1952

The Time of spawning age and secondary sex character of adult chubs (Mylocheilus caurinum) from Flathead Lake Montana as correlated with histological changes in their testes

Ruth Navarre Scott

The University of Montana

THE TIME OF SPAWNING, AGE, AND SECONDARY SEX CHARACTERS OF ADULT CHUBS (MYLOCHEILUS CAURINUM) FROM FLATHEAD LAKE, MONTANA, AS CORRELATED WITH HISTOLOGICAL CHANGES IN THEIR TESTES

by

Ruth Navarre Scott

B.A., Bates College, Lewiston, Maine, 1950

Presented in partial fulfillment

of the requirements for the degree of

Master of Arts

MONTANA STATE UNIVERSITY

1952

This thesis has been approved by the Board of Examiners in partial fulfillment of the requirements for the degree of Master of Arts in Zoology.

George F. Weisel
Chairman of Examining Committee

G B Castle
Dean of the Graduate School

Date Aug 18 1952

ACKNOWLEDGEMENTS

I wish to express my gratitude to Dr. G. F. Weisel for his aid and advice throughout this investigation, most particularly during the actual writing of the thesis. Without the assistance of Dr. R. B. Brunson and Richard Bjorklund in gathering the material at Flathead Lake, this work would have been impossible. The help which Drs. R. B. Brunson and J. Chapman gave made possible the photomicrographs of the testes. The services offered by Mr. Sumner Dow was invaluable in making photostats of the graphs. Dr. L. G. Browman was ever a counsel, with concrete suggestions and needed encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Aside from taxonomic descriptions, the Columbia River chub (*Mylocheilus caurinum*) has received little attention, although it is one of the largest and most abundant cyprinids in the Northwest.[1] It is the purpose of this investigation to add to the general knowledge concerning *Mylocheilus* by describing its breeding cycle in terms of histological changes occurring in its testes and macroscopical changes in its ovaries. Also, secondary sex characters which possibly might serve as indicators of sexual maturity are discussed and correlated with the testicular cycle, and dimorphic characters which might be used to distinguish males from females are evaluated. The age of the spawning fish is noted as well as such miscellaneous items as feeding activities and parasitism.

It is difficult to observe spawning activities of certain species of fish and so determine their breeding season. However, cyclic changes occurring in gonads of mature specimens usually can be used as a reliable criterion to determine the time of breeding. For instance, the histological changes which take place in the spermeries of *Perca flavescens* have been shown to conform with the breeding cycle (Turner, 1919). As spermatogenesis progresses to its final stage in *Perca*, the testes increase in size and weight so that the ratio of gonad weight can also be

[1] Drs. C. L. Hubbs and R. R. Miller advise that the spelling of the specific name *caurinus* be changed to *caurinum* to conform with the neuter gender of *Mylocheilus*.

used as an indicator of sexual maturity.

Another fish which exhibits a definite cycle in the tissue structure of the gonads is Cottus bairdii (Hann, 1927). The primordial germ cells of both sexes of this sculpin eventually give rise to the final sex products, there being no transition from somatic cells to germ cells at any time. After Cottus spawns, a number of spermatogonia lying dormant along the margins of the tubules commence to divide and fill up the tubules in preparation for the next spawning period.

Matthews (1936) determined the condition of the gonads of Fundulus at intervals during one year. He concluded that the differences in weight and in gross and microscopic appearance of both testes and ovaries at different periods are great enough to afford a definite index to the time of breeding.

A European cyprinid, Phoxinus sp., has an early production of primary spermatocytes during October and November, followed by a period of quiescence which is abruptly ended by rapid spermatogenesis just before spawning in May (Bullough, 1939).

Oncorhynchus nerka, a salmon which dies shortly after breeding, has obvious changes occurring in its testes preceeding spawning (Weisel, 1943). The histological appearance of this salmon's testes is similar to that of the rainbow trout, except that pycnosis and vacuolization occur in the germ cells and connective tissue of the salmon within a day or two after it has spawned.

There is a definite correlation between the breeding season of Lepomis macrochirus, as determined by field observations, and the cyclic alterations which appear in the structure of the testes (James, 1946). Lepomis has a series of milt discharges during May, June, and July. The lobules of the testes correspondingly contain maturing cysts of spermatocytes through-out this period which maintain a prolonged source of spermatozoa.

Seasonal variations are not striking in the testes of Gillichthys mirabilis, a gobiid fish of California. This is undoubtedly due to the fact that this marine teleost lives in an area of equitable climate and breeds over a long period - January to July. However, during the protracted spawning season the testes are packed with spermatozoa except when they are partially emptied during mating; in August and September the testes have few spermatozoa but the spermatocytes and spermatids increase; and by November the spermatocytes and spermatids are largely converted into spermatozoa. There is practically no seasonal variation in the size of the testes of Gillichthys (Weisel, 1949).

Secondary sex characters are an interesting adjunct to the sexual cycle of fish. Some characters appear and disappear, apparently controlled by seasonal changes which take place in the gonads, and can be used as indicators of sexual activity.

Breeding female Gasterosteus aculeatus develop a pinkish throat and belly, whereas breeding males have a carmine throat and belly, blue eyes, and greenish fins. By correlating the appearance and disappearance of these secondary sex characters with the varying degree of development of interstitial tissue within the testes, Courrier (1922) claimed that seasonal changes in coloration were dependent upon a hormone secreted by the interstitial cells. He further likened the role of the interstitial cells in fish to that in mammals. As support for his contention, he claimed no secondary sex characters appeared in fish which have no developed interstitial cells but do possess spermatogonia and spermatocytes.

Completely denying Courrier's conclusions, Champy (1923) claimed that some specimens of Gasterosteus in which no interstitial tissue could be seen histologically still exhibit the appearance of secondary sex characters. Impressed with the indirect indications that sexual dimorphism was dependent upon testicular hormones as claimed for Gasterosteus by Courrier and by the works of Aida (1921) on Haplocheihis latipes and of Winge (1923) on Lebistes retriculatus, Blacher (1926) demonstrated that upon atrophy of the testes, Lebistes lost its male sex colors. From this observation, he concluded that the intensiveness, shape, and development of the black and especially the red and yellow pigment spots depend upon hormones produced in the testis.

One of the most striking examples of secondary sex characters assumed just prior to the spawning period is that exhibited by the Pacific pink salmon, (Davidson and Shostrom, 1936). The males increase in length, a hump appears on their backs, they increase 19.11 per cent in height above the lateral line and their heads become greatly elongate and grotesque.

The black hue affected by Gillichthys during its mating is probably due to a nervous control of the chromatophores, as it appears and disappears in a few seconds (Weisel, 1949). This type of breeding color is not the same as those that are assumed gradually and are more or less permanent during the breeding season.

CHAPTER II

A REVIEW OF THE LITERATURE CONCERNING *MYLOCHEILUS CAURINUM*

The known range of the Columbia River chub in continental North America is in the Fraser and Skeena rivers of British Columbia, and in the Columbia River system of British Columbia, Idaho, Montana, Oregon, and Washington, It is limited in its distribution up the Snake River by Shoshone Falls (Miller, personal communication). With the exception of one Japanese cyprinid, *Mylocheilus* is the only member of its family known to enter the sea. It is this fact which probably explains,its presence on Vancouver and Nelson Islands (Carl and Clemens, 1948).

Mylocheilus is one of the few North American cyprinids that attains a size suitable for a pan fish. The largest specimen taken from Western Montana has a standard length of 28.5 cm. It was taken from Seeley Lake, Clearwater drainage, Montana. Chubs were frequently eaten by the Flathead Indians, although they were not considered as good as salmon or trout and had to be fried until crisp because of their many bones (Weisel, 1952). Lower down the Columbia River, where salmon were abundant, the Indians did not favor the chub for food. An early survey party at Fort Steilacoon reported them as being bony and insipid (Rept. Explor, etc., 1860). However, they have been served in hotels along the Columbia River as "whitefish" and have been peddled over the country as "trout" and "fresh-water herring" (Jordan and Evermann, 1934). At Flathead Lake, *Mylocheilus* has been called "whitefish" and has been served at hotels, while the true whitefish, which is not uncommon in the lake, did not seem to attract the attention of the local fisherman (Evermann, 1891). More recently, the chub was described as a food fish of some importance in Washington and Oregon, but as rarely utilized in British Columbia (Carl and Clemens, 1948).

The chub possesses considerable game qualities, rising to the fly and fighting vigorously for a time. Undoubtedly, as our natural resources in fish keep rapidly dwindling, the chub will become more valuable as a food and sport fish.

In spite of its use as food and its much greater potential for this use, practically nothing is known about the natural history of the Columbia River chub. Schultz (1935) gave some observations on its spawning behavior in a brief paper. Chubs in Lake Washington, Washington, were observed to spawn in 1935 at two times, on May 20 and on June 1. During the second spawning, the fish were distributed along 30 to 40 feet of the beach and within 8 feet of the shoreline. Several hundred fish, milling about, seemed to be concentrated in several main groups surrounding close assemblages of resting males. Distinguishing between the highly colored males and females by back coloration, the former dark green, the latter dark brown, Schultz observed that 4 to 8 males, upon spying a female, would persistently chase her until she was forced high up on the rocky rubble, about one foot from shore, and then would press their bodies hard against hers. Males aligned themselves so their vents were opposite the female's vent. As the males were approximately 2.8 cm. shorter than the females, their mouths were even with her operculum. Now with the males' tails arched over the female's caudal peduncle, all fish being more than half out of water, there occurred at once rapid vibrations or tremblings of the bodies in unison for about 1.5 seconds, followed by an equal period of rest, after which the trembling resumed. This spawning act took place about three times in succession. Each time the water around the fishes' tails became milky from milt and contained many pale greenish-gray eggs which were suspended for a second or two before settling to the bottom to attach in clumps or singly to the sides of rocks. The spawned female swam away and the spawned males rejoined the close assemblage of other males.

From his observation, Schultz concluded that deposition of eggs in such shallow water two feet from the shoreline is advantageous, although so far as known of fresh-water fish of northwest United States, it is peculiar to Mylocheilus. Some advantages listed were: wave action insures oxygenation and keeps eggs free from silt, but is not strong enough to turn rocks and thus expose the eggs; the eggs are firmly attached and so avoid danger of being swept away; suckers do not forage into such shallow water; upon hatching, the young fish are afforded ideal protection among the stones. However, there are these disadvantages: the eggs and larvae are subject to the attack from aquatic insect larvae; the eggs may be destroyed by a change in water level; and the spawning adults are exposed to danger from land predators.

In addition to the report on the spawning activity of the chub, the knowledge of its natural history has been supplemented by a study of its feeding habits and a study of the age-growth relationship of the chub.

The food of adult Mylocheilus differs essentially only in size from that of the young chub. Young chubs feed chiefly on water-fleas, copepods, midge larvae, and other small insects. The food of larger fish is composed largely of aquatic and terrestrial insects, water-fleas, and occasionally molluscs, and rarely fishes such as small sculpins (Carl and Clemens, 1948). Jordan and Evermann (1934) note that Mylocheilus preys to some extent upon salmon eggs.

The largest individual chub hitherto recorded in the literature was one from Okanogan Lake, British Columbia, in its 6th season measuring in fork length 24.4 cm. (Clemens, 1939). The growth of Mylocheilus in Okanogan Lake is rapid during the first two years of life, 5.0 and 5.5 cm. increase in fork length respectively, then tapers off so that in the 4th and 5th years it increases approximately 2.75 cm. per year in fork length.

CHAPTER III

MATERIALS AND METHODS

The chubs were taken in Flathead Lake, Montana, with a graded gill net. The majority of settings were made on a line south-southwest off the shallow rocky point which protects Yellow Bay from the northwest. Due to the large size of the smallest mesh, cyprinids under 12.5 cm. were not captured. One hundred and two male chubs ranging from 16.9 to 22.4 cm. in standard length and ninety-eight females ranging from 14.0 to 25.5 cm. were netted and used in this study. Three males were taken on May 20, one on May 28, ten every week between the 16th of June to the 10th of August with the exception of the weeks of July 12 and 27 and August 4 when nine, eight, and eight were taken respectively, one on September 6, ten on October 13, four on November 3, one on November 24, and one on December 8 (Table I). Three females were netted on May 20, two on May 28, ten every week between June 16 and August 10 with the exception of the weeks of June 16, July 27 and August 4 when nine, eight, and nine were taken respectively, five on October 13, six on November 3, and two on November 24 (Table II).

Immediately after removal from the net, each fish was slit the length of its belly and placed in 10% formalin. This proceedure insured fresh fixation. Standard lengths were recorded to the nearest millimeter on a standard measuring board. Total body weights were taken on a gram scale accurate to one-tenth gram. Preserved gonads were weighed to the nearest hundreth of a gram on an analytical balance. The quality and dis-

TABLE I

WEIGHTS AND LENGTHS OF MALE *MYLOCHEILUS*, 1951

Date of capture	Number of fish	Total body weight in gm.			Standard length in cm.			Weight of gonad in gm.		
		mean	min.	max.	mean	min.	max.	mean	min.	max.
5/20	3	112.0	95.5	128.5	19.1	18.2	19.6	0.66	0.2	1.3
5/28	1	118.5			19.1			3.7		
6/16	11	128.4	101.0	146.0	20.1	18.8	21.0	2.95	2.2	5.9
6/20	10	120.3	91.0	172.5	19.4	17.4	21.1	3.23	1.25	5.16
6/23	3	90.8	71.0	128.7	18.0	16.5	20.7	1.76	0.4	3.9
6/28	10	127.9	91.5	175.5	20.1	17.7	22.2	3.44	1.8	5.4
7/5	12	120.2	92.0	134.5	20.2	18.0	22.2	2.20	0.5	4.0
7/12	9	113.2	91.0	134.5	19.2	18.3	20.3	1.9	0.7	3.0
7/19	10	129.8	114.5	169.0	20.1	19.3	22.4	1.3	0.65	3.0
7/27	8	124.0	108.6	141.6	20.1	18.9	21.2	0.85	0.55	2.3
8/4	8	128.6	112.0	140.2	20.4	19.3	21.6	0.6	0.2	0.8
9/6	1	81.5			16.5			0.75		
10/13	10	96.8	42.7	117.8	17.7	13.4	19.5	0.42	0.09	1.12
11/3	4	122.7	98.3	140.0	19.2	18.0	20.3	0.91	0.5	1.15
11/24	1	134.3			19.7			1.6		
12/8	1	120.0			19.3			0.8		

TABLE II

WEIGHTS AND LENGTHS OF FEMALE MYLOCHEILUS, 1951

Date of capture	Number of fish	Total body weight in gm.			Standard length in cm.			Weight of gonad in gm.		
		mean	min.	max.	mean	min.	max.	mean	min.	max.
5/20	3	188.9	129.8	243.5	22.0	19.9	23.3	13.4	8.7	17.5
5/28	2	163.8	157.6	170.0	20.5	20.5	20.5	16.4	11.9	20.6
6/16	9	230.3	187.0	305.0	23.3	21.3	25.5	33.3	28.5	51.6
6/20	10	160.0	108.0	228.0	21.1	18.7	23.3	17.5	1.3	31.8
6/23	4	70.2	44.0	90.0	16.4	14.0	18.2	0.7	0.2	1.0
6/28	10	154.0	108.0	264.0	20.9	18.5	24.2	20.3	3.8	49.2
7/5	11	153.0	99.5	240.0	21.4	18.7	24.6	13.5	1.9	38.2
7/12	10	160.5	125.0	205.5	21.5	20.2	23.2	8.0	1.9	29.0
7/19	10	143.0	65.5	204.5	21.5	16.3	23.2	5.9	1.95	24.4
7/27	8	148.4	117.8	170.0	21.2	20.3	22.4	2.87	1.5	4.58
8/4	9	136.6	87.7	235.0	20.1	17.5	24.4	2.1	1.9	2.6
10/13	5	185.0	123.7	257.9	21.7	19.0	24.8	8.5	4.4	12.2
11/3	6	171.6	131.0	217.0	21.1	19.5	22.4	7.37	5.6	8.6
11/24	2	181.1	154.0	208.3	21.0	19.9	22.1	12.3	10.9	13.7

tribution of colors were noted on special field cards. The degree of tubercle growth was determined under a binocular scope, and recorded according to an arbitrarily set standard: tubercles above surface of epidermis, tubercles at surface of epidermis, tubercles below surface, tubercles barely distinguishable, and tubercles absent. Feeding activity and parasites were determined by macroscopic examination of the body cavity and of the stomach and intestine. When feasible, the food organisms and parasites were identified.

Scales for aging were removed from between the dorsal fin and the lateral line. These were placed in Bouin's picro-formal along with the testes.

After the testes were removed and weighed, they were cut in cross-section and placed in Bouin's picro-formal for continued preservation. Not every testis was examined microscopically, but testes from two fish in each of four length groups from each collection week were studied histologically. The four length groups into which the chubs were divided were 14.0 to 18.0 cm., 18.0 to 20.0 cm., 20.0 to 21.8 cm., and 22.0 to 22.4 cm. These length groups correspond quite closely to the age groups. Sections from the mid-region of those testes selected were embedded in parafin, cut at 5 to 7 microns, stained in Erlich's acid hematoxylin, and counter-stained in eosin. A few tissues were stained with Heidenhain's iron hematoxylin technique as used by Moore (1937).

CHAPTER IV

RESULTS

Description of the testes

The testes of Mylocheilus caurinum are paired organs situated in the dorsal part of the body cavity ventro-lateral to the swim bladder. Each testis is covered with a connective tissue sheath, and is attached to the swim bladder by a short mesorchium. The genital arteries and veins follow along the median dorsal hilus of the gonad. The primary sperm ducts from each testis unite to form a short common genital duct at the posterior ventral end of the coelom. This duct, in turn, leads to an opening in the genital papilla.

In cross section, the testes are triangular-shaped. They lack the central core of connective tissue from which septa of the lobules extend peripherally as described for perch by Turner (1919). Instead, their histological structure more closely resembles that of the salmon (Weisel, 1943), which is in order, inasmuch as salmonids are assumed to be more closely related phylogenetically to cyprinids than to centrarchids. The testes are divided into lobules by connective tissue strands which are continuous with the thicker connective tissue covering of the entire gonad. The lobules, or broad tubules, formed by the connective tissue strands, contain the germ cells and lead, presumably, by tortuous coils to the dorsally located sperm duct.

In order to evaluate the degree of maturity during different periods of the year, a series of arbitrary stages was established to describe the successive changes which occur in the testes. These stages are used in Figure 1 and in the discussion of the time of spawning.

1. Immature Testes: Immature testes contain long threads of spermatogonia which are partitioned into lobules by thin connective tissue strands. The spermatogonia are large with a dark staining nucleolus, or occassionally two or more nucleoli, surrounded by very dispersed threads and granules of nucleoplasm and a distinct nuclear membrane. The cell membrane is difficult to note, the cell limits being discernible only when viewed in its relation to the surrounding cells. There are only a few small lumena in the lobules (Fig. 4). It is by this last characteristic that an immature testis can be distinguished from a testis which is being reconstituted after spawning.

The only immature fish caught was netted in Polson Bay on June 23. The standard length of this fish was 16.9 cm.; its total body weight, 72.1 gm.; its gonad weight, 0.98 gm.; and the ratio of its gonad weight to total body weight, .00574.

2. Testes in Primary Spermatocyte Stage: Apparently, each spermatogonium undergoes repeated division before the first maturation division takes place since there are, on the average, 12 primary spermatocytes to each cyst. A cyst consists of a group of germ cells surrounded by a delicate membrane of connective tissue, the so-called follicle cells. The primary spermatocytes are distinguished from the spermatogonia by their smaller size and by the greater concentration of the nuclear material. At this stage of development, many spermatogonia are

present along the lobules of the testis, for not all of them develop cysts of spermatocytes. There is no stage in spermatogenesis later than primary spermatocytes in the testes at this time (Fig. 5).

Three fish netted on October 13 possessed testes at this degree of development. The measurements recorded on these fish were respectively; standard lengths, 19.5, 17.4, 18.1 cm.; total body weights, 116.7, 89.2, 97.0 gm.; gonad weights, 0.45, 0.09, 0.25 gm.; ratios of gonad weight to total body weight, .00386, .00102, .00257. One fish out of the three netted on November 3 also showed this stage of testicular development. It was 20.3 cm. in standard length; 140.0 gm. in total body weight; 1.15 gm. in gonad weight; and the ratio of gonad weight to total body weight was .00824.

3. Testes in Secondary Spermatocyte Stage: At this stage, cysts of both primary and secondary spermatocytes are present, but no spermatozoa have developed. Each cyst contains cells which are all in the same stage of maturation. One cyst may have primary spermatocytes only while an adjacent cyst may be all secondary spermatocytes. This condition has also been observed in *Cottus bairdii* (Hann, 1927), in *Lepomis macrochirus* (James, 1946), and in *Perca flavescens* (Turner, 1919). The chromatin of the nucleus stains more deeply as it becomes more condensed and the size of the cells diminish with each phase of maturation. (Fig. 6).

The earliest date at which the secondary spermatocyte stage was reached in 1951 was October 13. On this date one fish out of the four studied had testes in this stage of development. Two fish out of the three taken on November 3, the one fish netted on November 24, and the one fish netted on December 8 also had testes in this stage. The measurements of these fish were: range in total body weight, 116.8 to 135.7 gm., the average, 124.9 gm.; the range in standard length, 18.3 to 20.1 cm., the average, 19.3 cm.; the range in gonad weight, 0.8 to 1.6 gm., the average, 1.1 gm.; and the range in the ratios of gonad weight to total body weight, .00667 to .0119, the average, .00887.

The testes appear to remain in this condition throughout the winter as the testes of two fish netted on May 20 had not developed beyond this stage. The standard lengths of these fish were 18.2 and 19.5 cm.; the total body weights, 95.5 and 128.5 gm.; the gonad weights, 1.3 and 0.5 gm.; and the ratios of gonad weight to total body weight, .0136 and .00420.

4. Testes One-Quarter Filled with Spermatozoa: In a cross-sectional view of testes in this stage, approximately 1/4 of the area is filled with spermatozoa; the other 3/4 of the area contains cysts of spermatocytes in the various stages of development. The clusters of spermatozoa appear as dark masses in the center of the lumena of the lobules, surrounded by the cysts of spermatocytes and spermatids. The spermatozoa are readily distinguished by their small size, their dark condensed nuclei, and tail. There is no evidence that the first spermatozoa developed move to the duct for storage as described for <u>Phoxinus</u> by Bullough (1939).

Testes which had reached this degree of development were possessed by two fish studied, the first netted on May 28, the second on June 28.

The measurements recorded on these fish are respectively, 19.1 and 16.8 cm. in standard length, 118.5 and 101.0 gm. in total body weight, 3.7 and 1.9 gm. in gonad weight, .0310 and .0188 for the ratios of gonad weight to total body weight.

5. Testes Half to Three-Quarters Filled with Spermatozoa: A little later in development, the testes have the same general appearance as those described above, except that more cysts have completed maturation so that about half to three quarters of the total cross-sectional area of the testes contain spermatozoa. Dense masses of spermatozoa are contained in many of the lobules which are bordered only by thin strands of connective tissue as all their cysts have undergone maturation so that only spermatozoa are present. However, approximately 1/2 to 1/4 of the lobules still contain cysts which are not completely matured but contain spermatocytes. Also, within the connective tissue strands delimiting the lobules, spermatogonia may be seen - representing a reserve supply of germ cells potentially capable of replenishing the testes for another spawning period (Fig. 7).

Fish that had approximately 1/2 to 3/4 the cross-sectional area of their testes filled with spermatozoa were netted in late June or early July: four on the 16th of June, five on the 20th of June, two on the 28th of June, and one on July 5. The measurements of these fish considered as a group are as follows: the range in standard length, 17.4 to 21.7 cm., the average, 19.6 cm.; the range in total body weight was 91.0 to 172.5 gm., the average, 125.8 gm.; the range in gonad weight, 5.16 to 1.25 gm., the average, 3.22 gm.; the range in ratio of gonad weight to total body weight, .0111 to .0361, the average, .0258.

6. Testes Fully Ripened: Testes in this condition are composed of large lobules completely filled with large masses of compact spermatozoa. The large size of the lobules is probably due to their distension by numbers of sperm, allowed by the distribution of elastic tissue throughout the gonad. At this stage, there are no cysts undergoing maturation and only resting spermatogonia are seen lying along the connective tissue strands (Fig. 8). Some fish apparently commence spawning before this degree of development has been reached.

Fish having reached this ultimate stage in maturation were caught in late June and July: one fish on June 20, one fish on June 28, one on July 5, three on July 12, and one on July 19. Their combined measurements are: the range in total body weight was 104.0 to 175.5 gm, the average, 130.3 gm.; the range in standard length, 18.3 to 22.2 cm., the average, 20.1 cm.; the range in gonad weight, 1.60 to 4.0 gm., the average, 2.88 gm.; the range in ratio of gonad weight to total body weight, .0120 to .0310, the average, .0223.

7. Testes Partially Spent: these testes look flaccid, many of their lobules have a number of cysts undergoing maturation, but they still contain a large number of spermatozoa. As the fish may be partially stripped when pressure is necessarily exerted to remove them from the gill net, it is impossible to say whether the chub spawns a number of times during its breeding season, or whether the partially spent testes are artificially induced.

Relatively few fish possessed testes only partially spawned.

Three fish of six studied from July 5 and two fish of five from July 19 did have testes at this stage. The range in total body weight of these fish was 92.0 to 169.0 gm., the average, 130.9 gm.; the range in standard length, 18.4 to 22.4 cm., the average, 20.7 cm.; the range in gonad weight, 1.6 to 3.0 gm., the average, 2.19 gm.; the range in the ratio of gonad weight to total body weight, .00949 to .0260, the average, .0177.

8. Testes Fully Spent: Even in fully spent testes all of the sperm are not extruded. Some spermatozoa remain in the gonad to be resorbed. Also, not all cysts complete maturation, the spermatocytes being resorbed as well. Immediately after spawning, the testes are flaccid. Threads of spermatogonia begin to form, but spermatozoa and cysts of degenerating spermatocytes are still in evidence (Fig. 9). The gonad begins to resume a smaller size as the elastic tissue of the gonad constricts.

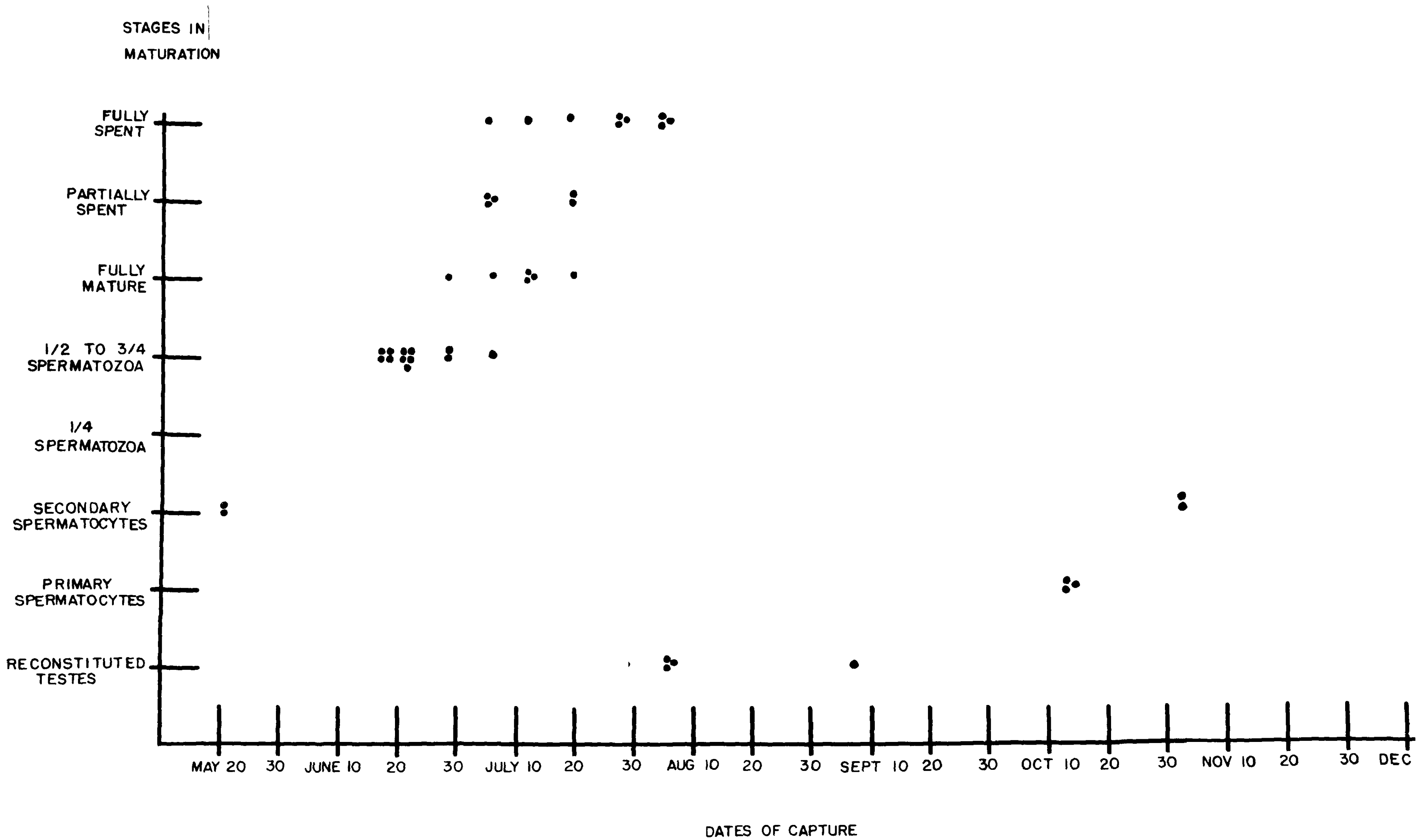
The first completely spent fish in 1951 was netted on July 5. Then the numbers of completely spent fish increased throughout late July and early August. One fish of four studied taken on July 12, two fish of five examined from July 19, three fish of four from July 27, and three fish of six from August 4 all are in this category. Their measurements combined are: range in total body weight, 108.6 to 140.0 gm., the average, 121.4 gm.; the range in standard length, 18.9 to 21.6 cm., the average, 19.9 cm.; the range in gonad weight, 0.55 to 0.9 gm., the average, 0.72 gm.; the range in the ratio of gonad weight to total body weight, .00486 to .00860, the average, .00598.

9. Reconstituted Testes: Within two weeks, completely spent testes can not be distinguished from immature testes, so complete is the reconstitution of the gonad. Macroscopically the gonad is a thin thread of tissue mostly covered by fat. Microscopical inspection of the gonad reveals that the spermatogonia have formed in threads along the connective tissue strands by division and that the lumena are again constricted. This general condition of reconstitution agrees with that described for *Lepomis* (James, 1946), and *Phoxinus* (Bullough, 1939).

One fish netted on July 27, three fish netted on August 4, and one fish caught on September 6 had testes that presumably were being reconstituted after spawning. Their combined measurements were: range in total body weight, 81.5 to 141.6 gm., the average, 111.3 gm.; the range in standard length, 16.9 to 21.2 cm., the average, 19.6 cm.; the range in gonad weight, 0.5 to 0.8 gm., the average, 0.7 gm.; the range in the ratio of gonad weight to total body weight, .00417 to .00920, the average, .00613.

The chronological occurrance of these nine phases in testicular development have a fairly definite pattern (Fig. 1). On May 20, the two chubs studied histologically had only progressed as far as the secondary spermatocyte stage. A chub from a May 28 setting showed further maturation so that approximately 1/4 of the testes contained spermatozoa. On June 16, half the volume of two pairs of testes consisted of spermatozoa and two others were 3/4 filled with sperm. On June 20, one pair of testes contained 1/2 spermatozoa, four pairs 3/4 spermatozoa, and one pair was fully matured. By June 28, there seemed to be no further general progress in

Figure 1.–Condition of testes of adult *Mylocheilus* at different times in the year. Each dot represents one fish examined.


STAGES IN MATURATION
FULLY SPENT
PARTIALLY SPENT
FULLY MATURE
1/2 TO 3/4 SPERMATOZOA
1/4 SPERMATOZOA
SECONDARY SPERMATOCYTES
PRIMARY SPERMATOCYTES
RECONSTITUTED TESTES
MAY 20
30
JUNE 10
20
30
JULY 10
20
30
AUG 10
20
30
SEPT 10
20
30
OCT 10
20
30
NOV 10
20
30
DEC
DATES OF CAPTURE

maturation, one fish having testes 1/4 filled with spermatozoa, one 1/2 filled, one 3/4 filled, and one fully matured. However, by July 5, maturation was complete in the majority of the fish studied, one out of six being fully spent, three partially spent, one fully matured, and one 3/4 filled with spermatozoa. On July 12, one fish out of four studied was fully spent, the other three being fully matured. This same situation continued for at least a week as on July 19, two out of five fish were fully spent, two partially spent, and one fully matured. But by July 27, a shift had occurred - all the fish were completely spent, one already having reconstituted its testes so that threads of spermatogonia were present in abundance throughout the lobules. On August 4 there was further division of germ cells. Three out of the sex fish examined were completely spent, the other three were reconstituted. Maturation in the testes from a fish netted on September 6 had progressed no further than the spermatogonia stage. However, by October 13, three of the four pairs of testes contained cysts of primary spermatocytes, the fourth pair of testes having matured to the stage containing cysts of secondary cytes as well as cysts of primary spermatocytes. By November 3, one fish had testes containing cysts of primary spermatocytes, but two others had gonads which had matured to the secondary spermatocyte stage. Both fish netted on November 24 and December 8 had testes developed to cysts of secondary spermatocytes, the same stage in development that the testes from fish caught on May 20 had attained.

Description of Secondary Sex Characters

Some cyprinids demonstrate striking sexual dimorphism. The most usual sexual dimorphisms are those of color, tubercular development, and relative size. These characters are used to distinguish between the males and females of some species.

Coloration: Mylocheilus is one of the most brilliantly colored minnows in the Northwest. Dorsally, it is dark olivaceous, its sides are washed with yellowish silver, and its belly flashes white. Upon this matrix of color, ventral to the lateral line, extends a reddish stripe, which thins towards the vent, then thickens towards the tail. The fins, lips, and operculum are tinged with red. Gold extends the length of the body in two stripes dorsal to the red line and blotches the operculum.

The quality of coloration was the factor measured, and in this quality the fish did not vary throughout the time of capture more than they varied from individual to individual in one netting. Intensity of coloration is a relative factor which can be varied by such artificial factors as the differing lengths of time the fish are in the nets. However, if there is an increase in intensity previous to spawning, it is not marked and cannot be used as a reliable criterion for time of spawning. Also, unlike many cyprinids, the male chubs cannot be distinguished from the females on the basis of this character, as both are highly colored. Therefore, coloration in Mylocheilus is not in the full implication of the term a sexual dimorphic character.

Tubercular Development: Tubercular development as well is not peculiar to one sex of Mylocheilus. Both males and females may have tubercles on the head, along the pectoral fin rays, over the dorsal surface of the back, and in some cases on the operculum. Counts of tubercles were made on the rectangular surface between the fish's eyes and nostrils. Male Mylocheilus have a higher total average of tubercles (39.1) than the female (25.0), and their average for any one date is consistently higher. However, there is a great deal of overlap between the individuals of the different sexes, so that the numbers of tubercles can not be used as a reliable criterion to distinguish sex (Table III).

On the other hand, tubercular development exhibits cyclic changes which correspond to the degree of maturity of the testes. The numbers and relative height of the tubercles in relation to the epidermal surface starts to increase in October when the first stages of maturation commence in the testes, reaches a peak in June just when the testes are becoming fully matured, and falls rather sharply in July when the testes become spent (Fig. 2).

Relative Size: The chubs exhibit their greatest sexual dimorphism in the relative size of the sexes (Tables I and II). Forty-one adult females and forty-one adult males caught between June 28 and July 19 have the following measurements:

Sex	Total body weight in gm.			Standard length in cm.		
	range	mean	mean deviation	range	mean	mean deviation
female	65.5 to 264.0	154.8	32.7	16.3 to 24.6	21.2	1.46
male	86.5 to 175.5	123.5	17.9	17.7 to 22.4	19.9	1.08

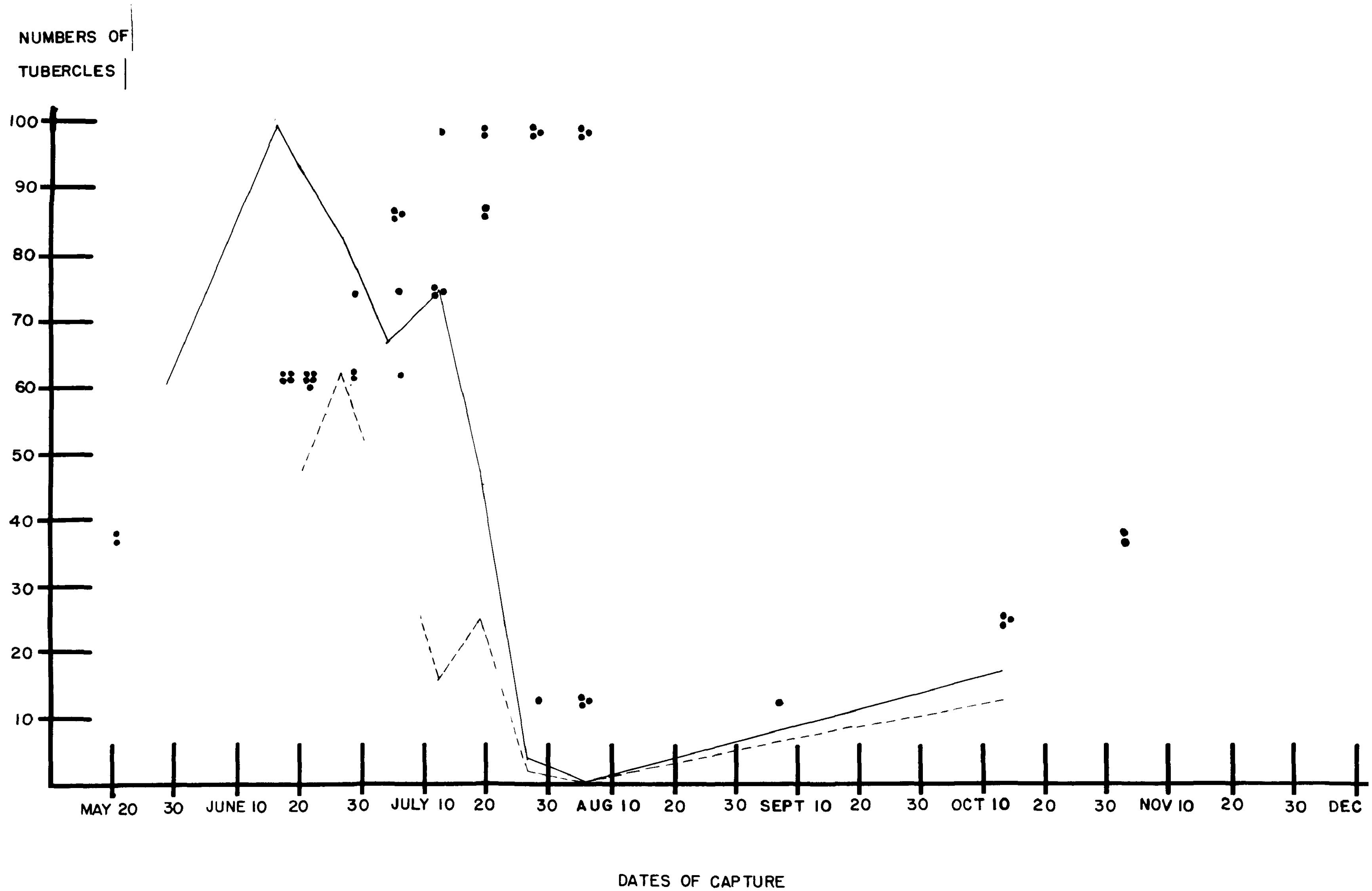
TABLE III

NUMBERS AND RELATIVE HEIGHT OF TUBERCLES

	MALES				FEMALES			
Date of capture	Number of fish	Range in numbers of tubercles	Average numbers of tubercles	Average height of tubercles to epidermis	Number of fish	Range in numbers of tubercles	Average numbers of tubercles	Average height of tubercles to epidermis
5/20	3	-	-	barely visible	3	0	0	none
5/28	1	57	57	below	2	0 to 97	48	below
6/16	11	63 to 134	100	above	9	0 to 133	50.8	below
6/20	10	53 to 132	95.5	at surface	10	0 to 102	44.7	below
6/28	10	54 to 111	83.2	above	10	43 to 79	62	at surface
7/5	12	0 to 94	68	at surface	11	0 to 107	43	below
7/12	9	0 to 123	77.8	at surface	10	0 to 81	14.8	below
7/19	10	0 to 142	46.9	below	10	0 to 135	22.5	barely visible
7/27	8	0 to 34	4.2	barely visible	8	0 to 25	3	barely visible
8/4	8	0	0	none	9	0	0	none
9/6	1	0	0	none	0	-	-	-
10/13	10	0 to 80	15	barely visible	5	0 to 55	11	barely visible
11/3	4	-	-	barely visible	6	-	-	barely visible
11/24	1	-	-	below	2	-	-	below
12/8	1	-	-	below	0	-	-	-
Average of totals			39.1				25.0	

Figure 2.–The average numbers of tubercles of male and female *Mylocheilus* in different seasons of the year.

Superimposed on this figure is the relative maturity of the testes as shown on Figure 1.



NUMBERS OF
TUBERCLES
100
90
80
70
60
50
40
30
20
10
MAY 20
30
JUNE 10
20
30
JULY 10
20
30
AUG 10
20
30
SEPT 10
20
30
OCT 10
20
30
NOV 10
20
30
DEC
DATES OF CAPTURE

Thus, females average 1.3 cm. longer than the males and are 31.3 gm. more in weight.

Age and Condition of Adult Chubs

The annuli on the scales of most Mylocheilus are difficult to determine. The circuli are evenly spaced, and in this respect resemble the scale of the cyprinid, Notropis cornutus chrysocephalus, as depicted by Lagler (1950). The annuli are marked off from one another most obviously by differences in the angle of the formation of the circuli for each successive year. On some scales, the angular demarkation is quite evident, on others it is not definite. This difference in circuli formation is considered valid for aging studies (Lagler, 1950).

To help overcome discrepancies in aging, the scales were first interpreted by the author and then, without knowledge of the author's opinions, by Dr. G. F. Weisel. If there was disagreement on the age of a fish, that specimen was not included in the data. The age determinations agree quite well with the lengths, even though they represent fish taken over a six month period.

The population of sexually mature male chubs caught in Flathead Lake in 1951 was represented by fish in their 3rd, 4th, and 5th season, the majority being in the first two classes (Table IV). One male in his 3rd season was immature, which indicates that not all fish spawn upon reaching their 3rd season, but that is the first probable age at which spawning can be achieved. Only one two year old fish was aged, an immature female of 15.7 cm. in standard length.

TABLE IV

AGE AND LENGTHS OF MALE *MYLOCHEILUS*

Age	Date of capture	Standard length in cm.	Total weight in gm.	Condition factor
III	June (immature)	16.9	72.1	1.49
III	October	17.4	89.2	1.68
III	June	17.7	91.5	1.64
IV	October	18.1	97.0	1.63
III	July	18.3	104.0	1.69
III	July	18.4	92.0	1.47
III	June	18.8	101.0	1.52
III	October	18.9	117.8	1.74
III	December	19.3	120.0	1.67
III	July	19.3	111.5	1.54
III	October	19.5	116.7	1.56
IV	July	19.8	123.0	1.58
IV	June	19.9	119.5	1.51
IV	July	20.3	134.5	1.61
IV	July	20.4	129.0	1.52
IV	June	20.7	138.5	1.56
IV	July	21.4	133.5	1.35
V	June	22.2	175.5	1.60
V	July	22.4	169.0	1.50

The condition factor, K, a constant in the relationship between form (standard length in millimeters) and specific gravity (total body weight in grams) is used widely by fishery investigators to express the "degree of well-being" of fishes (Lagler, 1950). Sharp changes in the value of K may be expected at spawning as the expulsion of spawn suddenly decreases the weight. In order to discover if the condition of Mylocheilus varied just prior to and after spawning, it is necessary to keep other factors as constant as possible. Therefore, the fish for which the condition factor was calculated were all adult males. These are listed separately on Table IV. Considered collectively by the month, the condition factors are:

Number of fish	Month of capture	Average condition factor	Age
6	June	1.55	3 in 3rd 2 in 4th 1 in 5th
8	July	1.53	3 in 3rd 4 in 4th 1 in 5th
4	October	1.65	3 in 3rd 1 in 4th
1	December	1.67	1 in 3rd

The trend in condition of Mylocheilus from Flathead Lake in 1951 shows a slight decrease in relative robustness during the spawning period with a gradual increase in "degree of well-being" in the period which corresponds to the initiation of the first stages of spermatogenesis.

Feeding Activities

The feeding activities of the chubs during the spring and summer are sporadic. They take food during the breeding season, in fact their alimentation during this period appears to be no different from the pre- and post spawning periods (Table V).

The stomach contents revealed that molluscs are taken in large numbers, the two principle species being Helisoma anceps and Lymnea sp. (identified by Dr. R. B. Brunson). Both adult and larval insects are eaten; on these, Coleoptera are taken most frequently, and some Diptera larvae and a few Hymenoptera are also included. Algae and detritus are found in abundance in some stomachs.

Parasitism

Fourteen per cent of the adult chubs studied were parasitised by intestinal cestodes and nematodes. Plerocercoid larvae of Ligula sp. (identified by Edmund Jeffers) were found around the gut in the body cavities of three of the two hundred fish examined. It is known that many fish including minnows, suckers, pike, and perch act as intermediate hosts of this parasite, the final host being gulls and other water birds (Hunter and Hunter, 1932). The life cycle of the parasite is not known, nor its effects on its hosts. However, if the sample taken was representative of the Mylocheilus population in Flathead Lake, 1.5% of the chubs are parasitised by Ligula.

TABLE V

NOTES ON FEEDING ACTIVITY

	MALES			FEMALES		
Date of capture	Number of fish	Range in feeding activity	Average feeding activity	Number of fish	Range in feeding activity	Average feeding activity
5/28	1	none	none	2	very active to active	active
6/16	11	none to very active	slight	9	none to very active	slight
6/20	10	slight to very active	moderate	10	none to very active	slight
6/28	10	none to active	almost none	10	none to very active	slight
7/5	12	none to active	moderate	11	none to active	moderate
7/12	9	none to moderate	almost none	10	none to very active	moderate
7/19	10	none to very active	slight	10	none to active	slight
7/27	8	slight to moderate	slight	8	none to moderate	slight
8/4	8	none to very active	slight	9	none to very active	moderate
9/6	1	slight	slight	0	——	——
10/13	10	none to slight	slight	5	none to very active	slight
11/3	4	none to very active	moderate	6	slight to very active	moderate
11/24	1	moderate	moderate	2	slight	slight
12/8	1	active	active	0	——	——

CHAPTER V

CONCLUSIONS

Time of Spawning

The testes of the Columbia River chub undergo definite cyclic alterations which reveal the time of year in which this fish breeds in Flathead Lake (Fig. 1). In early May of 1951, cysts of germ cells which had developed to secondary spermatocytes during the previous fall and winter months commenced their final maturation division. There was a period of quiescence during late winter and early spring since the cysts had reached the secondary spermatocyte stage by December. This period of quiescence is not peculiar to the chub. In Phoxinus there is a slow conversion of spermatogonia into primary spermatocytes during the winter, followed by a dormant period, and then in the spring - just before spawning - there is rapid formation of spermatozoa (Bullough, 1939). However, there is one principle difference between these two cyprinids, which is that the sex cells pass their period of dormancy in Mylocheilus as secondary spermatocytes, whereas they remain as primary cytes in Phoxinus.

By the last of May, maturation in the testes of the chubs had progressed so far that approximately 1/4 of the germ cells in the gonad were spermatozoa. In the middle of June, approximately half the fish had their testes 1/2 filled with sperm and the other half 3/4 filled. Towards the last of this month, a quarter of the fish examined had fully matured testes, packed with spermatozoa. In the first week of July, half the fish had partially spawned, one was spent, one was fully matured but had not spawned, and one was not yet fully ripened. On the 12th of July, three quarters of the fish were fully mature, the other quarter were completely spent. By the 19th of July, one fish out of five examined had not yet spawned, but was still fully mature. Of the other four, two were partially spent and two completely spent. This week marked the end of the spawning season, as the fish netted on July 27th were either completely spent or were in the process of reconstituting their testes.

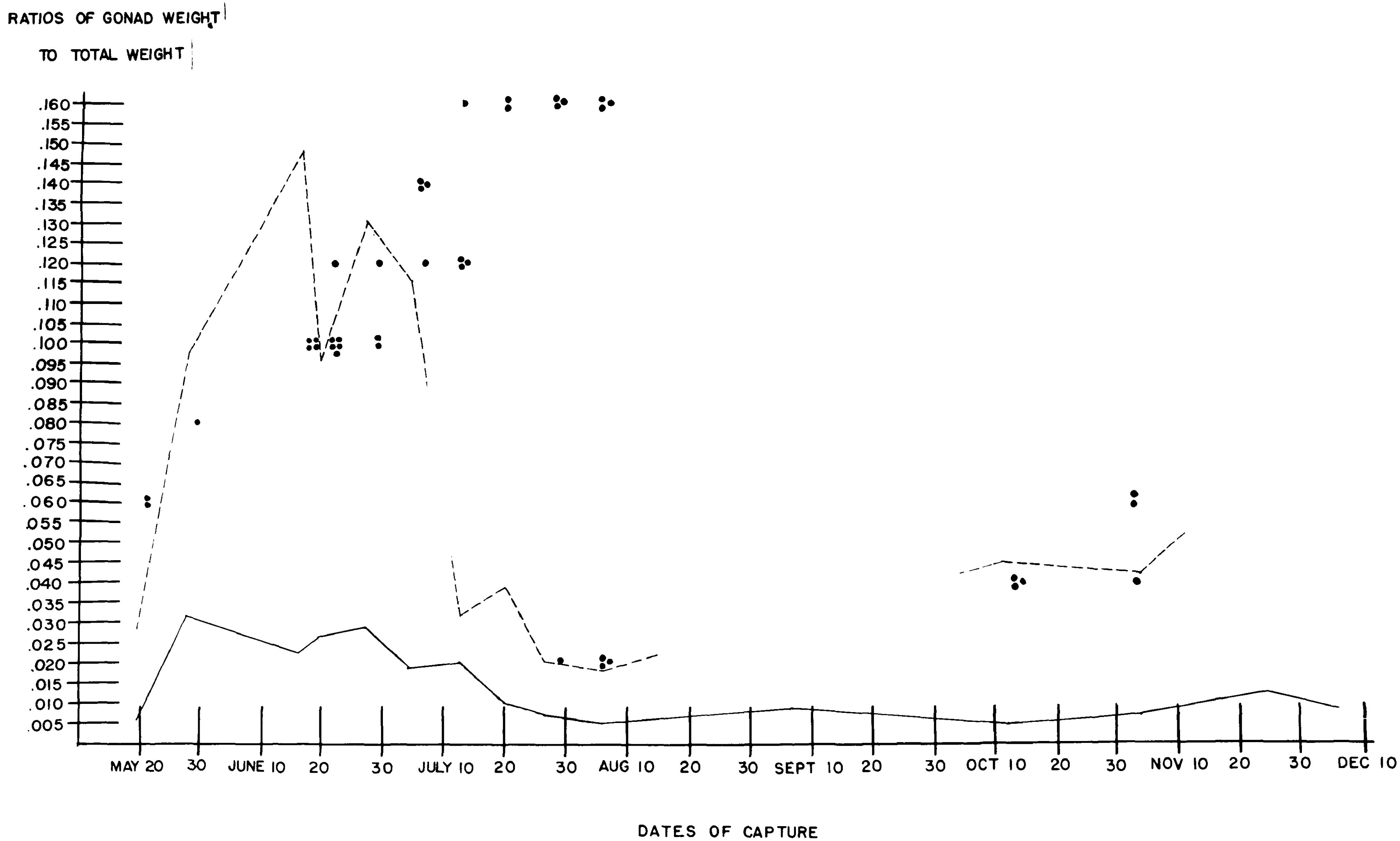
During active spermatogenesis, spermatogonia remain dormant along the connective tissue strands of the lobules, and it is from these cells that the testes are replenished directly after the sperm have been shed. This same condition has been described for Cottus, (Hann, 1927), Fundulus, (Mathews, 1938), Phoximus, (Bullough, 1939), and Salmo (Weisel, 1943).

By August 4, all fish studied had many well developed spermatogonia along the connective tissue strands. There was no advance in development of the testes by September 6 over the stage described in August. However, by the 13th of October, cysts of both primary and secondary spermatocytes had been formed in one pair of testes out of four, the other three pairs having developed only to cysts containing primary cytes. Testes taken on November 3 and 24 and December 8 showed gradual development toward the formation of cysts containing secondary spermatocytes. The testes must then become comparatively quiescent, as testes taken from fish in the first of May are hardly more advanced than those in November.

The weight of the testes alone cannot be used as a reliable criterion on which to base degree of maturity because of the variation in size of fish. However, the proportion between the total body weight and the weight of the testes is deemed reliable as it is fairly constant for a large number of individuals in any given season (Turner, 1919). By applying Turner's proceedure, it is evident that the average proportional weight of the testes increased in late May, reached a climax in late June, and gradually declined during July, several months after which the weight began to increase again (Fig. 3). The ovaries underwent a more spectacular proportional weight variation which, as expected, conformed to the male cycle. The gravimetric analysis agrees with the histological changes which occurred in the testes.

From the above evidence, spawning undoubtedly took place from July 5 to July 19 during 1951. However, 1951 had an exceptionally late spring. March was the coldest since state wide records began in 1895, having an average temperature of 27.2° F., 6.4 degrees below the normal expected average temperature. During April there was an extensive cold wave which, however, did not lower the average temperature of 41.3° F. more than 2.2 degree below the normal. May was a more typical month, having an average of 49.8° F. only 1.4 degrees below normal. June was the coldest since 1895 with an average temperature of 53.0° F., 4.9 degrees below normal (Climatological Data - Montana, 1951). As warmer water temperature might hasten the time of spawning in other years (Bullough, 1939), it is very possible that Mylocheilus often breeds earlier. However, it may be safely stated that the chub spawns in the very late spring or early summer in Flathead Lake. This agrees with Schultz's findings that chubs spawn during late May and early June in

Figure 3.-The ratio of gonad weight to total weight of male and female *Mylocheilus* in different months of the year. Superimposed on this figure is the relative maturity of the testes as shown on Figure 1.



RATIOS OF GONAD WEIGHT
TO TOTAL WEIGHT
.160
.155
.150
.145
.140
.135
.130
.125
.120
.115
.110
.105
.100
.095
.090
.085
.080
.075
.070
.065
.060
.055
.050
.045
.040
.035
.030
.025
.020
.015
.010
.005
MAY 20
30
JUNE 10
20
30
JULY 10
20
30
AUG 10
20
30
SEPT 10
20
30
OCT 10
20
30
NOV 10
20
30
DEC 10
DATES OF CAPTURE

Lake Washington, Washington.

From histological examination of testes taken during the period of spawning, it cannot be definitely stated that a fish spawns completely in one act. The testes of Lepomis, a fish that spawns throughout the summer months, is described as having loosely packed spermatozoa present in the lobules during this whole period (James, 1946). In general, the partially spawned testes of Mylocheilus correspond to the described condition of Lepomis, but the partially spawned condition could have been caused by the struggling of the fish in the net or by the partial stripping when handled in taking them from the net. If partially spawned testes are not artificially induced, some testes taken from fish in July indicate that the chubs spawn a number of times within approximately a two week period.

Age, Growth, and Condition

Fish in their 1st, 2nd, 3rd, 4th, and 5th seasons were taken from Flathead Lake in 1951. The seven yearlings were seined in Yellow Bay, Flathead Lake, in July, 1951. With this yearling material added to the data on mature fish, Table IV, the age and growth of Flathead chubs may be summarized:

Age	Number of fish	Range in standard length in cm.	Average standard length in cm.	Average Growth per year in cm.
I	7	4.4 to 5.5	5.11	5.11
II	0	——	——	——
III	10	16.9 to 19.5	18.50	——
IV	7	18.1 to 21.4	20.40	1.90
V	2	22.2 to 22.4	22.30	1.90

There must be rapid growth in the second year, after which it tapers off to a slow increase when sexual maturity is reached.

M. caurinum from Okanogan Lake, British Columbia, were measured and aged by Clemens (1939). Using fork length rather than standard length he determined the following relationship of age, size, and growth:

Age	Average fork length in cm.	Average growth per year in cm.
I	5.00	5.00
II	10.50	5.50
III	14.00	3.50
IV	16.75	2.75
V	19.50	2.75

No direct correlation is made with his findings and those for chubs from Flathead Lake, as the Montana fish were taken over a half-year period and their lengths were taken to the caudal base rather than to the caudal fork. No conversion factor to equalize standard length to fork length has been calculated for Mylocheilus. However, in comparison with the Okanogan fish, it would seem that there is faster growth in Flathead Lake for the first two years, but afterwards that growth is slower.

The condition of Mylocheilus varies slightly during the time of spawning and after spawning; in June the average condition factor is 1.55; in July, 1.53; in October, 1.65; and in December, 1.67. Probably because Mylocheilus apparently feeds throughout the year (Table V), it's condition is not as extremely affected by spawning as is the condition of fish such as Salmo salar that cease feeding during the breeding time (Belding, 1934).

Age at Spawning

The age at which the testes probably first ripen in Mylocheilus is 3 years. Although one male in his third season was not yet mature, over a third of the fish in spawning condition were in their 3rd season. No two year old males were known to spawn - none were caught with the spawning population. One female aged at two years was shown histologically to be immature which is further evidence that maturation usually occurs in the third year. Fish from 22.2 to 22.4 cm. long, the fewest in number, were aged at five years and were spawning or had spawned. The rapid reconstruction of the testes after spawning coupled with the fact that chubs in their 3rd, 4th, and 5th seasons were in the spawning condition indicates that they spawn in successive years.

Secondary Sex Characters

Coloration: Many cyprinids exhibit a definite difference in coloration between the sexes, particularly during the spawning season. In Richardsonius balteatus (Weisel and Newman, 1951), Hybognathus regius (Raney, 1939), Rhinichthys atratulus (Raney, 1940), and Notropis cornutus (Raney, 1940) the males are more brightly hued than the females. However, the quality of coloration of Mylocheilus did not vary from season to season or from sex to sex more than it varied from individual to individual. Coloration has been attributed to chromosomal inheritance in Lebistes (Blacher, 1926) transmitted by the X- and Y-chromosomes. The Lebistes females, even if possessing known genes for color on the X-chromosome, does not phenotypically exhibit the color character. It is thus hypothesized that the presence of the male hormones elaborated by the testis is necessary for the phenotypic expression of color. Other investigators (Zahl and Davis, 1932) observed the effects of gonadectomy on the secondary sexual characters of Amia calva and stated that the caudal ocellus in males is primarily independent of the testicular principle, but is secondarily intensified by it during the breeding season, and that the caudal ocellus is potentially present in females but that its appearance is inhibited by some principle, presumably an endocrine secretion elaborated by the ovaries. Although no breeding or gonadectomy experiments were performed on Mylocheilus, it is probable that the inherited color characters are relatively unaffected differentially by testicular or ovarian secretions, inasmuch as there is little or no seasonal variation and little or no sex differentiation.

Tubercular Development: On the other hand, there is a seasonal cycle exhibited by the appearance and disappearance of tubercles which can be correlated definitely with histological changes in the testes and with weight changes in the ovaries (Fig. 3). With the early start of maturation of the testis in the fall, the tubercle development was initiated. The tubercles increased in height and in number until the end of June. During and after spawning there was a rather rapid decline in both number and height until none were visible (Table III). Not only is the breeding cycle broadly indicated by tubercle growth, but also there is an average difference in numbers between males and females. However, there is an overlap in the range of numbers in males and females so that it is not a reliable point upon which to distinguish sex. It is possible that the tubercle development is initiated by gonadal secretions of both sexes but that there is a greater phenotypic response to the presence of the testicular principle.

Relative Size: It is in size that the greatest external difference between male and female *Mylocheilus* is illustrated. The females average 1.3 cm. longer and 31.3 gm. heavier than males. The following is a summary from Tables I and II:

Sex	Number of fish	Average weight in gm.	Average standard length in cm.
males	41	123.5	19.9
females	41	154.8	21.2

Schultz (1935) in his observations of spawning chubs on June 1, 1935, reported the following measurements of spawning chubs:

Sex	Number of fish	Average weight in gm.	Average standard length in cm.
males	25	123.8	19.7
females	34	203.6	22.4

A comparison of these two groups of data shows that spawning males from both areas are similar in both length and weight, but that there is considerable difference between the females. However, they both illustrate that the spawning females are larger in both weight and length than the spawning males.

Feeding

In general, the stomach contents of the chubs from Flathead

Lake coincide with the findings of Carl and Clemens (1948). These authors found the chubs feeding largely on aquatic and terrestrial insects, water-fleas, occassional molluscs and rarely on small fish such as sculpin. Although no fish remains were found in the stomachs of *Mylocheilus* examined by the author, one chub out of 87 examined from Flathead Lake by Dr. R. B. Brunson and Richard Bjorklund did have unidentified fish remains in its digestive tract (personal communication).

Jordan and Evermann (1896) state that the *Mylocheilus* "frequent the spawning beds of salmon where it devours their eggs." Although some chubs were netted during the salmon spawning season in October, November, and December there is no evidence of their eating salmon eggs.

CHAPTER VI

SUMMARY

1. One hundred and two male and ninety-eight female Columbia River chubs (Mylocheilus caurinum) were gill netted in Flathead Lake, Montana, during May 20 to December 8, 1951.

2. Histologically, their testes undergo a cyclic change. In early May the testes were packed with cysts of secondary spermatocytes. Towards the middle of this month spermatogenesis occurred rapidly and a few spermatozoa appeared. The spermatozoa increased in number through June so that by the end of June many of the testes were fully matured. From the first to the middle of July most of the cysts of spermatocytes disappeared, the testes either being packed with spermatozoa or spent. Spent testes were rapidly reconstructed in August and September by spermatogonia which had remained dormant within the connective tissue strands during the previous maturation of the testes. Corresponding to the reconstitution of the testes by spermatogonia was the reabsorption of spermatozoa and spermatocytes which had not been extruded during spawning. By November the reconstructed testes contained cysts of primary and secondary spermatocytes. There is evidently a period of quiescence from December to May, after which a flurry of spermatogenesis commences anew.

3. The cytological changes in the testes firmly imply that in 1951 the majority of the chubs spawned from July 5 to July 19. It was not determined whether they spawned once or several times during this

period. The spring of 1951 was exceptionally late, so in other years breeding may occur earlier. But, in general, it may be said that the chub is a late spring or an early summer spawner.

4. The gross appearance of the ovaries and the ratio of gonad weight to total body weight in both sexes, figured for each fish over the entire period of study, confirmed this time of spawning.

5. The majority of Mylocheilus spawned in their 3rd and 4th years. A few spawned in their 5th. No male chubs were netted that were over 5 years old.

6. The rapid reconstruction of the testes is evidence that the males, at least, spawn in successive years.

7. There is no accurate means of identifying the sexes by external characters. The color of the males and females was similar and they both possessed tubercles during the breeding season. The only pronounced sexual dimorphism was in the comparative lengths and weights. Adult females averaged 1.3 cm. longer and 31.3 gm. heavier than the males.

8. The colors were only slightly heightened during the spawning season and cannot be used as an indicator of sexual maturity. The tubercles, however, appeared in the late fall when the testes were undergoing the first stages in maturation, then commenced to disappear after the testes were fully matured. On the whole, the males were more heavily tuberculated than the females.

9. Food was taken during the spawning season. Molluscs, Coleoptera, larvae of Diptera, some plant material, and a few Hymenoptera constituted the bulk of the diet. There was no evidence of chubs eating

salmon eggs, although they were taken when salmon were spawning. Also, no fish remains were found in their stomachs.

10. Fourteen per cent of the chubs were parasitized by intestinal cestodes and nematodes, and 1.5 per cent by the plerocercoid larvae of Ligula.

LITERATURE CITED

Aida, T. 1921. On the inheritance of Color in a Freshwater Fish, Aplocheilhis latipes. Genetics 6: 554-572.

Belding, D. L. 1934. The cause of the high mortality in the Atlantic salmon after spawning. Trans. Amer. Fish. Soc. 64: 219-222.

Blacher, L. J. 1926. The dependence of sex characters upon testicular hormones in Lebistes. Biol. Bull. 50: 374-381.

Bullough, W. S. 1939. A study of the reproductive cycle of the minnow in relation to the environment. Proc. Zool. Soc. London, Ser. A. 109: 79-102.

Carl, G. C. and W. A. Clemens. 1948. The Fresh-water Fishes of British Columbia. Brit. Col. Prov. Museum, Dept. of Ed., Handbook no. 5. p. 76-77. Victoria, B. C.

Champy, C. 1923. Observations sur les caracteres sexuels chez les poissons. Compt. Rend. Soc. Biol., Paris. 88: 414-417.

Clemens, W. A. 1939. The fishes of Okanogan Lake and nearby water. Bull. Fish. Res. Bd. Canada 56: 27-38.

Courrier, R. 1921. Sur le conditionnement des caracteres sexue les secondaires chez les poissons. Compt. Rend. Soc. Biol. Paris. 85: 486-489.

Davidson, F. A. and O. E. Shostrom. 1936. Physical and chemical changes in the pink salmon during the spawning migration. U. S. Dept. of Commerce, Bur. of Fish., Invest. Rept. No. 33. II: 1-37.

Evermann, B. W. 1891. A reconnaissance of the streams and lakes of Western Montana and Northeastern Wyoming. Bull. U. S. Fish. Com. XI: 3-60.

Hann, A. W. 1927. The history of the germ-cells of Cottus bairdii. J. Morph. 43: 427-497.

Hunter, G. W. and Wanda S. Hunter. 1932. Studies on parasites of fish and of fish-eating birds. Suppl. 21st. Ann. Rept. N. Y. St. Cons. Dept., Biol. Surv. Oswegatchis and Black river systems. 1931: 252-271.

James, M. F. 1946. Histology of gonadal changes in the bluegill, Lepomis macrochirus Rafinesque, and the largemouth bass, Huro salmoides (Lacepede). J. Morph. 79: 63-89.

Jordan, D. S. and B. W. Evermann. 1934. American Food and Game Fishes. Garden City, New York. p. 72-73.

Lagler, K. F. 1950. Studies in Freshwater Fishery Biology. Ann Arbor, Michigan. p. 52-80.

Matthews, S. A. 1938. The seasonal cycle in the gonads of Fundulus. Biol. Bull. 75: 66-74.

Moore, G. A. 1937. The germ cells of the trout (Salmo irideus Gibbons). Trans. Amer. Mic. Soc. LVI (1): 105-112.

Raney, E. C. 1939. The breeding habits of the silvery minnow, Hybognathus regius Girard. Amer. Midland Naturalist. 21: 674-680.

Raney, E. C. 1940a. The breeding behavior of the common shiner, Notropis cornutus (Mitchill). N. Y. Zool. Soc. XXV: 1-20.

Randy, E. C. 1940b. Comparison of the breeding habits of two subspecies of black-nosed dace, Rhinichthys atratulus (Hermann). Amer. Midland Naturalist. 23: 399-403.

Rept. Explor. and Surveys to Ascertain the most Practicable and Economical Route for a Railroad from the Mississippi River to the Pacific Ocean ——— 1853-1855. Vol. 12 out of 12 vols. part 2, p. 359. Washington, D. C.

Schultz, L. P. 1935. The spawning habits of the chub, Mylocheilus caurinus - a forage fish of some value. Trans. Amer. Fish. Soc. 65: 143-147.

Turner, C. L. 1919. The seasonal cycle in the spermary of the perch. J. Morph. 32: 681-711.

U. S. Dept. of Commerce, Weather Bureau. March, 1951. Climatological Data - Montana. LIV (3). San Francisco, California.

U. S. Dept. of Commerce, Weather Bureau. April, 1951. Climatological Data - Montana. LIV (4). San Francisco, California.

U. S. Dept. of Commerce, Weather Bureau. May, 1951. Climatological Data - Montana. LIV (5). San Francisco, California.

U. S. Dept. of Commerce, Weather Bureau. June, 1951. Climatological Data - Montana. LIV (6). San Francisco, California.

Weisel, G. F. 1943. A histological study of the testes of the sock-eye salmon (Oncorhynchus nerka). J. Morph. 73: 207-230.

Weisel, G. F. 1949. The seminal vesicles and testes of Gillichthys, a marine teleost. Copeia 1949 (2): 101-110.

Weisel, G. F. 1952. Animal names, anatomical terms, and some ethnozoology of the Flathead Indians. Jour. Wash. Acad. Sci. (in press).

Weisel, G. F. and H. W. Newman. 1951. Breeding habits, development, and early life history of Richardsonius balteatus, a Northwestern minnow. Copeia 1951 (3): 187-194.

Winge, O. 1923. Crossing over between X- and Y-chromosomes in Lebistes. Jour. of Genetics 13: 254-276.

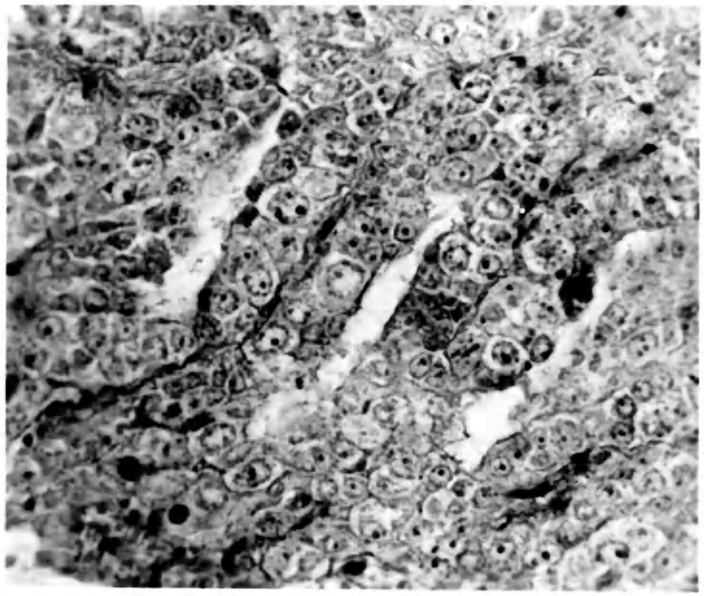
Zahl, P. A. and D. D. Davis. 1932. Effects of gonadectomy on the secondary sexual characters in the ganoid fish Amia calva. J. Exp. Zool. 63: 291-304.

Figure 4.–The immature testis

(magnification 400X)

Figure 5.–Testis in primary spermatocyte stage

(magnification 400X)



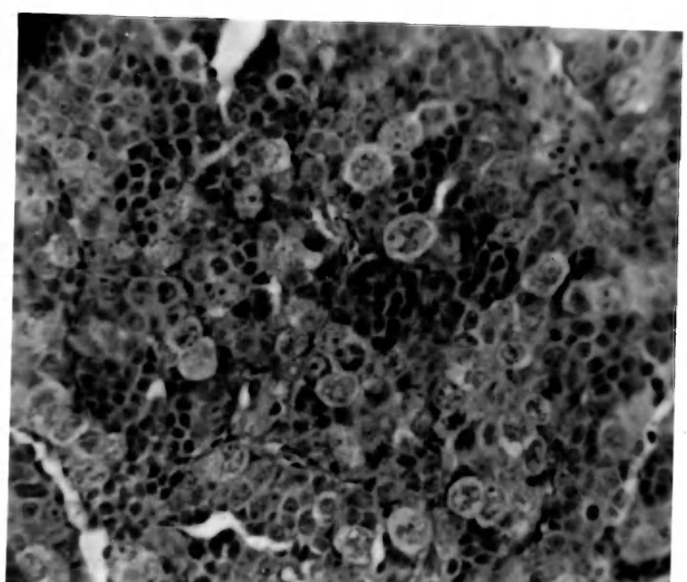
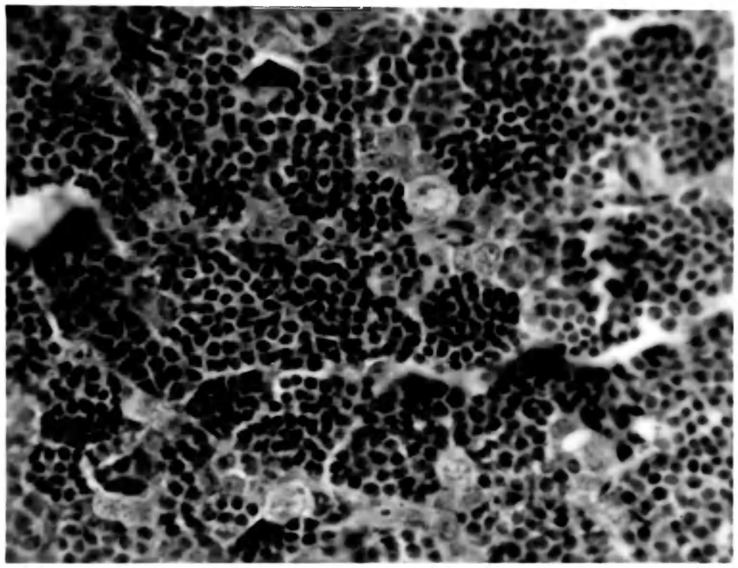


Figure 6.-Testis in secondary spermatocyte stage

(magnification 400X)

Figure 7.-Testis half to three-quarters filled with spermatozoa

(magnification 400X)



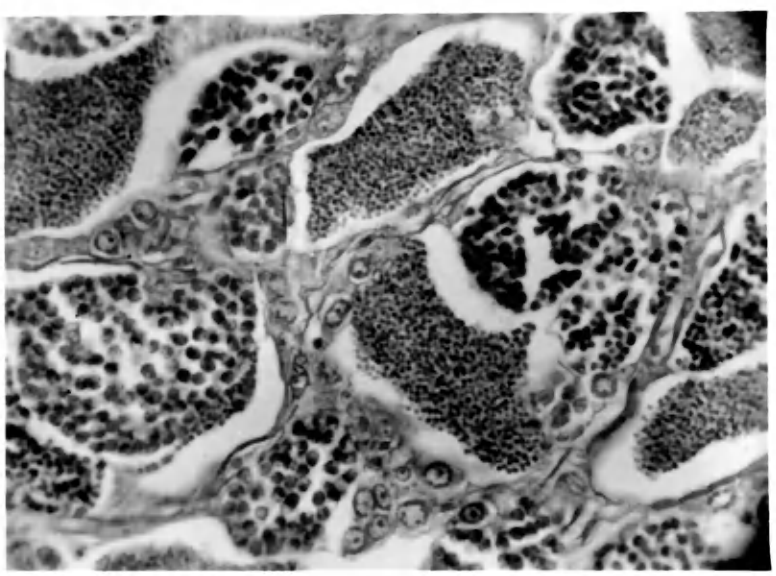


Figure 8.-Testis fully ripened

(magnification 400X)

Figure 9.-Testis fully spent

(magnification 400X)