SPAWNING OF YELLOWFIN TUNA IN HAWAIIAN WATERS

By FRED C. JUNE

FISHERY BULLETIN 77

UNITED STATES DEPARTMENT OF THE INTERIOR, Douglas McKay, *Secretary*
FISH AND WILDLIFE SERVICE, John L. Farley, *Director*

ABSTRACT

Frequency distributions of the diameters of ova from 112 yellowfin tuna (*Neothunnus macropterus*), captured by longline gear in Hawaiian waters during 1950, demonstrate that several developmental groups of ova are present in the ovaries of this species during the breeding season. Based on the position of the mode of the most mature group of ova present in the ovaries, 11 arbitrary stages of maturity are defined which trace the development of the ova from the immature, or resting, stage through the spawning stage.

A simple ovary-weight to fish-weight relation is given whereby spawning and nonspawning fish may be distinguished without laborious ova-diameter measurements. Over the size range of fish examined, 36.3 to 94.3 kilograms (80 to 208 pounds), the ovary-weight to fish-weight relation was found to be linear for ovaries in the immature, or resting, stage and in one of the maturing stages. However, the slopes and levels of the two regressions were significantly different.

The calculated number of ova produced at a single spawning by individual yellowfin tuna, over the size range 47.2 to 88 kilograms (104 to 194 pounds), increased with fish size and ranged from 2,370,000 to 8,590,000.

Spawning of the yellowfin tuna in Hawaiian waters during 1950 took place between mid-May and the end of October, and coincided with the period of best longline catches of the species.

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SPAWNING OF YELLOWFIN TUNA IN HAWAIIAN WATERS

By FRED C. JUNE, *Fishery Research Biologist*

The rapid growth of the Pacific tuna fisheries during the past several decades has focused attention on the biology of the various species that contribute to this industry. Studies are being made by the Pacific Oceanic Fishery Investigations, of the United States Fish and Wildlife Service, in those phases of the life history and habits of tunas which are basic to an interpretation of patterns of occurrence, movements, and dynamics of population numbers. Pertinent to such interpretation is the knowledge of the place, time, and extent of spawning.

Although records of spawning localities of tunas in the Pacific, based on collections of larvae and juveniles and the examination of gonads, have been furnished by various investigators (Marr 1948; Schaefer and Marr 1948; Wade 1950; Hatai, et al. 1941), knowledge of the extent of spawning in time for any particular locality and of the developmental changes that take place in the gonads concomitant with spawning is notably fragmentary.

The present paper deals with the spawning of the yellowfin tuna (*Neothunnus macropterus*) in Hawaiian waters, with particular reference to the reproductive process. Ova-diameter measurements have been used to demonstrate the developmental changes that take place in the gonads during the breeding season.[1]

Acknowledgment is made to Tamio Otsu, Richard Shomura, and Kenji Ego for their assistance in collecting data and making many of the ova measurements used in this paper, and to Tamotsu Nakata for preparing the various illustrations presented here. The author is also grateful to O. E. Sette and Dr. M. B. Schaefer for the time and advice they so freely gave him, and to Dr. A. L. Tester, University of Hawaii, for the many valuable suggestions offered him during the preparation of the paper.

COLLECTION AND TREATMENT OF OVARY SAMPLES

Because of the difficulty of determining and describing the relative maturity of the gonads from their external appearance, some objective measure of the growth of these organs that is associated with the breeding season was sought. The testes were not suitable because no quantitative measure of their sexual products could be found that would provide reasonably accurate estimates of their relative stages of development. While a relation was found between the size (weight) of the testes from individual fish and proximity of the spawning period in Hawaiian waters, variation in size of the testes among the fish examined was so great that no precise delineation of their growth changes could be obtained. Consequently, only the ovaries of female yellowfin tuna were used.

The material used in this study was obtained from yellowfin-tuna landings made in waters adjacent to the Hawaiian Islands by the commercial longline fishery. The ovaries were collected at the Kyodo Fishing Co., Ltd., of Honolulu, where the catches were weighed and sold at auction. Observations were made on 112 fish, or approximately 4 percent of the total number of yellowfin tuna landed at Honolulu between January and December 1950. The distribution, by dates, is given in table 1.

[1] The use of ova-diameter measurements to indicate the growth that takes place in the ovaries prior to spawning was introduced by Thompson (1915) in his life-history study of the Pacific halibut. Clark (1925, 1929, and 1934) later applied the technique to spawning investigations of the jack smelt (*Atherinops californiensis*), the grunion (*Leuresthes tenuis*), and the California sardine (*Sardinops caerulea*). Other investigators also have used the method in spawning studies of several other marine species (Schaefer 1936; Olsen and Merriman 1946).

TABLE 1.—*Weight and stage of maturity of 112 yellowfin tuna from the Hawaiian longline fishery, January–December 1950*

Date	Body weight	Fresh ovary weight	Stage of maturity [1]
	Pounds	*Grams*	
Jan. 11	(2)	132.0	A
11	98	154.0	B
11	99	154.0	A
11	97	149.0	A
26	110	154.7	A
26	181	448.6	C
Feb. 1	94	143.4	A
2	117	188.6	B
8	144	317.7	A
10	96	139.1	A
10	87	125.0	C
Mar. 17	119	293.1	B
21	116	240.0	A
24	92	191.4	B
24	89	(4)	B
31	80	109.5	A
Apr. 4	138	282.0	A
4	186	391.8	A
11	101	173.7	B
11	151	365.7	A
15	(2)	156.4	A
27	143	429.7	C
28	116	268.7	B
28	131	320.0	A
May 5	(2)	(4)	F
9	119	314.3	D
June 6	154	729.8	[3] F
8	113	1,176.0	G
8	130	1,047.5	G
13	126	1,121.5	F
14	121	1,712.0	J
14	109	357.5	D
20	172	2,565.2	F
20	135	(4)	F
22	125	1,317.3	F
23	131	872.6	F
26	124	1,010.9	G
26	126	988.5	F
28	137	1,326.8	H
29	136	1,084.9	F
29	157	1,404.4	I
July 3	104	751.6	F
5	116	981.7	E
7	130	1,571.6	F
11	107	1,127.6	H
12	143	1,620.7	F
12	105	1,112.3	F
14	124	1,364.4	F
19	132	1,386.1	F
25	121	848.3	E
25	134	996.2	F
28	153	1,536.8	G
Aug. 1	119	545.7	[3] F
3	147	1,236.0	F
3	177	1,628.4	F
10	173	2,791.9	I
Aug. 11	124	1,051.7	F
15	117	996.4	F
21	152	1,354.7	E
29	108	1,292.0	H
Sept. 6	121	1,122.1	F
8	118	740.2	F
18	194	2,823.3	F
25	171	1,121.3	F
Oct. 6	131	737.5	[3] F
6	191	1,146.3	[3] G
10	135	391.9	K
10	164	1.938.0	F
12	171	(4)	E
13	125	561.0	[3] F
13	122	(4)	K
13	160	656.1	[3] E
24	128	292.6	K
26	144	306.5	K
27	158	722.4	[3] F
31	130	235.3	A
31	124	254.2	A
Nov. 3	134	261.2	A
7	160	1,160.5	[3] G
9	138	321.7	K
10	122	182.1	A
16	162	479.4	A
16	124	193.4	A
24	150	250.3	A
24	147	231.6	A
26	149	247.2	A
29	174	290.0	A
29	146	229.5	A
30	107	185.5	A
30	119	228.6	A
30	130	231.2	A
30	142	272.7	A
Dec. 1	112	185.1	A
1	136	214.3	A
4	138	230.4	A
6	125	236.3	A
6	145	253.8	A
6	143	204.2	A
6	146	225.5	A
11	150	260.9	A
12	118	153.6	A
13	156	337.0	A
13	130	252.7	A
15	134	271.0	A
15	97	134.8	B
15	160	283.1	A
15	143	324.4	C
19	208	485.7	A
20	136	250.6	A
26	159	286.4	A
27	145	305.9	A
28	110	160.8	A

[1] For classification of the stages of maturity, see p. 51.
[2] Fish weights unavailable.
[3] Maturing ovaries in which some ova had been lost through previous spawning earlier in the season.
[4] Ovaries not intact.

Longline gear appears to select the larger, adult fish; hence, the smaller, sexually immature fish are not represented in the collections, which include the size range 36.3 to 94.3 kilograms (80 to 208 pounds).[2]

[2] Yellowfin tuna weighing between 15 and 60 lbs. are caught rather infrequently in Hawaiian waters. Longline gear, which is specifically designed to capture large fish, seldom takes yellowfin tuna smaller than 60 lbs. Trolling gear, which is less selective of fish size, catches very few of the 15-to-60-lb. size group, even though the amount of fishing effort is substantial throughout the year. Small yellowfin tuna from 2 to 20 lbs., but no larger, sometimes are captured in great numbers by the pole-and-line bait fishery for skipjack. The virtual absence of the maturing size group (15 to 60 lbs.) in the Hawaiian tuna landings suggests that most members of this segment of the population migrate elsewhere to mature, and only the adult sexually mature fish frequent local waters.

The date, weight of fish, locality of capture, and state of sexual maturity, based on the criteria established by Marr (1948), were recorded for each sample. The ovaries, together with attached peritoneal and vascular tissues, were carefully removed from the fish. The posterior end of each ovary was severed at its juncture with the oviduct, and after all superfluous tissues were removed, the ovaries were weighed on a triple-beam balance to the nearest 0.1 gram and placed in 10-percent formalin.

Preliminary test measurements were made on samples of 300 ova from the anterior, central, and posterior parts of both ovaries from a near-spawning fish to determine whether the ova matured at the same rate and in the same relative numbers throughout the ovaries. An analysis of variance and a chi-square test (Snedecor 1946) showed no significant differences either in the mean diameters of ova ($P>0.05$) or in the relative numbers of ova in each size group ($P=0.70$) from these three regions of an ovary. Furthermore, no significant differences were found in ova size between the two ovaries from the same fish. Similar measurements were made for an immature ovary, and no significant differences were found, either among the different parts of the same ovary or between ovaries from the same fish. Thus, it was concluded that the ova develop uniformly throughout the ovary and in both ovaries of a fish. All further ova samples for this study were taken from the middle of the left ovary and are considered representative of both ovaries.

The samples were taken after the ovaries had hardened in formalin. Ova from a sample were teased out under a binocular microscope to ensure complete separation from the follicles. After the ova were stirred in a watch glass, a small subsample was drawn with a pipette and placed on a glass slide for measurement. Parallel markings with 1-millimeter spacing on the slide were an aid in avoiding duplicate measurements of the same ovum. Measurements were made by an ocular micrometer in a compound microscope at a magnification of six diameters. Although the ova seldom were perfectly spherical, measurements were made on whatever axis fell parallel to the micrometer graduations. This method of measurement has been used by other investigators (Clark 1925, 1934; Schaefer 1936) and, on the basis of comparative test measurements of ova made before and after their preservation, it was found reliable in the present study.

Maturing ovaries contain not only the translucent, immature ova, but also several groups of larger sizes. To ensure adequate numbers of these latter groups without extravagant expenditure of time on the much more numerous immature ova, measurements were made on 300 ova larger than 0.16 mm. in diameter for all maturing ovaries. For ovaries in the immature stage, 200 ova were measured.

DESCRIPTION OF THE OVARIES

The ovaries of sexually mature yellowfin tuna are paired, elongated organs that extend nearly the length of the abdominal cavity. They are approximately round in cross section and almost symmetrical—the left ovary usually being slightly larger than the right. The ovaries are suspended from the dorsal wall of the body cavity by a fold in the peritoneum. Posteriorly, the ovaries unite, with the lumina joined to a short, wide oviduct that opens as a slit on a papilla behind the anus. Anteriorly, the ovaries receive their blood supply from two of the three branches of the coeliac-mesenteric artery; the No. I branch apparently nourishes the left ovary and the No. III branch the right ovary (Godsil and Byers 1944). As the time of spawning approaches, the superficial blood vessels supplying these organs become enlarged and very conspicuous.

DEVELOPMENT OF THE OVA

Within the ovaries of sexually mature fish, several developmental groups of ova may be distinguished at one time or another through the year. On the basis of the characteristics of the largest ova present, the ovaries were classified into the following stages of maturity:

Immature: Immature ovaries contain ova ranging from 0.01 to 0.18 mm. in diameter, with a mode of about 0.08 mm. Preserved in formalin, the ova are translucent, spherical bodies, and are invisible to the naked eye. Each ovum contains a rather large, vesicular, eccentric nucleus enclosed in a clearly defined nuclear membrane. Ova of this group are present in the ovaries throughout the year. Fresh ovaries at this stage are pinkish, elongated, and slender.

Intermediate: Ovaries at this stage contain ova which range from about 0.18 to 0.40 mm. in diameter. As the ovum increases from about 0.18 to 0.40 mm., the nucleus enlarges and the yolk granules appear. The cytoplasm containing the scattered, spherical yolk granules is enclosed by two membranes. The innermost of these, the vitelline, or fertilization, membrane, can be clearly distinguished only in the mature ovum. A comparatively tough, thin, and shell-like outer membrane, the zona radiata, is in direct contact with the surrounding ovarian follicle during development of the ovum. The zona radiata is transparent and sculptured with very fine striations on its outer surface. This membrane is clearly evident at all stages of maturation. Formalin-preserved ova in this group are semiopaque and are barely distinguishable without magnification. Fresh ovaries containing ova which have developed to the intermediate size are indistinguishable in appearance from those containing only immature ova.

Maturing: As the ova advance toward maturity, the group, beyond about 0.40 mm., becomes opaque and exhibits a characteristic yellowish color in formalin. The yolk granules appear as highly refractive, spherical bodies in the cytoplasm. At this stage of development, the fresh ovaries are conspicuously turgid, yellowish in color, and the ova are firmly embedded in the follicles.

To demonstrate the size distribution of the intermediate and maturing ova groups, a sample of tissue (0.5 gm.) was taken from a maturing ovary of a fish landed in early May. After separation of the ova from the follicles, measurements were made of all ova above 0.18 mm. in diameter in the sample. Their diameter frequency distribution, shown in figure 1, clearly indicates the presence of several groups which simultaneously are developing to maturity. Two chief groups are distinguishable: Intermediate, from 0.18 to about 0.40 mm.; and maturing, beyond 0.40 mm., which itself appears to consist of more than one group.

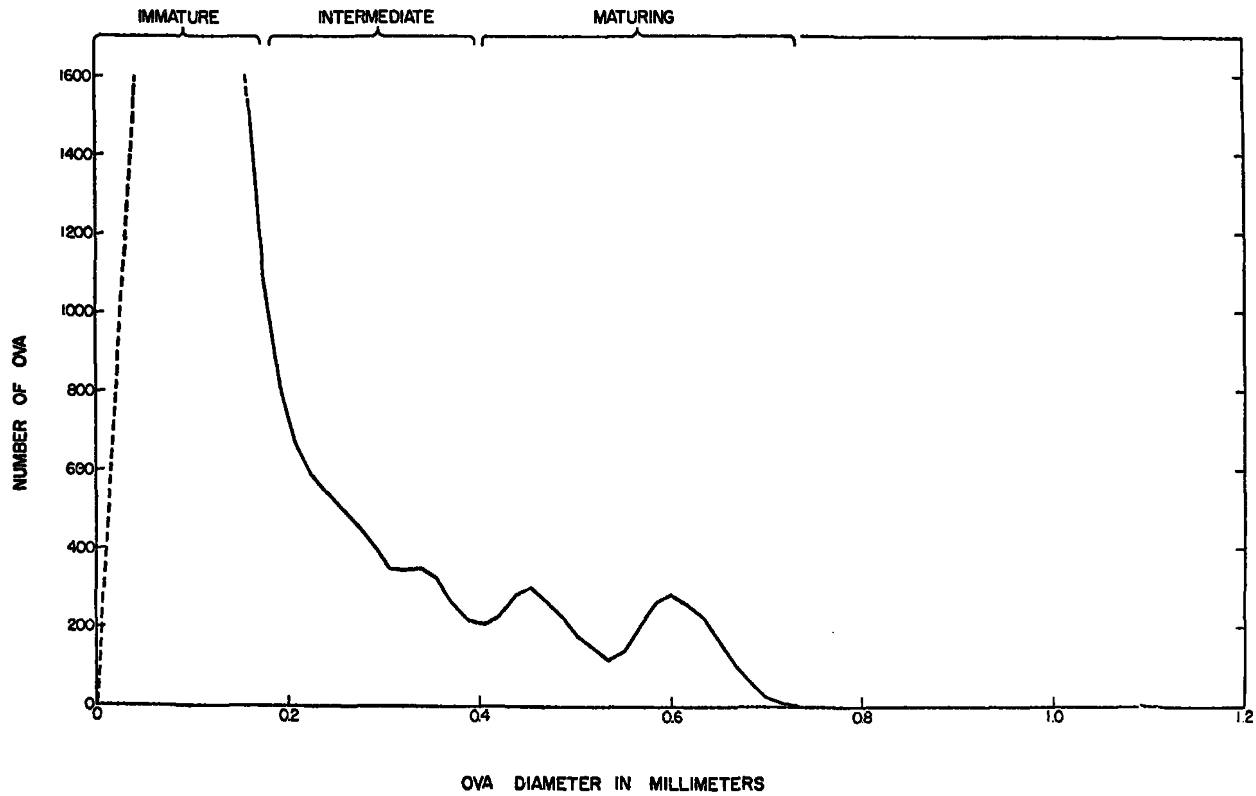

FIGURE 1.—Frequency polygon of 12,041 ova diameters measured from a maturing ovary of a yellowfin tuna taken in Hawaiian waters, May 5, 1950. Only ova measuring more than 0.18 mm. in diameter were included.

Ripe: On reaching the stage at which the ova are about to be spawned, distinct morphological changes occur in them, and they lose their opacity and assume a translucent, grayish color in formalin. Ripe ova measure about 0.76 to 1.23 mm. in diameter. Embedded in the yolk is a single, conspicuous, golden-yellow oil globule, which averages about 0.26 mm. in diameter. Ripe ova readily break from the follicles in which they develop, collect in the lumina of the ovaries, and flow freely from the oviduct when the sides of the fish are pressed.

To show further the size distribution of these developmental groups, a frequency distribution

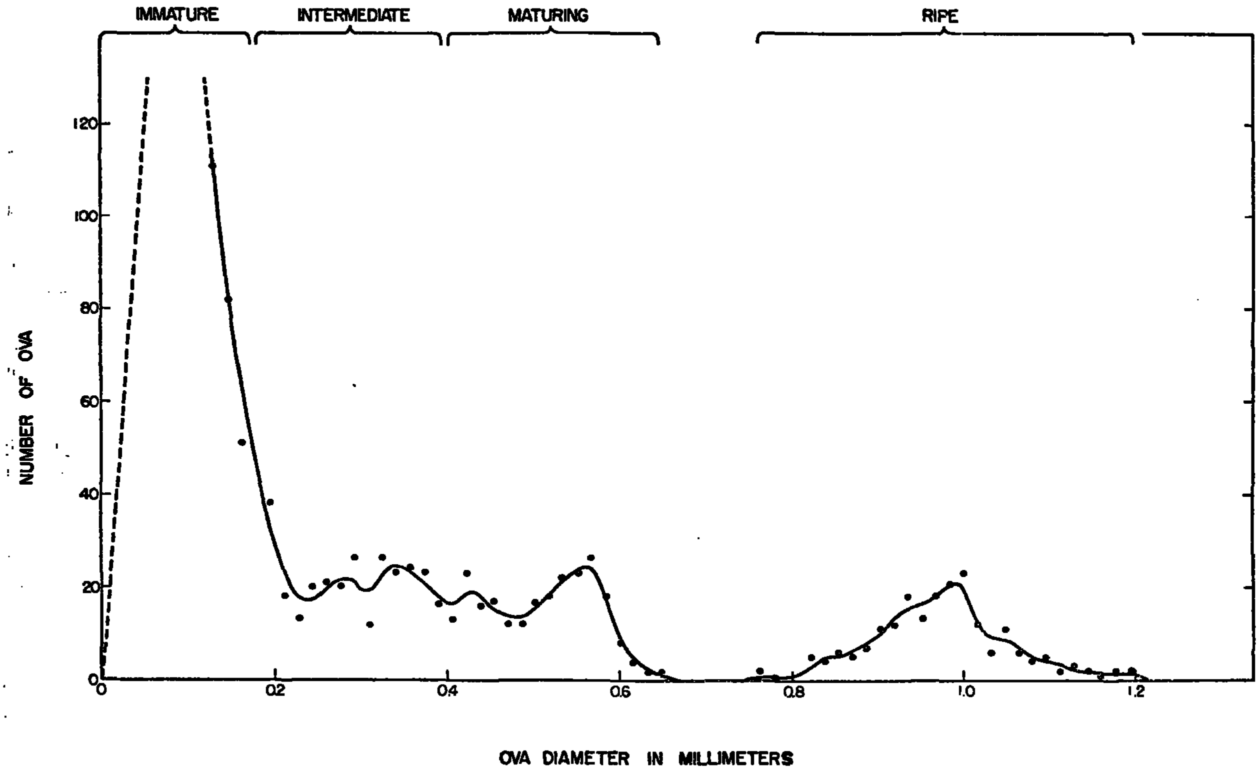

FIGURE 2.—Frequency curve of 1,000 ova diameters measured from a ripe ovary of a 121-lb. yellowfin tuna taken in Hawaiian waters, June 11, 1950. Only ova measuring more than 0.13 mm. in diameter were included. The line represents the original data (dots) smoothed by the formula $\frac{a+2b+c}{4}$

was made of 1,000 ova more than 0.13 mm. in diameter taken from a ripe ovary (fig. 2). The group with diameters measuring from 0.76 to 1.23 mm. includes ova almost ready for spawning, and it can be easily distinguished from the intermediate and maturing groups below 0.65 mm. in diameter. The immature group is indicated in figure 2 by a dotted line.

Spawned out: Toward the end of the spawning season the ovaries decrease in size, become hollow and flaccid, and gradually resume the appearance of those in the immature stage. Following spawning, ripe ova remnants in various stages of degeneration are found in the lumina of the ovaries. Ova in the intermediate and maturing groups which remain in the follicles at the conclusion of spawning take on a deep greenish or brownish color in formalin, and the yolk granules appear as dark specks, indicating that these ova are degenerating and undergoing absorption. During the spawning season ripe ova remnants often are present in the lumen of the ovary at the same time that the ovary is still very much enlarged and apparently maturing a group or successive groups of ova.

Since the previously noted developmental groups marking maturation of the ova were clearly evident, and since these groups showed intergradation in fish collected at different times through the year, the ova-diameter frequency polygons of each sample in the collection were classified according to the position of the mode of the most mature group of ova present. The following 11 arbitrary stages were defined:

Immature

Stage A: Mode at about 0.08 mm., no ova above 0.18 mm.

Intermediate

Stage B: Mode of the most mature group below 0.29 mm.; no ova above 0.34 mm.

Stage C: Mode of the most mature group between 0.29 and 0.39 mm.

Maturing

Stage D: Mode of the most mature group between 0.39 and 0.49 mm.

Stage E: Mode of the most mature group between 0.49 and 0.59 mm.

Maturing (Continued)

Stage F: Mode of the most mature group between 0.59 and 0.69 mm.

Stage G: Mode of the most mature group between 0.69 and 0.79 mm.

Stage H: Mode of the most mature group between 0.79 and 0.89 mm.

Stage I: Mode of the most mature group between 0.89 and 0.99 mm.

Ripe

Stage J: Mode of the most mature group at about 1.00 mm.; some ova segregated in the lumina of the ovaries.

Spawned out

Stage K: Ovaries with a few loose ova in the lumina, including some which are undergoing degeneration.

These stages are shown graphically in figure 3 which has no connotation of time, but simply illustrates the developmental stages from the immature, or resting, stage to the spawning stage. With the exception of stage J, which is represented by the ova from only one fish, the polygons are pooled frequencies of several fish of the same maturity classification. The number of individuals included in each frequency is indicated at the right. Except for stage A, the pooled frequencies were reduced to the basis of 300 ova (the number measured from each fish).

Ripe ovaries from only one female yellowfin tuna were present in the 1950 collections. Furthermore, examples of the preceding two stages of maturity, H and I, totaled only six among the fish sampled, suggesting perhaps that, during or just before spawning, yellowfin tuna bite less on the longline hooks or migrate to an area or a depth not being fished. Schaefer and Marr (1948) offered these hypotheses to account for the absence of spawning yellowfin tuna in the live-bait boat catches in the waters off Costa Rica during their investigations in 1947.

It may be seen in figure 3 that as the last group of ova evident in the frequency polygons progresses toward maturity, a second group becomes differentiated and also progresses toward maturity. The progression of the modes of these successive groups is more evident in figure 4, where the frequency distributions from figure 3 are expressed as deviations from the average frequency polygon of stages B through J. The deviations were smoothed twice by a moving average of 3 to remove chance fluctuations. In this form, the maturing group at about 0.30 mm. in stage C and at 0.45 mm. in stage D can be clearly distinguished by appearance from the immature group. Furthermore, the deficit of 0.20-mm. ova, beginning with stage E, suggests that fewer ova develop from the immature group into the intermediate group after stage D than before.

The progression of the two right-hand modes is compared in figure 5 by a scatter diagram wherein the position of the mode of the last group (Y) is plotted against the position of the mode of the preceding group (X) for each of 44 fish with ovaries in stages E through J. The locations of the modes were determined by inspection of the deviations of the individual frequencies from the average frequency polygon. A coefficient of correlation of 0.855 (P is considerably less than 0.01) emphasizes the close relation between the progression of succeeding modes.

The multiplicity of modal groups and the high correlation between modes of successive ova groups strongly indicate that individual fish spawn more than once a year.

Further support of the hypothesis that more than one group of ova is matured by individual fish during the spawning period arises from the presence of semitransparent, mature ova lying loosely in the lumina of the ovaries at the same time that a second group of ova is developing toward maturity. Among these remnants, some of the ova were grayish in color with the shell membranes partially collapsed; in a few, the oil globules were still discernible; and in others, only the transparent shell membranes or fragments of the shells remained. That these remnants are not carried over from the previous year's spawning is evidenced by the fact that ovaries in the resting, or immature, stage (stage A) and those in the intermediate stages of maturity (stages B and C) which were collected at the beginning of the year showed no signs of ova remnants in the lumina from recent spawnings. Furthermore, the condition of the mature ova remaining in the ovaries, as previously described, indicates that the group of which they were a part probably had been extruded only a short time before. Finally, among fish with ovaries in the maturing stages, eight samples in stages E, F, and G were found in which the ovaries were very much enlarged and turgid and in which remnants of mature ova were scat-

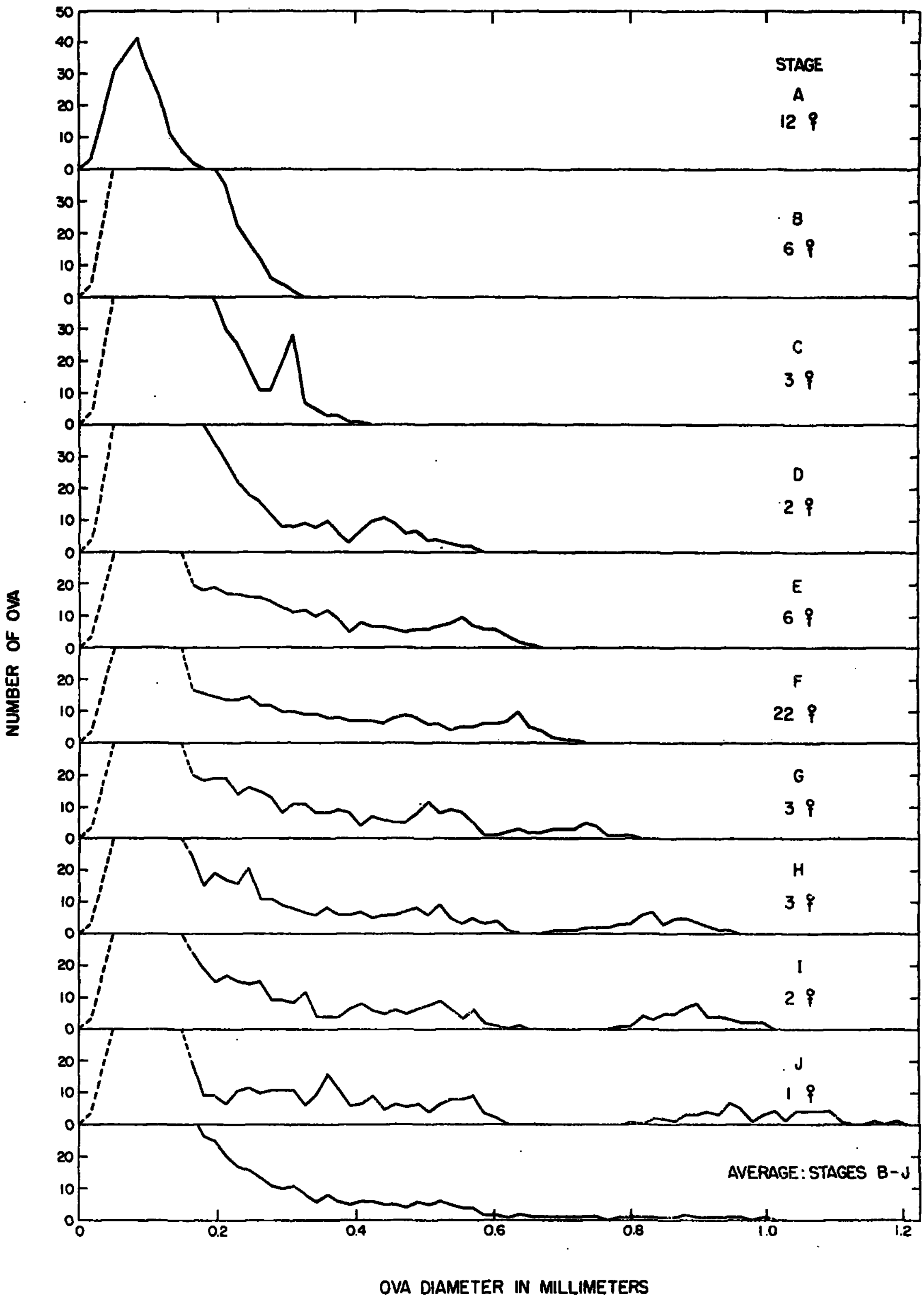

FIGURE 3.—Ova-diameter frequency polygons showing stages in development of the ova from the immature, or resting, stage to maturity.

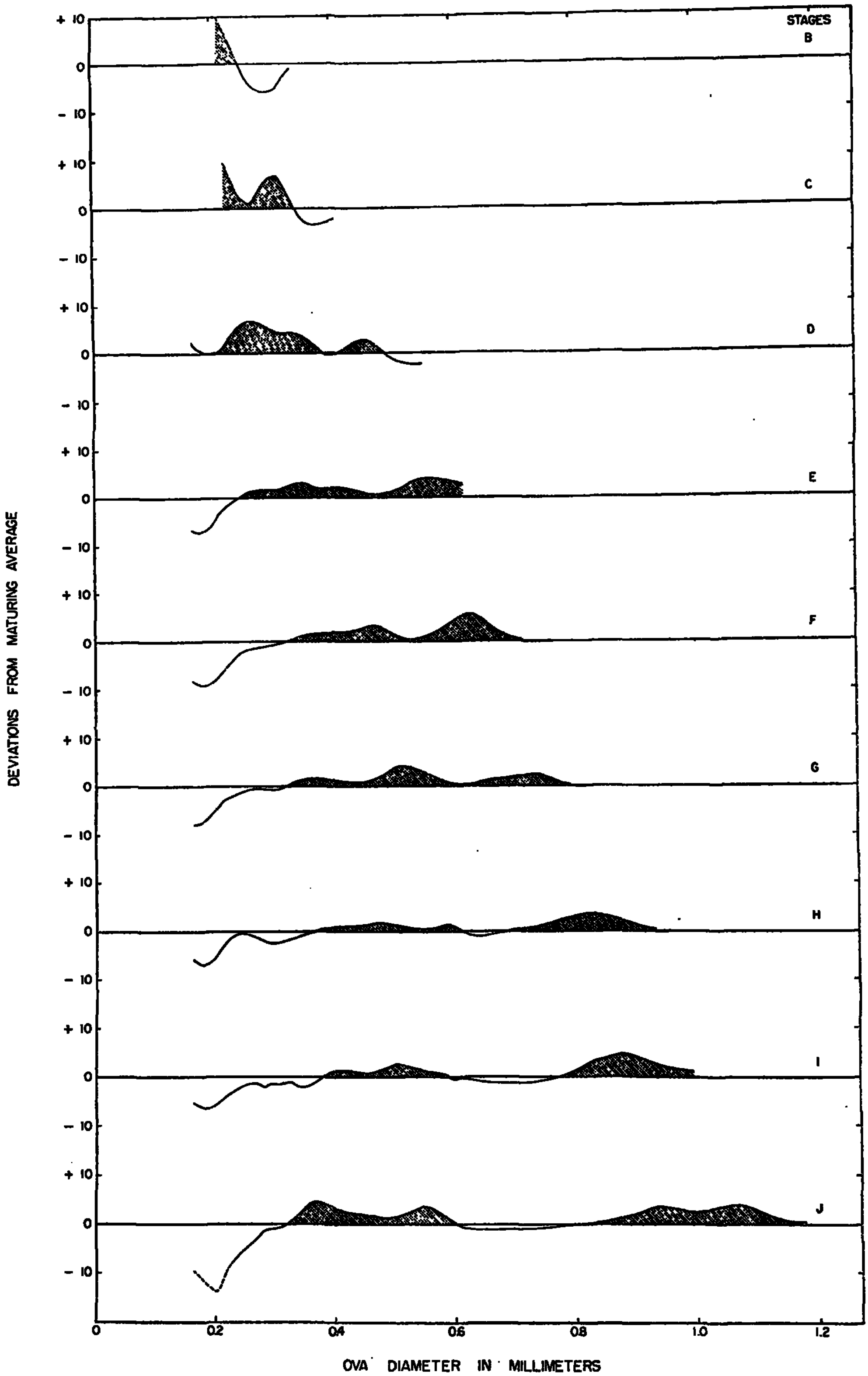

FIGURE 4.—Deviations from the average frequency polygon of stages B through J. The deviations were smoothed to minimize chance fluctuations.

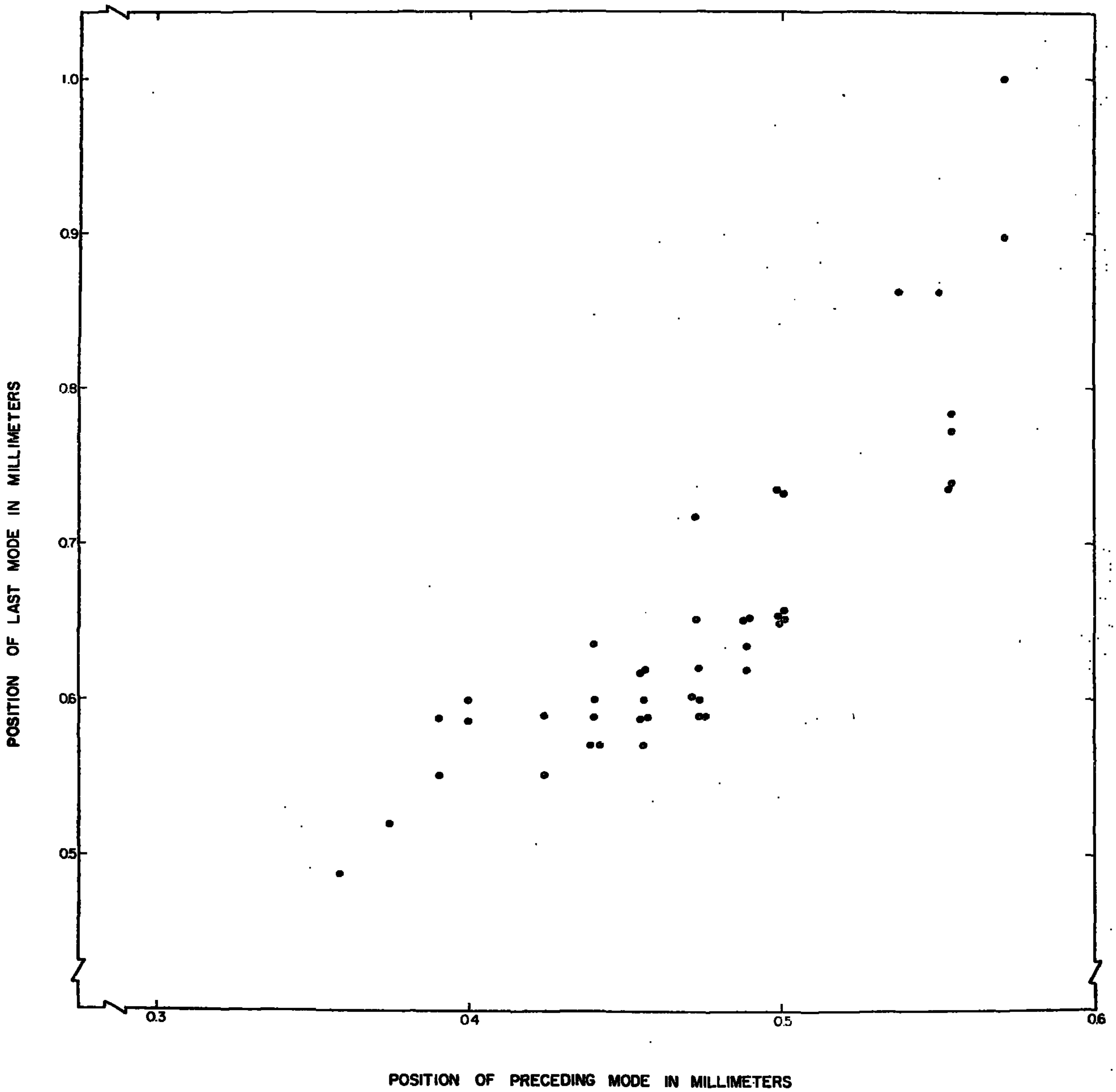

FIGURE 5.—Scatter diagram showing relation between modal groups of ova in the ovaries, stages E through J.

tered in the lumina. Of these eight samples, one was collected in June, one in August, five in October, and one in November. Thus, the presence of ova remnants in ovaries which show every indication of future spawning gives further evidence in favor of the hypothesis that more than one group of ova is matured and spawned by an individual fish in a single breeding season.

It is suggested in figure 3, by the trough at about 0.2 mm. in stages E to J, that many ova graduate from the immature to the intermediate group in stages A to D and few in stages E to J. In table 2 we find that fish with ovaries in stages A to D occur from November to May, tapering down to very few by June. This distribution of stages A through D suggests that the intermediate group should be found in largest number by early summer. Thereafter, if the intermediate group furnishes successive groups of ripe ova, the number of ova in the immature group should decrease. To determine whether the latter was true, comparisons were made of the ova-diameter frequency polygons for 23 fish in stage F according to the months in which they were collected. The number of ova larger than 0.55 mm. in diameter was compared with the number of those measuring between 0.18 and 0.53 mm. Ratios of the numbers of ova comprising intermediate and maturing groups for all fish in stage F are shown in table 3. It may be seen that in June the ratios ranged between 4.36:1 and 5.98:1, the average being 5.08:1. For July the average ratio, 4.50:1, was slightly less. It declined more in August (3.80:1) and still more in September (3.25:1).

To determine the consistency in each month of the ratios obtained for fish with ovaries in stage F, the chi-square test was used. No significant interaction (the smallest probability value obtained was about 0.70) was found among the individual samples collected during any one month; therefore, the pooled data for each month were used to test the consistency of the ratios among the 4 months, June through September. As shown by an interaction chi square of 27.72 ($P<0.01$), according to the method of Snedecor (1946, sec. 9.10), from the data presented in table 4, it is unlikely that the monthly ratios were drawn from a homogeneous population. Thus, there is a significant decline in the ratios of the maturing to the intermediate group through successive months.

This is not due to a change in the size of fish during the season. It is seen in table 3 that the mean weights of yellowfin tuna with ovaries in stage F did not change appreciably during the period considered. By an analysis of variance, it was found that among these samples, the difference between months was not more significant than the difference within months ($F=1.31$; $P>0.05$). In other words, the population did not change significantly as to size during the season. Thus, if the change is not connected with size of fish, it doubtless reflects a decline in proportionate numbers of ova composing the intermediate groups.

TABLE 2.—*Distribution by months of fish with ovaries in the various stages of maturity*

Month	Stages of maturity																						Total number
	A		B		C		D		E		F		G		H		I		J		K		
	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	
January	4	66.7	1	16.7	1	16.7																	6
February	3	60.0	1	20.0	1	20.0																	5
March	2	40.0	3	60.0																			5
April	5	62.5	2	25.0	1	12.5																	8
May							1	50.0			1	50.0											2
June							1	6.7			8	53.3	3	20.0	1	6.7	1	6.7	1	6.7			15
July									2	18.2	7	63.6	1	9.1	1	9.1							11
August									1	12.5	5	62.5			1	12.5	1	12.5					8
September											4	100.0											4
October	2	15.4							2	15.4	4	30.8	1	7.7							4	30.8	13
November	13	86.7											1	6.7							1	6.7	15
December	18	90.0	1	5.0	1	5.0																	20
Total	47		8		4		2		5		29		6		3		2		1		5		112

TABLE 3.—*Ratios of ova between 0.18 and 0.53 mm. to ova larger than 0.55 mm. for all fish with ovaries in stage F, June–September 1950*

Date	Fish weight	Ratio
	Pounds	
June 6	154	5.00:1
13	126	4.36:1
20	135	4.45:1
22	125	4.66:1
23	131	5.12:1
26	126	5.98:1
29	136	5.98:1
Average ratio		5.08:1
July 3	104	5.12:1
7	130	3.84:1
12	143	3.76:1
12	105	4.66:1
14	124	4.36:1
19	132	4.77:1
25	134	5.00:1
Average ratio		4.50:1
Aug. 1	119	3.84:1
3	177	3.48:1
3	147	3.84:1
11	124	4.17:1
15	117	3.69:1
Average ratio		3.80:1
Sept. 6	121	3.11:1
8	118	3.54:1
18	194	3.35:1
25	171	3.00:1
Average ratio		3.25:1

This decline is to be expected if there are successive spawnings by each fish.

TABLE 4.—*Proportionate numbers of ova more than 0.38 mm. in diameter for all fish with ovaries in stage F, June–September 1950*

Month	Total number of ova	Number of ova over 0.38 mm. in diameter	Probability of ova over 0.38 mm. in diameter [1]
June	2,100	349	0.166
July	2,100	385	0.183
August	1,500	313	0.209
September	1,200	283	0.236

[1] The ratio of pooled ova in the two groups for each month.

In summary, the presence of several groups of ova in the maturing ovaries as indicated by the ova-diameter frequency polygons, the significant correlation between successive groups of maturing ova, the presence of remnants of mature ova from a previous spawning in the lumina of the ovaries at the same time that successive groups are maturing, and the decrease in the proportionate numbers of ova composing the intermediate group as the spawning period progressed appear to be ample evidence that individual yellowfin tuna spawn more than once during the breeding season in Hawaiian waters. However, from this study, it is not possible to give any estimate of the number of times an individual fish may spawn during the season or the period of time that elapses between spawnings.

Among ovary samples collected during November and early December 1950, after spawning had terminated, were several with scattered remnants of mature ova in the lumina, indicating that the fish had spawned the previous summer or fall. Except for these remnants, which consisted of shells, some with the oil globules still discernible, only immature ova were present. Ovaries collected during January, February, and March, 1950, contained only immature ova, and no remnants were evident. Thus, it appears that ova in the intermediate stage at the close of the spawning period are absorbed and are not carried over to the following year.

RELATION OF OVARY SIZE TO FISH SIZE AS A MEASURE OF MATURITY

Having defined the stages of maturity according to the position of the mode of the most mature group of ova in the ova-diameter frequency distributions, the question then arises: Is there some simpler technique by which these stages might be approximated without the laborious measurements of ova diameters? If a relation exists between ovary weight at particular stages of maturity and fish weight (as a measure of fish size), it would be possible to compare the changes in the growth of the ovaries for fish of comparable sizes.

One means of estimating this relation is to determine the percentage that the ovary composes of the total weight of the fish. This method was used by Hoek (1895) as a measure of the degree of ripeness in Rhine salmon. Masterman (1913a and 1913b) later applied the method for estimating sexual maturity in European salmon and smelt (*Osmerus eperlanus*). Others investigating the spawning of the European salmon have followed Masterman. This method also has been used to a certain extent by Olsen and Merriman (1946) in their study of the spawning of the ocean pout (*Macrozoarces americanus*) in the North Atlantic.

While the relation of the ovary weight to the body weight gave a fair approximation of relative maturity in the yellowfin tuna, the ratio expressing this relation was not directly proportional over the size range of fish for a particular stage of maturity. When ovary weight was plotted against body weight,[3] great variation among the ovary weights was found for fish of a particular size. These observations appeared to fall into two generally distinct groups, one consisting of fish with ovaries in the immature stages (A, B, and C) and the other consisting of fish with ovaries in the maturing and ripe stages (D through J); although some of the values were intermediate between the two groups.

The relation between body weight and ovary weight for the immature and intermediate stages of maturity (A, B, and C) was found to be linear over the ranges of fish size and ovary size. Furthermore, when weights of ovaries in stages B and C were plotted against fish weights, the resulting regression was nearly identical with the linear regression obtained for fish with ovaries in stage A. Covariance analysis indicated that the two regression coefficients were sufficiently similar ($F=2.30$; $P>0.05$) to warrant the assumption that they arose from a single homogeneous group

[3] Both fish and ovaries were weighed while fresh.

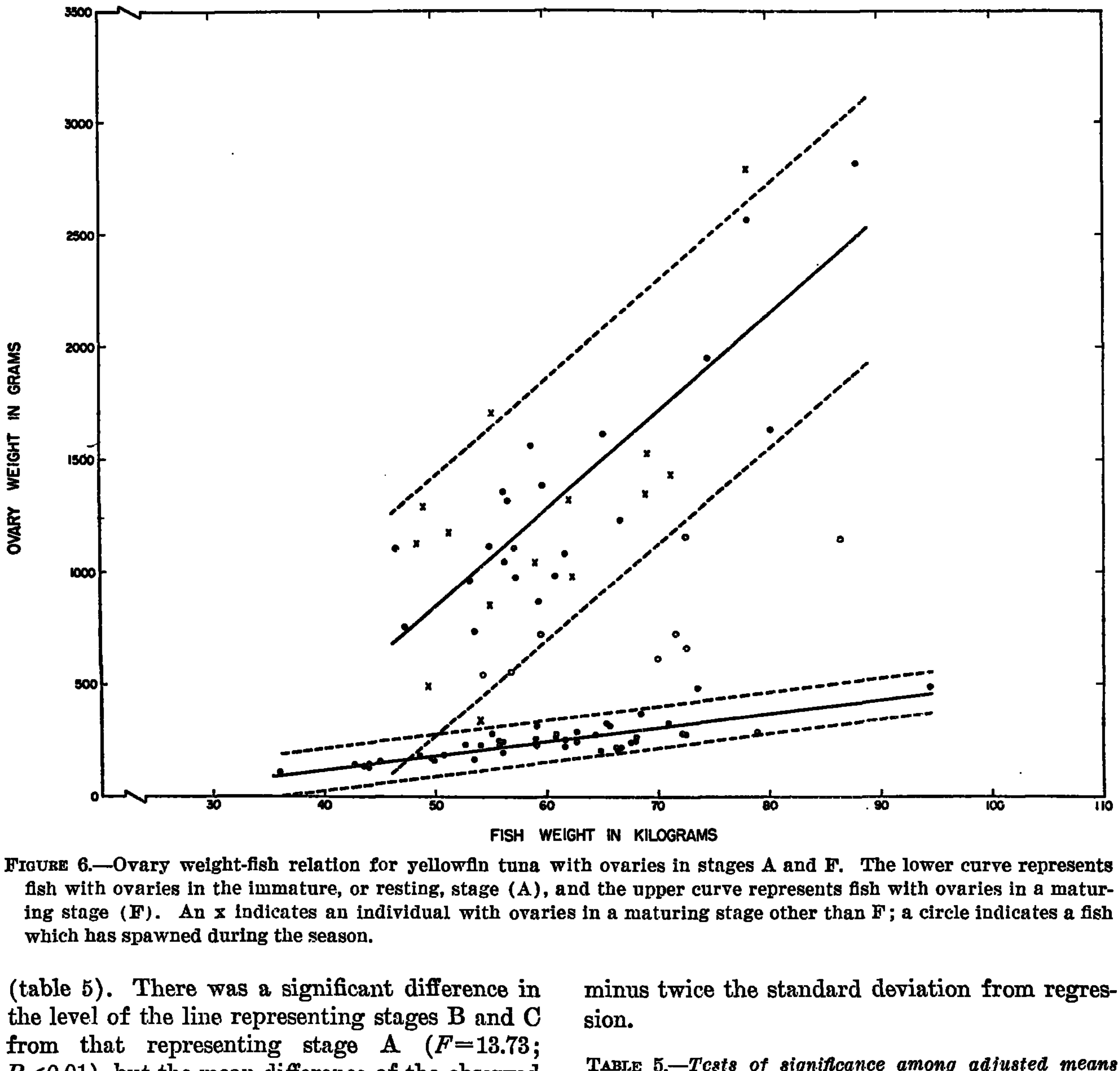

FIGURE 6.—Ovary weight-fish relation for yellowfin tuna with ovaries in stages A and F. The lower curve represents fish with ovaries in the immature, or resting, stage (A), and the upper curve represents fish with ovaries in a maturing stage (F). An x indicates an individual with ovaries in a maturing stage other than F; a circle indicates a fish which has spawned during the season.

(table 5). There was a significant difference in the level of the line representing stages B and C from that representing stage A ($F=13.73$; $P<0.01$), but the mean difference of the observed ovary weights was only 61.7 grams. Because of the significant difference in the levels of the two lines and because there were only eight ovaries in stage B and four in stage C, these were excluded from the linear regression of ovary weight on fish weight for the immature stage. The plotted data for stage A, shown in figure 6, were found to be best fitted by the equation

$$Y=6.0929X-124.35$$

where Y is ovary weight in grams and X is fish weight in kilograms. The dashed lines drawn parallel to the regression line represent plus and minus twice the standard deviation from regression.

TABLE 5.—*Tests of significance among adjusted means and between the regression coefficients of ovaries in stage A and in stages B and C*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total deviations from pooled linear regression	55	123.99888	----------
Deviations from average regression within samples	54	98.85509	1.83065
Deviations from individual regressions	53	94.74937	1.78772
Difference between regressions	1	4.10572	4.10572
Difference between adjusted means	1	25.14379	[1] 25.14379

[1] Highly significant.

As noted previously, there is an obvious increase in ovary weight as the ova develop to the maturing stages. The group of data shown in the upper part of figure 6 represents fish with ovaries in the ripe and maturing stages of development, D through J. Because stage F (shown by dots) was the only maturing stage which was well represented in the collection, it was used to describe the relation between ovary weight and fish weight at the approach of spawning. Over the ranges of fish size and ovary size, the regression of ovary weight on fish weight for ovaries in stage F[4] was found to be best described by the linear equation

$$Y=43.4811X-1327.8573.$$

The regression coefficient had a standard error of 6.060. The lines drawn parallel to the regression line indicate plus and minus twice the standard deviation from regression.

It is evident from figure 6 that the slopes and levels of the two regression lines, one representing fish with ovaries in the immature, or resting, stage (A) and the other representing fish in a maturing stage (F), are significantly different. The difference in slope is due to the fact that in stage F the larger fish tend to have relatively larger ovaries. The difference in level reflects the increase in size of the ovaries between stage A and stage F.

The values representing fish with ovaries in other maturing stages (D, E, G, H, and I) and the ripe stage (J), and those in which there was no evidence of previous spawning, are indicated in figure 6 by an x. Unfortunately, there are too few samples in any one of these stages to establish a regression with any degree of confidence. From the fact that most of the values for these stages lie within two standard deviations of the regression line for stage F, it appears that when the ovaries reach stage D they have already greatly increased in weight over stage A and that they increase very little while progressing through stages D, E, F, G, H, and I. The paucity of samples of all of these stages except F also suggests that the stages, other than F, probably are passed through very rapidly. The scarcity of some of the younger stages such as D and E might be ascribed to undersampling in May, but irregularity of sampling can scarcely explain the consistent dominance of stage F over all other stages during June to September. This dominance seems most reasonably explained by stage F being relatively long and stable and the other stages being transitory and rapidly passed. For practical purposes, then, but two groups need be recognized: (1) Fish with ovaries in stages A, B, and C, or in nonspawning condition; and (2) fish with ovaries in stages D to J, or in spawning condition.

For those ovaries in maturing stages in which some spawning had occurred during the season (remnants of mature ova were in the lumina), the points fall somewhere below the upper line but lie above the lower line. These are shown as circles in figure 6.

With respect to time of year, the regression for ovaries in stage A expresses the relation between ovary weight and fish weight during the off-breeding season (November through April), and the regression for ovaries in stage F expresses the relation during the spawning period (mid-May through October) as indicated by these data. (See Spawning Season, p. 61.)

Thus, by utilizing the scatter diagram of figure 6, it is possible, over the size range 36 to 94 kg. (80 to 208 lbs.), to determine whether an individual yellowfin tuna is in nonspawning or spawning condition by simply locating on figure 6 the coordinates of the weight of its ovaries and of its total weight. The coordinates will probably cross within one set of dashed lines or the other and the fish may be appropriately identified as nonspawning or spawning; some fish will fall at intermediate coordinate points—they are most probably in spawning condition and have already extruded some ova.

It is of interest that some discrepancy was found between the classification of the ovaries on the basis of their external appearance and their classification on the basis of the arbitrary stages of maturity established by ova-diameter measurements. It will be recalled (p. 48) that at the time of collection, the relative maturity of each ovary sample was recorded according to the stages established by Marr (1948). Among the 112 samples in the collection, this subjective method of classification resulted in 13 fish with maturing ovaries being incorrectly identified as spawned out, or roughly, in an error of about 10 percent. The greatest source of error was among those ovaries in stages E and F in which some spawning had taken place

[4] Only those ovaries in stage F which showed no evidence of previous spawning during the current year, as determined by the absence of remnants of ripe ova in the lumina, were used in the calculations.

during the season. On the whole, however, if Marr's definitions were grouped, placing his "immature" and "spawned-out" in "nonspawning" ("spawned-out" ovaries would have to be examined with a hand lens to eliminate those which still contained the yellowish, maturing ova in the follicles), and his "ripening" and "ripe" in "spawning," relatively few fish would be placed in different categories than those resulting from weighing the ovaries and the fish and plotting their coordinates on figure 6.

NUMBER OF OVA SPAWNED

To estimate the number of ova produced at a single spawning by yellowfin tuna of different sizes, calculations were made for 11 fish over the size range 47.2 to 88.0 kg. (104 to 194 lbs.). (See table 6.) While estimations for a greater number of specimens would have been desirable, the labor involved in separating the ova from the follicles and the subsequent ova measurements precluded the study of additional material.

TABLE 6.—*Estimated number of ova produced at one spawning by yellowfin tuna*

Weight of fish	Calculated number of maturing ova
	Thousands
47 kg	2,370
53 kg	3,390
56 kg	4,340
57 kg	5,170
59 kg	3,230
59 kg	6,510
61 kg	3,580
62 kg	5,610
69 kg	6,380
80 kg	6,000
88 kg	8,590

Ovaries were selected in which the most mature group of ova could be easily distinguished from ova being developed for subsequent spawnings and yet they were not sufficiently mature that any ova had been lost by spawning. Since it has been suggested that individual fish probably spawn more than once during a single breeding season, it is possible that subsequent spawnings would contain fewer ova; therefore, to make all estimations comparable, only ovaries in stage F which showed no evidence of previous spawning during the season were used. Accordingly, the estimates represent the number in the first of several possible spawnings. The method employed in calculating the number of ova was as follows: Ovaries from an individual fish were removed from the formalin preservative, and when sufficiently drained, they were weighed on a beam balance to the nearest 0.1 gram. A small sample of approximately 0.5 gram was removed from the ovaries and weighed on an analytical balance to the nearest 0.001 gram. The ova in the sample were teased out of the follicles, and measurements and counts were made of all ova composing the most mature group. The total number of ova in this group was calculated for each fish specimen by multiplying the ratio of the number of ova to the weight of the sample by the total weight of the preserved ovaries. Since the multiplier was in the order of 10,000, the estimate was recorded only to the nearest ten thousand ova.

The data shown in table 6 indicate that the number of ova produced at a single spawning increases with the size of the fish. This phenomenon has been demonstrated for many fishes, including the Volga-Caspian herrings, *Caspiolosa caspia*, *C. c. saposhnikovi*, *C. c. volgensis*, and *C. c. kesselri* (Kisselevitch 1923), the grunion, *Leuresthes tenuis* (Clark 1925), the California barracuda, *Sphyraena argentea*, (Walford 1932), the California sardine, *Sardinops caerulea*, (Clark 1934), and the surf smelt, *Hypomesus pretiosus*, (Schaefer 1936). The relation between number of ova and size of fish for the yellowfin tuna is shown graphically in figure 7. Assuming the

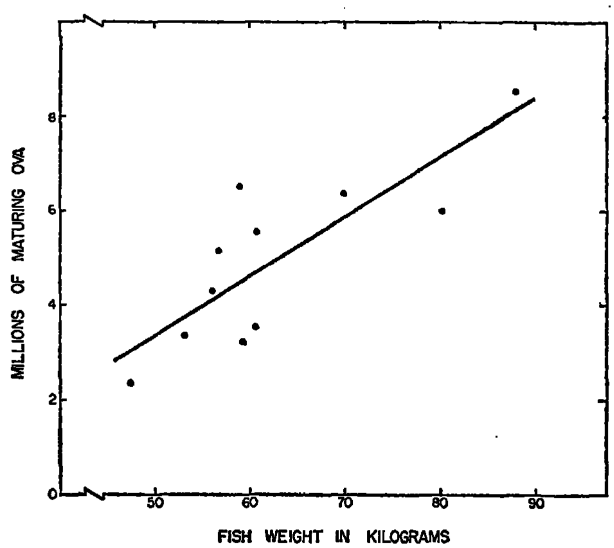

FIGURE 7.—Scatter diagram showing relation between the calculated number of ova produced for one spawning and the body weight of the fish.

relation to be linear, a straight line was fitted to the data by the method of least squares and is described by the equation

$$Y = 125{,}200X - 2{,}853{,}000,$$

where Y is number of maturing ova and X is fish weight in kilograms.

With 2 to 8 million ova produced by a spawning female in one of several possible spawnings a season, it is likely that a season's spawning of a small female exceeds 5 million ova and of a large female may be in the order of tens of millions.

SPAWNING SEASON

Until recently, the period over which spawning of the yellowfin tuna takes place in Hawaiian waters has been a matter of conjecture. There are no previous reports in the literature with regard to spawning of the species in this area. Commercial fishermen and local fish dealers had observed fish with enlarged gonads during the summer months, but could give no reasonably accurate information about the time of year these maturing ovaries occurred. It was not until 1949, when ovaries of yellowfin tuna first were sampled from landings of the longline fishery, that estimates of the onset and cessation of the spawning period were possible.

As pointed out previously, the ovary collections made during 1950 were continuous over the 12-month period. Furthermore, it has been shown that ovaries of yellowfin tuna from local waters pass through a maturing process which is consummated in spawning at some time during the 12-month interval. It remains, therefore, for us to determine the period during which spawning is most active and to establish its limits.

On the basis of the stages of maturity already described, the relative percentages of fish with ovaries in these various stages in each month give a good indication of the extent of development of the ovaries with respect to time of year. These percentages are presented in table 2, and show that fish with ovaries in the immature and intermediate stages were found in the samples only during the early months, January through April, and in the latter part of the calendar year, indicating that spawning did not occur in Hawaiian waters during the period mid-November through April. Beginning in May, fish with ovaries in stage F were present in the fishery. Unfortunately, the samples available during this period were few in number and, indeed, they may not give a true indication of all stages of sexual development represented in the fishery at that time. By June, however, all maturing stages with the exception of E were present in the samples, and during July and August most of the maturing stages were represented. The first spawned-out fish were taken in our collections in mid-October; yet, at that time the ovaries of more than half the fish sampled were in the maturing stages (E, F, and G). A single fish was found with ovaries in stage F that showed no indication of having spawned previously during the year. During the first week of November, a specimen with ovaries in stage G was taken; however, the ovaries of all other fish sampled were in either immature or spawned-out stages.

The data of table 2 may be summarized by combining stages A, B, and C to represent fish in nonspawning condition and by combining stages D through J to represent spawning fish. Fish with ovaries in stage K represent the spawned-out condition. The relative numbers of fish in each group are expressed as monthly percentages and are shown graphically in figure 8.

If our samples represent general spawning conditions, then from the data presented in table 2 and figure 8 it must be concluded that the spawning season occurred between mid-May and November.

To determine whether the increase in ovary size during spawning could be utilized to deliniate the spawning season, two methods of treatment were used: (1) A plot was made of the semimonthly ratios of ovary weight to fish weight (fig. 9); and (2) another plot (fig. 10) was made of the monthly percentages of fish in spawning condition as determined from the ovary-weight to fish-weight relation of figure 6. For the latter curve, all values which were excluded by the dashed lines representing two standard deviations from the regression for stage-A ovaries were considered to indicate fish in a spawning condition. This criterion agrees almost completely with the ova-diameter measurements which indicated only two nonspawning females with greatly enlarged ovaries—one taken in April and the other in November.

Both curves show that the spawning season extended from May to November. The curve (fig. 10) based on proportion of fish in spawning con-

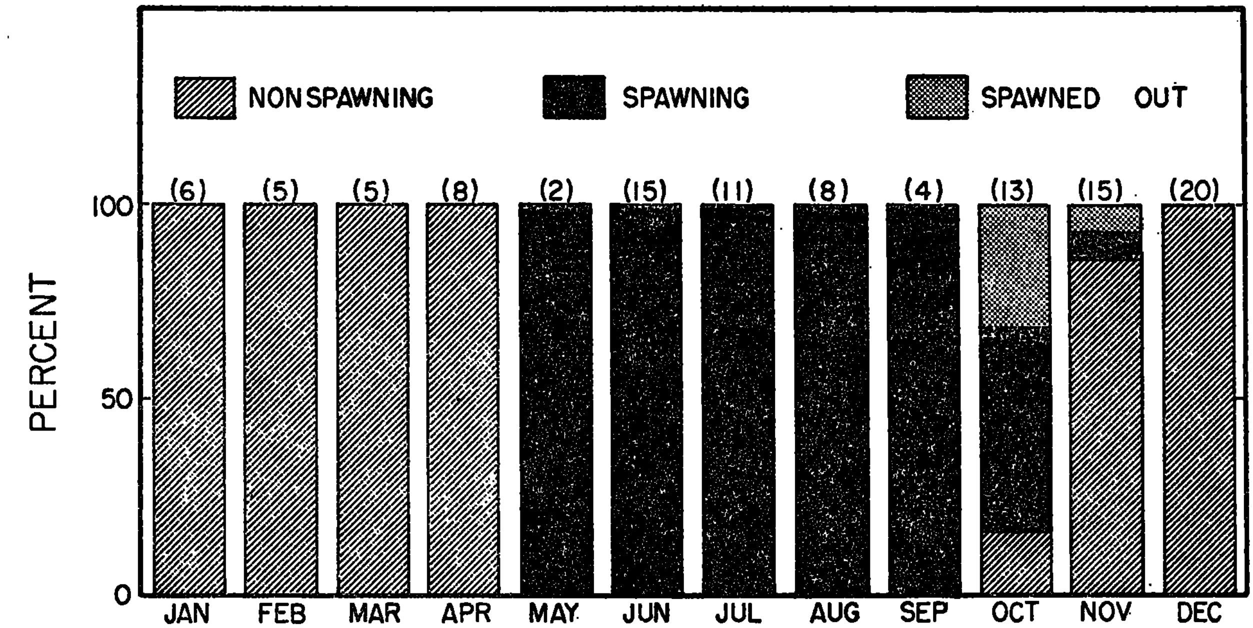

FIGURE 8.—Percentages of fish with ovaries in the nonspawning, spawning, and spawned-out conditions, by months. The number of fish included for each month is indicated at the top of each column.

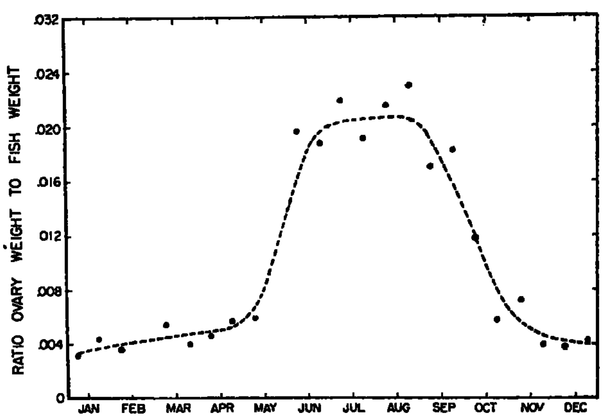

FIGURE 9.—The 1950 spawning season of yellowfin tuna as indicated by the mean ratios of ovary weight to body weight for all fish. The data are grouped into semimonthly periods.

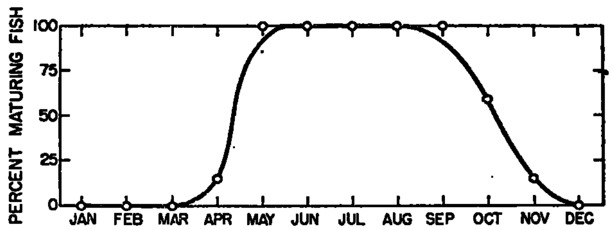

FIGURE 10.—The spawning season of yellowfin tuna represented by the percentages of maturing fish in each month, as determined by the ovary weight–fish weight regressions.

dition shows that no fish were in spawning condition from December through March, and that all were in that condition from May through September. The curve (fig. 9) based on ratios of ovary weight to fish weight, on the other hand, only shows that there was more spawning in the June to October period than in the rest of the year. Although interpretation of the ratios may make it possible to determine whether spawning involved all fish in the June to October period and none in the rest of the year, the regression method much more definitely establishes time and extent of spawning.

SPAWNING AND THE FISHING SEASON

The period of spawning and the peak of the fishing season so nearly coincide (fig. 11) that the fishery appears to be based on a spawning run. It should be remembered, however, that our use of the term "spawning fish" includes fish with well-developed ovaries that were not necessarily fully ripe for spawning. One fully ripe ovary was found among the samples taken. Fish that are actually spawning either do not take the hook or they migrate into deeper water outside the longlining grounds.

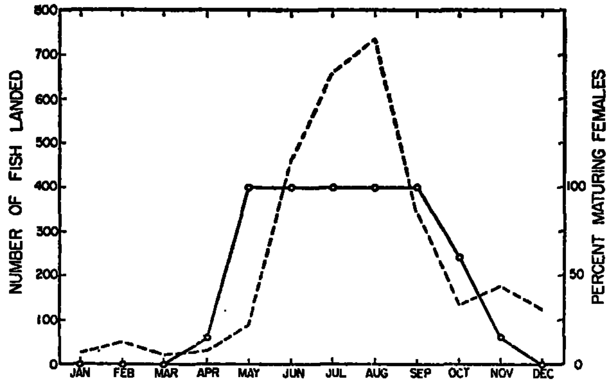

FIGURE 11.—Comparison of monthly yellowfin tuna landings for 1950 (– – – –) and the percentage of maturing females, as determined by the ovary weight-fish weight regressions (———).

SUMMARY

Ovaries from yellowfin tuna sampled from the longline catches landed at Honolulu during 1950 were used to determine the developmental changes that take place in the gonads during the breeding season, and data on the spawning of this species in Hawaii are presented.

It was found that several developmental groups of ova are present in the ovaries of individual fish during the breeding season. Based on diameter measurements of the most mature ova present, 11 arbitrary stages of maturity were established which trace the maturation of the ova from the immature stage through the spawning stage.

Several lines of evidence suggest that individual fish mature more than one group of ova and have several spawnings during the same season in Hawaiian waters. The number of ova produced in one spawning ranges from about 2,000,000 in small females to about 8,000,000 in large females.

Plotting ovary weight against fish weight yielded linear relations whereby the spawning condition of yellowfin tuna could be determined, thus providing a simple guide for distinguishing spawning from nonspawning fish without measuring ova.

The spawning period in Hawaiian waters during 1950 extended from about the middle of May to the end of October, and it was during this period that the best catches of yellowfin tuna were made in the longline fishery.

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