SEX-RATIOS AND SPERMATOGENESIS IN THE TOP-MINNOW, *GAMBUSIA AFFINIS*.*

By

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SEX-RATIOS AND SPERMATOGENESIS IN THE TOP-MINNOW, *GAMBUSIA AFFINIS*.

Introduction.

SEX RATIOS REPORTED IN GAMBUSIA AND OTHER POECILIIDS. A very considerable number of observations of the proportions of males and females in field-collections of *Gambusia* and of other viviparous species of the family *Poeciliidae* has been made. These observations show that in most collections of these fish the females were very largely in the majority. In Table I there is collected in convenient form data on the sex-ratios of collections noted in the literature. The uniform character of the evidence pointing toward an atypical sex-ratio among the adults:- a sex-ratio that varied widely from that observed in other groups of vertebrates - has convinced workers on these fishes that adult populations taken in the field nearly always possess a low percentage of males. It has been suggested in explanation of such a sex-ratio that this discrepancy may be owing to the loss of the smaller males through the meshes of the net, or explained by the greater agility of the males, who thereby avoided the net. However, in cases where the net used had a mesh so small that loss of the smaller males through the interstices was excluded, the collections still showed the

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*Forma *holbrooki*.

same inequalities of numbers of the sexes. In every collection on record of *Gambusia affinis*, and in nearly all (over 80 percent.) of the field collections of other poeciliids, the same striking disparity of the sexes is evident.

HILDEBRAND (1917) in his excellent paper on Gambusia states his conclusions on the proportions of the sexes in this species. He says (p.10):

> The proportions of males to females in this species has been discussed by various writers. In collections the males are generally much in the minority. It has been argued that this is due to the small size of the males which permits them to pass through the meshes of nets and thus to escape capture. However, when the writer has collected the minnows with mosquito netting of a mesh so small that even the tiniest male may pass through, the disparity in the numbers of the sexes has remained evident. Among the lots grown in the aquarium, the inequality is quite as great as it seems to be in nature. For example, on June 2, 1915, 60 of the young of the previous season had survived, and of these only seven were males. The indications are that the broods of 1915 the sexes are just as unequally represented, although * * * the sexes cannot be positively determined at this time. (October, 1915). Owing to the great or heavy loss during the early stages of life in the aquarium, the results as stated above may not afford a reliable criterion, although there is no apparent reason why aquarium life should not be as well suited to the male as to the female. Among the adults there is much fighting between the sexes and the males often suffer severely, but among the young these disastrous conflicts have not been observed. It seems entirely probable that the normal ratio of males to females is about 1 to 8 or 9".

HUXLEY (1931) gives us light on observations made by BOULENGER subsequently to the publication of his 1912 paper. HUXLEY notes that for nearly a year there was an atypical sex-ratio of 1♂:7♀. This condition occurred in nearly every litter. During the Winter time, then, and for a period of about six weeks, the sex-ratio changed to

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about 3♂:2♀. At the expiration of this period the succeeding generations for several years maintained an approximately 1:1 ratio. These observations were made the subject of restudy by HUXLEY in the course of this very suggestive paper.

BARNEY & ANSON (1921) made an exhaustive study of the sex-ratios of collections of _Gambusia affinis_ at different seasons of the year. In the course of their observations, which extended over a period of eighteen months, they found that the percentages of males in the catches in the different months varied from 11.1 percent., a sex-ratio of 100♂:888♀, to 74.8 percent., a sex-ratio of 100♂:186.9♀. With the largest monthly collection (August, 1919) there were 4,258 individuals in 44 catches, with a sex-ratio of 100♂: 1076♀. The sex-ratios of the total yearly collections were 100♂:747.3♀ for the year 1918, and 100♂:672.6♀ for the year 1919.* As these percentages were based on the examination of a total of 13,780 Gambusia, fairly distributed through 18 months, their work is worthy of consideration. Although there is some likelihood that their net was too coarse to hold the smaller individuals, mostly males, and that their criterion of maleness, a developed gonopod, is hardly adequate as has been pointed out elsewhere (GEISER, 1921) nevertheless, there is no question that the conclusions that they drew, both as to the fluctuation of the sex-ratio at differ-

*Recalculated.

ent seasons of the year, and as to the great preponderance of the females in numbers, are essentially correct.

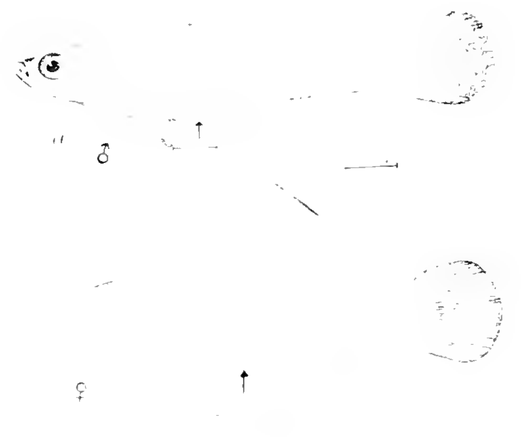
The writer has had populations under observation in which the percentage of males present was as low as 3.8 percent. By the method employed in catching them, it was not possible for a single individual, male or female, to escape, and thus one great source of uncertainty was avoided.

MAST (1921) found that in some populations of Gambusia that he studied, on dissection, a considerable proportion of the individuals which looked like females, and were counted as such, were males in which the secondary sex-characters were not developed. He designated these "sterile males" and suggested that the reports of excessive numbers of females might be due to the fact that many such males were counted as females. However, in the course of the writer's studies, when large numbers of females from populations showing low percentages of males were dissected to ascertain whether a considerable number of these 'females' might not really be sterile males, it was found that the percentage of sterile malesin such populations was too small to account, even in a small degree, for the very great disproportion in the numbers of the sexes.

The inequality in the sex-ratio is real, and cannot be

accounted for on the basis of faulty methods of collection or of sex-recognition (criteria).

CRITERIA OF SEX IN ADULT GAMBUSIA. In the whole sub-family Poeciliinae of the top-minnows, including Gambusia, there exists a well-marked sexual dimorphism. The males are much smaller in length than the females, and are much more slender in body. (Text-figure 1). The anal fin in the males is placed farther forward than in the females, and is modified into a conspicuous 'intromittent-' or copulatory-organ, the gonopod. So different is the Poeciliid male from the female that in one species male and female were originally described in different genera, and their identity was not discovered till some years later, when individuals of the two "species" were observed to copulate! This dimorphism gives a serviceable criterion of sex in adult Gambusia. In juvenile, immature forms it is not possible to easily ascertain the sex of an individual by these features. In the present study it was considered desirable to ascertain the limits of variation of these sex-characteristics, thereby gaining data which would permit one to ascertain readily, without dissection, or cytological study of the gonads, the sex of a given individual suspected of being a 'sterile male.' With this in mind, a study was also made of the variation in the position of the anal fin in males and females. A careful study was also made of the development in the males of the anal fin into the gonopod or intromittent organ. As this is one of the



TEXT-FIGURE 1.

ADULT GAMBUSIA, male and female. (After JORDAN & EVERMANN)

most striking criteria of sex, data concerning the time of its first appearance and its degree of development and differentiation in early stages were considered of the greatest importance and interest.

THE GONOPOD AND ITS DEVELOPMENT. The modified anal fin in the various groups of poeciliids became a spermatopod or a gonopod according to a definite structural plan. The gonopod is formed by the modification of the third, fourth, and fifth fin rays in species whose anal fin possesses from six to ten rays. The general plan of the gonopod, with very few exceptions is as follows. The first and second fin-rays are undivided and very short; the third ray was very much elongated; the fourth and fifth, also are considerably elongated; while the sixth to the tenth rays are normal, bifurcate, and segmented. The third to fifth fin-rays, also, possess modified ossicles. Genera and species, while following this general foundation plan, vary widely in the details of the structure of the gonopod, as LANGER (1913) has so beautifully shown. The primitive gonopod-form occurs in the genus Petalosoma. The apex of the gonopod may have a spoon-shaped process, the "prepuce" to aid in the sperm-ball transferrence to the genital papilla of the female during copulation. Such is the case in Petalosoma, Poecilia, and Mollienisia. Other modifications serving the same purpose are found in Phalloceros, in the form of hooks, and in Phallotorynus in the form of

a curious structure resembling a garden scoop or trowel.

The forms whose adult males possess gonopods are much more numerous than those having spermatopods. The spermatopod is made up of the modified first to fifth fin-rays of forms whose anal fin has more than 12 rays.

The writer's observations on the finer structure and progressive differentiation of the gonopod itself are reserved for future papers.

von IHERING (1883) was the first to present an account of the development into a gonopod of the anal fin of a poeciliid fish. He called attention to the fact that in the differentiation of the fin of <u>Phalloceros caudomaculatus</u> certain fin-rays (the third, fourth, and fifth) became modified to form a special apparatus to carry the sperm to the genital aperture of the female. He noted also the fact that the process of differentiation did not occur at any fixed time, or on the attainment of any definite body-length, but was a process that might be long delayed, or that, on the other hand, might occur precociously. He says in part:

> Merkwürdig ist die Entwicklung dieses ganzen Apparatus. An Männchen von unten 15 mm. Länge ist kaum ein Unterschied von Weibchen zu beobachten. Männchen von 17-18 mm. Länge - von der Schnautzenspitze bis zur Ende der Schwanzflosse gemessen - zeigen die Analflosse schon erheblich verlängert, allein weder von dem Zangenapparat des Endes

> noch von den Zähnen am hinteren Ast des 4. Flossenstrahles ist das geringste zu bemerken. Die Flosse ist verlängert, aber die drei verlängerten Strahlen enden nach vorn zugespitzt, resp. in einem Büchsel feinster Strahlen auslaufend, wie solche auch an den anderen Flossenstrahlen oft bemerkt werden. * * * * Männliche Tiere von 24 mm. Länge und darüber haben fast immer den Zangenapparat wohl entwickelt. (So namentlich zur Zeit des Beginnes der Geschlechts-Saison im Frühjahr. Ende des Sommers, resp. Herbstes, dagegen, trifft man auffallend grosse Männchen mit sehr langer und nicht differenzierter Analflosse an und wenig entwickelte Hoden. Es sind offenbar Junge derselben Saison entstandenen Tiere, welche erst im folgenden Sommer geschlechtsreif werden). Tiere von 20-21 mm. Länge zeigen das Stadium in welchem die Zähne sich ausbilden und bei Tieren von 21-22 mm. Länge findet man bald jenes Entwicklungs stadium vertreten. Die Entwicklung geht mit dem Längenwachstum nicht streng Hand in Hand, denn ich traf Tiere von 21 mm. mit völlig entwickelten Zangenapparate, und anderseit solche von 22 mm. Lange, an denen eben erst die Zähne zur Anlage kamen. Ausnahmsweise traf ich auch erheblich grössere Männchen von letzterem Stadium und es wäre daher wohl möglich, dass bei manchen Exemplaren der Zangenapparat atavistischer Weise niemals zur Ausbildung käme.

PHILIPPI (1908) in speaking of the same species (*fide* HENN, 1916) notes also the fact that occasionally the sex is not evidenced by the development of the gonopod. On p. 17 he maintains:

> Am neugeborenen Tier ist äusserlich das Geschlecht in keiner Weise zu erkennen. Zwar lassen sich bei Lupenbetrachtung Exemplare mit bögiger Uebergang des untern zum hintern Rand der Anale von solchen mit schrofferer Eckenbildung unterscheiden, bei welch letztern dann die Anale einen etwas längern Eindruck macht, doch hat die histologische Untersuchung der Gonaden solcher Tiere später gezeigt dass diese Differenz nicht mit einer solchen im Geschlecht korrespondiert, sondern eine sowohl bei Mannchen als bei Weibchen vorkommende Variation ist, deren Extreme übrigens durch alle Übergange verbunden sind. Im Laufe der Entwicklung Hand in Hand gehend mit der Ausbildung des Hodens, streckt sich die Anale in die Länge, und schliesslich trete die Wiederhaken an einem der Flossenstrahlen und am distalen Ende der ganzen Flosse der Klammerapparate auf.

And at a later place (p. 37) he states that he has found an individual of *Cnesterodon 10-maculatus* or *Phalloceros caudomaculatus* (it is not clear from the context which species is meant) 127 days old whose anal fin had not yet shown the least tendency to develope into a gonopod; yet this individual on cytological examination of the gonads proved to be a male. He agrees with GERLACH (1902) however, that 4-week old males of *Cnesterodon 10-maculatus* (15-20 mm. body-length*) are differentiated so that the sex may be ascertained by an examination of the anal fin.

LANGER's (1913) figures 31-33, illustrating the developmental stages of the gonopod of *Xiphophorus helleri* illustrate exceedingly well the great variability in the development of the modified anal fin, from the view-point of time. Fig. 31, his "Stadium 1" is of an animal 164 days old, while Figures 32 (Stage III) and 33 (Stage VI) are from individuals whose ages are 128 and 107 days, respectively. LANGER notes the fact that apparent females suddenly devel-

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*PHILIPPI, in his paper, always measured the fishes from the tip of the nose to the base of the caudal fin, although von IHERING (1883) had always measured total-lengths. In the course of the writer's work, he has recorded only total lengths. In comparing data gotten by the two methods, it is well to remember that the body-length is approximately 4/5 of the total length. The results of measurements to ascertain the relation of body-length to total-length in *Gambusia affinis* gave the following coefficients (mean values):

Total length........ 23 24 25 26 27 28 29 30 31 32 mm.
%-reduction to give
body-length......... 17 19 20 18 18 18 16 20 18 17 percent.

op into males, long after the other males have developed their gonopods. He has given the approximate average times of differentiation of the gonopods in different poeciliids. These are presented in Table 2. LANGER suggests on the basis of a casual experiment that an abundance of light may be the determining factor in the development of the gonopod. He reports that

> von einem Wurf <u>Pseudoxiphophori bimaculati</u> am 10. Juli nach 51 Tagen ein Mannchen (sich)entwickelt hatte, nach ungefahr gleicher Zeit zwei von einem Satz am 31. August. Als ich daraufhin die Aquarien an einen ungenugend belichteten Ort brachte, erfolgten keine weiteren Umbildungen. In der letzten Januarwoche wurden die Aquarien wieder ans Fenster gestellt, und jetzt entpuppten sich fast zu gleicher Zeit, ungefahr am 15. Februar, beinahe alle Tiere aus beiden Wurfen als Mannchen.

HILDEBRAND (1917) has called attention to the fact, also, that in young <u>Gambusia affinis</u> the anal fins of the male and females are similar, and of course, are so at birth, and that the typical gonopod is gradually developed, and at no definite age and length of the fish. When the young are only 13 mm. long and less than three months old, the gonopod is sometimes developed: on the other hand, the fish may be 17 mm. long and five months or even a year old, and it is still not developed. A lot of 43 young of which he writes, born in May, 1914, were examined on October 15 of that year (when the smallest was 17 mm. long) and it was thought that they were all females, as no gonopods had developed. On June 3, 1915, however, 6 of the surviving fish were easily recognized as males. The mortality was not

TABLE 2 .

Showing approximate ages at which the gonopodium develops in various Poeciliids.

Species	Age	Authority
Phalloceros caudomaculatus:	40 d.	LANGER.
Poecilia vivipara	40 d.	STANSCH.
Pseudoxiphophorus bimaculatus	50 d.	LANGER.
Platypoecilus maculatus	76 d.	"
Lebistes reticulatus	90 d.	"
Xiphophorus helleri	8-9 w.	"
Belonesox belizanus	30 w.	"
Mollienisia latipinna	16 W.	STANSCH.

stated, although he states elsewhere in his paper that the death-rate during early life in aquaria is inordinately high. From this he concluded that "the modification of the anal fin into an intromittent organ may take place when the fish reaches a length of 17 mm., or at any later stage until it attains its maximum normal growth of about 25 mm."

BELLAMY (1922) has noted that with his experimental litters of _Xiphophorus_ _helleri_ the gonopod developed in 136±60 days, while with _Platypoecilus_ _maculatus_, development took place in 141±80 days.

MAST (1921) as noted above, has given further evidence of the tardy development of the gonopod in the "sterile males" of Gambusia affinis. He found in these sterile males that the testes had already differentiated and were in a condition of advanced spermatogenesis. Even ripe spermatozoa were present in the form of sperm-balls. Yet with all this evidence of functional maturity, the anal fin gave no evidence as to the sex of the individual.

The writer's studies show that the gonopod develops rapidly during the summer, so that by the time the season is over, practically all the gonopods are developed and recognizable as such. The differentiation of the terminal portion of the gonopod into the characteristic hooks of , es-

pecially, the third, and the posterior branch of, the fourth fin rays does not, however, take place often until very late in the Fall, or during the Winter or following Spring.. This fact is well shown by a study of nearly 80 young males, 25+ mm. in length, killed Sept. 28, 1931. However, the percentage of 20+ mm. males which had not yet developed an elongated anal fin is so small that the atypical sex-ratios in the field collections cannot be explained on the basis of the undifferentiated males. The sex-ratios reported for adults appear to be essentially correct.

THE POSITION OF THE ANAL FIN AS A CRITERION OF SEX.

Some of the workers on poeciliid fishes have noted that in the male, the anal fin migrates forward during the period of sex-differentiation, so that when adulthood is reached the relative positions in the two sexes are quite dissimilar. The exact value of this index, i.e., the relation between the total length of the fish and the distance from the snout-tip to the anterior border of the anal fin, has never been calculated for these fish, although it is easily possible from some data given us in the literature to find a differential index. JORDAN & SNYDER (1901) have given us data which, when assembled and calculated, show characteristic indices for males and females. Thus, for Poecilia limantouri n.s., the average index for seven males is 2.496* as compared

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*I.e., $\frac{\text{total length of fish}}{\text{snout-anal distance}} = 2.496$.

with 1.771 for seven females; for Mollienisia latipinna the average index for two males is 2.374 as compared with 1.948 for three females. von IHERING (1883) gives data also for Phalloceros caudomaculatus from Brazil. Calculations based on his data result in an average index of 3.072 for four male and 2.432 for five females. Thus it will be seen that the index is always higher in the male (*i.e.*, the anal fin in the male is placed farther forward than in the female), although the ratios of the average values of the $\frac{\text{male-index}}{\text{female-index}}$ vary with the different species.

Tables 3,4, show the index values of two lots of Gambusia gotten from Beaufort, N. C., during 1920/21. These are the individuals whose total lengths are tabulated in Table From an inspection of these tables it will be seen that while there is considerable variation, the indices for both sexes have very definite norms. Thus, *e.g.*, in males the class 2.7-2.799 appears to be the mode, while in the females, class 2.1-2.199 is the mode. A comparison of the data of both tables, also, as they are assembled for comparison in Table 5 indicates that the index-values of the two sexes in the adults show very little overlapping. Graph I illustrates clearly the degree of variation. Thus, in this population, only 3.6 percent. of the males have an index value of less than 2.500, while, on the other hand, only 4.3 percent. of the females show an index in excess of 2.500. The findings in this population corroborate well the

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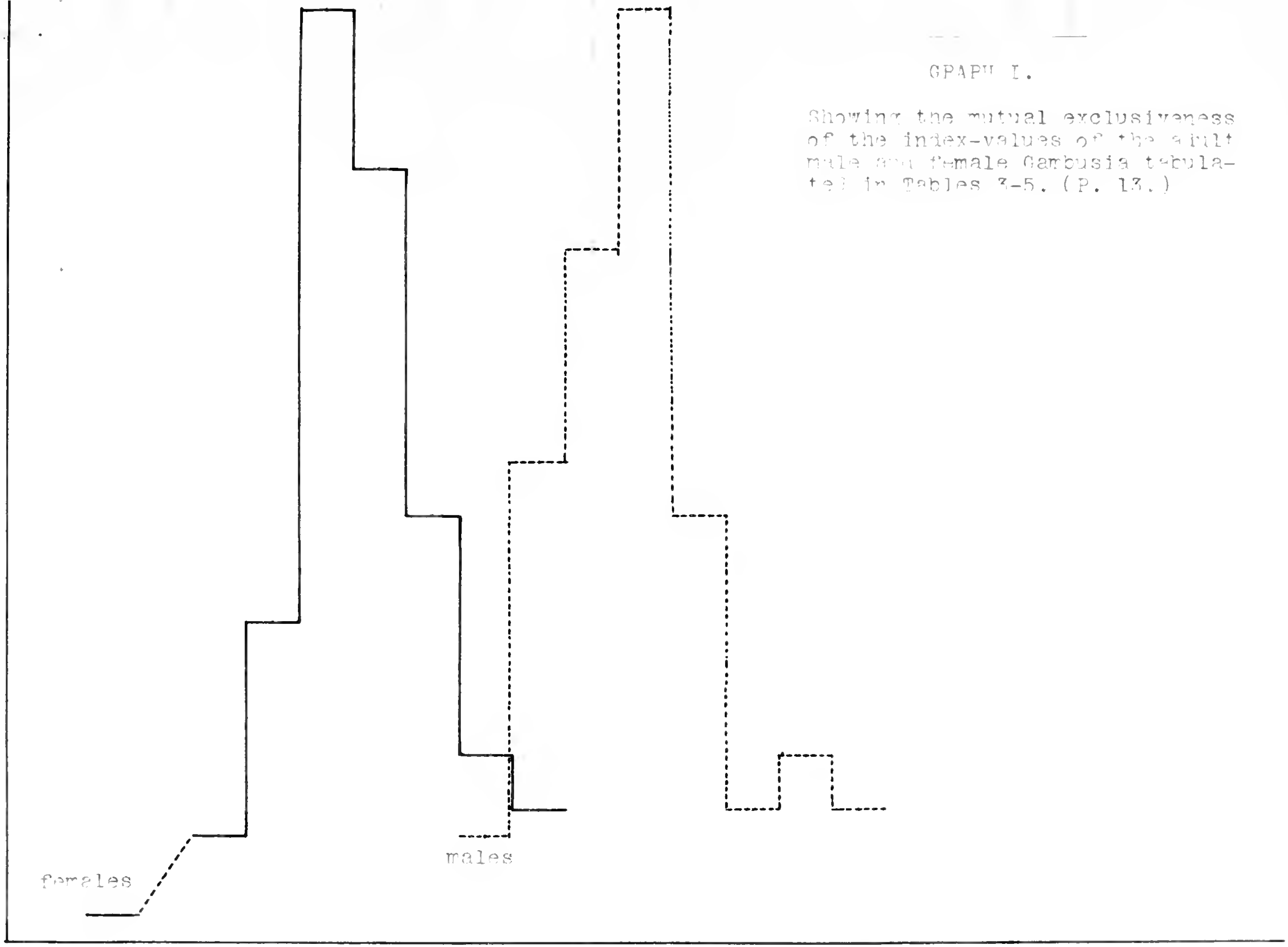
TABLE 5 .

Combined table, showing for comparison the distribution of the Indices of the males and females of Tables 3 and 4. .

Index values	Frequencies in the class Males	Females	% in Class. ♂	♀
1.700-1799		1		0.8
1.800-1.899				
1.900-1.999		4		3.4
2.000-2.099		12		10.3
2.100-2.199		35		30.1
2.200-2.299		29		25.0
2.300-2.399		16		13.8
2.400-2.499	4	7	3.6	6.0
2.500-2.599	18	5	16.2	4.3
2.600-2.699	26		23.3	
2.700-2.799	35		31.2	
2.800-2.899	16		14.6	
2.900-2.999	5		4.5	
3.000-3.099	7		6.4	
3.100-3.199	5		[illegible]	

GRAPH I.

Showing the mutual exclusiveness of the index-values of the adult male and female Gambusia tabulated in Tables 3-5. (P. 13.)



results of other series of measurements. Tables (omitted) give correlations between total -length and distance.

From this we see that the indexes for adult male Gambusia are such that by means of them the male can easily be distinguished from the females, in the lack of better criteria, even with rather old "sterile males" in which the gonopod is not well developed. The indices have rather sharply-defined limits, and these are nearly mutually exclusive. Applying these facts to the examination of suspected male Gambusia, we find that the number of sterile males is inconsiderable, and quite inadequate to explain the occurrence of unusual sex-ratios. The sex-ratios reported by field-workers appear to indicate a basic truth: there actually are in the field many more adult females than males.

From a study of the literature, it appears that but little work has hitherto been done on the sex-ratios of fishes of different groups, and what has been done refers practically only to adults. (FULTON, 1890, 1892, 1903). The reason is not far to seek. In sharp contrast with most mammals, among the lower vertebrates the sexes are indistinguisable at birth. Often a long period elapses before the young reptile, fish, amphibian or bird has developed secondary characteristics indicative of the sex of the individual. As a consequence the investigator of the sex-ratios of these

groups has usually contented himself with studying groups of adult fish. No account was taken of juvenile mortality. The connotation of the term "sex-ratio" then, has been 'sex-ratio of collection' in these groups.

DARWIN (1875) first called attention to the difficulties encountered in attempting to ascertain in fishes the proportions of the sexes at birth from data thus collected.

In nearly all reported sex-ratios of fishes the populations involved were not under experimental conditions, hence it is clear that the ratios reported - most of them varying markedly from the typical sex-ratio, in which the proportions of the sexes are practically equal - are complicated by unknown factors. Among these factors are two alternative ones that appear most likely: a, a possible differential death-rate during the juvenile and adult period, or, b, an atypical distribution of the sex-chromosomes to the two daughter cells in the maturation divisions of the germ-cells. As a result of this unequal distribution of the sex-determining chromosomes, a preponderance of one sex over another could conceivably be produced. The approximate proportions found, as 1♂:3♀ or 1♂:7♀ were suggestive of this explanation of the atypical sex-ratio.

The special chromosomes whose presence or absence ap-

pears in many groups of animals to be associated with a definite sex have not yet been reported on cytological grounds for any teleost fish. If we knew that such a chromosome (or chromosomes) existed in the sex-cells of a given species, thus giving rise to a dimorphic zygote, we could forecast approximately equal numbers of the sexes at birth (="secondary sex-ratio" of SCHULTZ, 1918) unless the gametes showed a differential viability. This cytological method of ascertaining primary and secondary sex-ratios is of course, the most dependable we have. Failing in this, practically sure conclusions may also be arrived at by taking large litters and raising them with as few mortalities as possible to a stage where the sexes may be distinguished, either by cytological study of the gonads, or by the development of the gonopod. Unless the early sex-ratios are ascertained by one of these methods, the results are quite untrustworthy. Apparently only one worker, EIGENMANN (1896) has done this. In his work on _Cymatogaster_ he ascertained the sex-ratios of litters before birth by microscopical examination of the gonads, and found it to be _ca._ 1:1. Only the ratios of adults can be learned by external observations of the individuals of a population. It is certain, of course, that if no differential death-rate exists in a given species, the sex-ratio of adults will give a trustworthy index to the sex-ratios at fertilization and at birth, as seems to be the case in the lake-whitefish, _Coregonus albus_, as reported by

PEARL (1916.)

As has been said, in Gambusia the females are greatly in excess of the males. The following cytological and experimental studies were undertaken with the view of ascertaining whether this is owing to peculiarities in the distribution of chromosomes, or to a differential viability in the gametes, or in the zygotes, either of embryonic or juvenile s stages.

II. THE GERM-CELL CYCLE, INCLUDING SPERMATOGENESIS, WITH ESPECIAL REFERENCE TO ITS BEARING ON THE EXPLANATION OF THE SEX-RATIO.

Introduction.

The illuminating genetical studies of SCHMIDT (1920) have demonstrated that *Lebistes* has an XX, XY chromosomal constitution. Since Gambusia and *Lebistes* are closely related, it is reasonable to assume that the chromosomal arrangement in these two forms is similar. If this is true, then we should expect in Gambusia equal numbers of potential males and females at the time of fertilization, and if this is what actually obtains, then the preponderance of females observed in adult populations must be due to a differential death-rate operating after fertilization. The importance of the investigation of spermatogenesis in this form is consequently obvious.

But little work has been done on the spermatogenesis of teleost fishes. The only title that the writer has been able to find is that by TURNER (1919) on the seasonal cycle in the perch. BÖHM (1891) and BEHRENS (1898) have investigated the oogenesis of the 'Forelle' and BLANC (1894) has studied the oogenesis of *Trutta lacustris*. Mrs. HARVEY

(1930) in her synoptic paper has noted a brief enumeration of other authors on diploid cleavage numbers in a number of other teleost fishes. But, except for the paper of TURNER, above noted no study appears to have been made of the maturation of the male sex-cells of teleosts. The explanation seems to lie in the fact that in this group of fishes the chromosomes are very numerous, small in size, and lack individuality.

MATERIALS. The Gambusia used in this study were from the P_1 stock of the experimental litters (q.v. in Section III of this paper). Thus the material for both cytological and breeding studies was from the same source. The stock had a very great preponderance of females, the males being outnumbered by the females nearly eight to one.

METHODS. Preliminary tests with FLEMMING's solution, and with many modifications of BOUIN's fluid, particularly ALLEN's (1918) modification, demonstrated the fact that the best fixation of prophase stages in the material was obtained by the use of BOUIN's fluid, modified by the addition of one percent. urea crystals (MERCK, C.P.). This was the fixing agent employed in all subsequent work on the cytology of Gambusia. With the best of care, however, it was not possible with any fixing solution to get good separation of the chromosomes in the spermatocyte metaphase plates, even though the earlier phases in the same section were

beautifully fixed. This difficulty in working with teleost testes is one that has troubled a number of workers. TURNER (1919) in his work on the perch was unable to obtain spread equatorial plates of chromosomes.

Both section- and smear-preparations were made. Sections were cut 5μ and 7μ thick. All preparations were stained by [illegible]'s (1910) long process in iron-hematoxylin. Occasionally counter-stains were used.

THE GENERAL ANATOMY OF THE TESTIS. In the adult fish the two testes are fused, forming a single gland lying just below the posterior portion of the swim-bladder, and anterior to the gonopod (Textfig. 2) The posterior portion of the intestine lies below and anteriorly to the combined testes (hereafter called the 'testis'). The large muscle controlling the movements of the gonopod lies just behind the testis.

The testis is whitish in color. It lacks the heavily pigmented investing membrane so characteristic of the ovary. It is suspended from the swim-bladder and the vertebral column by a very thin mesorchium. The surface of the testis when examined in the fresh condition shows a more or less favose network of cell-borders, under the thin investing membrane. These nettings are caused by the arrangement of the cysts in the testis.

TEXT-FIGURE 3.

a,
Male Gambusia, showing the position of the testes in relation to the other visceral organs. (After Kuntz.)

b, Free end of the intromittent organ, or gonopodium.

The testes vary with the season and the individual, in size. Thus, the median length of 19 taken at random on July 20, 1920 was 3.2 mm. and the median breadth 2.2 mm.; while the median length of five taken November 27, 1920 was 2.75 mm. and the median breadth 2.07 mm., a volume relation of approximately 1.6:1. The testes in individuals of the same litters vary but little in size, but they very greatly in individuals of the same size taken from different litters. (Tables 6,7.)

There is no prominent connective-tissue core in the testis of Gambusia such as TURNER (1919) described for the perch, and BROCK (1878, Taf. xxviii, Fig.3) figured for *Perca fluviatilis*. The testes of Gambusia consist of a tenuous, diffuse connective-tissue stroma, in the interstices of which the spermatogonia and cysts are closely packed. The structure of the adult testis resembles very closely that observed by von IHERING (1883, p. 486) in the South American poeciliid, *Phalloceros caudomaculatus*, and described by him in the following words:

> Die Zahl der Äste, in welche sich die Hodencanälchen resp. die Äste des Vas deferens spalten, ist eine sehr geringe. Die samenbildenden Zellen selbst, welche die Hauptmasse des Hodens bilden, liegen frei in dem Gerüstwerke feiner Membranen, welche die Hoden durchsetzen, und die gewissermassen die Ausläufer oder Fortsetzungen des Hodenkanälchen bilden. Diese selbst sind mit einem Cylinderepithel ganz wie das Vas deferens ausgekleidet. Es schien dasselbe mit Cilien ausgerüstet, allein die Erfüllung der Kanälchen mit coagulierter Flüssigkeit und zahlreichen Samenballen liess keine Gewiszheit gewinnen. Von den Samenbildungszellen liegen die grösseren und die grössten bis 0.03 mm., und mehr messenden mehr im Centrum des Querschnittes, in der Nähe des Vas deferens. Mehr gegen die Peripherie hin folgen

TABLE 6.

Showing the dimensions of five unselected Gambusia testes.
(Beaufort, N.C., Nov. 23, 1930)

Total l. of Fish	L. of testis*	*Br. of testis.
24.5 mm.	2.75 mm.	2.03 mm.
28.5	2.75	2.02
29.0	2.75	2.03
30.0	2.75	1.73
31.0	4.12	1.31

*Measured from camera-lucida drawings

TABLE 7 .

Showing the dimensions of 19 unselected Gambusia testes.
(Augusta, Ga., July 20, 1921).

No.	Length	Breadth
14	2.5 mm.	1.8mm.
16	2.5	2.3
5	2.6	2.0
4	2.8	2.0
6	2.9	2.2
9	2.9	2.0
11	2.9	2.3
13	2.9	2.5
17	3.0	2.7
15	3.2	2.2
3	3.3	2.8
2	3.5	1.1
8	3.5	2.4
10	3.5	2.0
7	3.65	2.6
18	3.7	3.0
12	4.2	2.2
19	4.5	3.0
1	4.8	2.5

kleinere und kleinste. Je kleiner die Zellen, um so geringer ist die Zahl der Kerne in ihnen, deren Zahl an den grossen Hodenzellen eine sehr hohe ist. Dieselben ordnen sich an der reiferen Hodenzelle an der Peripherie, und werden zum Kopf des Samenelementes. Dabei treten dieselben so an die Oberfläche, dass sie aus ihr ein wenig hervorragend der Oberfläche ein strahliges Aussehen verleihen. Die Spermatozoen bestehen aus dem erwähnten kurzen stark lichtbrechenden Körfe und einem daran ausschlieszenden Halsteile, welche allmählich feiner werdend in den auszerst zarten Schwanz übergeht. Die sämtlichen Körfe aller Spermatozoen welche in einer Hodenzelle entstanden, sind an der Peripherie der Zelle gelagert, indess der Fadenteil gegen das Centrum gerichtet ist. In diesem Zustande gelangen die reifen Hodenzellen aus ihrem Gerüstwerke in das Lumen des Hodenkanales resp. Vas deferens. Das Letztere ist in seinem letzten Abschnitte mit einer dicken Lage von Ringfäsern, jedenfalls wohl glatten Muskelfäsern, umgeben, zu denen noch eine dem Epithel anliegende Schicht von Langsfäsern hinzukommt. Die Zahl der Hodenkanäle ist am reifen Hoden sehr vermehrt, ohne dass indessen eine regelmässige Anordnung oder sehr hohe Zahl von Kanälchen, etc. einen rein tubulösen Bau zu Stande brachten wie es bei der Mehrzahl der Knochenfische besteht.

The histological picture drawn here is indeed remarkably similar to that portrayed by Gambusia. The writer's observations point to the general applicability to Gambusia of von IHERING's description of the occurrence of the larger cysts near the "vasa deferentia" The smaller cysts of multiplying spermatogonia are in the cortical portion of the testis. (Textf. 3). It is not correct for Gambusia to say, however, that the largest cysts occur in the inner, central portion of the testis. The cysts whose cells are in the spermatid stage are much larger than the mature spermatozeugmata, or sperm-balls as they are often called. The latter lie the closest to the center of the testis; the former, more peripherad. After the growth period preceding

TEXT-FIGURE 3.

Transverse section of a Spring testis, showing the zones of spermatogenesis. (Original.)

the first spermatocyte division, the cysts are larger than before. The 'Kerne' of the cells, which von IHERING mentions as being so much more frequent in the larger cysts are probably the plasmosomes of the earlier stages, and the spermatids and sperm-heads of the later stages. His decription of the formation of a spermatozeugma is quite correct for Gambusia.

As shall appear later, the relation of the ripe spermballs to the longitudinal testicular canal in Gambusia is not in accord with von IHERING's description, but his contention that there is no tubular structure in the testes of *Phalloceros* holds also for Gambusia. In the oviparous *Poeciliidae* we find testes with tubular structures (*cf*. *Fundulus heteroclitus*) but apparently with viviparity has come, in this family, fertilization of the eggs of the female by means of sperm-balls. These very probably are formed in essentially the same way in all the viviparous members of this family.

In the adults, the two testes are very closely united (Textfig.3): the double structure of the testis being evidenced only by the almost universal separation of the longitudinal testicular canal into two portions, one ramus on either side of the median line in the anterior four-fifths of the testis; and in the differential development of the

spermatocytes. (Textfig. 7.). Further discussion of this latter point will be given in another section.

EARLY DEVELOPMENT OF THE TESTIS. Embryos having a length of 1.5-3.0 mm. have the germ-glands as a paired structures lying in the typical location beneath the airbladder. (Textf. 4.) The germ-cells appear to be in resting stages. There is evidently at this period no high division-rate of the germ-cells. The gonad is enveloped with an investment of mesodermal cells. At birth (Textf. 5.) the gonads appear to be still undifferentiated. They are small paired cysts lying beneath the air bladder near the stomach, and they contain indifferent germ-cells. At this stage, the cells have prominent plasmosomes and appear to be in resting stages. Rather prominent granules of chromatin are scattered in a linin network.

About three weeks after birth, the primordial germ-cells begin to differentiate into male or female gonads. By the time the fish is four weeks old, temperature- and food-conditions being favorable, the sex of the young Gambusia can be ascertained cytologically. At this age the two testes are still separate (Textf. 6) except at their posterior ends, and are of variable, often unequal, size. A microscopic examination of the testes in section reveals

TEXT-FIGURE 4.

Germ-cells of a 1.75 mm. Carassius embryo. a), diagram showing the position of the gonads, b), the germ-cells surrounded by entoderm cells.

TEXT-FIGURE 5.

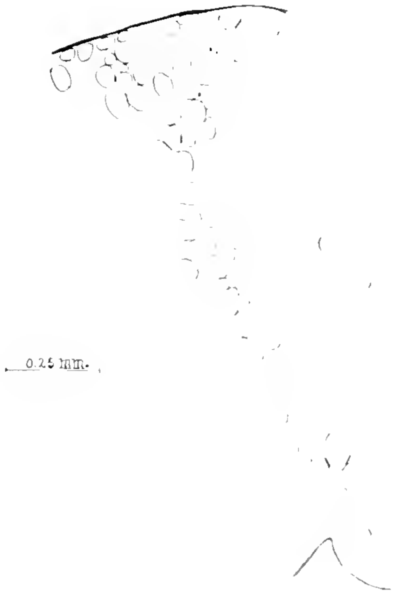
Germ-cells of a new-born [illegible]. a, diagram showing the position of the gonad, b, the germ-cells.

TEXT-FIGURE 6.

Longitudinal section of the testis of a 7½ weeks old Gambusia, showing the bilobed character of the young testis.

an increasing tortuousness of the longitudinal testicular canal, owing, probably, to unequal growth of the testis and the 'vas deferens.' The vas at this age possesses characteristic cuboidal or somewhat spherical lining cells, which in some sections appear to be ciliated. In the young individuals, there are conspicuous longitudinal strands of connective-tissue fibers running the length of the testis, which as the testis grows older, become inconspicuous and finally disappear.

In the changes which lead later to the adult condition, the vasa deferentia become larger and more convoluted, and the connective-tissue elements inconspicuously diffuse. The germ-cells mature; late mitotic stages appear, and with the production of spermatozoa, spermatozeugmata collect in the vasa deferentia, swelling them considerably. These canals, which in the early life of the individual are separate, may either fuse along a part of their length, or remain separate and wide apart, one branch in each half of the testis. Other evidences of their double structure are occasionally found in adult testes. (Textf. 7,). In the testis figured, the major portion of the gonad consisted of cysts containing cells in spermatid- and early spermatozeugmata-stages. These occupied the central portion of the testes, grouped about the paired longitudinal testicular canals. At the periphery are cysts whose cells were in early 1.-



TEXT-FIGURE 7.

A transverse section of a mature testis, showing evidence of the original bilobed character of the testis, thru a differential development of the cysts in spermatogenesis.

spermatocyte division progresses. Along the median plane there also occurred a band of cysts whose cells were in the same stages of mitotic division as those of the cysts occurring at the periphery of the testis. The line of fusion of the two original testes appears clearly in the vertical band of younger cysts running through the section figured.

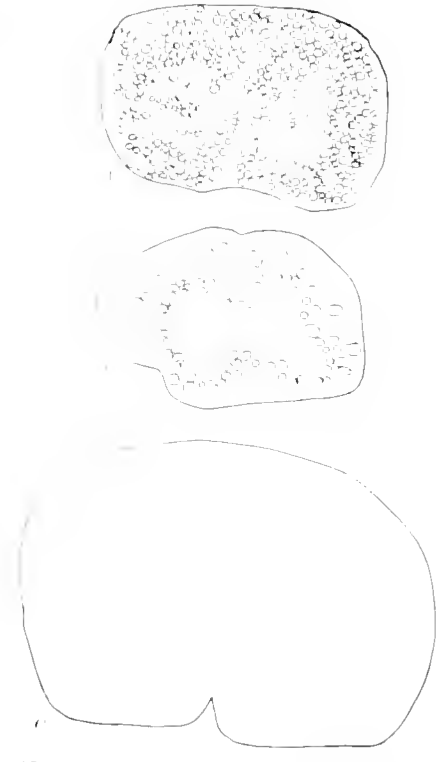
SEASONAL VARIATION IN THE ADULT GAMBUSIA TESTIS.

Volumetric variation: As we have already seen from Tables 6,7 (p.21) there is a seasonal volumetric variation in the testes of Gambusia; the volume of the testes being greater during the season when the testis is in active spermatogenesis, i.e., when there is an excess of early maturation stages. Although a large series of data was not collected with the intention of ascertaining the precise size-relations of testes in Summer and in Winter, yet all my observations point to a considerable volumetric increase in Summer (May/August) over Winter testes (November/February). (See Textf. 8,9.) The maximum volume difference noted was an eight-fold increase during the Summer season. This volumetric increase is associated with the reproductive activity of the male.

Variation in the number of spermatozeugmata at different seasons of the year: Observations made on the testes of Gambusia during November/June indicate that in the Fall and Winter, the testes are filled with spermatozeugmata. In February/March, the first wave of copulation occurs,

synchronously with the waves of warm weather; the testes are then freed of the great mass of the sperm-balls. Space is thus given for the development of the suppressed spermatogonia into cysts of spermatocytes, and spermatogenesis begins anew. In those males which have presumably copulated in February, the testes in May are already filled with newly-formed spermatozeugmata. These are discharged during this month, in the great wave of copulation that sweeps over the Gambusia population. By June/July the testes studied are in active spermatogenesis, the testes being filled with early phases. Smears taken in August/September show only spermatids and spermatozeugmata, together with late spermiogenesis stages. Sections from individuals killed in January/February have the testis filled with spermatozeugmata, and no earlier stages present. From these observations the conclusion is drawn that during the Summer there are several 'renewals' of the testis, i.e., that the gonad starts anew general spermatogenesis. It appears probable that the spermatozeugmata that have passed the Winter in the testis are available for the fertilization of the eggs of the females in the following Spring. Text-figure 8 gives a correct idea of the relative abundance of spermatozeugmata in testes at different seasons of the year.

Seasonal variation in the volume of the 'Vas deferens': Spermatozeugmata are produced by the further differentia-



reduce to [illegible]

TEXT-FIGURE 2. Showing the varying numbers of spermato[illegible] at different seasons of the year. A, [illegible] testis; B, June; C, May. (Camera-lucida drawings.)

tion and maturation of cysts of spermatocytes. Each of the latter types of cysts possesses from 1500-3250 spermatocytes, and these in turn give rise to about 6000-13,000 spermatozoa. These early spermatozoa later migrate to the outer portion ~~portio~~ of the cyst, where they come to rest. Their heads are directed peripherally and their tails, together with a very viscous colloidal material, almost fill the central portion of the cyst. The cyst-membrane persists for a short time, but later, with the ageing of the cyst, is dissolved. The mature balls of sperms thus lack all enveloping membranes. These~~s~~ sperm-balls are the spermatozeugmata, in which the spermatozoa are conveyed to the genital papilla of the female.

Within the testis, with the maturing of the spermatozeugmata, a most interesting change takes place. The wall of the 'vas deferens', hitherto a tortuous tube with but few diverticula, begins in different portions of the vas to show a differential growth-rate. From longitudinal sections most suitable to this study, the process seems clear. The cells of the vas deferens grow in such a manner that masses of ripe spermatozeugmata (12-32) are surrounded by invagination and evagination in such a way that they come to lie in a sack within the vas deferens. This sack later separates from the wall of the vas deferens. The walls of the sac later dissolve and set the sperm-balls free in the

longitudinal canal. The spermatozeugmata, thus, possess no investing membranes whatever in the lower part of the tube.

The spermatozeugmata when ripe are spheroid to ovoid bodies with a diameter (in Winter testes) of 210-320μ. KUNTZ (1914) in describing these bodies for *Gambusia affinis* says that

> The walls of the spermatophores are exceedingly delicate. If the spermatophores are ruptured under the microscope, the spermatozoa may be seen to escape freely, even though they are still immature and inactive.

In the passage above quoted it is clear that what KUNTZ has called the "spermatophores" are really spermatozeugmata, and they are so designated in the present paper. For the ripe sperm-balls of *Gambusia affinis* lack completely the investing membranes characteristic of spermatophores. As PHILIPPI (1908) has demonstrated for *Cnesterodon 10-maculatus* and as the writer has found for Gambusia, the sperm-ball~~s~~ in these forms is not a spermatophore, but a spermatozeugma, in which the sperms are merely aggregated and held in place by a cementing substance which undergoes rapid decomposition under the action of the secretion of the oviduct of the female, which thus liberates the sperms. Of course, the cysts containing immature spermatozoa possess in the testis a very thin cyst-wall, and it may be that KUNTZ observed these and consequently called them spermatophores. The present writer has worked with material from the same locality as KUNTZ, but has never been able to discover an investing

membrane in mature spermatozeugmata. In KUNTZ's figure of a 'spermatophore' the spermatozoa are irregularly arranged. This convinces one that KUNTZ has figured immature spermatozeugmata rather than mature ones, for as previously stated the spermatozoa in mature cysts lie at the periphery and have a definite arrangement.

Table 8 contains data which were gathered at different seasons of the year. The point in view was that of ascertaining quantitatively the winter increase in volume of the 'vas deferens.' Sections of representative testes at the different seasons appear in Textf. 9 . In each case, an average section of the series was taken for drawing and study.

It will be seen from Table 8 that in the Winter time the 'vas deferens' has a considerable volume. It appears that in Gambusia these ducts serve as storage places for sperm, somewhat as do the seminal vesicles in higher vertebrates. In March, after the early copulation-period, the volume is much diminished, and the testes become filled with cysts in early stages of spermatogenesis. In April/May, the volume of the vas deferens again greatly increases. The vas is emptied of the spermatozeugmata during the copulation period of May, and in May/June, the relative volume of the vas deferens is very small. Active spermatogenesis takes place in recurring cycles until the close of the Summer.

TABLE 8 .

Table showing the relation between the total cross-section-area of the testis, and the area of the cut vas deferens at different seasons of the year.

Individual	Actual area in square mm.		Percentage of total area occupied by the vas deferens.
	Total Area	Vas deferens.	
11-1-20/a2	1.130 mm^2	0.579 mm^2	51.2
12-4-20/b1	1.207	0.373	30.9
12-6-20/b3	0.912	0.188	20.3
1-7-21/a	Sagittal sections only cut:	no measurements.	
2-12-21/b3	1.544	0.965	62.4
3-16-21/2	1.281	0.074	5.8
4-?-19/a3	1.347	0.444	32.9
5-5-21/e2	0.736	0.115	13.2
5-2-21/c2	1.063	0.312	29.5
5-8-21/a4	4.436	0.069	1.5
6-10-21/2	2.062	0.025	1.2

NOTE: In ~~the~~ obtaining ~~of~~ the above data, the following method was employed. The median section was drawn with the aid of a camera-lucida at a magnification of 75 diameters; the respective projected areas measured by means of a planimeter, and the subsequent calculations made therefrom.

TEXT-FIGURE 9.

Showing the relative area taken up by the 'vas deferens' in testes at different seasons of the year. Note the Winter-testes (1-8), in comparison with the summer-testes (9, 10.)

There is, of course, considerable variation in the relative volume of the vasa deferentia of different individuals at any definite season.

OBSERVATIONS ON SPERMATOGENESIS. a). *Primary and Secondary Spermatogonia*. The definitive sex-cells by repeated divisions give rise to the final spermatogonia. It is not known how many fissions take place before the spermatogonia are produced which give rise to the final spermatogonial cyst. A computation based on the approximate total number of cysts produced during the lifetime of a male Gambusia, assuming that none of the daughter-cells of the fissions died or failed to produce in time cysts of spermatogonia, and that there is no transformation of mesodermal elements into germ-cells, gave the number of fissions as at least 16. Such a computation, however, is to be looked upon with mental reservation: it is indicated, however, that there are many cell-divisions from the time of the first segregation in the Keimbahn to the time when the cyst-forming spermatogonia are finally located in the testis.

The time when the first sex-cells are segregated is not known. My earliest Gambusia embryos have a snout-anus length of 1.53-3.05 mm. Definitive sex-cells are then present in the typical location beneath the swim-bladder. At birth the gonads are developed but slightly beyond the condition found in these embryos. The sexes are not readily distin-

guishable from each other. The gonads at birth have large, clear cells with a very clear nucleoplasm in which lies a prominent plasmosome. From the plasmosome, and apparently taking their origin from it, radiate delicate linin fibrils, upon which frequent granules of deeply-staining chromatic material may be seen. The filaments appear to extend to the inner surface of the nuclear wall. Occasionally the sex-cells in the gonad appear especially large and clear, and each cell is invested with a layer of mesoderm cells, but these have been observed in only a few individuals. (Textf. 5.). It is not improbable that such cells are developing oocytes, but the evidence in the present case is not completely convincing. In a few of the individuals, cells unmistakeably developing ova are found at birth. These cases are, however, rare. In those cells which have not yet differentiated there is usually a prominent plasmosome, with one or more other conspicuous irregular masses of chromatic material. In the later spermatogonia (Figs. 1, 2*) which are produced from localized peripheral germ-cells of the testis in the cyst in the early divisions of cyst formation, there are usually two prominent karyosomes. The final spermatogonia (Figs. 8, 9, 10)are approximately one-eighth the size of the germ-cell which started the cyst. These final spermatogonia possess a very fine reticulum of inconspicuous fibrils; the chromatin flakes are small and seen only with difficulty.

*Referring to figures on the plates.

The early spermatogonial chromosomes in multiplication-divisions in the cyst (Figs. 3-5) are simple rod-shaped elements, with an apparently terminal spindle-fiber attachment. In no case was a J- U- or V-shaped chromosome observed in a polar view of the plates. The chromosomes in the spermatogonial metaphase plates are usually so numerous and so closely pressed that a dependable count is almost precluded. The number, however, is not less than thirty nor more than thirty-six; a count that is corroborated by the fact that in a single widely spread plate 36 chromosomes were clearly seen. Further evidence lies in the fact that in the diakinesis stage preceding the first spermatocyte division, counts of the tetrads give 15 to 18 as the number of quadrivalents. The diploid number of chromosomes in the male germ-cells would then be 36 if no heterochromosomes were present, or 35 if one heterochromosome were present. On the latter point it is not possible to speak definitely, since the second spermatocyte plates are always too crowded and dense to show the exact number of chromosomes. It is apparent, however, that approximately 18 univalent chromosomes are present. Lagging chromosomes are frequently seen in anaphase stages viewed from the side, but not constantly enough to give a clue as to the number, size, and form of possible heterochromosomes. Occasionally precocious chromosomes appear in early maturation stages. If one were to trust the uncertain help of analogy, however, it might be claimed that the male Gambusia has one heterochromosome. (Cf. SCHMIDT,

1920, on Lebistes reticulatus). With this assumption, it would be quite within the bounds of probability that if the spermatogonial chromosome number is really 35, there are two classes of gametes in approximately equal numbers, containing 17 and 18 chromosomes, respectively.

By repeated mitotic divisions the cysts become filled with the final spermatogonia. (Figs. 8, 9, 10). The precise number of fissions of the original spermatogonium is not known; a series of computations of the final number of spermatogonia in a cyst indicates that at least 10, and often 12, fissions occur before the cysts are ready for the later stages of spermatogenesis. In a given cyst, the cells are all in the same stage of maturation and thus, as in insect testes, it is possible to find all stages of the process, each in a different phase, in a single section.

b). Later stages in Maturation. Leptotene. Synapsis. Pachytene. In the further maturation of the male germ-cells of Gambusia the process follows the usual path. There are no features worthy of special note. The filamentous chromosomes of the leptotene (Figs. 11-16) are produced by a condensation of the chromatin on the linin fibrils. There has not been any striking evidence of pairing of the chromosomes in the leptotene, owing to the difficulty of the material.
In the synaptene and pachytene stages (Figs. 17, 18) which follow upon the leptotene there is evidence that parasynap-

sis occurs. The chromatin threads in early synapsis become only half as numerous as in the leptotene. The pachytene shows the typical orientation of the thickened and shortened threads of this stage. No contraction-stages (*synizesis*) occurs in this species.

C). *Tetrads and Diakinesis.* The tetrads in their early stages appear to be of the open-ring type. (Figs. 20, 21). They contract, however, as they take on the peripheral position characteristic of diakinesis (Fig. 22) until all trace of their tetrad-character is lost. They then appear as large, spherical, deeply-staining bodies evenly distributed through the plasm just beneath the nuclear membrane. In this position they remain for some little time, after which they gather to form an equatorial plate. (Fig. 23).

The tetrads are halved in the first spermatocyte metaphase. The distribution of the dyads to the two daughter cells of the first spermatocyte division could not be made out. (Figs. 24-26.) The secondary spermatocyte division follows immediately upon the conclusion of the first division, with no nuclear reconstitution. After the secondary spermatocyte division is completed, the chromosomes clump together, the chromatin changes in reaction to stains, and becomes scattered throughout the nucleus. After the resultant spermatid nucleus has been reconstituted, a rather

long interkinesis ensues. This is followed by a stage in which the chromatin gathers at the periphery of the nucleus, and stains deeply.

d). Spermatozoa. The spermatids lose their cytoplasm; the nucleus becomes hemisperical (Fig. 31) then elongate (Fig 32, 33). As the spermatozoa begin to nearly attain to their mature form (Fig. 34) they migrate to the periphery of the cyst. The heads are directed outwardly, and the tails, together with a colloidal substance, fill the central cavity, precisely as shown by PHILIPPI (1907) for *Phalloceros caudomaculatus*(HENS.) These spermatozeugmata appear to retain their individuality until their discharge from the genital ducts of the male. The first spermatozoa are formed in May/June, and the process occurs until October. Their formation, then, is contemporaneous with the period of warm weather.

e). Dimorphism of the Spermatozoa. WILSON made the discovery that in certain insects, the males of which are heterozygotic, the spermatozoa can be divided into two groups which differ considerably in size, those in each group varying about a mode with but little overlapping. ZELENY & FAUST (1915, a, b) obtained similar results in several groups of insects, as did also WODSEDALEK (1920) in mammals. In Gambusia the writer was unable to find any such dimorphiam. It is possible that when spermatozoa

from different spermatozeugmata are measured, and the measurements grouped together, a complicating factor enters in the fact that the sperm-heads may thus be in different stages of maturity, and hence of varying volumes. In view of the facts, however, that the sex-chromosome, if it occurs, is not very different in size from the other chromosomes of the cell, and that the chromosome number is so large, thus making the relative increase in size of the gamete possessing the sex-chromosome slight, it is doubtful if such measurements of sperm-heads would furnish any clue to the existence of a sex-chromosome. Whether such a chromosome exists or not may be much more easily ascertained by breeding experiments involving sex-linked characters, as, e.g., SCHMIDT's work, already mentioned.

From the results obtained by the study of the spermatogenesis of Gambusia, no light is cast on the origin of the anomalous sex-ratio of adults. The evidence, while not completely satisfactory, appears to indicate the presence of heterochremosomes. The cytological condition of related forms would seem to indicate the production of equal numbers of male- and female-determining gametes. What the proportions of the sexes in experimental litters with known life-conditions would be, remains to be ascertained.

Summary.

Summary (On Spermatogenesis.)

The testis of adult Gambusia is formed by the median fusion of two original paired testes. This fusion takes place within the first two months of independent life. Traces of the double character of the adult testis are evidenced in the usual double-character of the longitudinal testicular canal, and in differential maturation along the median plane (fusion line).

The testes in Summer are somewhat larger than in Winter. The relative proportions vary markedly with different individuals. The fluctuation in volume is, however, not as great as it is in other teleosts.

The testis lacks any strongly-developed connective-tissue core.

There are no spermatic tubules.

The cysts of spermatozoa are the result of the continued fission of germ-cells which at the time of sexual activity migrate to the periphery of the gonad.

The sex-cells of Gambusia are early segregated from the somatic cells.

The gonads at birth are undifferentiated but sex is ascertainable within four weeks after birth - food- and temperature-conditions being favorable.

The male is capable of fertilizing the female's eggs when but $1\frac{1}{2}$-2 months old.

Ripe sperm are transferred to the female during copulation in the form of specialized sperm-balls (=spermatozeugmata).

During the Winter months (November/February) the testes are filled with spermatozeugmata, which very greatly swell the longitudinal testicular canals.

The period of active sexual-life (and active spermatogenesis) is the Spring and Summer months.

The 'vas deferens' in Winter constitutes approximately 60% of the volume of the testis as compared with a Summer relation of 1.2-1.5% of the testis-volume.

During the Summer about three waves of production of spermatozeugmata occur.

There are no special characteristic features of spermatogenesis in this form. The cysts are produced from 10-12

fissions of an original spermatogonium. The final spermatogonia pass through the usual prophase stages. Each cyst contains approximately 1500-3250 final spermatogonia, all in the same stage of development. During spermatogenesis, this same relation continues.

It appears from counts of spermatogonial metaphase plates and from diakinesis stages that the diploid number of chromosomes in male Gambusia is 35 or 36.

There is no evidence in the spermatogenetic process of an unusual method of chromatin distribution that would explain the occurrence of the atypical sex-ratio observed in the adults.

III. OBSERVATIONS ON SEX IN GAMBUSIA RAISED IN AQUARIA, WITH NOTES ON GROWTH AND DEVELOPMENT.

A. Observations on the Sex-Ratios of the Aquarium Populations

During the years 1919-1930 a series of experiments with gravid Gambusia was conducted with a view to ascertaining whether it were possible in these fish to alter the sex-ratios of the young at birth by the feeding of male gonads to the pregnant females. It was known that the males and females at birth cannot be distinguished by superficial observation, but it was assumed that they could be distinguished by cytological methods. Consequently the young of the experimental animals were taken at birth or several days later, fixed in BOUIN's fluid, and the entire animal later sectioned and studied. It was found on studying the sections thus obtained that while in some of the individuals cells unmistakably definitive oocytes occurred in the usual position just beneath the air-bladder, in others the gonads were still undifferentiated. No internal structures, such as genital ducts or other features gave an indication of the sex. It is consequently evident that the sex of Gambusia at birth cannot be ascertained by cytological methods.

The next endeavor was to raise the young in laboratory aquaria until the sexes were distinguishable.

These latter experiments came to naught. The death-rate of the young in small indoor aquaria was very great, so that even with the best of care and feeding the differentiation of the sex-glands did not occur during the maximum period that it was possible to keep any considerable portion of them alive. Microscopic study of those individuals which had died during the experiments cast no light on the proportions of the sexes of these fish.

During the Spring of 1921 it was found possible in large outdoor aquaria to raise young Gambusia and to keep them with very little mortality until the males could be distinguished from the females. Four litters were raised as follows:

On May 15, 1921 four females, each approximately 45 mm. long, and well advanced in pregnancy, were taken at random from a lot of gravid Gambusia which had been collected at Beaufort, N.C., March 22, and shipped to Baltimore. These were isolated in special breeding aquaria which consisted of ordinary 3.5 liter battery-jars in which were hung cages made of wire netting of 3.5 mm. square mesh. The cages were coated with beeswax to prevent rusting. The gravid females were put into these wire baskets, one in each, and the aquaria stocked with plants as usual. When the young were born they darted out of the mother's cage through the meshes and were thus able to escape her cannibalism.

Gambusia females very frequently eat their new-born young - a habit that is shared with some other viviparous cyprinodont fishes.

The females were fed a variety of foods: boiled white of egg, bread-crumbs soaked in beaten egg and dried, and finely chopped snails. They devoured eagerly microcrustacea, enchytraeid worms, and mosquito larvae, but these food materials were not always available.

The four females designated a, b, c, and d had young on the following days*: May 29, May 19, May 21, and May 21, respectively. Female a had a litter of 37 young; b, of 19; c, of 22; and d, of 34. The birth of all these litters except that of a was observed, and it is known with certainty that none of the young were eaten by the mothers. It is most probable in the case of Female a that none were eaten, for in the writer's stocks, 37 is a very good litter. As soon as parturition was completed the females were removed. The young were fed on Daphnia for a few days in the small aquaria where they were born; after which time they were removed and put into concrete aquaria 90 x 160 x 60 cm. These pools had been thoroughly cleaned out in the Spring and stocked with Elodea and Spirogyra. During the Summer the Elodea grew until it formed a thick forest of vegetation in the aquaria, offering excellent protection to the young Gambusia. Litter a was put into one of these concrete aquaria, while Litters b, c, and d were combined and put

into another. They were left in these aquaria without food except such as came to them in the form of insects, etc., until the first group was 26 days old, and the second group three and a half months old. They were then removed, killed and fixed in modified BOUIN's fluid. All of the 37 young in Litter a, and 58 of the 65 of the other litters, were recovered. In the aquarium containing the latter there were found in addition to the 58 individuals 12 which were relatively very small. These 12 were evidently offspring of some of the 65 individuals which had been put into the aquarium in May.

The proportions of the sexes in Litter a were learned by a cytological study of the gonads of all the individuals. The young were taken, after fixing was completed, the viscera dissected out in a mass, and these sectioned and studied. The gonads in all of these were differentiated to such an extent that the sexes could readily be ascertained by cytological investigation. In sub-section B, of this section, in discussing criteria of sex in young Gambusia, the writer has taken up in detail some of the most interesting findings gotten in the cytological study of the gonads of month-old Gambusia.

The sex of the young in the litters which were three and a half months old when killed could readily be ascertained with certainty by examining the gonads under a binocular

microscope. This was done in all of the specimens except those which had well-developed gonopods and were consequently unquestionably males. It was found that all the males in this lot had developed a gonopod, so that this would have been a completely satisfactory criterion of sex for the 3½-month old fish. However, to avoid any possible mistake, through the existence of sterile males the gonads themselves were examined.

In Litter a there were 19 males, 17 females, and one whose sex could not be ascertained because a mistake in technique lost the gonads during embedding. The individual whose sex was uncertain, however, was probably a female, as such a notation was made in the dissection-notes. In Litters b, c, and d, of the 58 surviving fish, 27 were males and 31 were females. For the total group of 94 young Gambusia whose sex was ascertained with certainty, there were 46 males and 48 females, a very close approximation to a 1:1 ratio.

It will be recalled that in Litters b, c, and d, seven were not recovered, and presumably died. If half these dead were males and half were females, the proportions of the sexes would then show a still closer approximation to equality. Even if all the dead were females, still the sex-ratio would not even begin to approach the great disproportion found in adults.

In these populations, taken all together, the percentage of individuals whose sex was unaccounted for is very low - only 8.3% - so that the approximate equality of the sexes in the litters cannot be accounted for on the basis of a differential death-rate. On the other hand, there is evidence to show that a differential death-rate in Gambusia may be responsible for the slight numerical inequality of the sexes in the older litters.

Table 13, p.57, records the results of a cytological examination of the gonads of the individuals of Litter a. As has been stated, the sex of only one individual was unascertained, due to loss by accident of the gonads. The table is presented because of the interest of the cytological findings. Some of these, e.g., the relation existing between the functional development of the testis and the development of the gonopod, will be dealt with shortly.

Corroboration of the conclusion reached that the numbers of males and females in Gambusia at birth are approximately equal is found in the results obtained by the examination of populations from two large pools kept under conditions as nearly ideal as possible. I. Into one of these pools there were put in May, 5 pregnant females. About the middle of October, 45 young, and 3 of the parents were recovered. Of these 45 young, 21 were males and 24 were females, again a very close approximation to equal numbers

of the sexes. II. In a pond known locally as the "Euglena pond", all of the Gambusia had died out during the winter. This pond was stocked in May, 1931 with 48 gravid females. In the following October 234 individuals were taken with a dip-net at random from the pond. Of these 234 Gambusia 94 were males, 60 were females, and 130 were so small that the sex could not be ascertained by external observation. Both of these collections were from the same parental stock as the females whose litters were studied. One is impelled to conclude as a result of these observations that the great excess of females in the parental stock (nearly 8 to 1) does not represent the proportions of the sexes at birth, but must have been due to a greater mortality of the males, during either the juvenile period or later.

The only careful piece of work that has hitherto been done in the attempt to ascertain the sex-ratio at birth of Gambusia is that by HILDEBRAND (1917), and his results are vitiated by a high mortality-rate. He found that five months after birth, of an original litter of 46 fish, none had developed a gonopod; and at the age of approximately 13 months, only six of the surviving fish possessed a gonopod, thus giving a very low ratio, even if a possible very high death-rate, which he mentions, is taken into consideration. But, as has already been seen, the factors of food and temperature are particularly potent in determining the rapidity of sexual development and the production of young in these

viviparous fish. This was shown clearly in the results of SCHMIDT's (1919, 1919a) work (_vide_ _infra_ p.60) as well as by the writer's studies on gonopod-development.

In confirmation of the correctness of the conclusion that the normal secondary sex-ratio in at least some Poeciliids is approximately 1:1, HENN's (1916) work may be brought forward. A total of 2,070 individuals of _Lebistes_ _reticulatus_ was obtained in a single collection, with a very-fine-meshed net, in the Barbadoes, under the direction of Professor C. H. EIGENMANN. The collection gave an approximately 1:1 ratio. In the lot were 520 males and 630 females, besides 920 fish less than 10 mm. long, and too small to permit ascertainment of the sex by superficial observation. HENN says on the point of the sex-ratio that

> it is quite certain that this count of males includes only members of that sex, while a few of the smaller specimens regarded as females may really have been immature males. It will thus be seen that the sex-ratio, when an adequate collection is at hand, does not materially differ from that found in other fishes.

He applies this conclusion to the Poeciliidae generally. SCHMIDT's (1920) experimental litters of Lebistes had 100♂: 116.6♀ (total of 78 young) and his other data also show an approximate 1:1 ratio for the sexes. Lebistes possesses very many physiological and cytological characteristics in common with its close relative, Gambusia. It would be a singular thing if certain closely-related species of a compact family such as the Poeciliidae possessed

an anomalous ratio of the sexes at birth, as has been assumed for Gambusia.

The Chromosomal Constitution of the Poeciliidae and its Bearing on the Sex-Ratio. It is unfortunate that the chromosomes of teleosts are so unsuited to investigation because of their extremely small size, lack of individuality, and tendency to clump on the equatorial plate. It may be proper, however, to mention in this connection some genetical investigations whose results give strong evidence of the type of genetical constitution possessed by Poeciliidae. SCHMIDT (1920) in breeding-studies was able to isolate a color-marking in a race of *Lebistes reticulatus* which was transmitted through the 'y'-chromosome. He undertook crossing experiments with two types of males on one type of females. The "new" male type, which we will call "*B*", possessed a brilliant dorsal-fin spot, which was entirely lacking in the "old" type, "*A*." Other color-characters made the two types instantly distinguishable. Crosses were made of Type A♂ x Type B♀, and Type B♂ x Type A♀,, and back-crosses were made with F_1 populations. Breeding-records were kept, with copious notes (and, frequently, with water color drawings) of a large number of individuals (*e.g.*, the registered males of $F_2 - F_5$ total 998.) It was found as a result of all these crosses that the dorsal fin-spot of Type B was carried through the male parent only. This fact was demonstrated very beautifully by SCHMIDT's experiments, and

caused him to conclude that the genetic constitution of this fish is of the XX, XY type, and that the Y-chromosome carries the factor for the fin-spot.

If, indeed, the genetic constitution of the Poeciliidae is of the XX, XY type it follows as a corollary that in the male, which is the heterozygous sex, approximately equal numbers of male- and of female-determining gametes are produced. Assuming that no differential chance of fertilization exists, we would infer that in the young, there would be nearly equal numbers of males and females. If, then, in the adults there is a pronounced excess of females, it would appear that this excess must be the end-result of a differential death-rate.

On the basis of the genetic evidence, we would expect approximately equal numbers of the sexes at birth, a condition which is found to be true from a study of young Gambusia kept under known conditions.

<u>Differential Death-Rate in Gambusia</u>. The males are much less resistant to harmful environmental factors than the females, and hence have a lower survival-value. This is shown by the results of several lines of experiment and observation.

In those cases where quantitative studies have been

male with analyzed factors, the males do not survive as well as the females. Thus, in the writer's experiments, it was found that high temperatures, high H-ion concentration, Oxygen-deficiency, and concentrations of KCN kill the males much more readily than the females. For example, in a collection of 233 young Gambusia which was killed with hot water, practically all the fish that died first were males. BELLAMY* states that in his experiments with other Poeciliids with high temperatures, O_2-deficiency, KCN, etc., he obtained results that are "in complete agreement" with the writer's contention that "males are more susceptible to 'difficult' conditions than the females."

Comm., 1-1921.

Unanalyzed deleterious influences in the environment also bring about a lethal selection to which the males succumb more readily than the females. The writer showed (GEISER, 1921 a) e.g., that males were less resistant than females to disturbances incident to shipment, during both cold and warm weather. Thus, in cold-weather shipments the male death-rate was 1½ times the female death-rate, and in warm-weather shipments, 2½ times the female death-rate. This latter result, also, was obtained when the females were heavily gravid. In aquarium catastrophes, such as epidemics of Icthyopthirius- and Saprolegnia-infestation, the males suffer much more severely than the females. This experience of the writer is confirmed by that of European aquarists generally. In catastrophes of unknown cause the same holds. Thus, one example out of many recorded in my notebook: "On 16. Nov.,

1921 in aquar. cont. 94♀, 20♂; 16 dead♂ and 14 dead ♀ found. No cause ascertained. No fungi. Life-conditions app. excellent. Death-rate for males 800-, and for females 148.8-per-thousand, _i_._e_., 5 3/8 to 1."

The greater ability of the females to survive is moreover evidenced in the proportions of males and females which in the writer's aquaria survive the winter. Thus, _e_._g_., in October, 1921, approximately 100 Gambusia, fairly equally divided as to sex, were left outdoors in a concrete aquarium to pass the winter; on April 10, 1922 all the survivors, 32 in number, were recovered. Of these, only one was a male. It is consequently evident that the females were more resistant to the weeding-out processes than the males, for only 40% of the females, as compared with nearly 100% of the males, had died.

There is still another way in which the numbers of males in a given lot of Gambusia are reduced. The males are smaller and hence are more liable to be devoured by small predaceous fish than the much larger females. Gravid female Gambusia in aquaria, also, frequently attack and kill the males. Records kept of dead adult fish taken from the aquaria show over ten times as many males as females. This fact lends support to CARBONNIER's (1866) contention (DARWIN, 1875, p. 375) that the males suffer from their small size since they are more liable to be devoured by females of their own species.

It is thus apparent that in Gambusia there is a differential death-rate, and that its operation explains the excess of females found in adult populations.*

It has also been noted in other animals that the males are less viable than the females. Thus JEWELL (1921) found a differential death-rate in foetal cattle. He suggests that the male of animals generally may be the less viable sex.

B. Other Observations on Sex in Parents and Litters. The Parental Stock. The parents of the experimental litters as has been seen, were taken from a lot of Gambusia affinis (f. holbrooki) shipped to Baltimore from Beaufort, N.C. In the population shipped the males were outnumbered by the females nearly 8 to 1. It will be seen that these parents might reasonably have been expected to also give in their progeny a very great excess of females, if the sex-ratio among adults were the same as it is at birth. In order to give an idea of the character of this stock, Table 9 is presented. This shows the total-lengths of two lots of fish from this shipment, i.e., 77 adult males and 95 adult females taken at random from those that had died in transit. The distribution of the population as to total length in these two lots of individuals represents fairly correctly the distribution of frequencies in classes in much larger populations measured. In this sense the frequencies may be

* Footnote on p. 52a.

[Footnote.]

*HUNTSMAN (1918) in his work on the Canadian plaice, *Hippoglossoides platessoides* Fabr., finds evidence also to show that in that fish the males have a greater mortality than the females. He says that

> a study of fish at different ages shows that for the first two or three years there are more males than females, but that the older the fish become the smaller is the relative number of males, and usually females alone are found among the oldest fish. In the gulf of St. Lawrence, where there are fish of many different ages, we found the males more numerous than the females among the three year olds, but for the later years fewer and fewer males, until finally after an age of fourteen years was reached there were only females and of these all ages up to 24 years with the single exception of the age of 21 years.
>
> In the Bay of Fundy no very old fish are found and both sexes appear to live equally long, the maximum age being ordinarily about eight years, but the same rule holds as to the young males being more numerous and the old males less numerous than the females of the same age. For example, we found the proportion of the former to the latter to be for the two year olds - 33 to 22, for the three-year olds - 11 to 9, for all the older fish, 10 to 14. There must surely be a higher death-rate among the females in early life and among the males later, and this is not influenced by the temperature, as similar proportions of the twosexes for the various years of life are found both where there is rapid growth and early maturity in warm water, and also where there is slow growth and late maturity in cold water.

This evidence would seem to indicate for the plaice a differential mortality for the sexes similar to that ascertained to exist in Gambusia,

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HUNTSMAN, A. G. (1918.) Histories of new food fishes. I. The Canadian plaice. Bull. of the Biological Board of Canada, No. 1.

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said to be 'normally distributed.'

It will be seen from this Table that with adult individuals, the males are less variable than the females* inasmuch as the range of variation in the male is through 11 classes only, while in the females it extends through 21 classes. The males are much smaller than the females, a fact that has been noted by practically every worker on the poeciliids. Thus, in this population, 63.3% of the males are included within the classes of 26-29 mm. total length, while with the females 47.2 % are found in the class 35-38mm. total length. Or, to put it in another way, in the males only 5.2 % have a total length of 30 mm. or above, while in the females only 9.4 % are less than 30 mm. total length.

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*The standard deviation of the 77 males from the mean length is 2.2237±.0543 mm.; for the 95 females it is 5.0616± .0489 mm. Upon comparing the coefficients of variation of males and females (2.39%, 5.33%) it is seen that the females appear to be 34% more variable than the males, in regard to total length, in this lot. When various dimensional relations ("indexes") are compared, the greater variability is much more marked. Thus, the total length/distance relation is over three times as variable in the female as in the male. It is possible that the lesser variability found in the adult males is the result of lethal selection.

For comparison with Table 9 in illustration of this point I have brought forward Table 10. Both males and females in this table confirm very well the statement of the greater variableness of the adult females over the adult males. The table shows this, however, a little more than would be ordinarily the case because of the fact that the measurements were made in the late Autumn, when the population had suffered the ingress of various broods of young during the Summer. On the other hand, Table 9 shows a lot made more homogeneous as a result of the greater maturity of the individuals after a period of sexual inactivity, and of the weeding out of the unfit and old during the Winter months. Other significant facts suggested by these two tables will be brought out in a later discussion, dealing with the differential growth-rate of the sexes.

Differential Growth-Rate in the Sexes. Table 11 gives the total lengths of the young of Litter *a* at death. From an inspection of the table, it will be seen that in 36 days a after birth the young had increased greatly in length. Thus, taking the population as a whole, 26 of the individuals had doubled in length during this period, and all except one had increased in length at least 87 percent. The mean length of the 19 males in the litter was 16.7 mm., while that of the 17 females also was 16.7 mm. A much larger percentage (96.3%) of the males than of the females (44.9%) have a length of 24+ mm. This indicates that for some time after birth the males grow more rapidly than the females. This contention is supported by obser-

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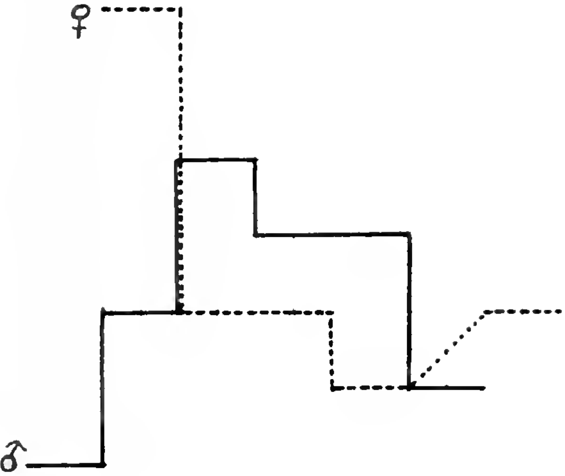
TABLE 11 .

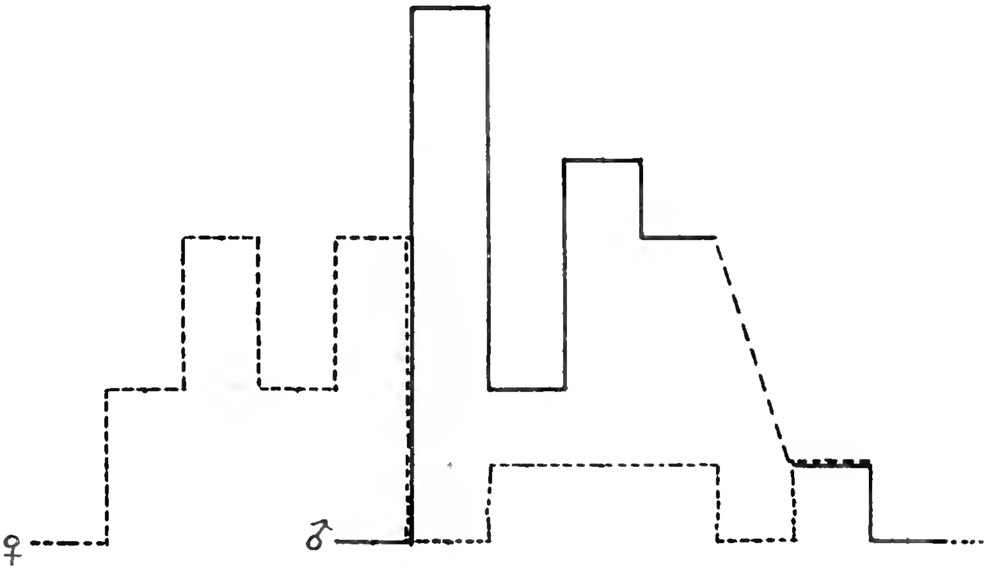
Lengths of individuals of Litter *a* at **death.**

Total l. in mm.	14	15	16	17	18	19	20	Total
No. of individuals								
males	1	3	5	4	4	2		19
females	-	7	3	3	2	-	3	18
males with gonop.			1	2	3	1		7

GRAPH II.

Showing lengths of individuals of litter a at death. (p. 54.)





GRAPH III.

Showing total lengths of individuals ~~of litters~~ of litters b, c, d, at death (age of 3½ months).

NOTE: 96.3% of the males are 34+mm. total length.
44.9% of the females " " " "

3.7% of the males are less than 34 mm.
55.1% of the females " " " "

vations on the rate of growth in litters b, c, and d are presented in Table 12. These results show that of the males of these litters 96.3 percent. as compared with only 44.9 percent. of the females, are 24 mm. or over in length. Or, to state the converse, 55.1 percent. of the females, as compared with only 3.7 percent. of the males, are less than 24 mm. in length.

This verifies the suggestion of LANGER (1913) that male Gambusia in early life exceed the females in rate of growth.

The differential growth-rate appears to set in as a result of gonadic differentiation.

On the question of the relative rates of growth of the s sexes in early life, the measurements of Fall and Spring populations (Tables 9,10) give instructive data in corroboration of the findings with the experimental litters. If we take the males and females of each population and plot their lengths together on the same graph it is seen that the two sexes in the Spring population (Graph 4) are almost mutually exclusive, when considered as to length, while in the Fall population (Graph 5) there is a great deal of overlapping, due to the presence in the population of a very considerable number of small female fish. If, now, we plot graphs which will show in comparison the lengths of the males

TABLE 12 .

Showing total lengths of individuals of Litter ~~II~~ b,c,d at death.
(Age of 3½ months)

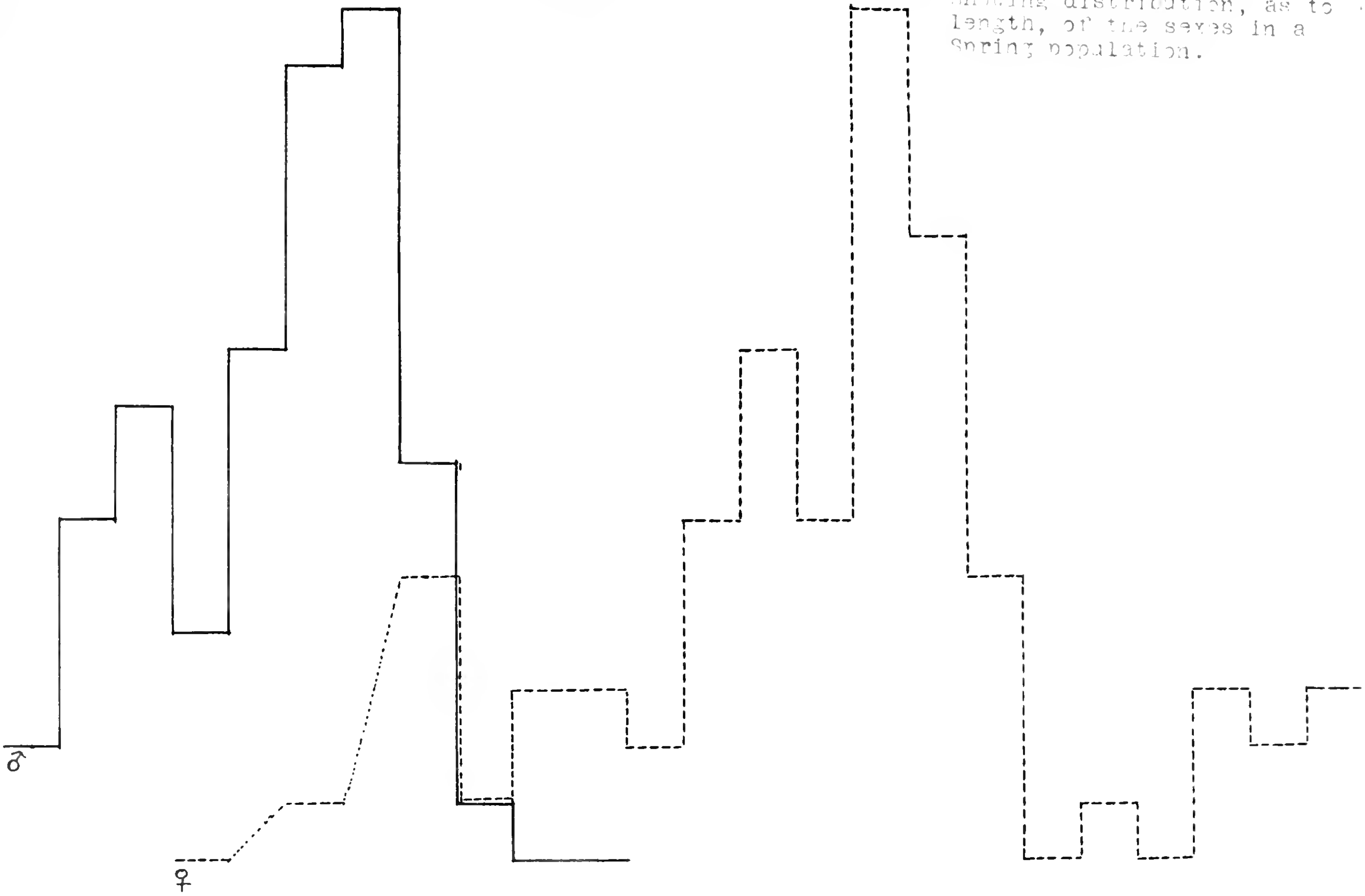
Total l. in mm.	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Frequencies															
males.........	1	3	5	3	5	1	2	2	2	1	2	-	-	3	1
females.......	-	-	-	-	1	3	3	6	5	-	2	1	-	1	-
Percentages															
males.........															
females........															

NOTE: 96.3% of the males are 24+ mm. in total length.
44.9% of the females " " " " " "

3.7% of the males are less than 24 mm. long.
55.1% of the females " " " "

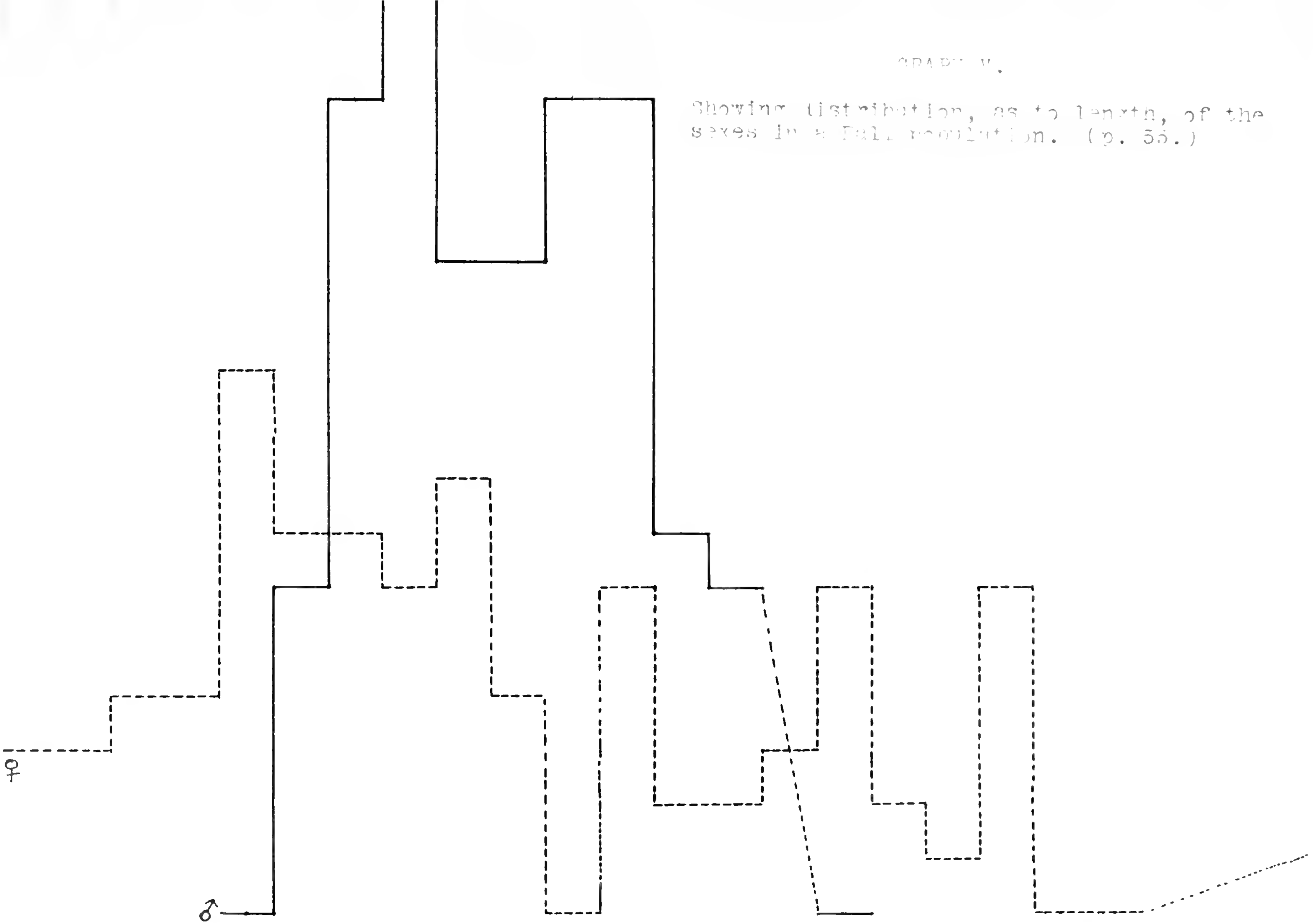
GRAPH IV.

Showing distribution, as to length, of the sexes in a Spring population.



GRAPH V.

Showing distribution, as to length, of the sexes in a full population. (p. 55.)

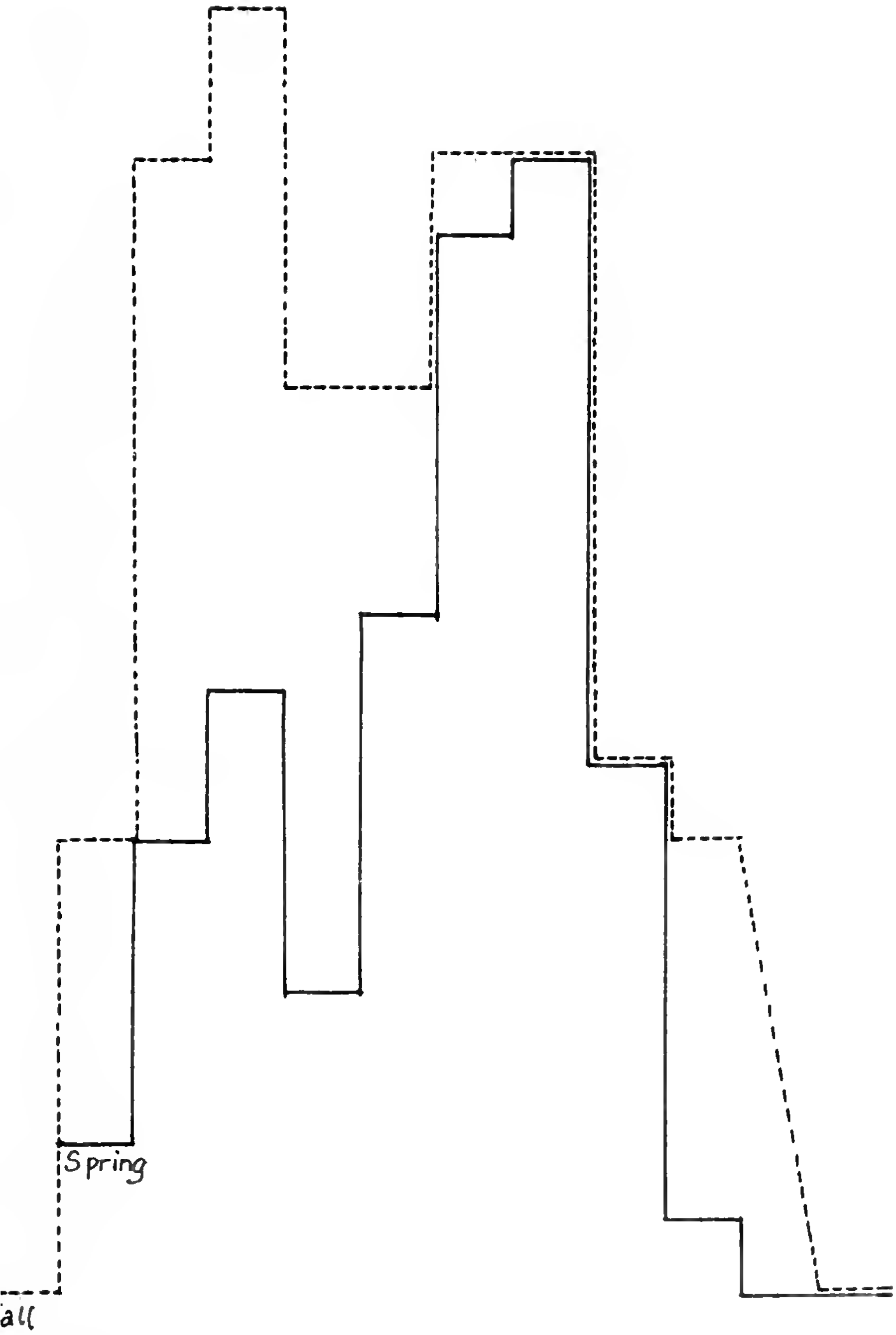


in Spring and Fall populations (Graph 6) and the lengths of the females in the same populations (Graph 7), the dissimilarity in relations becomes very marked. It must be remembered that in the Fall population we have a large number of young males and females, while in the Spring populations t the surviving fish are more mature. As Graph 6 shows, however, the difference in male total-lengths between the S Spring and Fall populations is very slight, relatively, and the ranges of variation are practically the same. On comparing with this graph the total lengths of the females in Fall and Spring (Graph 7) it is seen that the females in the Fall population are much smaller than those of the Spring population. This discrepancy in size is, indeed, very marked. It would appear most clearly, then, that the young males of the Fall population have grown much more rapidly than the females.

<u>Criteria of Sex in Young Gambusia.</u> <u>a.</u>) The development of the gonopod: In every individual except one of Litter <u>a</u> it was possible by cytological examination of the gonads to ascertain the sex with certainty. As previously stated, there were in this litter 19 males and 17 females. The external features of all the males were thoroughly studied before the individuals were sectioned. It was found that seven had well-developed gonopods, but that in twelve the anal fin was precisely like the anal in the female, and that there were no other observable distinguishing characters.

GRAPH VI.

Combined graph, showing length-distribution of the males in the spring and fall populations (Tables 9, 10.)



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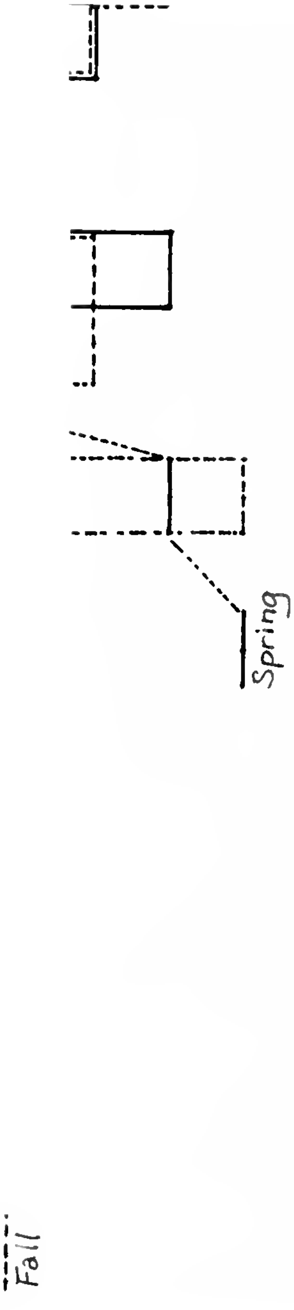

These males appeared on superficial observation precisely like females. When they were sectioned, however, fully developed testes were found. The development of the gonopod appeared to depend upon the antecedent differentiation of the testis to a condition of functioning, and this in turn, went generally hand-in-hand with increase in the length of the fish. (Cf. in Table 13 the cytological findings of Individuals 4, 9, 13, 16 with the smaller ones of this litter, e.g., 22, [illegible], 29, 32.) The data of Table 13 illustrates how widely the males of a single litter may vary in the degree of differentiation of the gonads.

En resumé, then, it may be said that in Litter a only 7 of the males had a gonopod at the age of 26 days; in Litters b, c, d, all of the males had well-developed gonopods at the age of $3\frac{1}{2}$ months. Thus, in the latter litters the gonopod was a satisfactory criterion of sex. On the other hand, in Litter a it was an extremely unsafe criterion of sex. It may be generally concluded, therefore, that in young Gambusia the sex may not be correctly ascertained by this superficial method~~s~~.

b.) The position of the anal fin in young Gambusia as a criterion of sex: As has been seen in an earlier section, the index-values for adult males and females are almost mutually exclusive. It now remains to be seen whether such a criterion of sex can be applied to ascertaining the sex of young, immature Gambusia - those less than 16 mm. in length,

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the measurement at which in Litter a we found the gonopod first developed. If this were possible, it would be a simple matter to ascertain the sex-ratios of collections of immature Gambusia without the laborious work of dissecting and sectioning individuals. To test out the applicability of such a criterion for ascertaining sex in undifferentiated young, we may study the indexes found in Litters a, b, c, d. These data are collected for comparison in Tables 14 and 15 .

Textfigure 10 gives an illustration of a new-born Gambusia. It will be seen from this figure that the anal fin in the new-born fish lies in position between that of the adult male and the adult female. The relative position of of the anal fin in new-born fish is rather close to that of adult females, *i.e.*, their indexes are similar.

On inspecting the data given in Table 14 it is seen that of Litter a, only 7 males (less than half of the male part of the litter) have an index as high as 2.500*, which is the

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*See Section I, pp. 12 *ff*. for a review of the significance, and method of determining of this index. It will be recalled that the "index" expresses the relative position of the anal fin. A 'high' index indicates that the individual has its anal fin placed farther forward than in an individual with a 'low' index. Thus, males have a 'high' and females a 'low' index.

TABLE 14 .

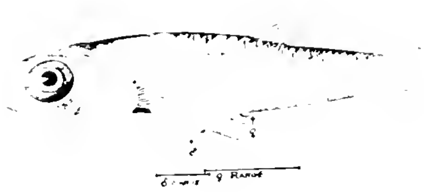
Showing the distribution of litters on the basis of the Total-length/distance Index. (Cf. Table 5.)

lass (Index)	1.6- 1.699	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	..	3.0
males					1	2	4	2	3	6		1
females	1		2		4	5	2	1	2	1		

TABLE 15 .

Showing the distribution of Litter ~~II~~ b,c,d on the basis of the Total-length Distance Index. (cf. Table)

.ass	2.1- 2.199	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9
males	-	-	-	1	- -	5	9	6	3
males	4	12	12	1	2				



TEXT-FIGURE 19.

Showing a new-born Gambusia. Note the mean position of the anterior border of the anal fin in adult male and female Gambusia (at the arrows), and the but slightly overlapping range of attachment-variation in the sexes.

threshold index in the adult male Gambusia of Table 3, while 19 of the males have an index less than this. Only 15.7 percent. of the males, as compared with 86.8 percent.of the females, have an index value of less than 3.200 - the mean index-value of new-born Gambusia measured. The adult females do not vary greatly from this mean determined for the Gambusia at birth, while, as it will be recalled, the males do vary extensively. It is concluded, then, that during sex-differentiation there has been a true forward-migration of the anal fin in the male a distance of at least 2 - 3 vertebrae. In Litters b, c, d, (Table 15) on the other hand, migration was complete, and there was very little overlapping in index-values. The indexes fall in very well with those previously determined for adult populations.

In view of these facts, we must conclude that in immature individuals it is not possible to ascertain the sex by merely determining the index-value.

Before leaving this subject it may be well to speak further, in extension of the discussion in Section I of the age at which Gambusia becomes sexually mature. SEALE (1917) notes the fact that two individuals of Gambusia affinis from the same litter copulated in his aquaria when they were only ten weeks old. The female was then removed to her own aquarium, and nine weeks and two days* after copulation had six young.

*SEALE's published statement corrected from his data.

He suggests later that 30 days is [illegible] the normal period of gestation for this species. Gambusia, according to SEALE, becomes sexually mature in from three to six months. HILDEBRAND (1917) to whose careful studies on this species we are deeply indebted, shows that in his experience the young fish became "sexually mature in the aquarium at the age of about four months; and quite probably in a shorter time under favorable conditions in their natural habitat." SCHMIDT (1919) found that the duration of pregnancy in Lebistes largely depends on the temperature at which the females are kept. Thus, at 25°, pregnancy lasted about 1 month, while at 18° it lasted more than 3 months. The same relation has been found by the writer to obtain in the time required for young Gambusia to come to sexual maturity: with a higher temperature, the young require a much shorter time for development.

In the combined Litters b, c, d, it will be recalled, some of the females of the litter had young approximately four weeks old (since 14.5 mm. young are 3 - 4 weeks old) hence it is evident that the female Gambusia is able to bear her first litter when she is only 2½ months old. If we consider 20 days as the average gestation period during the hotter part of the Summer, it would appear that the young must copulate at less than two months old. Hence, in the males, the testes must be functioning, while in the females the eggs must have been matured for fertilization at an age of 1½ to 2 months.

Summary.

The litters of Gambusia show an approximately 1:1 ratio at birth.

Male Gambusia are more susceptible than the females to harmful influences in the environment, resulting in a higher death-rate among the males.

The males at first grow faster than the females, but they stop growing before they have reached an average length of 27.5 mm.. while the females usually grow to an average length of 36.5 mm.

Observed length-maxima for males and females are 32 mm. and 45 mm., respectively.

Young Gambusia males may develop the gonopod within a month after birth, given suitable conditions of temperature, light and food. In the litters all the males had developed a gonopod at the age of 3½ months.

The degree of differentiation of the testis appears to be a function of the development of the gonopod, as is shown by Litter *a*.

In well-developing Gambusia, the sex may be ascertained by cytological study of the gonads when the young are less th than a month old.

Young Gambusia nearly double their length in approximately four weeks, and treble to quadruple it in the course of 3½ months.

Within 2½ months after birth, Gambusia females may bear their first litters. Hence, the males must have spermatozoa that are capable of fertilizing eggs at least at the age of 2 months.

IV. CONCLUSIONS.

1. Field collections of Gambusia almost invariably possess a great preponderance of females.

2. These sex-ratios vary with the different seasons of the year.

3. Studies on the spermatogenesis of Gambusia fail to reveal any unusual distribution of the chromosomes which would explain the atypical sex-ratios found.

4. Experiments with Gambusia raised in aquaria show the proportions of the sexes at birth to be approximately equal.

5. The males have a higher death-rate than the females, thus causing the atypical sex-ratios found in the adult populations.

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PLATE I.

Figs. 1, 2. Primary spermatogonial cells in young cysts.

Fig. 3. Spermatogonial metaphase, viewed en face.

Figs. 4, 5. Equatorial plates of spermatogonial metaphase, showing distribution of the chromosomes. Not all of the chromosomes shown in the figures.

Fig. 6. Clumped spermatogonial chromosomes, from an older cyst.

Figs. 8, 9, 10. Final spermatogonia, in resting stage.

Figs. 11, 12, 13. Leptotene stage from one cyst.

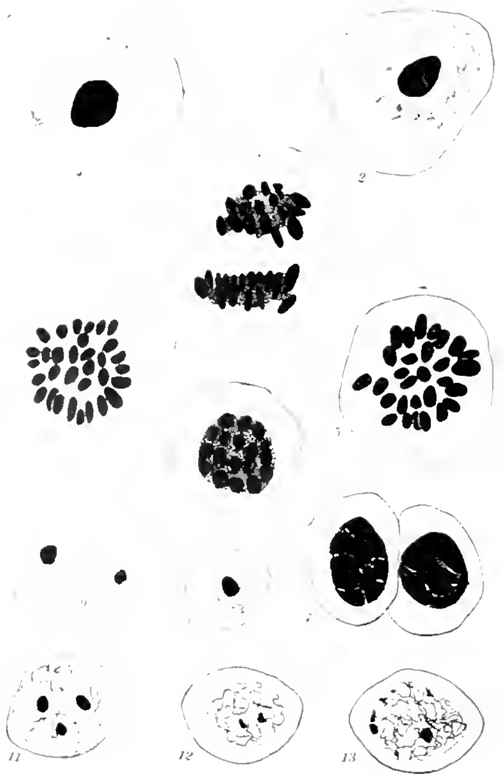
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PLATE II.

Figs. 14-16. Later stages of leptotene. Not all chromatin threads are shown.

Fig. 17. [illegible]

Fig. 18. [illegible]

Fig. 19. Same, cut across the threads, showing orientation of the filaments.

Figs. 20, 21. Tetrad formation.

Fig. 22. Diakinesis, showing peripheral arrangement of the tetrads.

Fig. 23. A [illegible]-cell of the first division, showing approximately 13 dyads.

Fig. 24. First spermatocyte division, viewed from the side.

Fig. 25. Second spermatocyte division.

Fig. 26. Plate of chromosomes from the second spermatocyte division.

Fig. 27. Same, with the chromosomes strongly agglomerated.

Fig. 28, 29. Resting stages.

Fig. 30. Spermatid, showing the chromatin [illegible] on a uniform peripheral distribution.

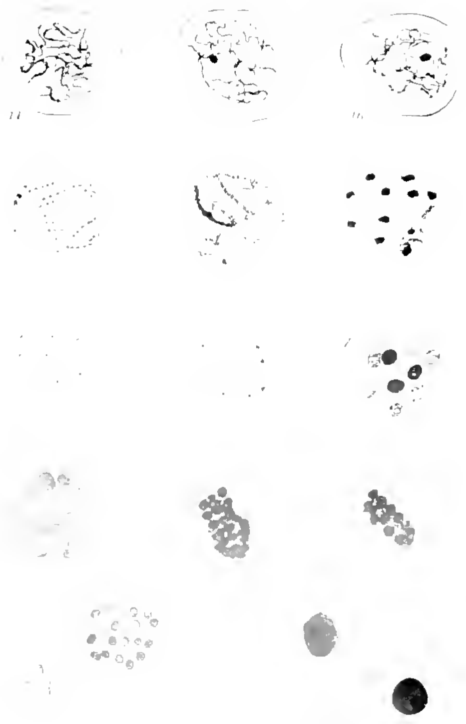

PLATE III.

Figs. 31, 32, 33. The [illegible] evolution of the sperm-[illegible]. (In these figures the spermatids are scattered indiscriminately throughout the cyst.)

Figs. 34, 35. Later stages in the development of the spermatozoon, showing the heads [illegible] peripheral-radial arrangement.

Fig. 36. Section cut tangential to the spermatozeugma, showing the closely-packed arrangement of the spermatozoa. (Iron-haematoxylin staining.)

Fig. 37. [illegible] from posterior portion of winter testis of [illegible].


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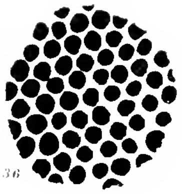
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VITA.

SAMUEL WOOD GEISER was born June 11, 1890, at Independence, Iowa. His elementary education was obtained in the public schools of Independence, from whose high school he was graduated in June, 1908. The following September he matriculated in the Upper Iowa University. From this institution in August, 1914, he obtained the degree of Bachelor of Arts, and in 1919 the honorary degree of Master of Arts. In October, 1919, he matriculated in the Philosophical Department of the Johns Hopkins University, as a candidate for the degree Ph.D. His Principal Subject was Zoology, with first and second subordinates in Plant Physiology and Paleontology, respectively.

He was Principal of the Consolidated Schools of Brandon, Iowa, 1912-13; Assistant in Zoology, Upper Iowa University, first semester, 1913-14; Instructor in Biology and Sociology in the State High School of Moorhead, Minn., second semester, 1913-14; Professor of Biology and Geology, Guilford College, North Carolina, 1914-16; Supervisor of Biology and Agriculture, in the Public Schools of Independence, Iowa, 1916-17; Professor of Biology in the Upper Iowa University, 1917-19. During the first two years of his study at Johns Hopkins, he served as Assistant in General Physiology, and Investigations, and was Bruce Fellow in Zoology during the Academic year of 1921-22. During the Summer of 1921, he occupied one of the Johns Hopkins tables at the Marine Biological Laboratory at Woods Hole, Mass.