have been attained in this photoperiod. The reliability of the data for the second and third experiments has been discussed previously.

Effect of Prior History of Photoperiod on Growth

The fourth experiment was extended for three weeks beyond the normal six weeks for an experiment. The photoperiod of each of the two varying photoperiods was held constant during this period (increasing photoperiod held at 16-hour day-length and decreasing photoperiod at an 8-hour day-length). This was done to permit a comparison of the growth of fish with a prior history of a varying photoperiod with growth of fish held at a constant photoperiod throughout the experiment. The mean increments in length and weight for the last three-week period of each photoperiod are given in Table 5. Differences between the two aquaria held at the same constant photoperiod were analyzed by means of the Student's "t" test. The fish having a prior history of an 8-hour photoperiod (Group 8C) had a significantly greater growth in length and weight ($t = 3.43 > t_{.995} = 2.771$ and $t = 4.29 > 2.771$, df = 27) than fish with a prior history of a decreasing photoperiod (Group 8V). Fish with a prior history of an increasing photoperiod (Group 16V) had a significantly greater gain in weight ($t = 2.75 > t_{.995} = 2.048$, df = 28) than fish having a prior history of a constant 16-hour photoperiod (Group 16C).

Table 5. Average increment in weight and total length of fish under each photoperiod for the third three-week period of the fourth experiment.

Description of Group		Weight in grams	Total Length in mm.
Group 8C	- 8-hour constant during entire expt.	3.21**	4.29**
Group 8V	- 8-hour constant previously decreasing 16-8 hour.	0.43	1.93
Group 16C	- 16-hour constant during entire expt.	1.93	3.07
Group 16V	- 16-hour constant previously increasing 8-16 hour.	3.62*	4.86

*Denotes significance at the 5% level (see text)

**Denotes significance at the 1% level (see text)

The difference in gain in length between the 16V and 16C groups was not significant by the normal two-tailed test for significance, however the gain was significantly greater by a one-tailed test for the group having a prior history of an increasing photoperiod (16V) compared to the 16-hour photoperiod (16C). The results show that the prior history of photoperiod does have an effect upon growth, an increasing photoperiod tending to stimulate growth and a decreasing photoperiod tending to suppress growth. These results offer further evidence of an effect of a varying photoperiod on growth as mentioned in the previous section.

Effect of Photoperiod on the Seasonal Growth Cycle of Fish

Since photoperiod has been shown to have an effect upon growth, it appears that it plays a role in the annual seasonal growth cycle of fish.

Temperature is probably primarily responsible for the seasonal cycle with photoperiod playing a less important role. It appears that the extremely rapid rate of growth which occurs in spring (April to June) may well be due to a synergistic action between increasing photoperiod and temperature. The decrease which occurs during the summer (July and August) during which temperatures are still increasing or are at a maximum may be due to the decreasing photoperiod or a combination of decreasing photoperiod and temperatures above the optimal level for growth. In view of the great regularity with which the seasonal cycle occurs and the variation in optimal temperature ranges of different species of fish, it may be that photoperiod is the dominant factor affecting the cycle. A study by Evans et al. (1962), on respiration of trout tissue, showed that photoperiod was temperature dependent and its effect evident only at high temperatures. The rapid decrease in growth rate of fish during autumn is probably due to an interaction of both decreasing photoperiod and falling temperatures. In winter, temperature appears to be the predominant factor since substantial growth occurred at low photoperiods and high temperature in the laboratory. The above explanation of the annual seasonal growth cycle in fish is largely conjecture. Further studies are necessary to resolve the interaction of photoperiod and temperature on growth.

Effect of Photoperiod on Food Consumption

The amount of food consumed by a fish increases with the size of a fish. As a result, food consumption was calculated as a percentage of the body weight by the formula previously given. The rate of food consumption for each experiment is given in Table 6. The rate of food consumption as calculated for each tank of fish is given in Appendix B. The results given in Table 6 are not comparable between experiments due to the feeding of different types of food. The second and third experiments are the only two in which conditions were similar.

Table 6. The average rate of food consumption (percent body weight) of fish under each photoperiod of each six-week experiment.

Expt.	8 hr. percent	8-16 hr. percent	16 hr. percent	16-8 hr. percent
1	38.78	42.64	57.24	44.67
2	83.70	77.06	87.11	67.60
3	76.55	91.70	103.01	79.05
4	44.35	55.15	58.74	55.74

In all four experiments, the rate of food consumption was greatest in the 16-hour photoperiod. In three of the four experiments, the rate of food consumption was lowest in the 8-hour photoperiod. These results do not agree with the general growth pattern of the fish over the 6 weeks of each experiment (Table 6 and Figures 1 and 2). It is believed that the variability may be due to differences in activity of the fish in the various photoperiods. In the 16-hour photoperiod the longer light condition allows greater activity over a 24-hour period. This increase in activity increases the overall energy expenditure thereby increasing the maintenance requirements. Roberts, as reported by Anderson (1959), found a 30% reduction in respiration of sunfish held at a 9-hour photoperiod compared to fish held at a 15-hour photoperiod.

The rate of food consumption is directly related to photoperiod. This is apparent from a view of Table 6 as well as from the comparison of the rate of food consumption between the two 3-week periods of varying photoperiods given in Table 7. In general, low rates of food

Table 7. Rates of food consumption (expressed as percent body weight) for the first and second three-week periods of increasing and decreasing photoperiods in each experiment.

Expt.	8-16 hr. Photoperiod		16-8 hr. Photoperiod	
	1st 3 Wks. (8-12 hr.) percent	2nd 3 Wks. (12-16 hr.) percent	1st 3 Wks. (16-12 hr.) percent	2nd 3 Wks. (12-8 hr.) percent
1	20.79	23.60	24.00	20.13
2	37.64	42.72	40.35	27.46
3	52.52	39.93	44.87	33.45
4	31.67	24.67	39.58	14.76

consumption are associated with short photoperiods and high food consumption with longer photoperiods. Exceptions to this relationship occur in the increasing photoperiod of the third and fourth experiments. Few studies of the effect of photoperiod on growth described in the literature have involved an analysis of food consumption. Anderson (1959) found no significant difference in the food consumption of fish held at a 10-hour or 15-hour photoperiod.

Although the rate of food consumption for the six-week periods did not appear closely correlated with increment in length and weight, there is a close correlation with increment in weight, This can be seen in a comparison of increment in weight and rate of food consumption between the two 3-week periods of the varying photoperiods (Figure 1 and Table 7). The results of a correlation analysis of rate of food consumption with gain in weight and with gain in length of each tank of fish in each experiment is given in Table 8. The correlation between food consumption and increment in weight is significantly different from zero in every experiment. Total length was not closely correlated with the rate of food consumption; possibly some other factor may be involved in determining increment in length.

Table 8. Coefficients of correlation between rate of food consumption and average increment in weight and total length per tank of fish in each six-week experiment.

Expt.	Correlation Coeff. Ave. Weight Increment	Correlation Coeff. Ave. T. Length Increment
1	.787**	.576
2	.750**	.025
3	.766**	.613*
4	.814***	.205
	N = 12	

*Denotes significance at the 5% level.

**Denotes significance at the 1% level.

***Denotes significance at the 0.5% level.

The results indicate that the effect of photoperiod on growth, particularly increment in weight, is partially mediated through increase in the appetite of the fish. This is shown by the direct relationship of food consumption and photoperiod and by the relationship of food consumption and increment in weight.

Effect of Photoperiod on Efficiency of Food Conversion

The efficiency of food conversion was calculated on a per-tank basis and is expressed as the percentage of food which was utilized by the group of fish for growth. The higher the value the greater is the efficiency of conversion. The average efficiency of food conversion for the three tanks of fish under each photoperiod of each experiment is given in Table 9. The values calculated for each tank of fish are given in Appendix C. The efficiency of food conversion among experiments are not comparable because of the different types of food fed. The efficiency of the first and fourth experiments appear very great because of the feeding of dried commercial pellets. The values in the second and third experiments are lower and more reasonable since the liver fed in these experiments was weighed on a wet weight basis.

Table 9. Average efficiency of food conversion (percent) for each photoperiod of each experiment over the entire experiment, and the rank of the values in order of decreasing efficiency for each experiment.

Expt.	8 hr percent	Rank	8-16 hr. percent	Rank	16 hr. percent	Rank	16-8 hr. percent	Rank
1	57.05	III	59.25	II	68.72	I	41.03	IV
2	26.60	II	28.59	I	23.94	III	17.11	IV
3	7.44	IV	23.48	I	20.97	III	21.63	II
4	70.73	III	82.38	I	74.16	II	62.27	IV

In general, it appears that efficiency of food conversion is directly related to photoperiod, fish under longer photoperiods having greater efficiency. This is shown in the greater efficiency of food conversion attained by fish in the 16-hour photoperiod compared with fish in the 8-hour photoperiod in three of the four experiments (Table 9). Except for the increasing photoperiod of the third experiment and decreasing photoperiod of the second experiment, greater efficiency is attained by fish in the three-week period with the longer photoperiod in both the increasing and decreasing photoperiods (Table 10). Differences in efficiency of food conversion between the two 3-week periods of varying photoperiod were not statistically significant. A two-way analysis of variance, performed in conjunction with the effect of thyroid, revealed that differences in efficiency of food conversion between photoperiods of each of the first three experiments were significantly different only in the third experiment. In this experiment, the significant difference was due to the unexplainable low value obtained for the 8-hour photoperiod group. A one-way analysis of variance of the data of the fourth experiment also revealed no significant difference. Although statistically there are no differences in efficiency of food conversion, the consistant response obtained in the separate experiments certainly suggest that real differences between photoperiod groups may exist.

Table 10. Efficiency of food conversion (percent) for the first and second three-week periods of each photoperiod of each experiment

Expt.	3-wk. period	8 hr. percent	8-16 hr. percent	16 hr. percent	16-8 hr. percent
1	1	47.07	40.60	74.03	53.47
	2	68.71	74.17	65.17	27.71
2	1	24.13	19.63	30.68	15.95
	2	29.44	35.95	13.06	18.72
3	1	19.29	25.60	32.27	32.58
	2	--*	21.58	--*	7.69
4	1	68.46	76.25	76.54	78.40
	2	73.65	88.63	71.51	25.49

*Loss of weight occurred, no calculation of food conversion possible.

The data indicate that growth is directly related to efficiency of food conversion. This is shown by a comparison of the average efficiency of food conversion for the six week period (Table 9) with growth in weight for the same period (Figure 1). The same relationship is shown in similar comparisons of the two 3-week periods of the varying photoperiods. Comparable results are not as apparent for comparisons with total length. However, a correlation analysis of the efficiency of food conversion with gain in total length shows that a close correlation exists. The results of correlation analyses are given in Table 11. The correlation coefficient was significantly different from zero in all experiments for both length and weight. The close correlation of efficiency of food conversion with total length indicates that growth in total length is primarily influenced by this factor.

Table 11. Coefficients of correlation between efficiency of food conversion and average increment in weight and total length per tank of fish in each six-week experiment.

Expt.	Correlation Coeff. Ave. Weight Increment	Correlation Coeff. Ave. Total Length Increment
1	.904***	.888***
2	.944***	.821***
3	.860***	.899***
4	.674*	.668*
	N = 12	

***Denotes significance at the 0.5% level.

*Denotes significance at the 5% level.

It appears that the effects of photoperiod are mediated through an increase in efficiency of food conversion as well as an increase in the appetite of the fish. This is not supported by statistical evidence, but the relationships between length of photoperiod, increased growth in length and weight and increased efficiency of food conversion certainly suggest that the effect of photoperiod on growth of fish is also initiated through increased efficiency of food conversion. Evidence that photoperiod may influence the respiratory metabolism is given by Evans et al. (1962), in which studies of oxygen consumption of tissue of rainbow trout showed a 16% higher metabolic rate in fish acclimated to an 8-hour photoperiod than fish acclimated to a 16-hour photoperiod at 16° C. These differences were not reflected in trout acclimated to the same photoperiods at 8° C. indicating that the effect is temperature dependent.

A comparison of the rank of each photoperiod in each experiment for all four experiments suggests that varying photoperiod may have a greater effect on efficiency of food conversion than a constant photoperiod (Table 9). In three of the four experiments, data from the increasing photoperiod groups showed the greatest efficiency of food conversion. Conversely, the decreasing photoperiod groups had the poorest efficiency. This situation is also reflected in a comparison of the efficiency of food conversion for the first and second 3-week periods of each experiment (Table 10). The increasing photoperiod group had an increased efficiency the second 3-week period compared with the first 3-week period in three of the four experiments. It also had a greater efficiency of conversion than any other photoperiod the second three-weeks. Under decreasing photoperiod, there was a general decrease in efficiency of the group the second 3-week period. In the first and fourth experiments, which are perhaps most reliable, the efficiency of food conversion was poorest during the second three-weeks in the decreasing photoperiod groups. These results are in keeping with the previous suggestion that varying photoperiod may have a greater effect upon growth than a constant photoperiod. Further studies with better controlled conditions are necessary to resolve this issue.

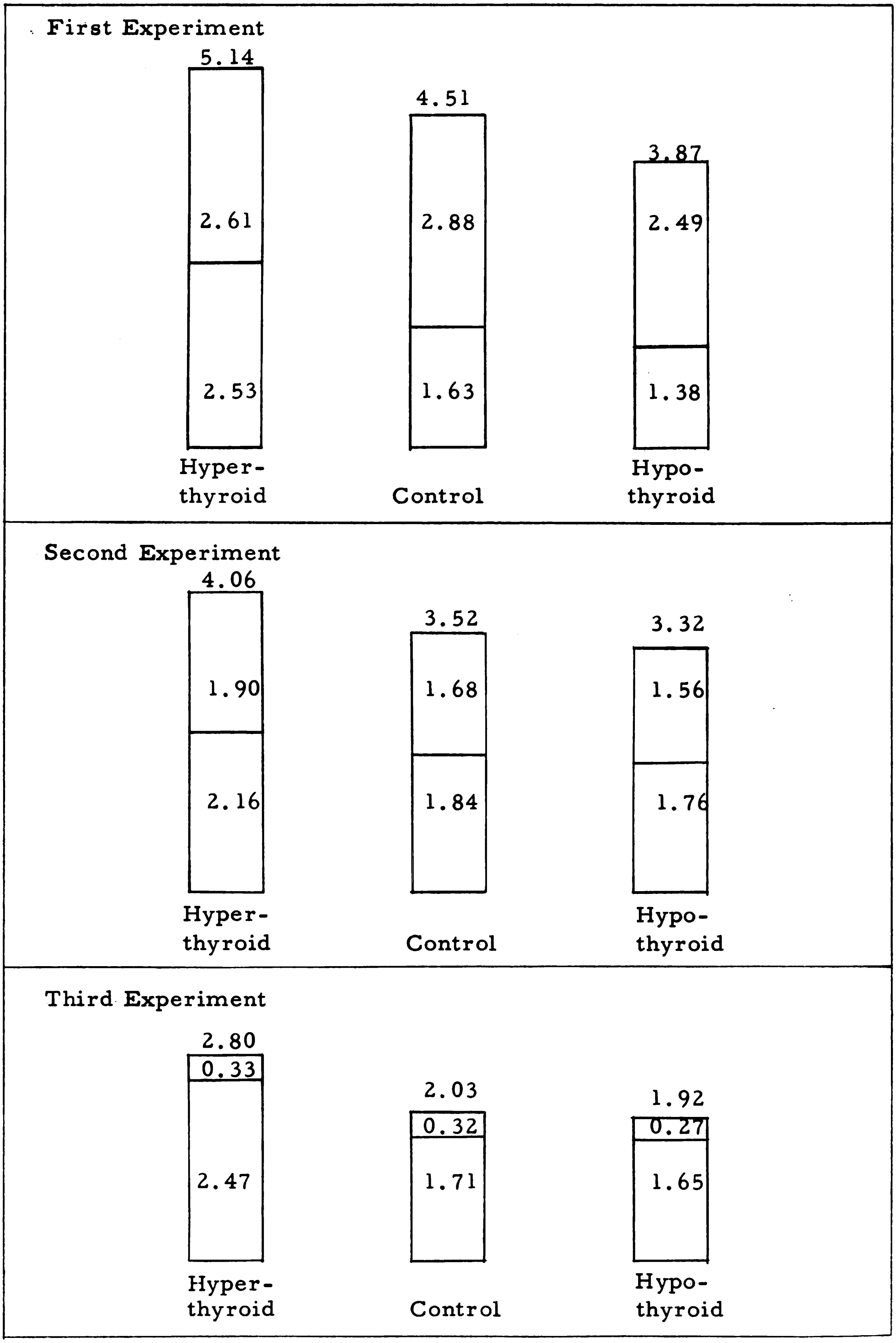
Effect of Thyroid Activity

The effect of thyroid gland upon growth in length and weight, food consumption, and efficiency of food conversion was evaluated in the first three experiments. All values are an average of the respective "thyroid" groups under each of the four photoperiods. The results are therefore based upon a larger sample than the results for photoperiod.

Results of Increment in Weight

The average increase in weight of a fish under each thyroidal condition is presented in Figure 5. It is assumed that factors which resulted in inhibition of growth (second and third experiments) affected each thyroidal condition equally and did not give rise to differences in growth between groups. The results of the effect of thyroid activity on increment in weight of a fish were consistent throughout the three experiments. The greatest increase in weight occurred in the hyperthyroid group and the smallest in the hypothyroid group; the control group had an intermediate growth. Differences in increment in weight of fish in each thyroidal group were tested by a two-way analysis of variance in conjunction with the effect of photoperiod. There was no significant difference in gain in weight between thyroidal groups in any of the three experiments. The relationship of increase in weight with increasing thyroid activity persisted in all periods except the second 3-week period of the first experiment. The increase in weight of the hyperthyroid group was 14.0%, 15.3%, and 37.9% greater than that for the controls in each experiment. The increment in weight of the hypothyroid group was 14.0%, 5.7%, and 5.4% less than that of the controls.

Figure 5. Average increment in weight (grams) of a fish under each thyroidal condition of the three experiments. The lower portion of the bar and the value enclosed represents the gain in weight the first three-week period; the upper portion and the value enclosed represents the gain in weight the second three-week period. Values given at the top of each bar represents the gain in weight over a six-week period.


First Experiment
5.14
2.61
2.53
Hyper-
thyroid
4.51
2.88
1.63
Control
3.87
2.49
1.38
Hypo-
thyroid
Second Experiment
4.06
1.90
2.16
Hyper-
thyroid
3.52
1.68
1.84
Control
3.32
1.56
1.76
Hypo-
thyroid
Third Experiment
2.80
0.33
2.47
Hyper-
thyroid
2.03
0.32
1.71
Control
1.92
0.27
1.65
Hypo-
thyroid

Results of Increment in Total Length

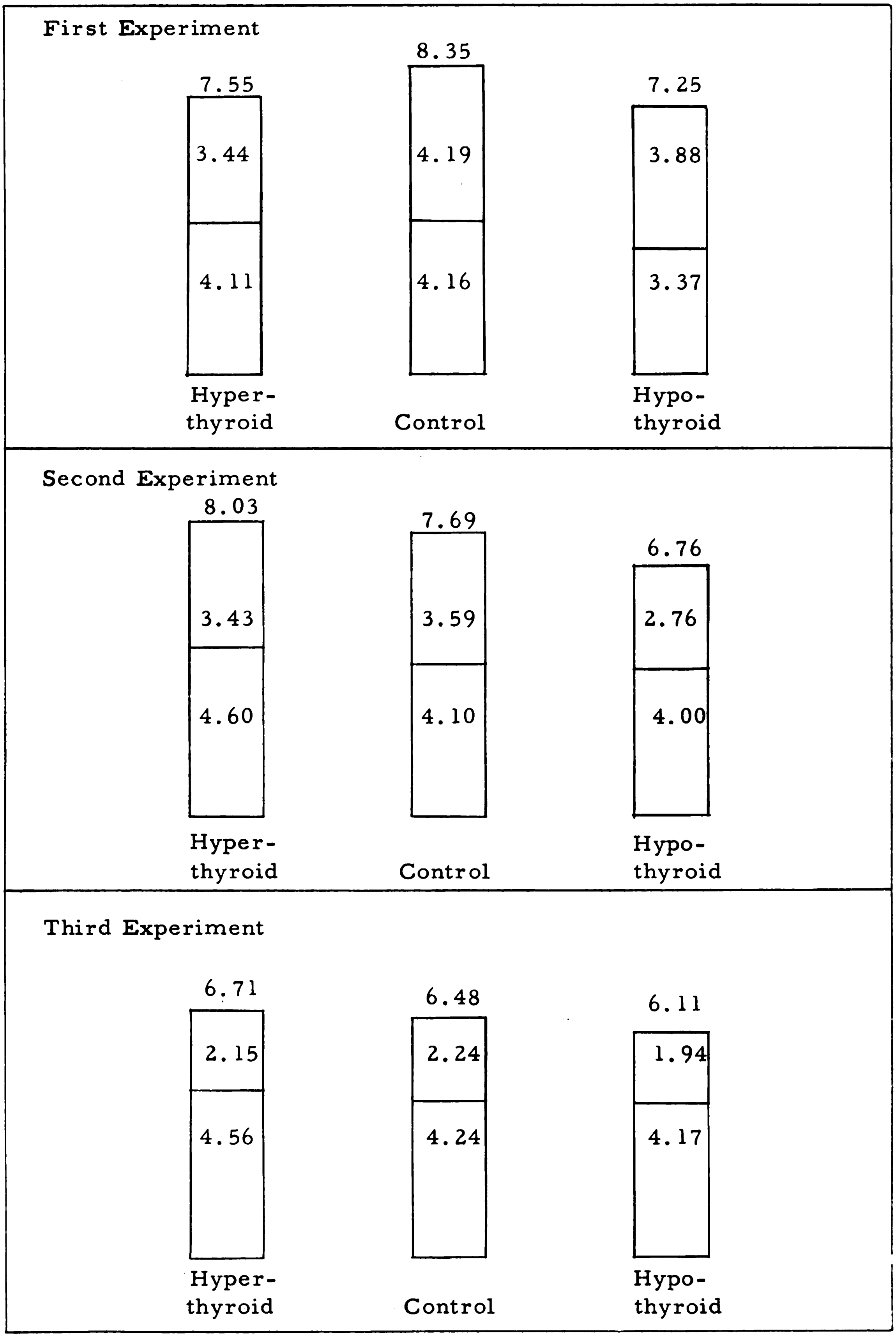
The average increment in total length of a fish under each thyroidal condition of the three experiments is given in Figure 6. In the second and third experiments, increase in length was directly related to thyroid activity; the greatest increase occurring in the hyperthyroid group, least in the hypothyroid group, with the controls intermediate. In the first experiment, the control group attained the greatest increment in length, but the hypothyroid group still showed the smallest growth in terms of body length. A two-way analysis of variance performed in conjunction with the effect of photoperiod showed no significant difference in increment in length between the three thyroidal groups in any of the three experiments. The increase in total length of the hyperthyroid group compared with the control group was 9.5% less in the first experiment, 4.4% greater in the second experiment and 3.6% greater in the third experiment. The increase in total length of the hypothyroid group was uniformly less (13.1%, 12.1%, and 5.7% respectively) than that of the controls in the three experiments. The data suggest that growth in terms of increase in body length may be directly related to thyroid activity. However, this relationship is not as evident as for weight.

Effect of Thyroid Activity on Growth in Length and Weight

Much work has been done on the effect of the thyroid gland on growth and differentiation in fish using different methods and contraditory results have been obtained.

With the use of antithyroid drugs, Dales and Hoar (1954) retarded the growth of chum salmon fry and Scott (1953) obtained similar results with zebra fish. Fortune (1955) reared *Phoxinus laevis* from the egg in 0.5% thiourea and found no effect upon growth. Effects of antithyroid drugs on physiological processes other than growth are reported by Pickford and Atz (1957).

Figure 6. Average increment in total length (millimeters) of a fish under each thyroidal condition of the three experiments. The lower portion of the bar and the enclosed value represents the gain in length the first three-week period; the upper portion and the enclosed value represents the gain in length the second three-week period. Values given at the top of the bar represent the gain in length over the six-week period.


First Experiment
7.55
3.44
4.11
Hyper-thyroid
8.35
4.19
4.16
Control
7.25
3.88
3.37
Hypo-thyroid
Second Experiment
8.03
3.43
4.60
Hyper-thyroid
7.69
3.59
4.10
Control
6.76
2.76
4.00
Hypo-thyroid
Third Experiment
6.71
2.15
4.56
Hyper-thyroid
6.48
2.24
4.24
Control
6.11
1.94
4.17
Hypo-thyroid

Other investigators have studied the effect of the thyroid using artificial thyroxine. Dales and Hoar (1954) found a retardation of growth of chum salmon fingerlings given thyroxine. Smith and Everett (1943) found no difference in growth of normal and thyroxine-treated immature guppies (Lebistes). Barrington et al. (1961), obtained an increased growth in length and weight of yearling rainbow trout immersed in a thyroxine solution.

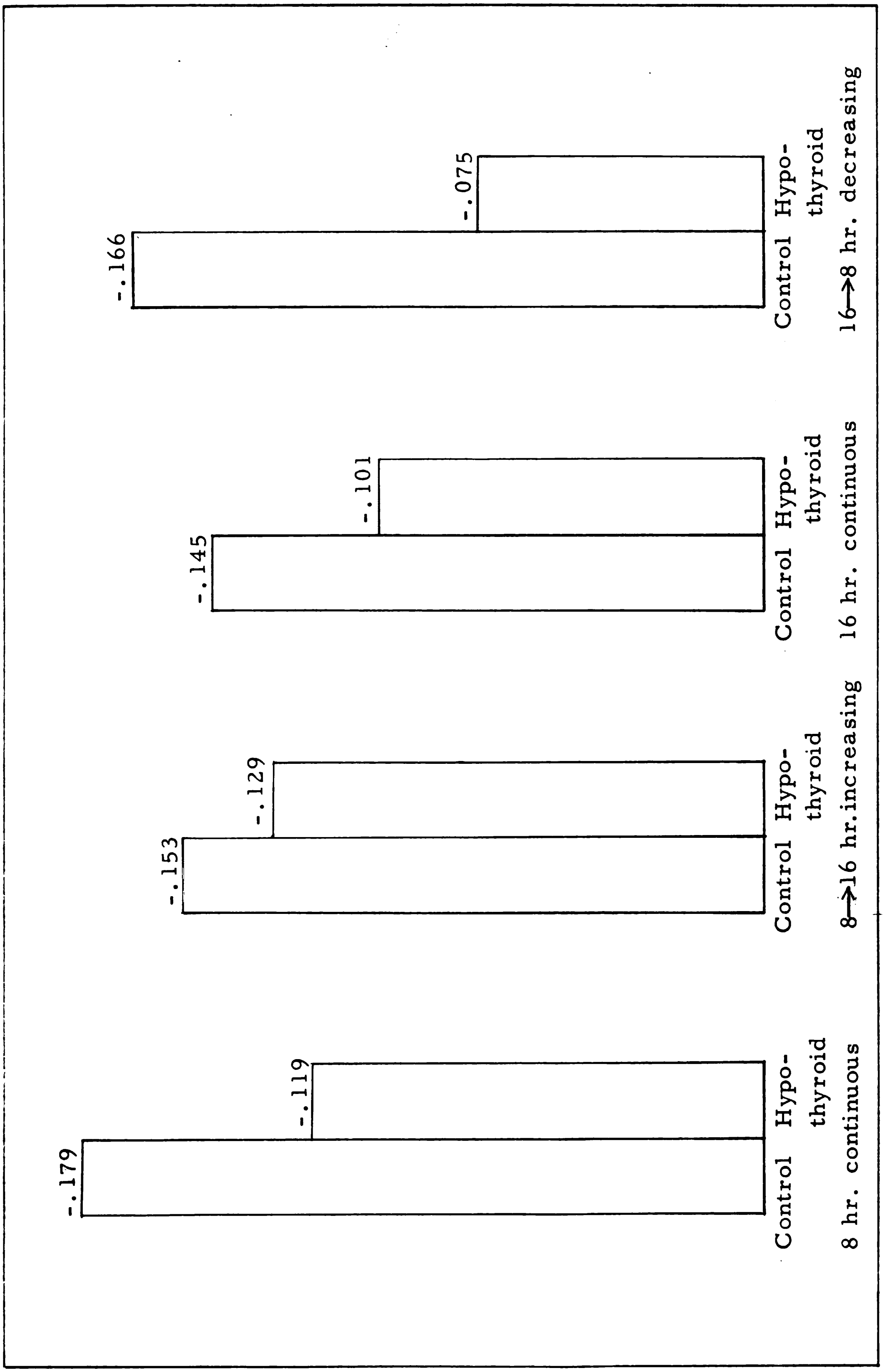
Treatment with mammalian thyroid powder has also produced conflicting results. Grobstein and Bellany (1939) reported an inhibition of growth. Smith and Everett (1943) found no effect upon growth. Hooper (1961) found no effect upon growth of immature guppies from feeding mammalian thyroid powder. Hooper (1952) obtained an increased growth in guppies immersed in water to which thyroid powder had been added. Barrington et al. (1961), obtained a marked stimulating effect upon growth in length and weight from feeding of thyroid powder.

The results of this study using thyroxine are generally contradictory to those found in the literature. This discrepancy may be due to the method employed. In the studies cited above, using artificial thyroxine, the fish were immersed in a solution of water and thyroxine. Although Pickford and Atz (1957) state that it makes little difference if thyroid hormone is injected, administered orally or added to aquarium water, such a difference may actually exist. Positive results were obtained in this study by injection. Bjorklund (1958) also obtained a transitory increase in length and weight of goldfish injected with thyroxine and triiodothyronine in two independent experiments.

Only one study in the literature relates to the effect of radiothyroidectomy on growth of fish. LaRoche and Leblond (1953) found no difference in growth (in weight) of thyroidectomized fish and controls after 10 months. However, an analysis of his data shows that after 5 months, slight differences did exist between the controls and radiothyroidectomized fish. The mean body weight for two groups of controls was 40 grams, and the mean body weight for two groups of thyroidectomized fish was 36 grams. This difference amounts to a 10% reduction in growth of the radiothyroidectomized fish. At the end of the experiment, one lot of radiothyroidectomized fish still had 10% lesser growth than the controls. There was no difference in the second radiothyroidectomized lot and controls. Although thyroidectomy was persistent to the end of the experiment, the difference in the two lots of radiothyroidectomized fish may be explained by the diet fed the fish. The group of fish which had been radiothyroidectomized and which had attained the same growth as the controls were fed a diet containing iodine; whereas the group of thyroidectomized fish which had a lesser growth than the controls were on an iodine-free diet. Pickford and Atz (1957) state that total removal or destruction of the thyroid gland does not wholly abolish the synthesis of thyroid hormone. It appears that a small amount of thyroxine can be synthesized in the total absence of thyroid tissue, although the site of this extra thyroidal function has not been identified. In view of the diet fed the fish, one might conclude that radiothyroidectomy did retard growth as was found in the present study.

Since a histological analysis of the lower jaw of radiothyroidectomized fish in the second experiment revealed the presence of thyroid follicles, a measurement of thyroid activity was made of the control and hypothyroid fish in this experiment in conjunction with a study of the effects of photoperiod on thyroid activity. The rate of loss of radioiodine from the head region was used as an index of thyroid activity. The fish were marked so that measurements could be made of each fish. The average thyroid activity of each group under each photoperiod is presented in Figure 7. Values for the individual fish are given in Table 15. The radiothyroidectomized group had a lower thyroid activity than the control group under each photoperiod. The differences in thyroid activity between the two groups are 33% less in the 8-hour photoperiod, 30% less

Figure 7. Average index of thyroid activity of the control and hypothyroid groups under each photoperiod of the second experiment. Values given at the top of each bar are the average regression coefficients obtained for each group.


-.179
-.119
Control
Hypo-
thyroid
8 hr. continuous
-.153
-.129
Control
Hypo-
thyroid
8→16 hr.increasing
-.145
-.101
Control
Hypo-
thyroid
16 hr. continuous
-.166
-.075
Control
Hypo-
thyroid
16→8 hr. decreasing

in the 16-hour photoperiod, 15% less in the increasing photoperiod, and 54% less in the decreasing photoperiod. A two-way analysis of variance performed in conjunction with photoperiod showed a significant difference at the 1% level ($F = 11.08 > F_{.99}$ $(1, 55) = 7.08$). Although complete thyroidectomy was not attained in the second and third experiments, it is assumed that a hypothyroid condition did exist in all experiments. Had the investigator been able to accomplish complete thyroidectomy, it is felt that greater retardation of growth may have been attained. One factor which is unexplained is the fact that the increased dose of 200 microcuries failed to have a greater inhibitory effect upon growth than in the first and second experiments when less radioiodine was used.

The fish which received injections of thyroxine did not have a "true" hyperthyroid condition. Sections of the lower jaw of these fish revealed the presence of thyroid follicles. If control of the thyroid occurs through the thyroid-stimulating hormone - thyroxine (TSH-TH) balance in fish as in mammals, as postulated by Chavin (1956), one would expect the epithelial cells to atrophy or become very squamous, which did not appear to be true in this study. Nevertheless, a hyperthyroid condition was evident in this study. The dose of exogenous thyroxine was based on the thyroid secretion rate for trout and was corrected for size of the fish and temperature. It may be that a sufficient period of time was not allowed for the degeneration of the thyroidal follicular cells. It is possible that if a hyperthyroid condition had been achieved to a greater degree, even greater growth may have been attained.

The mechanism by which the thyroid may affect growth in fish is unknown. A study by Pickford reported in Pickford and Atz (1957) points to a synergistic action with growth hormone such as occurs in mammals. She refers to an experiment in which hypophsectomized *Fundulus* were given injections of hake growth hormone, mammalian TSH, and a combination of the two hormones over a five week period. Growth in length and weight was stimulated by the hake growth hormone but not by TSH. Greater growth was attained by the group given a combination of the two hormones. The percent increase for growth hormone alone was 21.5% for weight and 5.24% for length compared to 35.8% for weight and 7.18% for length in the group receiving a combination of the two hormones. The difference in the percent increase in length between the two groups was statistically significant. A synergistic action between these two hormones may exist, or as Hoar (1957) postulates, thyroxine may stimulate the release of endogenous growth hormone in some manner.

The Effect of Thyroid Activity on Food Consumption

The average rate of food consumption for a tank of fish under each thyroidal condition is given in Table 12. The results are not comparable between experiments because of the use of different diets.

Table 12. The average rate of food consumption (percent body weight) of a tank of fish under each thyroidal condition of each six-week experiment.

Expt.	Hyperthyroid percent	Control percent	Hypothyroid percent
1	50.02	44.07	44.14
2	84.79	74.07	75.87
3	97.01	88.94	78.14

The rate of food consumption was greatest for the hyperthyroid group in all three experiments. However, there was very little difference in the rate of food consumption between the control and hypothyroid condition, except for the third experiment. A two-way analysis of variance (performed for each experiment in conjunction with the effect of photoperiod) showed no significant difference between food consumption in the three thyroidal conditions. Several investigations (reviewed by Pickford and Atz, 1957) showed increased locomotor activity after treatment with thyroxine. The increase in the rate of food consumption may be due to an increase in appetite as a result of increased activity. Thyroid activity *per se* does not appear to influence food consumption since decreases in food consumption did not always occur in the hypothyroid condition.

No data on the effect of thyroid activity on the rate of food consumption of fish could be found in the literature. Most studies have involved the effects of thyroid activity on fat, protein, and carbohydrate metabolism. Hoersch *et al.* (1961), in studies of the thyroid secretion rate in sheep, found no correlation between the thyroid secretion rate and food consumption. The present study indicates that the effect of the thyroid in promoting growth of fish is not mediated through an increase in appetite (food consumption).

Effect of Thyroid Activity on Efficiency of Food Conversion

The efficiency of food conversion (percent) for a tank of fish under each thyroidal condition is given in Table 13. The results are not comparable between experiments for reasons stated previously. The hyperthyroid group showed the greatest efficiency of food conversion in all three experiments and the hypothyroid the poorest efficiency. A two-way analysis of variance (performed in conjunction with the effects of photoperiod) showed no significant differences between the thyroidal groups in any of the three experiments. Although the differences are not great, it appears that the effect of the thyroid is mediated through increased efficiency in food conversion. The greater efficiency of the

Table 13. Average efficiency of food conversion for a tank of fish under each thyroidal condition of each six-week experiment.

Expt.	Hyperthyroid percent	Control percent	Hypothyroid percent
1	60.78	60.49	51.31
2	25.12	24.73	23.12
3	21.97	17.83	17.14

hyperthyroid group further substantiates the theory that the increased food consumption of this group is due to the increased activity and that there is no effect of the thyroid on appetite (food consumption) of fish.

Only one reference was found which related the effect of thyroid activity on efficiency of food conversion. Bjorklund (1958) calculated a coefficient of growth ($\frac{\text{gain in weight}}{\text{food consumed}} \times 100$) which is the same as the efficiency of food conversion determined in this study. Bjorklund (op. cit.) found an increased coefficient of growth in fish treated with thyroxine and in fish treated with triiodothyronine (40.8% and 42.3% respectively) compared with controls (31.7%) over a 20-day period. Over a 70-day period, these differences in the coefficient of growth were not apparent. In a second experiment, a similar increased efficiency was found for about 20 days following injection of thyroxine and triiodothyronine (44.3% and 44.2%, respectively) compared with saline injected controls (30.2%). The transitory nature of the efficiency of growth can probably be explained by the feed-back mechanism between thyroxine and thyroid-stimulating-hormone which is believed to control thyroid activity.
A positive correlation between efficiency of food conversion and thyroid secretion rate has been found in sheep (Hoersch et al., 1961).

Whether the thyroid exerts a calorigenic effect in poikilotherms is still a matter of conjecture. The results of this study on the effect of thyroid activity on the efficiency of food conversion certainly indicate that the thyroid gland does have some metabolic effect in fish. How this effect occurs awaits further study on the manner in which thyroxine and its analogs enter the biochemical chain.

Effect of Photoperiod on Thyroid Activity

The investigator was interested in determining if there was an effect of photoperiod on thyroid activity. It is possible that the effect of photoperiod on growth may be mediated through the thyroid gland. It was not possible to test for an interaction between photoperiod and thyroid activity in this study because the growth of individual fish was not known. Only a mean measurement was obtained for a tank of fish and no estimate of the variance within a group was possible, thus preventing a measurement of interaction. It was therefore decided to estimate the thyroid activity of the fish in the second experiment using radioiodine. The use of the rate of loss from the head region of fish has been reported by other investigators (Swift, 1955, 1959; Fromm and Reineke, 1956; Hoffert and Fromm, 1959).

Thyroid activity was measured for 7 fish, 4 acclimated to an 8-hour photoperiod and 3 to a 16-hour photoperiod, prior to the start of the second experiment. At the end of the six-week experiment, measurements of thyroid activity were made using 73 fish (36 control and 37 hypothyroid).

Measurements of the radioactivity in the fish were not made until the ninth day after injection of the tracer dose in the group of 7 fish and not until the tenth day after injection in the 73-fish group. This lapse of time between injection and measurements of activity was provided to allow for the loss of extra-thyroidal iodine taken up by the fish. Data presented by Hoffert and Fromm (1959) indicate that the loss of radioiodine from the head region of trout shows an initial rapid loss followed by a less rapid loss. The initial rapid loss is believed to involve chiefly extra-thyroidal iodine and the less rapid loss represents a true rate of release of radioiodine from the thyroidal tissue. Fromm and Reineke (1959) suggest that to obtain true iodine output rates, only data collected subsequent to the eighth day after injection should be used.

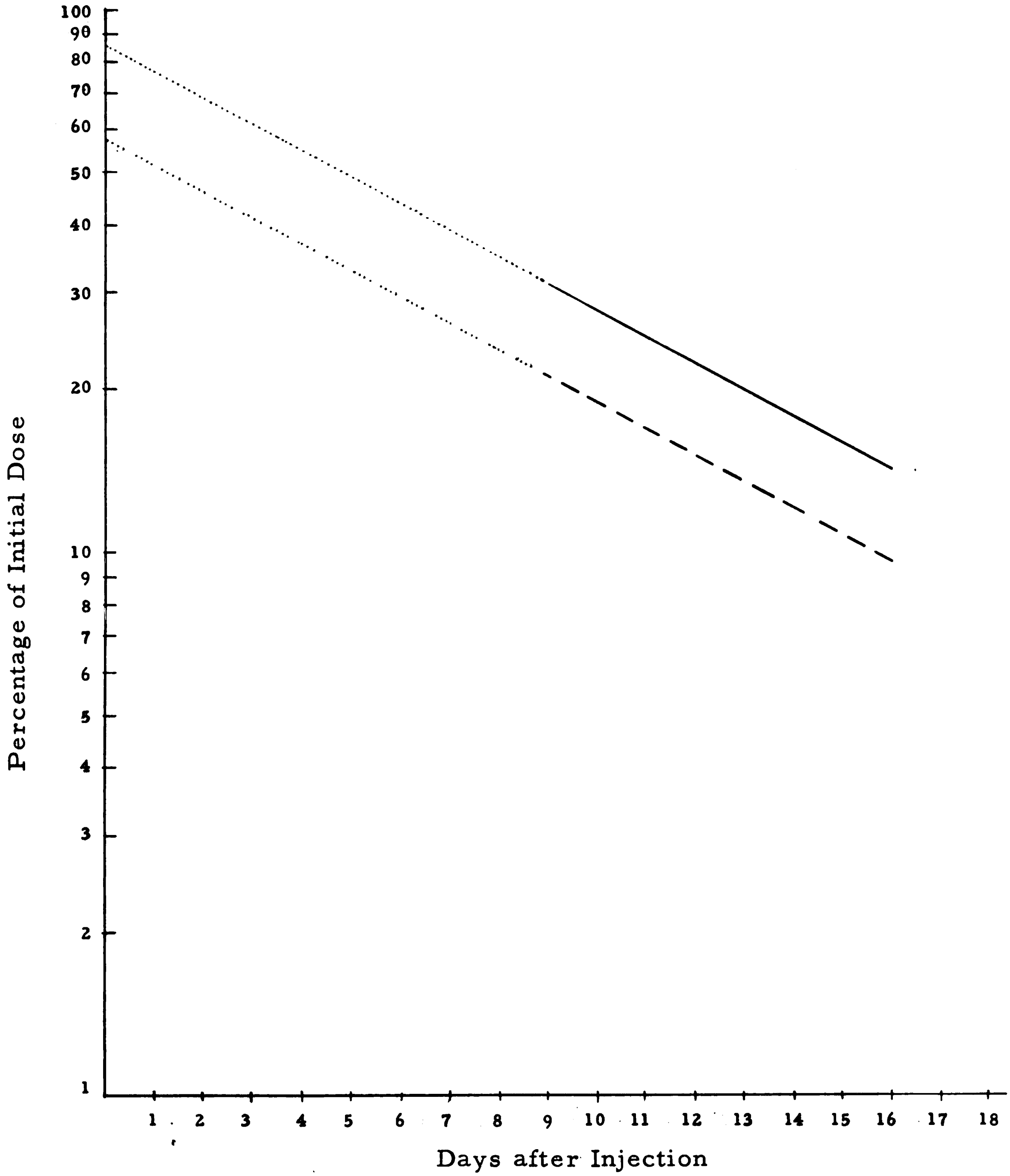
Studies of thyroid activity in fish prior to the beginning of the second experiment showed no difference between fish acclimated for two weeks to a 8-hour photoperiod and those acclimated to a 16-hour photoperiod. A semilog plot of the loss of radioiodine for the two groups is given in Figure 8. Extrapolation of the output curve to zero time indicates that a slightly greater uptake occurred in the 8-hour photoperiod than in the 16-hour photoperiod.

The regression coefficient (index of thyroid activity) and output halftime for each fish and the mean for each group is presented in Table 14.

Table 14. Regression coefficient and output halftime of each fish and the mean value for each photoperiod.

Fish		Regression Coeff.		Output Halftime (days)
8-Hour Photoperiod				
4832		-.1389		4.99
4833		-.0920		7.54
4842		-.1662		5.96
4843		-.1064		6.52
	Mean	-.1134	Mean	6.14
16-Hour Photoperiod				
4836		-.0772		8.98
4838		-.1892		3.66
4839		-.0711		9.75
	Mean	-.1125	Mean	6.16

Figure 8. Semilog plot of the output of radioiodine from the head region of green sunfish maintained under constant 8-hour photoperiod (solid line) and constant 16-hour photoperiod (broken line) for two weeks. Counting was started the ninth day after injection. Dotted line represents extrapolation back to zero time.



100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
Percentage of Initial Dose
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Days after Injection

The output halftime refers to the time required for the removal of one-half of the radioiodine (corrected for decay) originally present in the thyroidal area. The results of one fish under the 16-hour photoperiod (4838) appears to be inconsistent with the other two fish. Except for this fish, it would appear that the thyroid activity is lower for fish maintained on a 16-hour photoperiod.

The regression coefficients for the individual fish at the end of the second experiment are given in Table 15. The average regression coefficient of fish under each photoperiod and the two thyroid conditions are given in Figure 9. The results of the control groups indicate that photoperiod may have an effect upon thyroid activity. The fish under constant 8-hour photoperiod had the highest average index of thyroid activity and the constant 16-hour photoperiod the lowest. The values for the two varying photoperiod groups were intermediate. The increasing photoperiod group, which had been maintained at a 16-hour day-length since the end of the experiment, had an average index of thyroid activity lower than that of the decreasing photoperiod group and approached the value obtained for the 16-hour photoperiod. The decreasing photoperiod group, which had been maintained at a 8-hour day-length since the end of the experiment, had an average index of thyroid activity which approached that of the 8-hour photoperiod group. Extrapolation of the output curves back to zero time indicated a greater theoretical uptake in the 8-hour photoperiod group than in the 16-hour photoperiod group (129% and 105%, respectively).

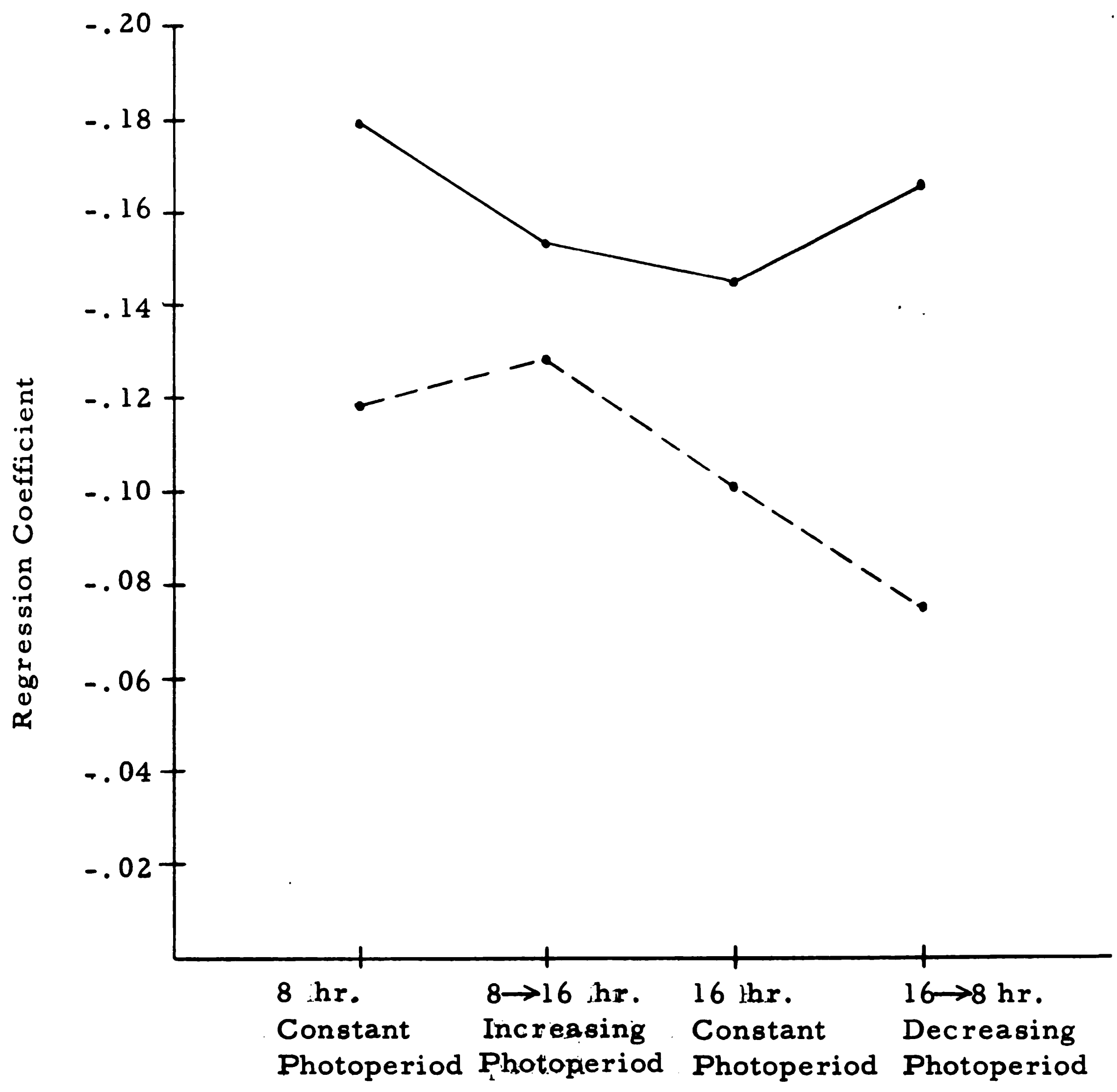
Interpretation of the data for the hypothyroid group regarding the effect of photoperiod on thyroid activity is questionable because of differences in the degree of thyroidectomy that may have been attained in the various fish.

A one-way analysis of variance on the data for the control fish showed there was no significant difference in thyroid activity of the fish under the various photoperiods.

Table 15. Regression coefficients (b) for each fish of the control and hypothyroid groups under each photoperiod in the second experiment.

Continuous 8 hr. Photo. Fish	b	Increasing 8-16 hr. Photo. Fish	b	Continuous 16 hr. Photo. Fish	b	Decreasing 16-8 hr. Photo Fish	b
Control Fish							
4828	-.204	4914	-.118	4944	-.089	4934	-.169
4829	-.132	4915	-.146	4945	-.152	4935	-.163
4830	-.159	4916	-.218	4946	-.148	4936	-.140
4831	-.141	4917	-.225	4947	-.231	4937	-.141
4832	-.202	4918	-.108	4948	-.175	4938	-.151
4833	-.198	4919	-.106	4949	-.150	4939	-.116
4834	-.174	4920	-.124	4950	-.148	4940	-.131
4838	-.238	4921	-.103	4951	-.119	4941	-.254
		4922	-.150	4952	-.093	4942	-.243
		4923	-.172			4943	-.148
Mean	-.1786	Mean	-.1525	Mean	-.1450	Mean	-.1656
Hypothyroid Fish							
4839	-.135	4905	-.169	4953	-.097	4924	-.056
4840	-.124	4907	+.010	4954	-.075	4925	-.157
4841	-.098	4908	-.162	4955	-.107	4926	-.084
4842	-.154	4909	-.130	4956	-.081	4927	-.039
4901	-.008	4910	-.192	4957	-.183	4928	-.072
4902	-.144	4911	-.073	4958	-.035	4929	-.113
4903	-.086	4912	-.166	4959	-.085	4930	-.199
4904	-.145	4913	-.150	4960	-.091	4931	-.008
4906	-.181			4961	-.115	4932	+.051
				4962	-.138	4933	-.076
Mean	-.1195	Mean	-.1294	Mean	-.1009	Mean	-.0753

Figure 9. Graph of the average regression coefficients of the control fish (solid line) and hypothyroid fish (broken line) under each photoperiod of the second experiment.



Regression Coefficient
-.20
-.18
-.16
-.14
-.12
-.10
-.08
-.06
-.04
-.02
8 hr. Constant Photoperiod
8→16 hr. Increasing Photoperiod
16 hr. Constant Photoperiod
16→8 hr. Decreasing Photoperiod

Results of studies of the effect of light on thyroid activity found in the literature are inconsistent although most of them indicate light may have an effect. Only one study has been directly concerned with the effect of photoperiod on thyroid activity. Grant et al. (1961) studied the effect of light on thyroidal uptake of injected radioiodine by newts. Groups of experimental animals were kept in continuous darkness, a 12-hour day, and constant illumination. Those kept under constant illumination had a very low uptake (0.8-2.8%) whereas those kept on a 12-hour diurnal condition and in continuous darkness showed initial high uptakes (6-22%). The latter two groups exhibited a leveling trend after a week and after four weeks they approximated the levels of animals kept under continuous illumination. Using histological criteria, Rasquin (1949) and Rasquin and Rosenbloom (1954) demonstrated that a hypertrophy of the thyroid occurred in Astanax mexicanus kept in darkness but that normal thyroid follicles were restored by exposure of the fish to light. Baggerman (1960), in studies of the migration of four species of Pacific salmon, felt that photoperiod was the external stimulus affecting the thyroid-pituitary system which in turn effected the induction of the migration disposition. Hoar (1959) stated that both thyroid activity and photoperiod affect temperature resistance in goldfish although it is not known whether there is an interaction between these two factors. Work of Robertson (1958) as quoted from Hoar (1959) indicates that there may be an interaction. She found that fish maintained on a short-day basis (8 hours of light) had a greater uptake of radioiodine than fish maintained on a long-day basis (16 hours of light). Since high uptake of radioiodine is associated with greater thyroid activity, her findings are in agreement with those presented here for green sunfish. Berg et al. (1959) felt that the seasonal pattern of thyroxinogenesis found in Fundulus may have been influenced by photoperiod although this was not studied separately. Temperature was not a factor since all fish used were acclimated to and maintained at a constant temperature. Swift (1955) found that thyroid activity was more closely correlated with photoperiod than temperature although further studies by him led him to draw other conclusions. To the investigator's knowledge, this study is the first in which an attempt has been made to evaluate the effects of different photoperiods on thyroid activity in fish. Studies have been carried out on the effect of different photoperiods in sheep. Hoersch et al. (1961) found that 4, 8, and 12 hours of light per day suppressed thyroid activity whereas increased light beyond 12 hours stimulated thyroid function. Their data are contrary to the data presented here for fish although measurements were not made using the same technique or at comparable photoperiods.

A comparison of the thyroid activity of the fish held at the two constant photoperiods was made prior to the start and at the end of the second experiment. The mean regression coefficients and output halftimes are given in Table 16. An increase in thyroid activity occurred in

Table 16. Mean regression coefficients (b) and output halftimes ($T\frac{1}{2}$) for control fish of the 8-hour and 16-hour photoperiods at the beginning and the end of the second experiment.

	Beginning of Experiment		End of Experiment	
Photoperiod	b	Output $T\frac{1}{2}$	b	Output $T\frac{1}{2}$
8-hour	-.1125	6.14 days	-.1786	3.88 days
16-hour	-.1134	6.16 days	-.1450	4.78 days

both photoperiod groups during the experiment. A greater increase in activity occurred in the 8-hour photoperiod group. A Student's "t" test was performed to determine if the differences in thyroid activity were significant. The increase in thyroid activity in the 8-hour photoperiod group was significant at the 1% level ($t = 3.48 > t_{.995} = 3.169$, df = 10). The difference in thyroid activity in the 16-hour photoperiod group was not significant. It appears that short photoperiods stimulate thyroid activity and that there is an inverse relationship between photoperiod and thyroid activity. This is contrary to the findings of the effect of photoperiod on growth where greater growth occurs in fish under longer photoperiods. One might infer that the effect of photoperiod on growth is not mediated through the thyroid gland.

The conclusions are tentative because the values obtained at the beginning of the experiment are based on a small sample size and a greater size range was involved in the determinations at the end of the experiment. Further study, involving a larger sample and determinations on individual fish are necessary to resolve this issue. It would be of interest to determine the thyroid activity of individual fish at different intervals during a varying photoperiod.

Comparison of Thyroid Activity of the Green Sunfish with Other Fish

The percent uptake of radioiodine obtained in this study is inconsistent with those reported in the literature for many fresh water teleosts. Berg *et al*. (1959) made a study of I^{131} uptake by eight fresh water teleosts and obtained values varying from 1.6% for *Lepomis gibbosus* to as high as 33.1% for *Umbra pygmaeus*. Some of the variation may have been due to seasonal factors. Of particular interest were the results obtained for *Lepomis gibbosus* (pumpkinseed) which is closely related to the green sunfish. Berg *et al*. (*op. cit.*) found maximum uptakes for this species of 1.6% and 3.5% at 12 and 20 hours, respectively, after injection. Because of the low uptake of radioiodine by this species, the investigators felt that it had one of the least efficient thyroids of fresh water fish.

Gorbman (1959) inferred from these data that thyroxine was produced very slowly in this species if at all.

In the study prior to the beginning of the second experiment, the average percent injected dose remaining in the green sunfish nine days after injection was 31% for the 8-hour photoperiod group and 21% for the 16-hour photoperiod group. Extrapolating the output curve back to 24 hours and ignoring any extra-thyroidal accumulation of iodine, the percent uptake for the 8-hour group and 16-hour group is 76% and 52% respectively.

The study performed at the end of the second experiment was under almost identical conditions as those of Berg <u>et</u> <u>al</u>. (1959). They obtained an uptake of 1.6% for July and 3.5% for August at temperatures of 23° and 24° C. in the pumpkinseed. This green sunfish study was made during early September (Sept. 2-9) at temperatures of 74°-78° F. (23.3°-25.6° C.). Extreme variability (4.6%-47%) occurred in the percent injected dose remaining 10 days after injection; the mean percent injected dose remaining was 25%. Extrapolating the output curve back to 24 hours after injection, the values ranged from 35% to over 100%; the mean percent injected dose was 100%. The results of this indicate that the green sunfish has a very efficient thyroid gland. If one considers the uptake 24 hours after injection, such as when the maximum uptake occurred in the pumpkinseed, the green sunfish has a greater efficiency of I^{131} uptake than any of the fresh water teleosts studied by Berg <u>et</u> <u>al</u>. (<u>op</u>. <u>cit</u>.). Greater values were obtained for hypothyroid fish in this study than those obtained by Berg <u>et</u> <u>al</u>. (<u>op</u>. <u>cit</u>.). The average content of I^{131} for the hypothyroids 10 days after injection was 18% of the injected dose and 33% when extrapolated back to 24 hours after injection. Variations are known to occur between species, but the differences found here between the green sunfish and pumpkinseed which occur together in many waters appears so extreme that some other factor such as experimental methods must be responsible.

The biological halftime of radioiodine obtained for the green sunfish was very short. The average output halftime for the controls under each photoperiod is given in Table 17. Few studies reported in the

Table 17. Average output halftime for control fish under each photoperiod at the end of the second experiment.

Continuous 8 hr. Photoperiod	Increasing 8-16 hr. Photoperiod	Continuous 16 hr. Photoperiod	Decreasing 16-8 hr. Photoperiod
3.88 days	4.54 days	4.78 days	4.18 days

literature have involved *in vivo* determinations of thyroid activity in fish. Fromm and Reineke (1959) studied iodine output rates of rainbow trout in conjunction with the effects of thyroidectomy on oxygen consumption and obtained an output halftime of 15.5 days at approximately 15° C. Hoffert and Fromm (1959) determined the halftime output rate of rainbow trout and obtained a value of 37 days at approximately 15°C. Differences between the results reported in the literature and those reported here for the green sunfish may be due to the habitat preferences of these two species. The green sunfish is a warm-water fish whereas the rainbow trout is typically a cold-water fish. The influence of temperature on the thyroid function of fish is controversial (Leloup and Fontaine, 1960); however it appears reasonable to believe that warm water fish may have a greater thyroid activity than cold water fish. Further study of this matter is warranted.

Summary

Three of the four independent experiments performed involved a determination of the effect of photoperiod and thyroid activity on growth in total length and weight, rate of food consumption and efficiency of food conversion. The fourth experiment involved only the effect of photoperiod on the above measurements. There were four photoperiods (constant 8-hour, constant 16-hour, variable increasing 8 to 16 hours and variable decreasing 16 to 8 hours) and three thyroidal conditions (hyperthyroid, hypothyroid and control) used in this study. Differences between the effects of a varying photoperiod and constant photoperiod were determined. The effect of photoperiod on thyroid activity was determined by measuring the rate of loss of radioiodine from the head region of the fish in the second experiment.

Conclusions

Although, for the most part, statistical evaluation of the data obtained in this series of experiments indicated few significant differences, the repetition of trends in the data warrants the following conclusions:

1. Photoperiod does influence growth of fish held at constant temperature. Growth expressed as gain in body weight or percent gain in weight as well as gain in total length varied directly with the length of the photoperiod. Gain in total length appeared to be less dependent upon photoperiod than did weight gain.

2. The rate of food consumption and efficiency of food conversion were highest in fish held at longer photoperiods suggesting that the effect of photoperiod on growth is mediated through an increase in appetite and efficiency of food conversion.

3. A varying photoperiod had a greater influence upon growth than a constant photoperiod. Growth was enhanced by increasing the photoperiod and depressed by a gradual decrease in photoperiod.

4. Growth of green sunfish appeared to be related to the availability of thyroxine. Hyperthyroid fish exhibited greater growth and hypothyroid fish less growth than normal controls. Differences in growth measured by gain in total length showed a lesser correlation with the three "thyroidal" groups than gain in weight.

5. No differences in food consumption occurred between the hyperthyroid, hypothyroid and control groups.

6. Efficiency of food conversion was found to be greatest in the hyperthyroid fish and least in the hypothyroid fish, although the differences are small.

7. Determinations of the rate of loss of radioiodine from the head region of normal intact fish revealed that photoperiod has an effect upon thyroid activity. Short photoperiods stimulate thyroid activity and longer photoperiods depress thyroid activity.

8. The effect of photoperiod on growth does not appear to be mediated through the thyroid gland. A paradoxical situation existed in which growth was directly related to photoperiod and to thyroid activity based upon the artificially induced thyroid conditions; yet an inverse relationship was found between photoperiod and thyroid activity based upon the output of radioiodine from the head region of normal intact fish.

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APPENDICES

APPENDIX A

Mean measurements and increments in total length and weight of fish in each tank of the first three experiments. Initial total length and weight and increment in total length and weight of each fish of the fourth experiment.

Mean Measurement and Increment in Total Length and Weight of a Fish in Each Tank of the First Experiment

	Mean Measurement						Mean Increment					
	Initial		3rd Week		6th Week		1st 3 Weeks		2nd 3 Weeks		Over 6 Weeks	
	T.L.	Wt.	T.L.	Wt.	T.L.	Wt.	T.L.	Wt.	T.L.	Wt.	T.L.	Wt.
Condition	mm.	gm.	mm.	gm.	mm.	gm.	mm.	gm.	mm	gm.	mm.	gm.
Continuous 8-Hour Photoperiod												
Hyperthyroid	89.78	13.82	94.22	15.90	98.56	19.52	4.44	2.08	4.34	3.62	8.78	5.70
Control	88.67	13.03	92.33	14.16	95.11	16.16	3.66	1.13	2.78	2.00	6.44	3.13
Hypothyroid	91.25	14.48	93.62	14.93	96.25	16.78	2.37	0.45	2.63	1.85	5.00	2.30
Increasing 8-16 Hour Photoperiod												
Hyperthyroid	90.25	13.56	93.50	14.99	97.00	17.79	3.25	1.41	3.50	2.80	6.75	4.21
Control	88.44	13.62	92.44	14.97	97.11	18.61	4.00	1.35	4.67	3.64	8.67	4.99
Hypothyroid	89.63	14.08	93.38	15.20	98.13	18.38	3.75	1.21	4.75	3.18	8.50	4.30
Decreasing 16-8 Hour Photoperiod												
Hyperthyroid	89.30	13.66	93.90	16.55	96.10	17.46	4.60	2.89	2.20	0.91	6.80	3.80
Control	91.11	14.66	95.22	16.29	98.33	17.46	4.11	1.63	3.11	1.17	7.22	2.80
Hypothyroid	89.77	13.43	92.89	14.66	95.11	15.46	3.12	1.23	2.22	0.80	5.34	2.03
Continuous 16-Hour Photoperiod												
Hyperthyroid	90.67	14.17	94.67	17.92	98.56	21.12	4.00	3.75	3.89	3.20	7.89	6.95
Control	88.50	13.35	93.30	15.68	99.30	20.21	4.80	2.33	6.00	4.53	10.80	6.86
Hypothyroid	89.10	13.58	93.20	16.06	98.90	20.03	4.10	2.48	5.70	3.97	9.80	6.45

Mean Measurement and Increment in Total Length and Weight of a Fish in Each Tank of the Second Experiment

	Mean Measurement						Mean Increment					
	Initial		3rd Week		6th Week		1st 3 Weeks		2nd 3 Weeks		Over 6 Weeks	
	T.L.	Wt.	T.L.	Wt.	T.L.	Wt.	T.L.	Wt.	T.L.	Wt.	T.L.	Wt.
Condition	mm.	gm.	mm.	gm.	mm.	gm.	mm.	gm.	mm.	gm.	mm.	gm.
Continuous 8-Hour Photoperiod												
Hyperthyroid	95.50	16.63	102.50	19.95	107.90	22.64	7.00	3.32	5.40	2.69	12.40	6.01
Control	97.50	17.60	102.22	19.44	107.33	21.70	4.72	1.84	5.11	2.26	9.83	4.10
Hypothyroid	92.50	14.52	95.30	15.41	98.70	16.76	2.80	0.89	3.40	1.35	6.20	2.24
Increasing 8-16 Hour Photoperiod												
Hyperthyroid	97.00	17.25	101.00	18.54	104.80	21.84	4.00	1.29	3.80	3.30	7.80	4.59
Control	97.80	17.24	101.70	18.83	107.00	21.96	3.90	1.59	5.30	3.13	9.20	4.72
Hypothyroid	98.38	17.34	102.25	18.88	106.25	21.75	3.87	1.54	4.00	2.87	7.87	4.41
Decreasing 16-8 Hour Photoperiod												
Hyperthyroid	97.30	17.41	99.20	17.95	101.60	18.49	1.90	0.54	2.40	0.54	4.30	1.08
Control	97.10	17.58	101.40	19.28	104.70	20.59	4.30	1.70	3.30	1.31	7.60	3.01
Hypothyroid	94.60	16.01	97.10	17.16	100.20	18.18	2.50	1.15	3.10	1.02	5.60	2.17
Continuous 16 Hour Photoperiod												
Hyperthyroid	95.20	16.84	100.70	20.33	102.80	21.40	5.50	3.49	2.10	1.07	7.60	4.56
Control	97.40	16.78	101.00	18.99	101.80	19.07	3.60	2.21	0.80	0.08	4.40	2.29
Hypothyroid	95.00	15.81	101.80	19.22	102.60	20.47	6.80	3.41	0.80	1.25	7.60	4.66

Mean Measurement and Increment in Total Length and Weight of a Fish in Each Tank of the Third Experiment

	Mean Measurement						Mean Increment					
	Initial		3rd Week		6th Week		1st 3 Weeks		2nd 3 Weeks		Over 6 Weeks	
	T.L.	Wt.	T.L.	Wt.	T.L.	Wt.	T.L.	Wt.	T.L.	Wt.	T.L.	Wt.
Condition	mm.	gm.	mm.	bm.	mm.	gm.	mm.	gm.	mm.	gm.	mm.	gm.
Continuous 8-Hour Photoperiod												
Hyperthyroid	89.40	11.40	92.30	12.72	92.60	12.21	2.90	1.32	0.30	-0.51	3.20	0.81
Control	88.75	11.64	91.63	12.49	92.63	12.10	2.88	0.85	1.00	-0.39	3.88	0.46
Hypothyroid	90.80	12.22	93.40	13.10	94.00	12.76	2.60	0.88	0.60	-0.34	3.20	0.54
Increasing 8-16 Hour Photoperiod												
Hyperthyroid	90.33	11.99	97.33	15.60	101.44	17.30	7.00	3.61	4.11	1.70	11.11	5.31
Control	89.80	12.14	94.20	13.46	97.30	14.75	4.40	1.32	3.10	1.29	7.50	2.61
Hypothyroid	91.20	12.95	94.70	13.82	97.50	14.72	3.50	0.87	2.80	0.90	6.30	1.77
Decreasing 16-8 Hour Photoperiod												
Hyperthyroid	87.70	10.54	91.30	12.51	94.70	12.96	3.60	1.97	3.40	0.45	7.00	2.42
Control	89.10	11.45	93.20	13.35	96.30	13.78	4.10	1.90	3.10	0.43	7.20	2.33
Hypothyroid	90.44	11.90	94.33	13.50	96.44	13.62	3.89	1.60	2.11	0.12	6.00	1.72
Continuous 16-Hour Photoperiod												
Hyperthyroid	89.40	11.52	94.40	14.60	95.40	14.44	5.00	3.08	1.00	-0.16	6.00	2.92
Control	88.33	11.08	93.60	13.70	94.70	13.50	5.30	2.62	1.10	-0.20	6.40	2.42
Hypothyroid	88.00	11.64	93.90	14.51	95.50	14.59	5.90	2.87	1.60	0.08	7.50	2.95

itial Weight and Increment in Weight for Each Fish of the Fourth Experiment

ag	Initial	1st 3 wk	2nd 3 wk	First 6 wk	3rd 3 wk	Initial	1st 3 wk	2nd 3 wk	First 6 wk	3rd 3 wk	Initial	1st 3 wk	2nd 3 wk	First 6 wk	3rd 3 wk
ontinuous 8-Hour Photoperiod															
		Tank 1						Tank 2					Tank 3		
PC	16.4	4.8	5.9	10.7	9.0	13.2	2.0	2.6	4.6	2.8	17.5	1.5	3.8	5.3	3.4
PC	9.1	1.2	0.1	1.3	0.0	12.4	2.4	1.9	4.3	2.8	9.1	4.1	0.1	4.2	1.5
	10.6	0.7	0.0	0.7	0.6	11.6	0.3	2.1	2.4	2.8	14.8	3.6	3.4	7.0	4.5
PV	14.4	5.0	2.2	7.2	4.8	12.0	1.9	2.1	4.0	5.9	14.7	6.2	5.5	11.7	3.3
PV	10.0	----------	died	------------		12.7	4.6	2.3	6.9	2.2	12.4	0.6	0.9	1.5	1.4
Mean		2.93	2.05	4.98	3.60		2.24	2.50	4.74	3.30		3.20	2.74	5.94	2.82
creasing 8-16-Hour Photoperiod															
		Tank 4						Tank 5					Tank 6		
PC	9.5	3.3	2.2	5.5	-1.9	14.0	5.6	9.1	14.7	9.8	10.2	9.4	8.0	17.4	6.2
PC	12.0	3.1	5.3	8.4	1.5	11.4	0.8	0.8	1.6	3.1	9.5	2.5	2.8	5.3	3.7
	11.5	4.5	5.3	9.8	9.0	11.0	2.3	2.5	4.8	2.6	8.7	1.4	0.6	2.0	1.2
PV	9.5	1.3	1.1	2.4	0.8	12.4	4.9	7.7	12.6	7.3	8.9	2.2	1.2	3.4	2.4
PV	12.1	1.7	2.9	4.6	3.3	12.3	4.5	5.0	9.5	2.6	8.7	2.2	2.1	4.3	2.7
Mean		2.78	3.36	6.14	2.54		3.62	5.02	8.64	5.08		3.54	2.94	6.48	3.24
ecreasing 16-8 Hour Photoperiod															
		Tank 7						Tank 8					Tank 9		
PC	12.6	1.1	0.0	1.1	-1.9	13.4	1.9	0.0	1.9	0.1	20.4	9.7	1.4	11.1	0.9
PC	12.1	1.3	0.1	1.4	0.0	18.6	9.5	0.8	10.3	1.1	13.7	9.3	3.4	12.7	0.2
	15.5	2.2	-0.4	1.8	0.0	15.6	10.4	1.1	11.5	2.1	9.1	2.7	0.6	3.3	0.3
PV	17.8	7.5	0.2	7.7	0.3	14.0	0.8	0.1	0.9	0.6	15.2	9.5	2.9	12.4	1.8
PV	18.0	7.3	0.2	7.5	0.0	16.3	7.9	1.1	9.0	0.9	12.8	1.6	0.1	1.7	0.1
Mean		3.88	0.02	3.90	-0.32		6.10	0.62	6.72	0.96		6.56	1.65	8.28	0.66
ontinuous 16-Hour Photoperiod															
		Tank 10						Tank 11					Tank 12		
PC	10.9	2.8	2.0	4.8	0.7	13.3	6.6	5.8	12.4	3.7	11.2	3.7	4.2	7.9	2.6
PC	9.9	1.6	1.3	2.9	0.8	13.1	5.4	3.5	8.9	3.2	10.0	0.5	1.2	1.7	0.9
	11.9	2.4	2.4	4.8	0.7	14.1	5.9	3.0	8.9	2.4	14.0	4.9	3.6	8.5	3.3
PV	9.0	1.4	0.6	2.0	1.2	10.3	3.2	3.3	6.5	2.7	11.7	5.7	4.1	9.8	2.7
PV	14.8	8.2	4.1	12.3	1.5	9.0	0.6	1.9	2.5	0.5	11.4	1.3	2.6	3.9	2.1
Mean		3.28	2.08	5.36	0.98		4.34	3.50	7.84	2.50		3.22	3.14	6.36	2.32

nitial Total Length and Increment in Total Length (Millimeters) for Each Fish of the Fourth Experiment

ag	Initial	1st 3 wk	2nd 3 wk	First 6 wk	3rd 3wk	Initial	1st 3 wk	2nd 3 wk	First 6 wk	3rd 3wk	Initial	1st 3 wk	2nd 3 wk	First 6 wk	3rd 3 wk
ontinuous 8-Hour Photoperiod															
			Tank 1					Tank 2					Tank 3		
PC	97	10	9	19	7	95	4	3	7	4	99	3	6	9	4
PC	85	1	1	2	0	92	7	2	9	4	85	6	1	7	2
	86	4	0	4	1	85	4	4	8	6	95	7	5	12	5
PV	96	10	4	14	6	90	5	3	8	9	96	10	7	17	5
PV	--------died-------------------					95	9	3	12	4	90	3	1	4	3
Mean		6.25	3.50	9.75	3.50		5.80	3.00	8.80	5.40		5.80	4.00	9.80	3.80
ncreasing 8-16 Hour Photoperiod															
			Tank 4					Tank 5					Tank 6		
PC	87	7	4	11	-3	95	9	12	21	10	102	12	9	21	5
PC	91	7	7	14	2	88	1	1	2	6	95	5	4	9	6
	89	10	8	18	10	87	5	4	9	4	87	4	1	5	7
PV	85	3	3	6	1	91	9	11	20	9	89	5	3	8	4
PV	89	4	4	8	4	90	9	8	17	4	87	5	4	9	4
Mean		6.20	5.20	11.40	2.80		6.60	7.20	13.80	6.60		6.20	4.20	10.40	5.20
ecreasing 16-8 Hour Photoperiod															
			Tank 7					Tank 8					Tank 9		
PC	89	3	0	3	0	90	5	1	6	1	103	12	4	16	2
PC	88	3	0	3	1	101	13	3	16	3	93	14	6	20	2
	93	4	1	5	1	97	14	4	18	3	85	4	1	5	1
PV	101	9	3	12	2	90	3	1	4	2	93	14	6	20	5
PV	101	11	1	12	2	99	12	4	16	2	88	4	1	5	2
Mean		6.00	1.00	7.00	1.20		9.40	2.60	12.00	2.20		9.60	3.60	13.20	2.40
ontinuous 16-Hour Photoperiod															
			Tank 10					Tank 11					Tank 12		
PC	90	4	3	7	2	95	11	7	18	6	88	7	5	12	4
PC	85	3	2	5	1	94	8	5	13	5	86	2	2	4	1
	89	4	4	8	2	94	7	5	12	3	93	7	5	12	4
PV	85	3	2	5	1	86	6	5	11	4	92	10	7	17	5
PV	95	12	6	18	2	84	2	2	4	2	88	4	3	7	4
Mean		5.20	3.40	8.60	1.60		6.80	4.80	11.60	4.00		6.00	4.40	10.40	3.60

APPENDIX B

Rates of food consumption (per cent body weight) for each tank of fish for each three week period and the six week period of each experiment.

Data from First Experiment

Food Consumption (Percent Body Weight) for the 6-Week Period

Condition	8 hr.	8-16 hr.	16 hr.	16-8 hr.	Weighted Mean Thy.
Hyperthyroid	45.12	43.15	62.93	47.60	50.02
Control	35.99	43.72	55.76	39.33	44.07
Hypothyroid	34.56	41.06	53.05	47.00	44.14
Weighted Mean Photo.	38.78	42.64	57.24	44.67	

Food Consumption (Percent Body Weight) for the First 3-Week Period

Condition	8 hr	8-16 hr.	16 hr.	16-8 hr.
Hyperthyroid	21.83	19.54	29.40	26.35
Control	17.32	22.36	23.03	20.97
Hypothyroid	17.67	20.36	23.52	24.61

Food Consumption (Percent Body Weight) for the Second 3-Week Period

Condition	8 hr.	8-16 hr.	16 hr.	16-8 hr.
Hyperthyroid	25.47	24.00	32.60	20.14
Control	21.39	25.22	33.51	18.19
Hypothyroid	18.41	21.71	30.09	22.17

Data from Second Experiment

Food Consumption (Percent Body Weight) for the 6-Week Period

Condition	8 hr.	8-16 hr.	16 hr.	16-8 hr.	Weighted Mean Thy.
Hyperthyroid	99.56	81.40	99.94	56.15	84.79
Control	71.48	76.53	73.80	74.35	74.07
Hypothyroid	78.51	72.95	86.71	72.06	75.87
Weighted Mean Photo.	83.70	77.06	87.11	67.60	

Food Consumption (Percent Body Weight) for the First 3-Week Period

Condition	8 hr.	8-16 hr.	16 hr.	16-8 hr.
Hyperthyroid	58.72	42.74	64.56	31.05
Control	39.16	37.75	46.63	46.17
Hypothyroid	43.36	32.16	53.38	43.77

Food Consumption (Percent Body Weight) for the Second 3-Week Period

Condition	8 hr.	8-16 hr.	16 hr.	16-8 hr.
Hyperthyroid	41.37	40.91	34.07	25.19
Control	33.14	40.15	25.69	28.49
Hypothyroid	37.08	48.18	32.18	28.63

Data from Third Experiment

Food Consumption (Percent Body Weight) for the 6-Week Period

Condition	8 hr.	8-16 hr.	16 hr.	16-8 hr.	Weighted Mean Thy.
Hyperthyroid	83.78	117.12	110.17	71.40	97.01
Control	82.47	84.94	99.92	88.39	88.94
Hypothyroid	63.14	72.13	98.82	76.86	78.14
Weighted Mean Photo	76.55	91.70	103.01	79.05	

Food Consumption (Percent Body Weight) for the First 3-Week Period

Condition	8 hr.	8-16 hr.	16 hr.	16-8 hr.
Hyperthyroid	47.10	72.06	74.04	35.23
Control	42.40	45.39	64.89	52.33
Hypothyroid	46.98	39.67	67.69	46.33

Food Consumption (Percent Body Weight) for the Second 3-Week Period

Condition	8 hr.	8-16 hr.	16 hr.	16-8 hr.
Hyperthyroid	33.77	42.31	31.89	34.00
Control	46.16	39.77	31.18	34.35
Hypothyroid	18.17	32.72	28.25	31.87

Data from Fourth Experiment

Food Consumption (Percent Body Weight) for the 6-Week Period

Tank	8 hr.	8-16 hr.	16 hr.	16-8 hr.
1	45.60	51.63	57.43	48.13
2	40.56	58.98	60.77	54.59
3	46.61	54.29	57.79	64.04
Weighted Mean	44.35	55.15	58.74	55.74

Food Consumption (Percent Body Weight for the First 3-Week Period

Tank	8 hr.	8-16 hr.	16 hr.	16-8 hr.
1	28.87	30.38	34.86	33.56
2	23.60	31.90	36.09	38.75
3	28.10	32.58	37.76	44.40

Food Consumption (Percent Body Weight) for the Second 3-Week Period

Tank	8 hr.	8-16 hr.	16 hr.	16-8 hr.
1	19.75	22.65	22.52	11.31
2	17.40	28.77	25.35	14.81
3	18.99	22.22	24.93	18.38

Food Consumption (Percent Body Weight) for the Third 3-Week Period

Tank	8 hr.	8-16 hr.	16 hr.	16-8 hr.
1	21.13	24.01	13.21	9.23
2	18.19	28.98	18.31	9.36
3	16.32	23.69	18.84	10.42

APPENDIX C

Efficiency of food conversion (percent) for each tank of fish for each three-week period and six-week period of each experiment.

Data from First Experiment

Percent Efficiency of Food Conversion for the 6-Week Period

Condition	8 hr.	8-16 hr.	16 hr.	16-8 hr.	Weighted Mean Thy.
Hyperthyroid	72.78	57.20	61.39	51.30	60.78
Control	51.63	63.96	73.31	44.17	60.49
Hypothyroid	39.94	56.11	72.34	27.33	51.31
Weighted Mean Photo.	57.05	59.25	68.72	41.03	

Percent Efficiency of Food Conversion for the First 3-Week Period

Condition	8 hr.	8-16 hr.	16 hr.	16-8 hr.
Hyperthyroid	63.06	51.18	79.49	72.59
Control	39.45	37.97	69.70	50.33
Hypothyroid	20.48	34.01	71.14	34.17

Percent Efficiency of Food Conversion for the Second 3-Week Period

Hyperthyroid	80.30	61.53	47.90	26.57
Control	61.69	86.04	75.31	37.66
Hypothyroid	58.27	74.50	73.11	20.20

Data from Second Experiment

Percent Efficiency of Food Conversion for the 6-Week Period

Condition	8 hr.	8-16 hr.	16 hr.	16-8 hr.	Weighted Mean Thy.
Hyperthyroid	30.74	28.85	23.86	17.61	25.12
Control	28.22	31.47	17.31	21.21	24.73
Hypothyroid	18.24	25.04	29.62	10.71	23.12
Weighted Mean Photo.	26.60	28.59	23.94	17.11	

Percent Efficiency of Food Conversion for the First 3-Week Period

Condition	8 hr.	8-16 hr.	16 hr.	16-8 hr.
Hyperthyroid	30.91	16.86	29.08	9.84
Control	23.39	23.35	26.50	19.98
Hypothyroid	13.71	18.87	36.47	15.84

Percent Efficiency of Food Conversion for the Second 3-Week Period

Condition	8 hr.	8-16 hr.	16 hr.	16-8 hr.
Hyperthyroid	30.53	39.95	15.05	11.76
Control	33.38	38.22	1.64	23.06
Hypothyroid	23.32	29.37	19.59	20.16

Data from Third Experiment

Percent Efficiency of Food Conversion for the 6-Week Period

Condition	8 hr.	8-16 hr.	16 hr.	16-8 hr.	Weighted Mean Thy.
Hyperthyroid	8.19	28.03	20.42	28.84	21.97
Control	6.65	21.93	19.71	20.90	17.83
Hypothyroid	7.47	17.74	22.76	16.24	17.14
Weighted Mean Photo.	7.44	23.48	20.97	21.63	

Percent Efficiency of Food Conversion for the Second 3-Week Period

Condition	8 hr.	8-16 hr.	16 hr.	16-8 hr.
Hyperthyroid	23.24	32.37	31.85	48.52
Control	19.03	22.72	32.59	29.28
Hypothyroid	15.41	16.38	32.43	25.17

Percent Efficiency of Food Conversion for the Third 3-Week Period

Condition	8 hr.	8-16 hr.	16 hr.	16-8 hr.
Hyperthyroid	****	21.98	****	10.39
Control	****	22.99	****	9.23
Hypothyroid	****	19.27	1.95	2.83

**** Weight loss occurred; no calculation of food conversion possible.

Data from Fourth Experiment

Percent Efficiency of Food Conversion for the 6-Week Period

Tank	8 hr.	8-16 hr.	16 hr.	16-8 hr.
1	61.25	85.02	66.75	47.24
2	73.62	88.56	80.72	64.99
3	76.45	73.40	73.66	70.42

Percent Efficiency of Food Conversion for the First 3-Week Period

Tank	8 hr.	8-16 hr.	16 hr.	16-8 hr.
1	60.34	74.33	72.70	63.65
2	70.31	80.88	84.50	84.49
3	74.42	73.44	71.33	84.32

Percent Efficiency of Food Conversion for the Second 3-Week Period

Tank	8 hr.	8-16 hr.	16 hr.	16-8 hr.
1	62.60	96.44	59.12	0.93
2	77.49	95.08	76.49	19.87
3	78.96	73.35	76.59	43.24

Percent Efficiency of Food Conversion for the Third 3-Week Period

Tank	8 hr.	8-16 hr.	16 hr.	16-8 hr.
1	88.02	57.70	43.25	*****
2	98.21	74.90	64.83	44.99
3	82.07	64.75	64.19	27.75

*****Weight loss occurred; no calculation of food conversion possible.