THE SPAWNING FREQUENCY OF SKIPJACK TUNA, *KATSUWONUS PELAMIS*, FROM THE SOUTH PACIFIC

J. Roe Hunter,[1] Beverly J. Macewicz,[1]
and John R. Sibert[2]

ABSTRACT

Histological criteria to age postovulatory follicles were developed from examination of laboratory-spawned skipjack tuna; the criteria were used to estimate the frequency of spawning of skipjack tuna from the South Pacific. Examination of 87 skipjack tuna from field collections taken in October-November indicated that spawning occurred nearly every day. The fraction of mature females with postovulatory follicles, <24 hours old, was 0.85 (standard deviation = 0.071) indicating that the mean interval between spawnings was only 1.18 days.

Estimates of the frequency of spawning of multiple spawning fishes are essential for understanding their reproductive biology. To estimate annual reproductive effort or fecundity, and how these variables are related to size or age structure of a population requires knowledge of the frequency of spawning and the number of eggs produced per spawning. Batch fecundity, the number of eggs produced per spawning, has been estimated for skipjack tuna a number of times (see review by Matsumoto et al. 1984) but the spawning rate of the skipjack is unknown. Thus spawning frequency is one of the missing links in an assessment of the reproduction of skipjack populations.

It has long been recognized that skipjack tuna spawn more than once in a season because more than one mode of advanced oocytes are found in active ovaries (Brock 1954; Buñag 1956; Joseph 1963; Raju 1964; Simmons 1969; Batts 1972; Cayré 1981; Goldberg and Au 1986). The frequency of occurrence of female black skipjack tuna, *Euthynnus lineatus*, throughout the spawning season with ovaries containing hydrated oocytes led Schaefer (1986) to conclude that the average interval between spawnings of black skipjack in the eastern tropical Pacific was 2.1-5.7 d depending on the region.

Over the last 6 years, two methods have been developed for measuring the spawning rate of multiple spawning marine fishes: One method is based on the frequency of ovaries containing hydrated oocytes and the other is based on the frequency with which they contain postovulatory follicles of known age (Hunter and Macewicz 1985a). These methods have been used to measure the rate of spawning in a number of marine fishes: *Engraulis mordax* (Hunter and Goldberg 1980; Hunter and Macewicz 1980); *Engraulis ringens* (Alheit et al. 1984); *Hypsoblennius jenkinsi* (Present 1985); *Sardinella brasiliensis* (Isaac-Nahum et al. 1985); *Seriphus politus* (DeMartini and Fountain 1981); and *Euthynnus lineatus* (Schaefer 1986). Postovulatory follicles were used in most studies, but DeMartini and Fountain (1981) and Schaefer (1986) used the incidence of females with hydrated oocytes to estimate spawning frequency. The hydrated oocyte method may produce a biased estimate in some species because of increased vulnerability of hydrated females to netting gear (Alheit et al. 1984).

The objective of this paper was to estimate the spawning rate of South Pacific skipjack tuna by applying some of these techniques. It was not possible to use the hydrated ovary method in our study because fish were not caught during the period of the day when the ovary was hydrated. Instead, we used the incidence of females having ovaries containing postovulatory follicles to estimate the frequency of spawning of skipjack tuna. This method requires ovaries to be preserved immediately in formaldehyde solution when the fish is caught, a histological examination of the ovary, and the development of a staging system for estimating the age of the postovulatory follicle. Our histological classification included not only an assessment of spawning frequency but also an assessment of the extent of ovarian atresia. The atretic condition of the ovary is a sensitive index of the reproductive state of

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females during the spawning season and can be used to identify females approaching the end of their spawning season as well as those in postspawning condition (Hunter and Macewicz 1985b).

METHODS

Skipjack tuna were captured either by pole and line or were catches associated with moored fish attraction devices or free floating natural flotsam. Two sets of collections of skipjack tuna were analyzed: a group of 12 females taken near Noumea, New Caledonia on 23 February 1984; and a group of 87 females taken in 8 different collections at various locations in the South Pacific from 20 October to 30 November 1984 (Table 1). Our samples were opportunistically taken and spanned a great latitudinal range (0°-23°S). At present the peak spawning months of skipjack tuna are poorly defined over this range of latitudes. Spawning is known to occur throughout the year in some areas (Nishikawa et al. 1985), but regional differences may exist in the peak months of spawning, and the spawning season also varies with skipjack size (Naganuma 1979). Naganuma concluded from analysis of gonosomatic indices (GSI) that peak spawning period for small skipjack tuna (40-60 cm) in the South Pacific is October to December. Argue et al. (1983) examined 11,000 adult skipjack tuna for cannibalism of juveniles (15-70 mm) and for GSI over the same latitude range as this study, but covering 80° of longitude (140°W-140°E). They found that cannibalism and female GSI was highest between October and March in this broad area. More data are needed to identify the regional variation about this general pattern.

The 8 collections of gonads (collections 2-9, Table

1) were treated statistically as 8 "clusters" of random samples of unequal size. The mean proportion of postovulatory follicles <24 h old was calculated as the total number of females with such follicles divided by the total number of mature females. Cochran (1977) pointed out that estimation of variance by the simple binomial probability formula can produce serious errors. The variance was calculated by the appropriate formula recommended by Cochran (1977).

Three female skipjack tuna were spawned in captivity (23°-24°C; June 1985) at the Kewalo Research Facility of the National Marine Fisheries Service using the stress spawning technique of Kaya et al. (1982). One fish (48 cm fork length [FL]) was sacrificed at the time of spawning, another (43.8 cm FL) 12 h later and the third (44 cm FL) 24 h after spawning. The ovaries of these females were used to establish histological criteria for the aging of the postovulatory follicles of the sea-caught females.

Ovaries were preserved in 10% Formalin[3] and embedded in Paraplast. Histological sections were cut at 5-6 μm and stained with Harris hematoxylin followed by eosin-phloxine-B counter stain (H&E).

Histological Classification

To estimate reproductive condition of skipjack tuna, we used two histological classification systems: one for estimating spawning frequency and the other for assessing the likelihood that a female will continue to spawn (atretic state of the ovary). Each ovary was classified histologically according to both systems. These classification systems were developed for northern anchovy, *Engraulis mordax*, by Hunter and Goldberg (1980) and Hunter and Macewicz (1980, 1985a, b) and are used here with a few modifications appropriate to skipjack tuna ovarian structure and their rates of postovulatory follicle resorption. The descriptions of postovulatory follicles of different ages are from the three captive Hawaiian skipjack tuna. As these fish resorbed their postovulatory follicles much more rapidly than did the northern anchovy, we used stages of shorter duration. The atretic classification system remains unchanged, except for a few minor details of histological structure based on our observations of sea-caught fish. We believe that the reproductive interpretations we associate with the atretic classes are

TABLE 1.—Characteristics of 9 collections of female skipjack tuna taken in the South Pacific in 1984.

Collection number	Date	Time of day (h)	N	Fork length Mean (cm)	Fork length Range (cm)	Gear[1]	Lat.[2] S	Long.[2] E
1	2-23-84	0800	12	47	44-51	PL	23.00	167.00
2	10-20-84	0745	7	49	46-50	PS	16.	178-179
3	10-23-84	0700	6	48	46-52	PS	16.	178-179
4	10-24-84	0700	8	49	46-52	PS	16.	178-179
5	10-25-84	0700	7	50	47-52	PS	16.	178-179
6	10-26-84	0700	14	49	45-51	PS	16.	178-179
7	10-27-84	0645	8	48	46-50	PS	16.	178-179
8	11-19-84	0755	25	50	44-62	PS	03.41	144.08
9	11-30-84	1955	12	56	49-60	PS	0.03	147.46

[1]PL = pole and line; PS = purse seine catch of skipjack tuna attracted to either a fish attraction device moored in waters of 350-450 m deep or natural flotsam.
[2]Latitude and longitude given in degrees and minutes when available.

meaningful because the oocyte resorption seems to follow a similar sequence of stages in most teleosts (Bretschneider and Duyvene de Wit 1947; Lambert 1970). The rate a skipjack tuna ovary passes from one atretic state to another is not specified and would require an additional study of captive fish. The characteristics of the two classification systems are outlined below.

Spawning Frequency

Hydrated and Migratory Nucleus Stages

Ovaries with many translucent hydrated oocytes (oocytes enlarged by fluid uptake just prior to ovulation) are classified in the hydrated stage. Spawning is considered to be imminent. In northern anchovy, spawning takes place in <12 h after the onset of hydration. No skipjack tuna with hydrated oocytes were taken in our field collections. Female skipjack tuna were taken with ovaries in the migratory nucleus stage. This stage occurs just before the onset of hydration and is characterized by the migration of the nucleus to the animal pole of the oocyte
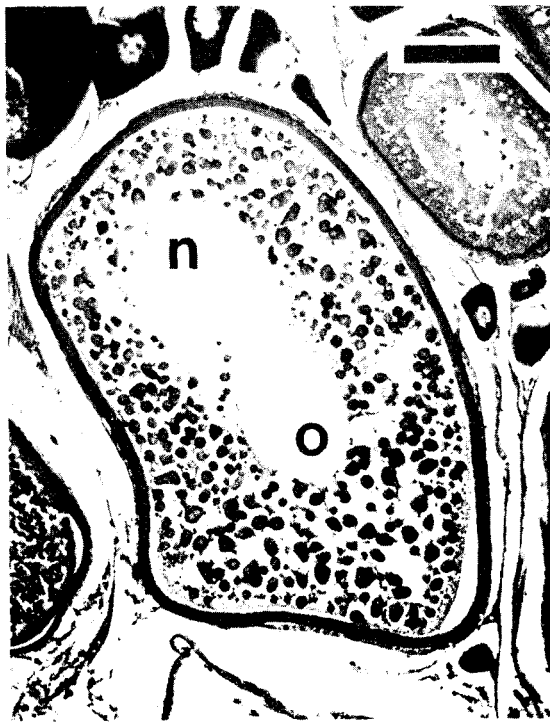

FIGURE 1.—Skipjack tuna oocyte with migratory nucleus (n) and large oil droplet (o); bar = 0.1 mm.

and the beginning of the fusion of its yolk globules (Fig. 1).

Age 0-H Postovulatory Follicles

Ovaries with new postovulatory follicles with no signs of follicle degeneration are classed as age 0-h postovulatory follicles. Hydrated oocytes may occasionally be present. Estimated elapsed time from spawning is 0-2 h. No skipjack tuna taken at sea were in this stage, but from the laboratory specimen (Fig. 2a, b) we can discern the following histological characteristics: The new postovulatory follicle has an irregular, convoluted shape. The granulosa epithelial cell layer of the follicle appears as an irregularly looped cord of slightly hypertrophied cuboidal cells with prominent healthy nuclei linearly arranged. The granulosa appears only loosely attached to the thecal connective tissue layer. Although the theca is less convoluted than the granulosa layer, it is distinct, contains blood capillaries and appears thicker than the thecal layer seen in northern anchovy.

Age 12-H Postovulatory Follicles

Twelve-hour-old postovulatory follicles (Fig. 2c, d) show signs of degeneration similar to that observed in northern anchovy after about 24 h. Histological characteristics include the follicle which is smaller with fewer convolutions; a lumen which is evident; the degenerating granulosa which is no longer a recognizable unbroken cord of cells, but rather the cells are scattered in clumps in the lumen or may be irregularly attached to the theca; and some pycnotic or irregular nuclei which are evident. The theca has begun to disintegrate although it still remains thick and distinct. Deterioration of the theca is indicated by its overall smaller size, a more filamentous rather than cohesize cellular arrangement, and some irregular nuclei.

Age 24-H Postovulatory Follicles

Ovaries containing 24-h-old postovulatory follicles showed pronounced signs of degeneration similar to that observed in northern anchovy 48 h after spawning. At this stage the follicle is much smaller than that at 12 h but a lumen is still evident (Fig. 2e, f). Only few granulosa cells remain; they usually have pycnotic nuclei and generally are loosely attached to the thecal layer. The thecal layer is still fairly thick although it contains some pycnotic
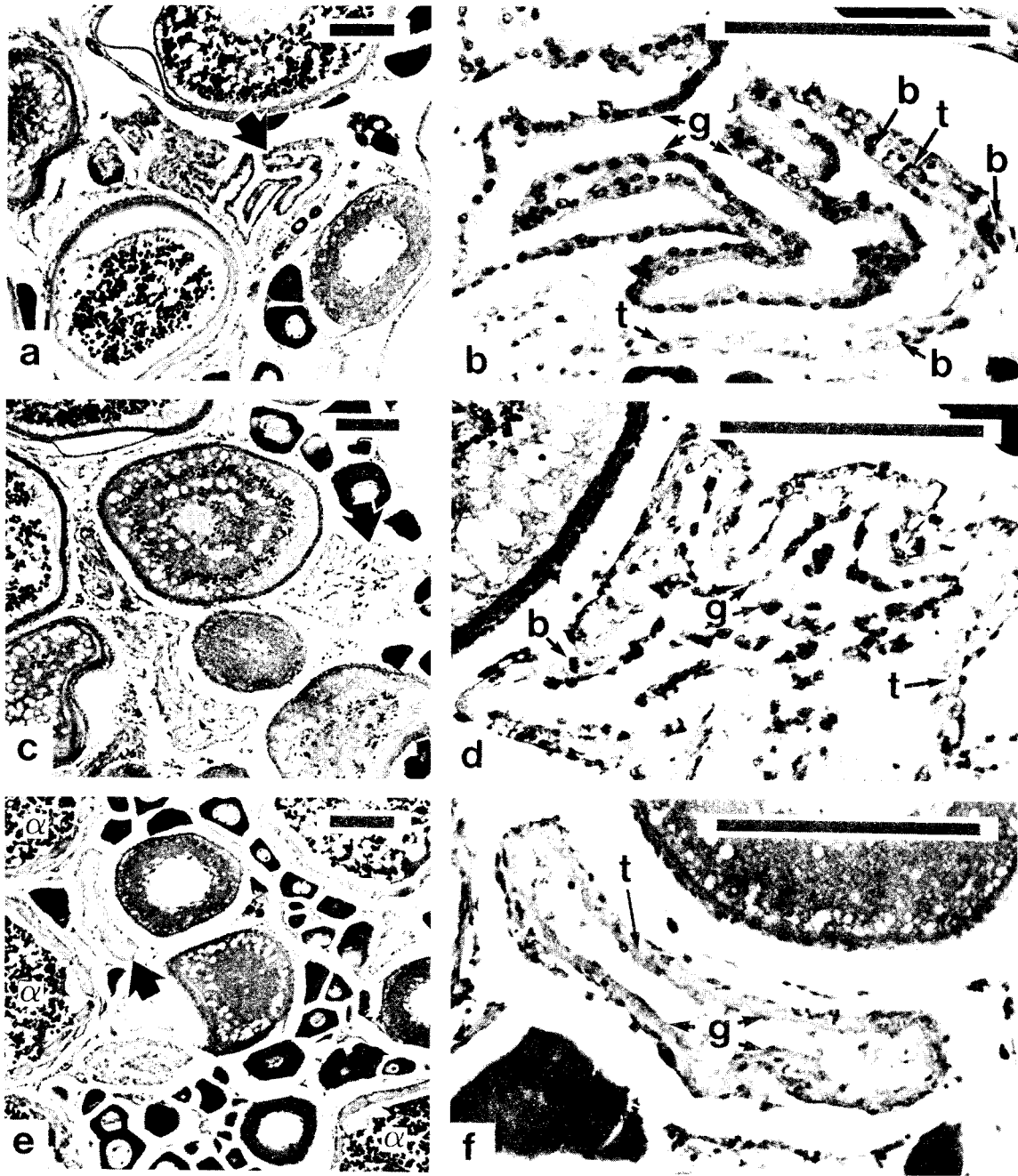
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FIGURE 2.—Degeneration of postovulatory follicles of skipjack tuna spawned in the laboratory. Arrow in left panel indicates the postovulatory follicle that is seen under a higher magnification in right panel. a and b, 0 h after spawning (no deterioration); c and d, 12 h after spawning (pronounced degeneration); and e and f, 24 h after spawning (little remains of the degenerating postovulatory follicle). Bar = 0.1 mm; g = granulosa epithelial cell layer; t = thecal cell layer; b = red blood cell(s); and α = early alpha stage atretic oocytes.

nuclei, and lymphocytes, and has a more filamentous composition.

Nonspawning (mature)

Ovaries with many yolked oocytes and containing no hydrated oocytes or postovulatory follicles were classified as nonspawning. They may contain postovulatory follicles in advanced stages of degeneration which cannot be readily distinguished from late stage corpora atretica. Elapsed time from spawning was more than 24 h. Also classified as nonspawning (mature) were females in postspawning condition. The ovaries of such females contained no yolked oocytes, but atretic follicles (beta stage) were present indicating that the ovary was active recently (see next section).

Immature

Ovaries containing no yolked oocytes and no α or β stage atretic structures were classed as immature.

Atretic States

It is well known in seasonal spawning fishes that a low incidence of atresia (resorption of the oocyte and its follicle) occurs throughout the spawning season, but it becomes marked as the spawning season closes and the remaining advanced oocytes in the ovary are resorbed. During the initial atretic phase (α), the oocyte is resorbed and any yolk globules are broken down and resorbed by the hypertrophying granulosa cells of the follicle (Bretschneider and Duyvene de Wit 1947; Lambert 1970). In the next stage (β), all the yolk is gone, and there remains a small, rather compact structure with one or more cavities. The structure is composed of granulosa and theca cells with penetrating blood vessels. Further stages of follicle resorption have been described by the same authors, but the incidence and extent of α and β stages have proven to be the most useful in the classification of atretic states of ovaries (Hunter and Macewicz 1985b). The characteristics of α and β atretic structures are described and illustrated for northern anchovy by Hunter and Macewicz (1985b) and α atretic oocytes of skipjack tuna are essentially similar. However, β atresia differs from northern anchovy in containing numerous spherical vacuoles scattered throughout the follicle. The vacuoles are the remnants of the oil droplet which takes longer than yolk to resorb and in H&E sections appear empty. Occasionally,

a large beta stage follicle may be seen in which the granulosa and thecal cells have proliferated.

Listed below are the characteristics of the four atretic states we used to classify skipjack tuna ovaries along with what is known regarding the spawning potential of northern anchovy classed in these states.

Atretic State 0

Yolked oocytes present, with no α atresia of yolked oocytes; β stage atresia may be present, but it cannot be distinguished with certainty from late stage postovulatory follicles (>24 h old). Female northern anchovy in this state have a high potential of spawning.

Atretic State 1

Less than 50% of the yolked oocytes are in the α stage of atresia. The frequency of spawning for northern anchovy classed in this state is less than half of that for females classed in atretic state 0. Thus, atretic state 1 indicates a decline in spawning rate.

Atretic State 2

Fifty percent or more of the yolked oocytes are in the α stage of atresia. The frequency of spawning for female northern anchovy classed in this state is very low and indicates that cessation of spawning is imminent.

Atretic State 3

Ovaries contain β stage atresia and no yolked oocytes. Such fish have completed their spawning season since they have no yolked oocytes. The presence of β stage atresia indicates that oocyte resorption has taken place and thereby distinguishes such recently mature but postspawning fish from immature females. In northern anchovy, atretic state 3 may persist for 30 d.

RESULTS AND DISCUSSION

All postovulatory follicles in sea-caught skipjack were less degenerated than those observed in a laboratory specimen examined 24 h after spawning, indicating that all of those in the sea collections were <24 h old. The fraction of mature females with postovulatory follicles <24 h old ([55 + 18]/86, Table 2) was 0.85 with the standard deviation estimated to

TABLE 2.—Numbers of female skipjack tuna in various spawning and atretic states. The 8 collections taken in the South Pacific between 20 October and 30 November 1984.

Collection number	Atretic state[1]	Age (A) Postovulatory follicles (h)		Non-spawning	Total mature females
		A ≤ 12	12 < A < 24		
2	0	0	0	2	2
	1	1	0	2	3
	2	0	0	1	1
	3	0	0	1	1
	Total	1	0	6	7
3	0	1	0	1	2
	1	4	0	0	4
	2	0	0	0	0
	3	0	0	0	0
	Total	5	0	1	6
[2]4	0	3	0	0	3
	1	3	0	0	3
	2	1	0	0	1
	3	0	0	0	0
	Total	7	0	0	7
5	0	3	0	0	3
	1	2	0	0	2
	2	1	1	0	2
	3	0	0	0	0
	Total	6	1	0	7
6	0	9	0	0	9
	1	5	0	0	5
	2	0	0	0	0
	3	0	0	0	0
	Total	14	0	0	14
7	0	3	0	0	3
	1	5	0	0	5
	2	0	0	0	0
	3	0	0	0	0
	Total	8	0	0	8
8	0	8	5	0	13
	1	5	2	1	8
	2	[3]1	0	2	3
	3	0	0	1	1
	Total	14	7	4	25
9	0	0	[4]4	[5]2	6
	1	0	[6]6	0	6
	2	0	0	0	0
	3	0	0	0	0
	Total	0	10	2	12
2-9	0	27	9	5	41
	1	25	8	3	36
	2	3	1	3	7
	3	0	0	2	2
	Total	55	18	13	86

[1]Atretic State 0 = no alpha stage atresia of yolked oocytes.
State 1 = alpha stage atresia of yolked oocytes present, but <50% affected.
State 2 = alpha stage atresia present, 50% or more yolked oocytes affected.
State 3 = no yolked oocytes present and beta stage atresia present.
[2]One female skipjack tuna in collection 4 was immature.
[3]A female with hydrated oocytes and age 0 h postovulatory follicles.
[4]Three of these females had oocytes in migratory nucleus stage.
[5]Two of these females had oocytes in migratory nucleus stage.
[6]Five of these females had oocytes in migratory nucleus stage.

be 0.071 (Cochran 1977; see methods). This means that the average interval between spawnings (1/0.85) was only 1.18 d. Only one female was immature, reducing the denominator for the above fraction spawning from 87 to 86. If we consider only those females with yolked oocytes and no or minor atresia (atretic states 0 and 1) the fraction spawning is 0.90, implying a mean interval of 1.11 d between spawnings. This indicates that the spawning rate of female skipjack tuna in prime reproductive condition is very close to daily.

High levels of ovarian atresia were much more common among the 12 females taken in February than those taken in October-November, indicating that the February fish were nearing the end of their spawning season. Females with highly atretic ovaries (state 2) and postspawning ovaries (state 3) constituted 66% of the fish in the February collections (Table 3), but they made up only 10% of the fish taken in October-November. The February collection was the only one taken by pole and line. It is possible that pole-and-line fishing may be selective against spawning fish (Iverson et al. 1970; Matsumoto et al. 1984) although some spawning fish were taken in this collection.

The most unusual feature of the February collection was that the spawning fraction was high, 0.25 for a group where 50% of the fish were in postspawning condition, had no yolked oocytes, and were incapable of spawning (atretic state 3). The spawning fraction was 1.0 for the three females with no or minor atresia because all three had postovulatory follicles. Thus skipjack tuna with active ovaries appear to spawn nearly every day. It appears that those unable to maintain this rate may discontinue spawning and resorb the ovary because females with active ovaries, showing no evidence of spawning, were rare in all collections. Postspawning females

TABLE 3.—Numbers of female skipjack tuna in various spawning and atretic states. This single collection was taken 23 February 1984.

Collection number	Atretic state[1]	Postovulatory follicles		Non-spawning	Total mature females
		12 h	24 h		
1	0	2	0	0	2
	1	1	0	1	2
	2	0	0	2	2
	3	0	0	6	6
	Total	3	0	9	12

[1]Atretic State 0 = no alpha stage atresia of yolked oocytes.
State 1 = alpha stage atresia of yolked oocytes present, but <50% affected.
State 2 = alpha stage atresia present, 50% or more yolked oocytes affected.
State 3 = no yolked oocytes present and beta stage atresia present.

might reactivate their ovary sometime later in the year if their physiological condition favored reproduction. Evidence for northern anchovy indicates that the transitions from spawning to postspawning states and vice versa can occur rapidly. In the laboratory at 16°C, northern anchovy can resorb all advanced oocytes within a few weeks (Hunter and Macewicz 1985b) and can produce an active ovary in 30 d (Hunter and Leong 1981). Owing to the higher water temperatures and high metabolism of skipjack tuna they are probably capable of even faster reproductive cycling.

Histological examination of females taken late in the day (1955 h, collection 9) provided additional evidence for daily spawning. Eight of 10 females with postovulatory follicles in this collection also had oocytes in the migratory nucleus stage. This stage is the precursor to hydration. Thus, fish which had spawned <24 h before were beginning to hydrate a new batch of eggs which presumably would be spawned in <12 h. The migratory nucleus stage was observed only in this collection probably because it was the only one taken in the evening, whereas all others were taken in the morning (0645-0755). The rarity of females with hydrated oocytes in our collections and the age of the postovulatory follicles imply that spawning usually took place at night. Spawning in daylight hours has been observed by fishermen and scientists, however (Iverson et al. 1970; Matsumoto et al. 1984).

A single female taken during the morning (collection 8) had small (0.70 mm) early stage hydrated oocytes (hydrated oocytes in which the yolk globules had not fully fused). This female, the only one with hydrated oocytes in our collections, also had new postovulatory follicles despite the fact that the hydrated oocytes were not fully advanced. This female may have been induced to hydrate and spawn by the stress of capture or may be simply an exception to the rule. To capture significant numbers of females with hydrated oocytes would probably require sampling after 2100 h. It is important to capture eventually some females in the hydrated stage because it is the best way to confirm that all oocytes in the most advanced modal group, the group of oocytes considered to be the next spawning batch (Hunter and Goldberg 1980), are in fact spawned. Counts of hydrated eggs are also the easiest and most accurate method of estimating batch fecundity (Hunter et al. 1985).

The "stress" spawning technique of Kaya et al. (1982) was used to produce the spawned skipjack tuna for the aging of postovulatory follicles. In this technique females captured at sea and placed in a tank spawn spontaneously, usually about 8 h after capture presumably because of the stress of capture and handling. Spawning typically takes place at about 2400 h, which, by our estimate, appears to be close to the usual time of spawning. It now seems likely that many of these fish are naturally expressing their daily spawning activity. On the other hand, eggs less than the normal size range, 0.8-1.17 mm (Matsumoto et al. 1984), are occasionally spawned, indicating that stress may induce premature hydration in some individuals. That the skipjack tuna do not continue to spawn in the tanks is due probably to the stress of captivity. Our examination of a captive skipjack 24 h after spawning indicated that nearly all remaining oocytes containing yolk were in the early stages of alpha atresia (Fig. 2e). Similarly, female northern anchovy nearly always resorb their advanced oocytes a few days after capture although they will subsequently mature and spawn (Leong 1971; Hunter and Macewicz 1985b).

If female skipjack tuna spawn at the frequency we observed (85% of the females per day), the cost of reproduction and annual fecundity will be high because skipjack tuna appear to have a long spawning season. The relative batch fecundity of skipjack (number of eggs per spawning per body weight) is about 100 eggs per gram (Matsumoto et al. 1984; Goldberg and Au 1985). Skipjack tuna eggs are about the same size as those of *Scomber japonicus* which average in weight 0.04 mg (unpubl. data, National Marine Fisheries Service, Southwest Fisheries Center). We estimate the cost of a single spawning (excluding the metabolic cost of egg maturation and reproductive behavior) to be about 2% of the body weight per spawning (*Scomber* egg dry weight × relative batch fecundity × conversion to wet weight; $4 \times 10^{-5} \times 100 \times 5 = 0.02$). If a female spawned every 1.18 d over 3 mo (90 d), it would produce about 7,600 eggs per gram body weight at an average daily cost of 1.7% of the body weight per day; a 4 kg skipjack tuna would spawn about 30 million eggs over this period.

If the collections used in this study were an unbiased sample of the South Pacific skipjack tuna population, then little doubt exists that spawning occurs almost daily when they have active ovaries. This preliminary study provides the tools necessary for a population-wide assessment of reproduction. We established the time-specific, histological criteria for assessment of spawning rate, and the method was applied to a small sample. A great deal more remains to be done for a proper assessment of reproduction in skipjack tuna. Specifically, many more samples at different times of day, using a

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variety of fishing gears, are needed to insure that sampling biases do not exist; a wide range of skipjack tuna sizes or ages need to be sampled so that the age-specific reproductive effort can be estimated; and females with hydrated oocytes need to be collected to verify that nearly all oocytes in the most advanced mode are hydrated and spawned. The last point seems particularly important because our estimated body weight cost of reproduction is high and is very sensitive to the estimate of batch fecundity. It may never be practical to analyze histologically sufficient numbers of specimens to estimate spawning frequency for all months and ages since some spawning occurs the year around (Nishikawa et al. 1985). On the other hand, it may be practical to calibrate the gonosomatic index (GSI) in peak spawning months using histological criteria and to use the GSI as a calibrated index of spawning frequency during months of low spawning frequency. We do not intend to continue this work but we encourage those working on the biology of tunas to include such studies in their research plans.

ACKNOWLEDGMENTS

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