

# SPAWNING HABITS OF SOME PHILIPPINE TUNA BASED ON DIAMETER MEASUREMENTS OF THE OVARIAN OVA<sup>1</sup>

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SIX TEXT FIGURES

## INTRODUCTION

The ecology of the tuna, its large size, and extensive migratory habits precludes a direct study of much of its life history. Its spawning habits have, consequently, been largely approached by such indirect methods as collecting juvenile stages and planktonic eggs, size at its first sexual maturity, and year classes based on the commercial catch, etc. The present study is an attempt to add to our knowledge of the spawning behavior of Philippine tuna through the statistical analysis of growth of the ova in the ovary by measuring their increasing diameters. This method was developed by Thompson (1915) and successfully employed by such investigators as Clark (1925, 1929, 1934); De Jong (1940); Hickling and Rutemberg cited by De Jong (1940); and Mane (1929, 1934). The following commercially important species of Philippine tuna were considered in the present study: *Neothunnus macropterus*, *Katsuwonus pelamis*, and *Euthynnus yaito*.

## MATERIAL AND METHODS

### COLLECTION OF MATERIAL

Material was obtained from two sources, namely, tuna caught by trolling operations of exploratory vessels of the Philippine Fishery Program<sup>2</sup>, and tuna caught in fish corrals for the fresh fish markets and collected by market teams of the Program in Iloilo City, Iloilo Province, and Batangas, Batangas Province.

<sup>1</sup> This paper was prepared when the author was connected as Aquatic Biologist with the United States Fish and Wildlife Service, Philippine Fishery Program, 1947-1950. While the author has had the benefit of some suggestions from some technical personnel of the Service, the paper is entirely of his own planning and execution and he assumes full responsibility for it.

<sup>2</sup> A portion of the Philippine Rehabilitation Program authorized by the Philippine Rehabilitation Act of 1946, title 50, App. U. S. Code Sec. 1789.

The ovaries were preserved in 10 per cent formalin. Specimens from the catch of the exploratory vessels were preserved immediately after the fish were landed; those obtained by the market teams came from fish varying from strictly fresh to fish iced for a day or more. The ovaries were taken from females irrespective of size, season, place of capture, or condition of maturity.

#### MEASUREMENT OF THE OVA

To measure the diameter of the eggs, a binocular microscope, fitted with an eyepiece micrometer calibrated at 0.0325 mm for each micrometer unit, was used. All measurements were made directly from formalin-preserved specimens. Test measurements were made of eggs from the anterior, central, and posterior portions of the ovary. Slight, statistically insignificant, variations were noted but to minimize any possible error from this source, each ovary was sampled uniformly from the three sections, and the frequency of the total measurements was used to represent the ovary.

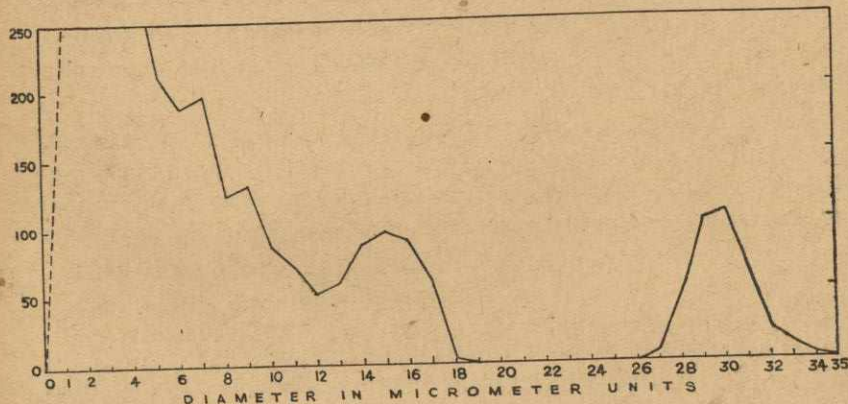


FIG. 1. Ova diameter frequency polygons of six ovaries of *Katsuwonus pelamis* of Stage V maturity.

Clark's (1926) method of measuring the diameters of the eggs by whatever axis they occur on the eyepiece micrometer was used with slight modification. De Jong (1940), in pointing out the disadvantage of this method, showed that "in the frequency polygon of the diameters of the ova, the difference between two groups of eggs appears less than they are in reality. Indeed, in some cases, the largest diameter of an egg of the group of the younger eggs exceeded the smallest diameter of an egg of an

older group." In the present study, the upper diameter limit of the maturing group of eggs at 14–17 micrometer units (0.45–0.55 mm) and the upper diameter limit of the ripe group at 33–34 micrometer units (1.07–1.10 mm) were shown to be of special significance. The former establishes the point where ripening starts and to which the spent ovary reverts after each fractional spawning. The latter may well be the basis for determining the size of the planktonic eggs of the tuna under study. Without modification, Clark's method of measuring the eggs would result in an upper diameter limit for these groups larger than is actually the case. To get true measurements for these groups, the average diameter of the egg was taken.

To preserve the peculiarity of each ovary, no measurements of eggs from ovaries of similar stage of maturity were combined for the purpose of producing a single smooth curve. Each ovary was treated as a distinct unit, and enough eggs were measured so that it was sufficiently sampled. An ovary was considered as sufficiently sampled when, in the resulting frequency polygon, no glaring artificial irregularities were present which would disappear if the curve were smoothed by a running average method.

It was observed in this study that the method of measuring a uniform number of eggs for all stages of maturity was defective in that the younger ovaries with few size groups were either sufficiently or excessively sampled, while the maturer ovaries with more size groups and larger eggs were inadequately represented. In order to obtain an adequate representation for all size groups at all stages of maturity, more eggs were measured in more mature ovaries in the following schedule:

Maturity of ovary	Number of eggs measured
Stages I and II <sup>a</sup> .....	500
Stage III .....	1,000
Stage IV .....	2,500
Stages V and VI .....	2,000
Stage VII .....	1,000

For the purpose of this study, the ovary was considered important only when it began to develop eggs larger than the immature. Hence, except for immature ovaries, only ova beyond

<sup>a</sup> Maturity scale adopted by the International Council for the Exploration of the Sea as cited by Clark (1934).

the immature stage were measured. By this method, more than 70,000 eggs from 145 ovaries of *Neothunnus macropterus*, *Katsuwonus pelamis*, and *Euthynnus yaito* were measured.

All diameter measurements of eggs are given in the original micrometer units by which they were measured and can be converted into millimeters by multiplying by 0.0325.

#### NEOTHUNNUS MACROPTERUS

##### CLASSIFICATION OF THE OVARIES AND OVA

In this paper the ovaries were classified into the various stages of maturity as immature, maturing, mature, ripe, and spent. These stages were then fitted to the International maturity scale as follows:

	Diameter of longest egg in micrometer units	International Maturity Scale
Immature .....	4	Stage I
Early maturing .....	10	Stage II
Late maturing .....	17	Stage III
Mature .....	27	Stage IV
		to Stage V
Ripe .....	34	Stage VI
Spent .....	16 or 17	Stage VII

With the exception of the spent stage, all the other stage designation may be applied to either the ovary or the largest egg contained therein. In the case of the spent fish, however, the ovary is classified as spent but the largest eggs are of the late maturing stage.

The following are the general descriptions of the external appearance of the different stages of the ovaries of *Neothunnus macropterus*:

The immature ovaries are small, much elongated, round in cross-section, with no apparent vascularization, fairly turgid.

From the maturing to the ripe stage the ovary is externally not sharply distinguishable from one stage to the next, inasmuch as the characteristic features, size and degree of ramification of blood vessels, increase imperceptibly from one stage to the next. The ovary becomes increasingly rounded and tightly packed. Towards the ripening stage, however, the ovary turns soft and delicate, the thin skin showing the large eggs inside. The blood vessels are much branched and deeply gorged. The spent ovary is soft and flabby and may contain a number of large degenerating eggs.

The different classes of ova show distinct structural differences as follows: The immature eggs are transparent and colorless, the diameter ranging between 0 and 4 micrometer units. The maturing group refers to eggs with a diameter range of from 3 to 16 or 17 micrometer units and which have turned opaque, the yolk starting to acquire a light orange color. This maturing group is subdivided into an early maturing group to designate eggs from 3 to 10 micrometer units, and to late maturing group to designate eggs from 10 to 17 micrometer units. Eggs beyond 17 micrometer units up to 27 micrometer units are classified as mature. These eggs are also opaque with a singular, well-developed, deep-yellow oil globule. The ripe eggs range in diameter from 27 to 34 micrometer units with the single oil globule ranging in diameter from 7 to 8 micrometer units. These ripe eggs are soft and delicate with a finely granular yolk of light orange color, and somewhat transparent. They have burst free from their follicles and can be easily pressed out from the ovary. Degenerating eggs of from 5 to 34 micrometer units have been observed in many ovaries of *Neothunnus macropterus* and more especially in the spent ovaries. These eggs showed the disintegration of the perivitelline membrane and the thinning of the yolk material which takes on a light to medium gray color. In the case of ripe degenerating eggs, the oil globule is often broken up into many small globules scattered unevenly on the yolk mass.

##### THE LARGEST EGG DIAMETER METHOD OF CLASSIFYING TUNA OVARIES

The literature on tuna available to the writer does not show of any standardized method of classifying tuna ovaries and for this reason various workers (Matsui, 1942; Schaefer and Marr, 1948) adopted systems of their own with the result that the data presented are not immediately comparable. For a widely distributed species as the tuna, the problems of which must necessarily be solved on the basis of joint efforts of many investigators from many countries, a uniform method of classification, especially of the ovaries, is badly needed. So far, the tuna ovaries have been classified on the basis of external appearance alone. Based on studies of 145 ovaries of the three species, the writer found that external appearance alone is inadequate and misleading even for gross classification of gonads. The characters extensively used in the

classification of the gonads are size, degree of softness or turgidity, and degree of ramification and gorging of blood vessels.

The size of the ovary is an undependable guide to its maturity. A small, slender ovary that looks immature may actually be a spent ovary preparing for a rest period. The size of the ovary depends not only on the state of maturity but also on the size of the fish because the fish continues to grow even after the first spawning and it goes through a number of spawning seasons during its lifetime.

Likewise, the degree of turgidity or softness of the ovary can also be misleading. A soft, and from all external indications, a spent ovary may actually be in a maturing stage, and may contain more mature eggs than those of ovaries that appear round and turgid. This happens, for instance, in the case of fish preparing to develop the first batch of eggs during its first spawning season (first spawner) and of fish that has just spawned a batch of eggs and is preparing to develop the next batch (repeat first spawner).

The number of blood vessels ramifying the walls of the ovary proved, in many cases, to be also a poor criterion for classifying the maturity of tuna ovaries. The generally accepted belief is that as the ovary matures vascularization becomes more pronounced. In all three species, many ovaries far advanced in maturity and identified as "first spawners" were observed to have smooth, clean, ovarian walls, with only faint traces of blood vessels. On the other hand, ovaries of "repeat first spawners" or "repeat second spawners" may be very heavily vascularized and deeply purpled even at an earlier maturity.

Again, the age of the fish complicates the matter, vascularization apparently becoming more thickly matted and deeply grooved as the fish gets older. These markings probably become more or less permanently set in the larger ovaries and may become increasingly less affected by the alternate cycle of spawning and sexual inactivity.

The numerous exceptions to the classification of tuna ovaries by their external features only tend to emphasize the insufficiency of this method. It is believed that the maturity of tuna ovaries can only be accurately determined by correlating external characteristics with the diameter measurements of the ova. To make this method practical, only the largest eggs are measured from a representative portion of the ovary by means

of an eyepiece micrometer in a microscope. No ova diameter frequency is taken and the upper size limit of the largest size group alone is used as the measure of maturity; hence, in this paper this method is termed the largest ova diameter method. The method, however, presupposes that the largest egg diameter range of the different stages of maturity in a species has been previously established by taking the diameter frequencies of the ova.

The use of the largest egg diameter method to determine the maturity of the developing ovary is based on the assumption that egg diameter is a fixed characteristic uninfluenced by internal or external factors and that it varies only with change in the position of the mode of the largest egg group. The position of the mode of the largest egg group would be the most accurate measure of maturity; however, this would necessitate the taking of the ova diameter frequency of the ovary and in determining maturity of a large number of ovaries this method, which would involve the measurement of a large number of eggs, would be impracticable.

To test the reliability of the largest egg diameter method, a comparison was made of measurements obtained by means of diameter frequencies. Six ovaries of Stage V maturity of *Katsuwonus pelamis* with the largest eggs measuring 23 or 24 micrometer units, were selected and 1,000 eggs as random

TABLE 1.—Ova diameter frequencies of six ovaries of *Katsuwonus pelamis* of stage V maturity.

Diameter in micrometer units	Egg diameter frequency					
	1	2	3	4	5	6
3	3	7	13	26	3	19
4	33	76	30	188	40	104
5	118	124	123	159	110	117
6	130	108	120	118	158	100
7	101	127	81	72	126	91
8	88	69	83	54	113	56
9	71	65	54	41	80	46
10	56	49	61	42	51	53
11	68	41	59	35	38	50
12	59	34	53	43	35	55
13	44	39	46	38	29	50
14	36	38	47	40	27	26
15	35	28	15	14	25	12
16		10				2
17			4	3		
18	7	4	21	1	6	
19	31	15	40	13	23	13
20	48	53	75	29	37	46
21	50	69	46	37	56	65
22	17	33	18	30	29	60
23	5	11	6	14	11	27
24				3	3	8
Total	1,000	1,000	1,000	1,000	1,000	1,000

sample from each ovary were measured (Table 1). The frequency polygons of these ovaries were then plotted together as in fig. 1. A close similarity is at once evident in the six polygons. The modes of the oldest group of eggs fall consistently at around 21 micrometer units, while the new batch of eggs forms a fairly distinguishable size group with the modes falling between 10 and 11 micrometer units. Grouped together as Stage V ovaries by the largest egg diameter method, the six ovaries showed almost identical frequency polygons which further confirm their closely identical maturity, and graphically show the high degree of reliability of the method.

The method may not be easily adapted for field studies especially on board small vessels; however, it is believed that for a more comprehensive study of the spawning behavior of the various species of tuna, accurate determination of the condition of maturity of the gonads is necessary, and this can only be done by correlating the size of the largest eggs in the ovary with the external appearance of the ovaries. This requires laboratory treatment of all the gonads.

#### HISTORY OF THE RIPENING OVA

##### THE RIPE OVARY

A nearly ripe ovary of *Neothunnus macropterus* was secured from Lipa City Market, Batangas Province, on July 31, 1949. This yellowfin was caught in a shallow water fish corral near Daet, Camarines Norte. It had a fork length of 567 mm. The pair of ovaries weighed 129 grams. During the period from October 1947 to November 1949, when the exploratory vessels of the Philippine Fishery Program were trolling in Philippine waters, not a single ripe female yellowfin was collected. Schaefer and Marr (1948) had the same experience with yellowfins and skipjacks in the Eastern Pacific Ocean. However, Sanzo, cited by Ehrenbaum (1924), obtained eggs from spawn-ripe *Ocrynus* (*Thunnus*) *germo* Lacépède, caught in fish traps in Sicilian waters.

The nearly ripe ovary of *Neothunnus macropterus* was well vascularized, and had a thin wall through which the developing eggs could be seen. Preserved in formalin, the ripe eggs appeared somewhat translucent and light orange in color, and many of them were wrinkled, having a somewhat collapsed appearance. The yolk was finely granular, but sometimes had a sprinkling of fairly spherical granules. Each egg had a characteristic deep-yellow oil globule, with the diameter rang-

ing from 7 to 8 micrometer units, or from 0.2275 mm. to 0.2600 mm. The nearly ripe eggs ranged in diameter from 27 to 34 micrometer units, or from 0.8775 mm. to 1.1050 mm. From measurements of degenerating ripe eggs encountered in newly spent ovaries of *Neothunnus macropterus* it is deduced that the size of the running ripe eggs of this species would probably not be very different from the above diameter range. No degenerating ripe eggs larger than 34 micrometer units were observed, and, therefore, the upper size limit of 34 micrometer units or 1.1050 mm. may be considered as fairly well established for ripe yellowfin eggs.

Figure 2 shows the frequency polygon of the diameters of 2,000 eggs measured at random from the nearly ripe ovary of *Neothunnus macropterus*. There are four groups of eggs apparent in this curve. The first group, between 0 and 4 micrometer units, is the most numerous and represents the immature, transparent eggs present in the ovary throughout the year. The second group, with a diameter of from 3 to 10 micrometer units, represents the early maturing eggs which have turned opaque. The third group, the late maturing eggs, are more opaque and larger, ranging from 10 to 17 micrometer units. The fourth group, from 27 to 34 micrometer units and shown to be widely detached from the rest, represents ripe or nearly ripe, semitransparent eggs which to all appearances would be spawned soon.

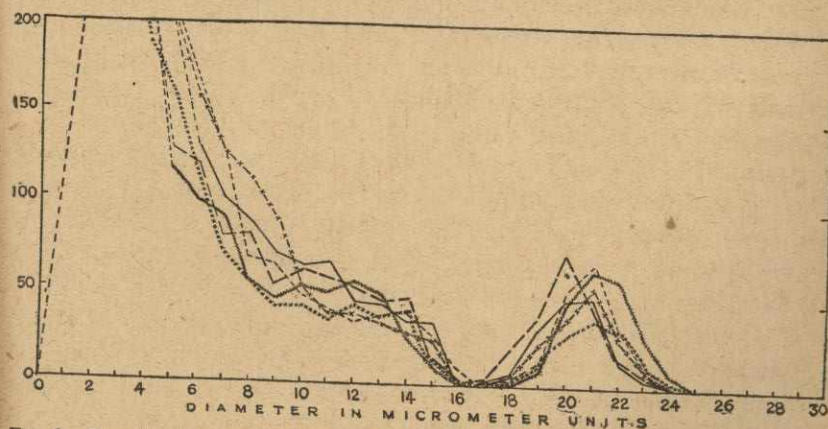


Fig. 2. Diameter frequency polygon of 2,000 eggs of a nearly ripe ovary of *Neothunnus macropterus*.

#### MATURATION OF EGGS IN THE YELLOWFIN OVARY

Wade (1949) in a study of spawning periodicity of Philippine tuna has shown that *Neothunnus macropterus* spawns

throughout the year in Philippine waters, although a period of intense spawning activity was suggested. Because of the absence of strictly periodic spawning, short and definite, it was not possible in the present study to establish the absolute duration of spawning, either in the species as a whole or in the individual of the species. For the purpose of demonstrating the growth of the ova in a spawning fish, the diameter of eggs from many ovaries was measured, and from the resulting frequency polygons a few were selected to illustrate the various stages in what would appear to be the logical order of growth. Figure 3 based on Table 2 shows the selected frequency polygons illustrating the composite picture of growth of ova to maturity in *Neothunnus macropterus*. The stage represented by each polygon has been designated in accordance with the maturity scale adapted by the International Council for the Exploration of the Sea as cited by Clark (1934). Except in the immature stages, the immature eggs, which were not measured, were indicated in the polygons by dotted lines.

TABLE 2.—Diameter frequency measurements of ova of *Neothunnus macropterus* at different stages of Maturation.

Diameter in micrometer units	Stage I	Stage II	Stage II-A	Stage III	Stage III-A	Stage IV	Stage VI	Stage VII	Stage VII-A
1	89								
2	276								357
3	109	11	62	254	40	182	12	36	273
4	26	109	345	164	141	371	142	239	182
5		212	243	114	148	249	212	204	84
6		135	169	84	121	226	188	146	51
7		33	92	79	104	140	198	96	31
8			53	70	69	109	124	51	17
9			24	57	58	79	132	49	5
10			10	56	42	74	88	34	
11			2	61	40	62	72	25	
12				49	53	70	52	22	
13				13	56	78	60	23	
14					53	54	88	30	
15					46	33	98	22	
16					23	14	92	19	
17						28	62	4	
18						61	88		
19						69			
20						15			
21						2			
22									
23									
24									
25									
26								8	
27								50	
28								104	
29								110	
30								66	
31								22	
32								12	
33								4	
34									
Total number of eggs measured	500	500	1,000	1,000	1,000	2,000	2,000	1,000	1,000

Stage I shows the frequency polygon of an immature yellowfin ovary. Only one group of eggs, the immature group, is present. At the start of spawning, a number of eggs from this immature egg-stock begins to develop. The initial growth is characterized by an increase in diameter of the eggs and a loss of translucency.

In Stage III, when the largest egg diameter reaches 13 micrometer units, the maturing batch begins to develop into a modal group. In Stage III-A, this group becomes sufficiently established as to show a definite mode at 13 micrometer units and the diameter of the largest eggs had increased to 17 micrometer units.

In Stage IV the mode of the maturing group has shifted to 19 micrometer units, and the largest eggs now measure 22 micrometer units. At this stage, a new group of eggs had started to develop between 10 and 16 micrometer spaces.

No Stage V ovaries of *Neothunnus macropterus* were collected. However, by referring to Stage V in figs. 4 and 5 representing the growth of ova in *Katsuwonus pelamis* and *Euthynnus yaito*, respectively, a fairly good picture can be constructed representing what can be reasonably expected as the developmental characteristics of the mature group in *Neothunnus macropterus*. In these two frequency polygons, the maturing group, now designated as mature, with the egg diameter ranging from 19 to 24 micrometer units, is shown to have been separated completely from the younger groups. At the same time, the second batch of eggs has developed more distinctly as a group.

Stage VI (fig. 3) is a reproduction of the nearly ripe yellowfin ovary illustrated in fig. 2 and shows the now translucent ripening group further shifted to the right, with an egg diameter range of from 27 to 34 micrometer units. As demonstrated by indirect evidences, the upper diameter limit of running ripe ova of *Neothunnus macropterus* should be about 34 micrometer units. A close examination of the ripening group in the nearly ripe ovary shows that a few eggs on the lower diameter limit of 27 micrometer units were still opaque while all the larger eggs have turned hyaline. It appears that 27 micrometer units is the diameter at which the mature eggs of yellowfin turn translucent and become ripe.

Of the 60 ovaries of *Neothunnus macropterus* measured, no ovaries beyond Stage VI, with the sole exception of the

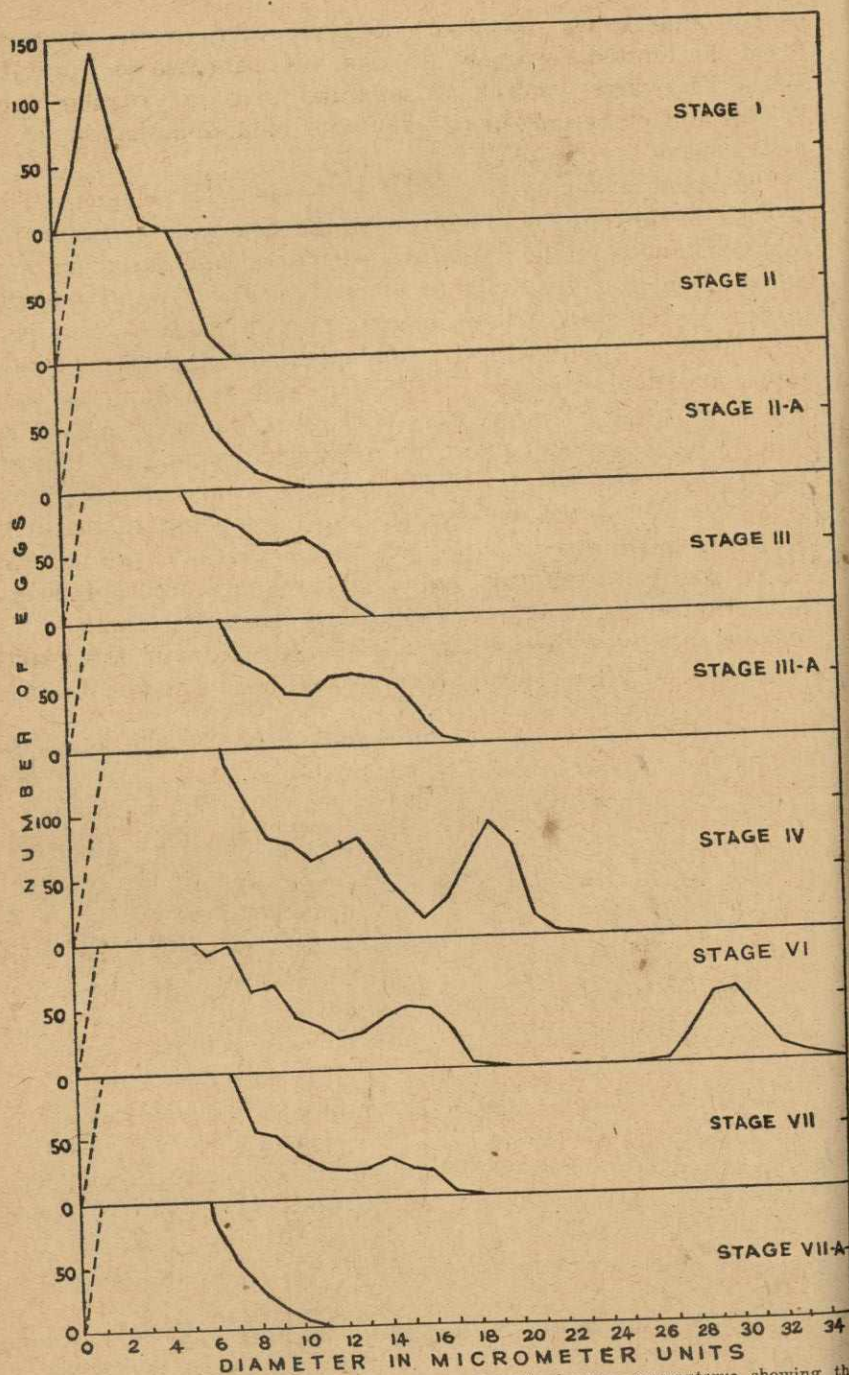


FIG. 3. Ova diameter frequency polygons of *Neothunnus macropterus* showing the various stages of development of the ova to maturity.

nearly ripe ovary collected in Lipa City, were observed. From the limited evidences on hand, yellowfin females carrying Stage VI ovaries appear to separate from the regular tuna schools and/or become more wary with baits and shallow water contraptions.

The spent ovary is presented in Stage VII polygon. This resembles the frequency polygon of the ripe ovary without the ripe eggs and contains immature, early, and late maturing eggs up to 17 micrometer units in diameter. The type of polygon similar to the spent ovary appears first in Stage V and persists through Stage VI, with the maturing eggs as a second batch, and finally back to Stage VII, with the maturing eggs as a first batch. The position of the new batch of eggs from Stage V through Stage VII is strikingly unchanged, indicating arrested growth of the ova beyond 17 micrometer units, while the first batch of eggs is being developed to maturity and final deposition. If growth takes place at all in the second group during this period, it probably occurs between the immature egg-stock and the early maturing stage.

From the above discussion, it is indicated that in the nearly ripe ovary of *Neothunnus macropterus* there are two batches

TABLE 3.—Diameter frequency measurements of ova of *Katsuwonus pelamis* at different stages of maturation.

Diameter in micrometer units	Stage I	Stage III	Stage III-A	Stage V	Stage V-A	Stage VII	Stage VII-A
1	85						
2	173						
3	138						32
4	104	25	32	34		95	382
5		83	189	182	206	268	250
6		106	199	204	204	200	118
7		146	141	175	211	131	100
8		130	125	160	191	87	68
9		113	87	98	135	57	27
10		80	69	80	113	27	15
11		61	66	92	104	31	8
12		58	58	87	94	19	
13		52	43	110	105	18	
14		59	38	87	95	24	
15		61	49	46	56	20	
16		22	69	8	23	15	
17		4	50	4	7	8	
18			29		2		
19			6				
20				23			
21				80	5		
22				113	11		
23				105	42		
24				48	102		
25				14	156		
26					94		
27					36		
					8		
Total number of eggs measured...	500	1,000	1,250	1,750	2,000	1,000	1,000

TABLE 4.—Diameter frequency measurements of ova of *Euthynnus yaito* at different stages of maturation.

Diameter in micrometer units	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage V-A	Stage VII
1	159						
2	227						113
3	102		11	39	6	209	208
4	12	66	99	132	271	395	136
5		216	154	162	258	200	92
6		175	113	199	241	167	92
7		43	103	170	155	146	53
8			94	134	107	108	63
9			87	126	87	97	50
10			53	88	91	65	53
11			60	89	112	68	42
12			56	38	116	63	42
13			81	13	106	68	42
14			66	28	37	76	51
15			23	75	13	37	25
16				89	1	5	
17				72			
18				36			
19					8		
20					30		
21					58	5	
22					107	12	
23					101	60	
24					66	72	
25					26	100	
26					3	38	
27						9	
Total number of eggs measured...	500	500	1,000	1,500	2,000	2,000	1,000

of developing eggs, one nearly ripe and the other half ripe. The half ripe group is expected to take the place of the ripening group as soon as the latter is spawned. The composite picture of growth of the ova shown in fig. 3 tells nothing as to the absolute time interval between spawning, but it can reasonably be stated that the time between two spawnings would be less than the time necessary for the eggs to grow from the immature egg stock to maturity. The time it takes an egg to grow and mature is unknown.

#### FREQUENCY OF SPAWNING DURING A SEASON

##### EVIDENCE FROM THE MULTIPLICITY OF MODES IN THE RIPENING OVARY

The preceding discussion on the nature of spawning in yellowfin presumes that several batches of eggs are laid by a spawning fish during its spawning season; that is, the developmental cycle from Stage VII to Stage III and back to Stage VII shown in fig. 3 is repeated several times until the end of the season when Stage VII develops into Stage VII-A and the ovary goes into a resting period.

That the fish spawns more than once during a season is strongly suggested by the several modes in the ripening ovary.

Stage II through Stage IV shows the progressive formation of a new group of eggs even before the maturing group has completely detached itself from the younger groups. Whether this new group of eggs will mature and be eventually spawned, or degenerate and be resorbed, or whether this represents eggs which would be spawned in a succeeding season, is a problem that warrants discussion.

##### EVIDENCE FROM THE SPENT OVARY

That the new group of eggs does not represent those which would be spawned in a succeeding season is demonstrated by the following observation: The growth of the upper size limit of the second batch of eggs has been shown to be arrested at 16 or 17 micrometer units during the period the first batch is being ripened. The spent yellowfin ovary goes through a rest period with only immature eggs or with immature and early maturing eggs not exceeding 10 micrometer units in diameter. If the second batch of eggs is to be spawned during the next spawning season instead of during the current spawning period, all spent fish should be going through a rest period with Stage VII ovaries with the diameter of largest eggs ranging from 16 to 17 micrometer units.

##### EVIDENCE FROM THE PRESENCE OF RIPE DEGENERATING EGGS IN MATURING OVARIES

Degenerating ripe eggs have been frequently observed in the spent and maturing ovaries of *Neothunnus macropterus*. Even in ovaries that have reached Stage V maturity there may still be a few ripe eggs in a degenerating state. The degenerating eggs are obviously remnants from a previous spawning. They acquire the status of extraneous bodies as soon as the ovaries are spent and the process of resorbing or eliminating them can be expected to start immediately after the fish has spawned. The fact that they are encountered even in Stage V ovaries indicates the relatively short time it takes for the maturing group of 12 to 17 micrometer units in the spent ovaries to develop to the distinct batch in Stage V ovaries with a diameter range of 17 to 24 micrometer units. Since it takes such a short time for the maturing batch of eggs to grow to full maturity, new batches of eggs must be matured and spawned at frequent and short intervals allowing the ovary only a brief rest period between spawning. This phenomenon has been observed by Clark (1925, 1929,



1934) and by the existence of these degenerating ripe eggs, she demonstrated the multiplicity of spawn in the grunion, the jack smelt and the California sardine.

#### THE SPENT AND THE RESTING OVARIES

Of the 60 ovaries of *Neothunnus macropterus* examined, only 3 were found to be spent. The scarcity of spent females was also noted by Wade (1949) who failed to observe a single spent fish.

A newly spent ovary is fairly soft but looks still well filled with unspawned eggs in various stages of disintegration and could be mistaken for a ripening, ripe, or spawning ovary. Eventually it clears itself of most of the debris of the last spawning and becomes a typical spent ovary, soft, flabby, and deeply vascularized. It may still have a few degenerating ripe eggs by which it is positively identified as spent. It contains two groups of developing eggs, the early and the late maturing with a frequency polygon strikingly similar to that of Stage III ovaries with the largest egg measuring 16 to 17 micrometer units in diameter.

Since the yellowfin is shown to spawn several batches of eggs during a season, the ovary must go through an equivalent number of cycles of development from Stage III to Stage VII and back to Stage III. At the end of the spawning period when the last batch of eggs is laid, the ovary goes through from Stage VII to Stage VII-A. Stage VII-A, in fig. 3, shows a spent ovary preparing for a rest period. At this stage, the ovary has shrunk a little but otherwise looks externally like Stage VII. It has lost the larger, late maturing group of eggs perhaps by resorption and now shows only a smaller, early maturing group with an upper diameter unit of this 10-micrometer unit which may be further reduced as the degeneration of the larger eggs progresses.

It appears that at the end of the spawning period, when the last batch of eggs has been spawned, the spent ovary begins to clear itself of the eggs that by the inertia of growth have continued to develop beyond the immature size. The process consists of first resorbing the older group of eggs of 10- to 17-micrometer unit diameter followed by the early maturing eggs below 10 micrometer units.

Table 5 shows that of the 60 ovaries of *Neothunnus macropterus* examined, 14 ovaries, or over 20 per cent of the

total, were in recognizable preparatory resting stage. The table shows the spent ovaries in various stages of eliminating the maturing group of eggs from 5 to 12 micrometer units

TABLE 5.—Length and weight of fish, weight of ovaries and degree of maturity of ovaries of *Neothunnus macropterus*.

Specimen number	Fork length	Weight of fish	Weight of ovary	Largest egg diameter	Maturity of ovary
	mm.	kgm.	gm.	micro-unit	
1	554	3.12	26.5	16	Maturing.
2	512	2.66	8.0	3.5	Immature
3	587	4.00	6.8	4.0	Do.
4	769	9.60	103.4	16	Maturing
5	673	6.25	131.4	20	Mature.
6	610	4.43	99.2	20	Do.
7	604	4.20	106.0	20	Do.
8	1,422	57.98	721.0	17	Maturing.
9	806		142.0	20	Mature.
10			80.0	17	Spent.
11	745	7.84	77.2	16	Maturing.
12	800	8.52	47.0	11	Early maturing.
13	587	4.14	76.2	20	Mature.
14	608		104.0	16	Spent.
15	570		18.0	17	Maturing.
16	520		93.0	17	Spent.
17	554		36.0	16	Maturing.
18	706		87.0	11	Early maturing.
19	669		66.5	16	Maturing.
20	741		116.2	21	Mature.
21	689		64.7	13	Early maturing.
22	567		129.0	34	Ripe.
23	640	5.00	90.0	18	Maturing
24	580	3.86	97.0	19	Do.
25	546	3.06	7.8	3.5	Immature.
26	620	4.09	21.6	4	Resting.
27	810		107.5	16	Maturing.
28	610		37.5	7	Resting.
29	627		41.0	9	Early maturing.
30	602		88.4	17	Maturing.
31	582		44.9	11	To resting stage.
32	570		31.2	9	Do.
33	566		36.5	12	Do.
34	534		42.5	15	Maturing.
35	542		66.5	6	To resting stage.
36	606		50.0	11	Do.
37	592		61.9	12	Do.
38	562		51.8	11	Do.
39	612		38.0	5	Do.
40	600		29.0	6	Do.
41	568		31.3	7	Early maturing.
42	702		146.6	17	Maturing.
43	616		60.5	10	To resting stage.
44	577		30.2	6	Early maturing.
45	521		12.0	3.5	Immature
46	542		36.4	10	Early maturing.
47	587		74.0	10	To resting stage.
48	572		32.8	9	Early maturing.
49	545		27.2	6	Do.
50	533		20.6	5	Resting stage
51	633		68.2	15	Maturing.
52	566		44.0	9	Early maturing.
53	581		28.2	9	To resting stage.
54	621		67.0	10	Do.
55	611		80.6	13	Early maturing.
56	582		122.0	16	Maturing.
57	771		156.9	10	Early maturing.
58	657		116.1	15	Maturing.
59	587		46.4	13	Early maturing.
60	580	3.52	76	12	Do.

preparatory to sexual deactivation. The ovary that might be considered as in the most advanced stage of preparation for rest still contains maturing eggs of 3 to 5 micrometer units. As to whether this ovary is already in a "stable" resting stage or that these remaining early maturing eggs will also degenerate and leave only the immature class of eggs, could not be determined in the present study.

#### ELASTICITY OF THE OVARIAN WALL OF THE SPENT OVARY

The ovaries of *Neothunnus macropterus* appear to contract very little, if at all, to the size before spawning during the successive maturation of the several batches of eggs up to the end of the spawning season. Some of the larger Stage IV ovaries of *Neothunnus macropterus* were observed to be still somewhat flabby, and a finger or pencil could be inserted in the half-empty lumen. The same phenomenon was suspected by Marr (1948) in the eastern Pacific yellowfin. However, at the end of the spawning season, the ovary starts to shrink fairly rapidly through the various preparatory stages of the resting ovary, and an ovary in the resting stage could hardly be distinguished from an immature ovary. The shrinkage is accompanied not only by the resorption of the maturing eggs but also by the decrease in size and number of blood vessels. The resting stage, however, retains some degree of vascularization which is absent in the original immature ovary. It is also quite soft even in the full shrunken state. It can be identified from the immature ovary by considering its size and the size of the fish against size at sexual maturity. Because the egg composition of an ovary in a resting stage could not be definitely established with the available materials, it is difficult to determine if an ovary with early maturing eggs of from 3 to 5 micrometer units with no signs of disintegration, goes through or comes out of a resting stage.

The above observation on the resting ovary is based entirely on young specimens caught on trolling lines. Very few specimens of the size range caught by longline were available for the present study. In all probability, the elasticity of the ovarian wall would gradually decrease with age and with repeated spawning of the fish and the resting ovaries of these older fish would show characteristics quite easily distinguished from those of the original immature ovary.

#### KATSUWONUS PELAMIS

##### CLASSIFICATION OF THE OVA AND THE OVARIES

The size and appearance of the ovaries and the ova of different stages of development of *Katsuwonus pelamis* are very similar to those of *Neothunnus macropterus*. The only significant difference noted is that the yolk of the ripe egg of *Katsuwonus pelamis* appears to be more granular than that of *Neothunnus macropterus*. Because of this close resemblance, the classification of the ova and the ovaries for *Neothunnus macropterus* may be reasonably considered also for *Katsuwonus pelamis*.

Figure 4 (Table 3) shows the diameter frequency polygons of seven ovaries of *Katsuwonus pelamis* selected and so arranged as to approximately illustrate the various stages of growth of the ova to maturity. By a superficial comparison of fig. 3 and 4 it will be observed that the ova of *Katsuwonus pelamis* follow the same pattern of growth as those of *Neothunnus macropterus*. At the onset of spawning, some immature eggs turn opaque and increase in size through Stages II and III. As early as Stage III, a distinct mode towards the upper size limit of the maturing group of eggs is noted. A fairly discernible younger group has started to work itself up to another mode on the 8 to 16 micrometer range. In Stage V, the first group of eggs is completely separated from the rest and the second group has already developed a distinct mode resembling the polygon of Stage II without the first maturing group. As in *Neothunnus macropterus* the growth of the second group is held at 16 or 17 micrometer limit while the first batch is being matured and spawned.

Out of the 55 ovaries of *Katsuwonus pelamis* measured, not a single ovary with maturity beyond Stage V was observed, and those with eggs larger than 24 micrometer units were extremely rare. The diameter frequency polygon of the most mature ovary of *Katsuwonus pelamis* available for the present study is illustrated by Stage VI-A, fig. 4. This particular polygon shows the first batch of eggs (20 to 27 micrometer units) completely separated from the second and younger stage. The ova in this mature group have each a fully developed oil globule, with a yolk that has not yet turned translucent, except in a few eggs belonging to the upper diameter limit of 27 micrometer units. The largest ripe degenerating eggs of *Katsuwonus pelamis* observed in several

spent ovaries had a diameter of 32 micrometer units. This indicates that the ripening group of eggs in Stage V-A would move further to the right and would be ready for spawning

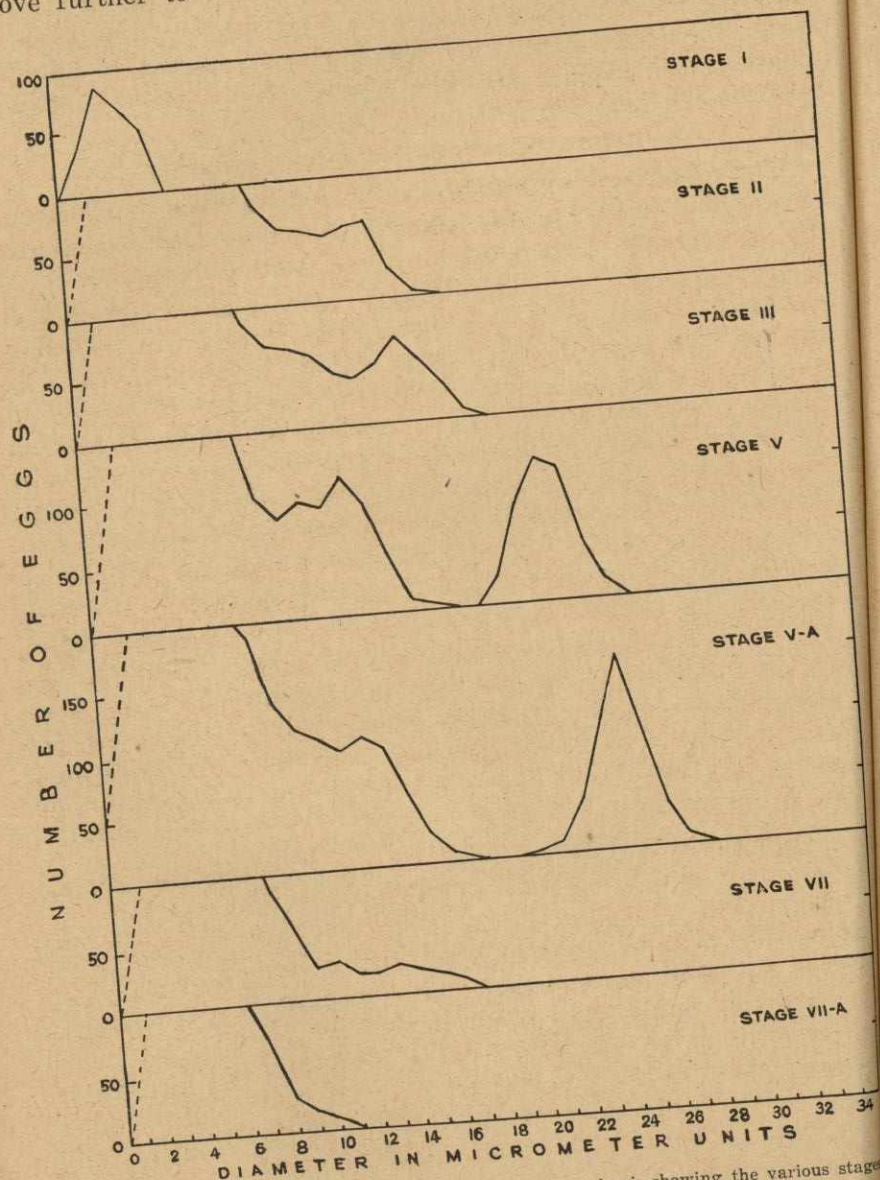


FIG. 4. Ova diameter frequency polygons of *Katsuwonus pelamis* showing the various stages of development of the ova to maturity.

when it falls within the diameter range of 26 to 32 micrometer units.

From the above observation, it is evident that the type of egg development in *Katsuwonus pelamis* is similar to that of *Neothunnus macropterus*; that as soon as the first batch of eggs is formed, a second batch begins to appear to take its place. This process evidently results in a fractional spawning of the type observed in yellowfin.

#### FREQUENCY OF SPAWNING DURING A SEASON

That *Katsuwonus pelamis* spawns more than once during a season is shown by the same evidences used to demonstrate the multiplicity of spawning in *Neothunnus macropterus*.

Although no frequency polygon of a ripe ovary of *Katsuwonus pelamis* could be shown, the mature ovaries represented by Stages V and V-A in fig. 4 already show at least two distinct modal groups developing one after the other indicating maturation of eggs in successive batches similar to *Neothunnus macropterus*. Many spent ovaries of *Katsuwonus pelamis* were examined which still contained a number of ripe degenerating eggs from the recently spawned batch.

Stages VII and VII-A, fig. 4, show two phases of the spent ovary, the first with the largest eggs at 16 micrometer units, and the second with the largest eggs at 10 micrometer units. The first ovary may probably develop another batch of eggs within the spawning season, but the second goes through the preparatory phases of the resting stage, having eliminated the late maturing group of eggs from 10 to 16 micrometer units, and may eventually eliminate the remaining early maturing group.

Table 6 shows that out of 55 ovaries of *Katsuwonus pelamis* examined, only three were in the process of preparing for a rest period. One ovary contained only early maturing eggs with the largest diameter at 10 micrometer units, while the other still contains two late maturing eggs of from 10 to 16 micrometer units but most of them were in various stages of degeneration. As in *Neothunnus macropterus*, no ovary in recognizable resting stage was observed containing immature eggs only; hence, the egg composition of the ovary of *Katsuwonus pelamis* in the "stabilized" resting stage has not, likewise, been established in the present study.

## EUTHYNNUS YAITO

## Classification and Description of Ova and Ovaries.

The ovaries and the ova of the different stages of development of *Euthynnus yaito* are practically similar in appearance to those of *Neothunnus macropterus* and *Katsuwonus pelamis* with the following minor exceptions: The mature and spent ovaries of *Euthynnus yaito* are more thickly ramified with blood vessels than those of *Neothunnus macropterus* and the yolk of the ripe egg is more granular. In both these characteristics *Euthynnus yaito* and *Katsuwonus pelamis* are very close to each other. With respect to size of the ovarian eggs, *Euthynnus yaito* differs with both *Neothunnus macropterus* and *Katsuwonus pelamis* in that upper size limit of the maturing eggs of *Euthynnus yaito* ranges from 14 to 16 micrometer units while that of the two latter species ranges from 16 to 17 micrometer units.

Taking all similarities and differences together, the ovaries and ova of *Euthynnus yaito* show more similarities to *Katsuwonus pelamis* than to *Neothunnus macropterus*.

## HISTORY OF THE RIPENING OVA

Figure 5 based on Table 4 shows the diameter frequency polygon of seven ovaries of *Euthynnus yaito* of different stages selected and so arranged as to illustrate in a logical way the growth of the ova to maturity.

As the eggs grow in size from the immature group (Stage I) to Stage III, a batch of eggs begins to form a modal group around diameter 13 micrometer units with the upper size limit at 15 micrometer units. By Stage V, this group has developed further and has separated from the rest. By this time, another batch of eggs has developed into a distinct class with the mode at around 12 micrometer units and with an upper size limit at 15 micrometer units. This stage shows two modes of almost equal prominence, the first representing the mature group (left side), and the second (right side), representing the late maturing group.

Stage V-A illustrates a slightly older ovary than Stage V. In this stage the late maturing group has grown larger, the mode of the mature group has moved from 22 to 25 micrometer units and the size range has increased from 19-26 to 21-27 micrometer units; the mode of the second group of eggs has shifted from 12 to 14 micrometer units and the

batch has started to crowd towards the upper diameter limit of 16 micrometer units. This crowding appears to be due to the further growth of the maturing batch at its lower limit and its arrest at diameter 15 and 16 since Stage V.

No ovary was available to represent the ripe ovary or Stage VI. However, from the appearance of the spent ovary represented by Stage VII, it appears probable that little change takes place in the appearance of the polygon from Stage V-A to Stage VI, except in the further shifting of the mode of the mature group to the right.

Stage VII polygon shows that even after the ovary has spawned the last batch of eggs, no growth takes place in the maturing group beyond 16 micrometer units.

In the absence of ripe ovaries for this study, the size and appearance of the ripe eggs of *Euthynnus yaito* can only be inferred from the ripe degenerating eggs found in the ovaries of the spent fish. The largest measurable degenerating eggs of *Euthynnus yaito* were 33 micrometer units in diameter, translucent, with a single oil globule measuring from 7 to 8 micrometer units, and a granular, light orange yolk. The largest eggs in the ovary illustrating Stage V-A, fig. 5, measured 27 micrometer units and several of them were already turning transparent. It is probable, therefore, that mature eggs begin to ripen when they have grown to 27 micrometer units in diameter. This suggests that the polygon of the ripe ovary of *Euthynnus yaito* would not be very different from the polygon of the nearly ripe ovary of *Neothunnus macropterus* illustrated in fig. 2.

Based on the above analysis of the growth of the ova, spawning in *Euthynnus yaito* may be said to be fractional as in *Neothunnus macropterus* and *Katsuwonus pelamis*. It appears that fractional spawning of the type shown for the three species of tuna under study is common to other tuna species. Schaefer and Marr (1948) and Marr (1948), citing Frade and Manacas, suggested that the type of fractional spawning the latter demonstrated in *Thynnus thynnus* (Linnaeus) is similar to that in *Neothunnus macropterus*. Preliminary ova diameter measurements from several ovaries of *Kishinouella tonggol* and *Auxis tapeinosoma* show similar type of spawning periodicity.

De Jong (1940) appears to be the first investigator to apply the diameter frequency method in the study of the

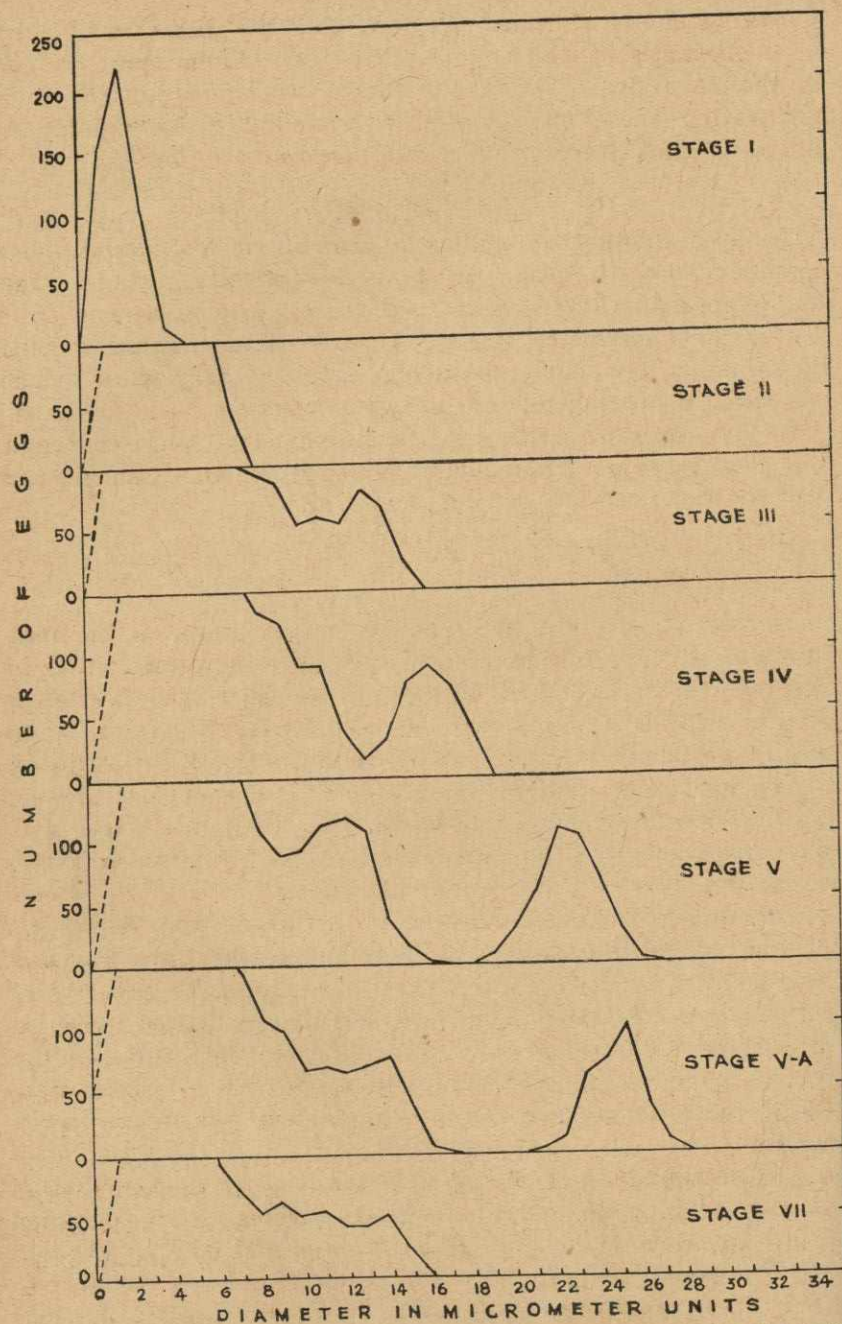


Fig. 5. Ova diameter frequency polygons of *Euthynnus yaito* showing the various stages of development of the ova to maturity.

spawning habits of tuna. He measured the diameter of eggs in two ovaries of *Euthynnus alleteratus* (Rafinesque) caught in the Java Sea. The two frequency polygons of De Jong illustrating these two ovaries were found to be similar to Stage IV and Stage V of *Euthynnus yaito* in fig. 5.

#### THE SPENT AND THE RESTING OVARIES

Table 7 shows that of the 30 ovaries of *Euthynnus yaito* examined, 6 were spent and 3 were in a resting stage. The diameter of the largest eggs in the spent ovaries ranged from 14 to 15 micrometer units. All the three resting ovaries contained a few early maturing eggs the largest of which measured 5 micrometer units. As to whether the ovaries will finally degenerate these few remaining early maturing eggs, and deactivate with only immature eggs, is not established in the present study.

#### Correlation of Length of Fish, Weight of Ovaries and Degree of Maturity.

In general, the weight of the ovary increases as the maturation of the eggs progresses. It also increases with the increase in the size and/or age of the fish. The possibility that these relationships may be used as indexes of maturity of the gonads was suggested by Matsui (1942) for skipjack.

To determine roughly the degree of correlation between length of fish, weight of ovaries, and their maturity, scattered diagrams of these three variables as compiled in Tables 5, 6, and 7 were prepared as shown in fig. 6. The weight of the ovary is represented as the ordinate and the length of the fish as the abscissa. To represent the third variable, degree of maturity of the ovary, the intersecting points of all variates of the first and second the variables were indicated by various marks corresponding to the degree of maturity of the ovary. Thus, mature ovaries were designated by black circles, ripe ovaries by stars, spent ovaries by white circles, etc.

The scattered diagram for *Euthynnus yaito* is based on 30 ovaries varying in weight from 8.4 grams to 205.0 grams secured from fish varying in length from 430 mm. to 622 mm. The scattered diagram for *Katsuwonus pelamis* is derived from 55 ovaries varying in weight from 8.5 grams to 168.2 grams from fish varying in length from 430 mm. to 632 mm.

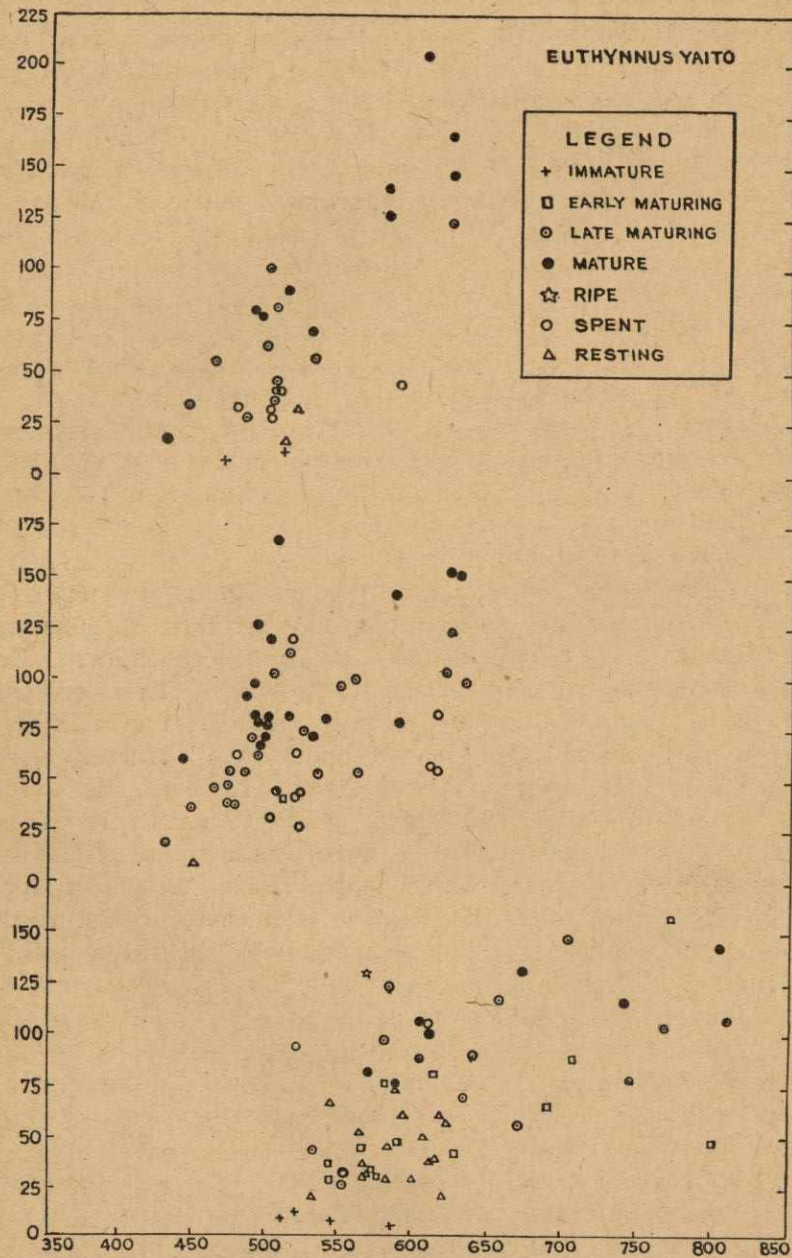


Fig. 6. Scatter diagrams showing correlation of body length and gonad weight with maturity of the ovaries of *Euthynnus yaito*, *Katsuwonus pelamis*, and *Neothunnus macropterus*.

The scattered diagram for *Neothunnus macropterus* is based on 60 ovaries varying in weight from 6.8 grams to 721 grams from fish varying in size from 512 mm. to 1,422 mm.

That the weight of the ovary is a function of the size of the fish may be seen from the positive correlation broadly indicated in all three scattered diagrams. However, the wide dispersal of the points from a regression line that can be drawn through these points, indicates the existence of factors tending to disturb the normal ratio of gonad weight to body length. Because the ovaries represented were of different degrees of maturity, it was immediately presumed that the cause may be largely traced to this third factor.

The degree of influence of this maturity factor may be roughly determined from the distribution behavior of the different maturity signs in the scattered diagrams. The scattered diagrams for the three species show some faintly recognizable pattern of distribution of like signs; that is, similar degree of maturity. For instance, the black circles representing the heavy mature ovaries, tend to distribute themselves on the upper side of the regression line, while the lightweight spent ovaries tend to congregate below this line. These groupings appear to indicate some definite and direct correlation between the three variables throughout the observed size range in the three species. On the whole, however, the tendency to form a general pattern of distribution has been much obscured by many ovaries whose maturity distribution appears irregular. It is possible that a clearer picture of this distribution pattern could be obtained with more ovaries than the admittedly inadequate number available in the present study.

The correlation may have been muddled by such cases as the following: For instance, a mature ovary of a young tuna would be heavier than a spent ovary of an older fish. A newly spent ovary may weigh as much as a maturing ovary of the same size of fish. Food, degree of parasitism, body constitution, season, even errors in measurements, etc., may further modify the relationship of weight of ovaries to weight of fish, to the extent that the obvious relationship of the gonad weight to its maturity may be obscured or even altogether obliterated in the actual measurements of these variables. Matsui (1942) noticed similar wide variations in skipjacks, and because he was puzzled by their existence, he suggested

that his data did not show correlation between the progress of maturation of the gonad and its weight. Considering the many factors that tend to alter this relationship it may be considered as un dependable as a criterion in determining the degree of maturity of the ovaries of tuna.

TABLE 6.—Length and weight of fish, weight of ovaries and degree of maturity of ovaries *Katsuwonus pelamis*.

Specimen number	Fork length	Weight of fish	Weight of ovary	Largest egg diameter	Maturity of ovary
	mm.	kg.	gm.	micro. unit	
1	500	2.72	119.9	20	Mature.
2	505	2.84	168.2	24	Do.
3	485	2.50	90.5	20	Do.
4	491	2.50	126.1	27	Do.
5	476	2.01	38.5	18	Do.
6	493	2.44	77.1	21	Do.
7	627	5.00	150.5	23	Do.
8	614	5.08	82.3	16	Spent.
9	503	4.14	101.0	15	Maturing.
10	621	3.57	123.5	18	Do.
11	585	3.57	141.0	24	Mature.
12	504	2.44	44.0	15	Spent.
13	549	3.69	95.9	17	Maturing.
14	493	2.27	60.8	16	Do.
15	609	4.60	56.2	16	Spent.
16	510	1.93	28.0	10	To resting stage.
17	490	2.38	15.0	17	Early maturing.
18	587	4.24	18.0	22	Mature.
19	430	1.59	78.0	20	Do.
20	620	4.09	17.9	14	Maturing.
21	515	2.25	123.0	16	Do.
22	515	2.25	120.0	16	Spent.
23	513	2.66	112.6	19	Maturing.
24	538	2.78	79.3	23	Mature.
25	528	2.47	62.3	20	Do.
26	498	2.55	76.5	19	Maturing.
27	463	1.90	45.6	16	Do.
28	477	1.98	62.3	16	Spent.
29	517	2.80	62.3	16	Spent to resting stage.
30	496	2.72	79.0	20	Mature.
31	541	3.60	59.4	20	Do.
32	471	1.90	47.4	16	Maturing.
33	448	1.50	36.4	16	Do.
34	527	3.18	70.5	24	Mature.
35	472	2.47	38.0	15	Maturing.
36	497	2.72	69.5	20	Mature.
37	472	2.04	53.2	18	Maturing.
38	482	2.01	52.7	16	Do.
39	520	2.25	41.3	14	Do.
40	487	2.25	69.2	19	Do.
41	560	2.25	52.2	16	Do.
42	513	3.06	80.0	19	Do.
43	523	3.18	73.0	17	Do.
44	632	5.22	97.0	17	Do.
45	492	2.72	65.8	20	Mature.
46	581	3.18	51.7	14	Maturing.
47	490	2.72	97.0	20	Mature.
48	498	2.55	76.5	19	Maturing.
49	559	3.75	99.9	18	Do.
50	500	3.05	30.5	16	Spent.
51	612	4.88	53.6	4	Do.
52	450	1.81	8.5	4	Immature.
53	520	2.95	90.7	19	Spent.
54	520	2.50	25.2	16	Spent to resting stage.
55	622	151.0	22	Mature.	

TABLE 7.—Length and weight of fish, weight of ovaries and degree of maturity of ovaries of *Euthynnus yaito*

Specimen number	Fork length	Weight of fish	Weight of ovary	Largest egg diameter	Maturity of ovary
	mm.	kg.	gm.	micro. unit	
1	503	2.15	41.7	15	Spent.
2	444	1.56	35.0	16	Maturing.
3	504	2.25	41.7	14	Spent.
4	529	58.0	58.0	16	Maturing.
5	586	44.6	44.6	15	Spent.
6	527	71.6	71.6	16	Maturing.
7	517	32.5	32.5	5	Resting.
8	482	27.9	27.9	12	Maturing.
9	510	1.93	17.0	5	Resting.
10	510	11.8	11.8	5	Do.
11	500	101.0	101.0	15	Maturing.
12	505	2.25	82.4	15	Do.
13	499	2.25	64.0	15	Do.
14	622	166.2	166.2	21	Mature.
15	605	205.0	205.0	27	Do.
16	580	141.0	141.0	24	Do.
17	464	1.81	56.6	17	Maturing.
18	477	1.93	34.3	14	Spent.
19	470	1.93	8.4	4	Immature.
20	500	32.6	32.6	14	Spent.
21	502	37.0	37.0	14	Maturing.
22	500	28.0	28.0	14	Spent.
23	621	4.09	123.5	14	Maturing.
24	580	3.29	127.5	19	Mature.
25	512	92.0	92.0	16	Maturing.
26	504	2.27	46.3	14	Do.
27	430	1.59	17.6	14	Do.
28	622	4.88	147.0	22	Mature.
29	495	2.47	79.0	21	Do.
30	490	2.41	81.0	22	Do.

## SUMMARY

1. The spawning periodicity in the three species of Philippine tuna, *Neothunnus macropterus*, *Katsuwonus pelamis*, and *Euthynnus yaito*, are remarkably similar. Before the first batch of eggs has reached maturity, a second batch develops from the immature egg-stock, so that just before spawning the frequency curve shows two completely separated and distinct size groups.

2. All three species spawn more than one batch of eggs during a spawning season. How many batches are spawned by an individual fish per season, and whether or not the number or size of the batches increases with the age of the fish, could not be established with the statistical method used.

3. Each species apparently does not have a well-defined spawning season as shown by the fact that, except for the ripe stage, ovaries of all stages of maturity could be collected in a random sample at any time of the year, but the individual fish has a distinct spawning periodicity with a well-established rest period. The duration of the spawning cycle has not been established.

4. All resting ovaries in the three species still contain early maturing eggs from 3 to 5 micrometer units. As to whether the resting stage attains "stability" with such early maturing eggs or they eventually degenerate and leave the resting ovary with immature eggs only, has not been determined for any of the three species.

5. No running ripe ovaries of any of the three species were collected. However, on the basis of a single nearly ripe ovary of *Neothunnus macropterus* collected, and supplemented by newly spent ovaries with degenerating ripe eggs, the size of the ripe eggs of *Neothunnus macropterus* probably has size range of from 27 to 34 micrometer units, or from 0.8775 mm. to 1.1050 mm. On the basis of the degenerating ripe eggs observed in newly spent ovaries of *Katsuwonus pelamis* and *Euthynnus yaito*, the ripe eggs of these two species would probably fall within the size range of the ripe eggs of *Neothunnus macropterus*.

6. The measurement of maturity of tuna ovaries on the basis of their external physical features alone has been shown to be unreliable. To supplement this method a rapid and fairly accurate method is suggested which consists in measuring maturity by the largest egg diameter in the ovary.

7. Although the weight of the gonad is a function of the body weight of the fish, a correlation of the two based on a random sample of a population of any of the three species, is largely obscured or obliterated because the ovaries represented are in various degrees of maturation.

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## ILLUSTRATIONS

### TEXT FIGURES

- FIG. 1. Ova diameter frequency polygons of six ovaries of *Katsuwonus pelamis* of Stage V maturity.
2. Diameter frequency polygon of 2,000 eggs of a nearly ripe ovary of *Neothunnus macropterus*.
3. Ova diameter frequency polygons of *Neothunnus macropterus* showing the various stages of development of the ova to maturity.
4. Ova diameter frequency polygons of *Katsuwonus pelamis* showing the various stages of development of the ova to maturity.
5. Ova diameter frequency polygons of *Euthynnus yaito* showing the various stages of development of the ova to maturity.
6. Scattered diagrams showing correlation of body length and gonad weight with maturity of the ovaries of *Euthynnus yaito*, *Katsuwonus pelamis*, and *Neothunnus macropterus*.