Abstract.—Spawning of yellowfin tuna, *Thunnus albacares*, around Clipperton Atoll, in the eastern Pacific Ocean, occurred between 2230 and 0330 h on the basis of the presence of migratory-nucleus and hydrated-stage oocytes and new postovulatory follicles in ovaries of females sampled at different times of the day. Histological criteria were developed to estimate the ages of the postovulatory follicles and used to estimate spawning frequency. Histological examinations of testicular tissues provided criteria on the structural characteristics of the sperm duct useful for estimation of spawning frequency. The mean interval between spawnings was 1.14 days for females and 1.22 days for males. The average batch fecundity was 1.57 million oocytes, or 68 oocytes per gram of body weight. The average daily cost of spawning, excluding behavioral activities, is estimated to be 0.97% and 0.28% of the body weight per day for females and males, respectively.

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Spawning time, frequency, and batch fecundity of yellowfin tuna, *Thunnus albacares*, near Clipperton Atoll in the eastern Pacific Ocean

Kurt M. Schaefer

Inter-American Tropical Tuna Commission, Scripps Institution of Oceanography
8604 La Jolla Shores Drive, La Jolla, California 92037-1508

Knowledge of the reproductive biology of yellowfin tuna, *Thunnus albacares*, is essential for a comprehensive understanding of the population dynamics of this species. Previous investigations on the reproductive biology of yellowfin tuna have addressed the topics of spawning distribution, sex ratios, length at maturity, and fecundity (Wild, 1994). However, fundamental information on maturation and spawning has not previously been elucidated for either male or female yellowfin tuna.

Knowledge of the appearance and longevity of postovulatory follicles in ovaries after spawning is necessary for estimating spawning frequency. The frequency of ovaries containing postovulatory follicles has been used to estimate spawning frequency in some scombroid fishes, including skipjack tuna, *Katsuwonus pelamis* (Hunter et al., 1986); yellowfin tuna (McPherson, 1991); bigeye tuna, *Thunnus obesus* (Nikaido et al., 1991); and chub mackerel, *Scomber japonicus* (Dickerson et al., 1992). However, the age and longevity of postovulatory follicles have been determined only for skipjack tuna and chub mackerel held in captivity (Hunter et al., 1986; Dickerson et al., 1992). The use of histological methods generally provides more precise criteria than do gonosomatic indices or oocyte diameter measurements for assessing reproductive status of individuals (Hunter and Macewicz, 1985; West, 1990).

Although structural changes over seasonal cycles have been described from histological examinations of teleost testes (Grier, 1981) and used to estimate length at maturity and define spawning seasons, there have been no investigations of diel testicular changes in relation to rhythmic spawning activity. Information on testicular histology in tunas is scarce because most researchers have been satisfied with gross morphological or gonosomatic indices to measure the reproductive activity of males. Although histological examinations of the testes from albacore tuna, *Thunnus alalunga* (Ratty et al., 1990), and bigeye tuna, *Thunnus obesus* (Nikaido et al., 1991), have provided descriptions of some aspects of spermatogenesis and characteristics of sexual maturity in male tunas, there have been no descriptions of histological characteristics of tuna testes which can be used to determine when a male has spawned and which would thus be useful for estimating the spawning frequency and reproductive effort of males.

Yellowfin tuna, like many subtropical and tropical pelagic fishes, continuously produce batches of hydrated oocytes (Hunter et al., 1985). Their annual fecundity is indeterminate and exceeds the standing stock of oocytes within the ovaries at any given time. Annual

fecundity can be estimated from batch fecundity (the number of oocytes released per spawning) and spawning frequency. However, only at the final stages of oocyte maturation, beginning with the migratory-nucleus phase and followed by hydration, is there a distinct hiatus in the distribution of oocytes from which the batch fecundity can be determined. Previous batch fecundity estimates for yellowfin tuna in the eastern Pacific (Joseph, 1963) were based upon counts of yolked oocytes or upon more advanced stages from ovarian tissue samples that had been placed in Gilson's fluid. This methodology, however, creates a potential bias in batch fecundity estimates because Gilson's fluid causes significant shrinkage of oocytes and compaction of the oocyte size distribution, which then complicates partitioning of the most advanced group of oocytes from the adjacent group of smaller oocytes.

The objectives of this study are 1) to determine the time of day that yellowfin spawn and to develop histological criteria for estimating spawning frequency; 2) to estimate batch fecundity from migratory-nucleus or hydrated-stage oocytes in order to provide an estimate of total fecundity; and 3) to estimate energetic investment in spawning.

Materials and methods

Sampling and processing

Yellowfin tuna were caught by rod and reel aboard the MV *Royal Polaris*, a San Diego-based long-range sportfishing boat, around Clipperton Atoll in the eastern Pacific Ocean (lat. 10°118'N, long. 109°13'W) during the first two weeks of May during both 1986 and 1987 throughout the diel period (Fig. 1). Clipperton Atoll is located in an area in which large catches of yellowfin tuna are taken by purse seiners (Bayliff, 1991). Sea-surface temperatures during these periods were 83° to 84°F (28° to 29°C).

Specimens close to or greater than 900 mm fork length (FL) were measured to the nearest millimeter and their gonads removed immediately after capture (Fig. 2). The decision to sample only yellowfin greater than about 900 mm FL was based on earlier research by Orange (1961), who found that about 70% of fish greater than 900 mm FL were reproductively active. A 2-cm cross section from the central region of the ovaries or testes of each specimen was fixed in 10% neutral-buffered formalin (Hunter, 1985). The ovaries or testes from each specimen, minus the section, were frozen at sea, thawed in the laboratory, blotted dry, and weighed to the nearest centigram on a Mettler electronic balance. A portion of each

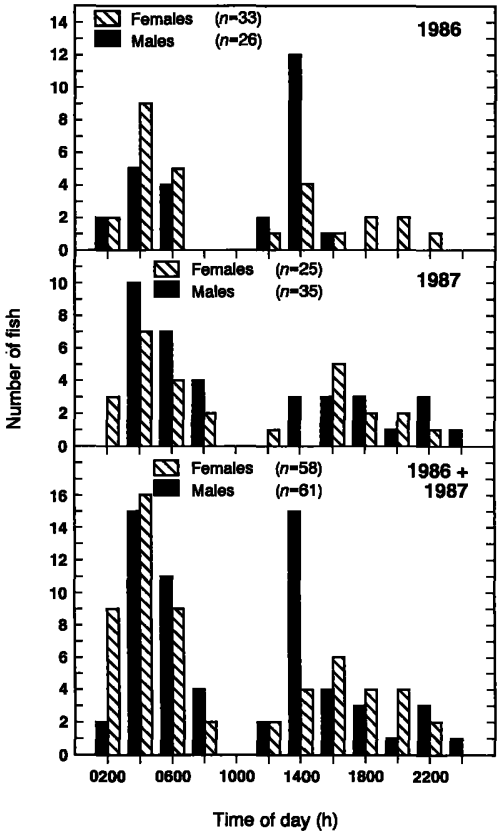

Figure 1
Number of yellowfin tuna, *Thunnus albacares*, sampled for reproductive data during May of 1986 and 1987 from around Clipperton Atoll in the eastern Pacific Ocean by times of day.

sample of preserved gonadal tissue was embedded in Paraplast, sectioned at approximately 6 μm, and stained with Harris hematoxylin followed by eosin counterstain. The slides were viewed by light microscopy with a Nikon optiphot microscope fitted with a microflex HFX-II photographic attachment and Kodak T-max 100 film.

Histological classification

The classification of yellowfin tuna ovaries was based on a modification of the system of Hunter and Macewicz (1985). For each ovary the most-developed oocytes were classified as follows: 1 = unyolked, 2 = partially yolked, 3 = fully yolked, 4 = migratory-nucleus stage, and 5 = hydrated. Identification of ovarian atresia (resorption of oocytes) was restricted to advanced yolked oocytes (stage 3). Ovaries were classified into one of three categories of alpha (α)

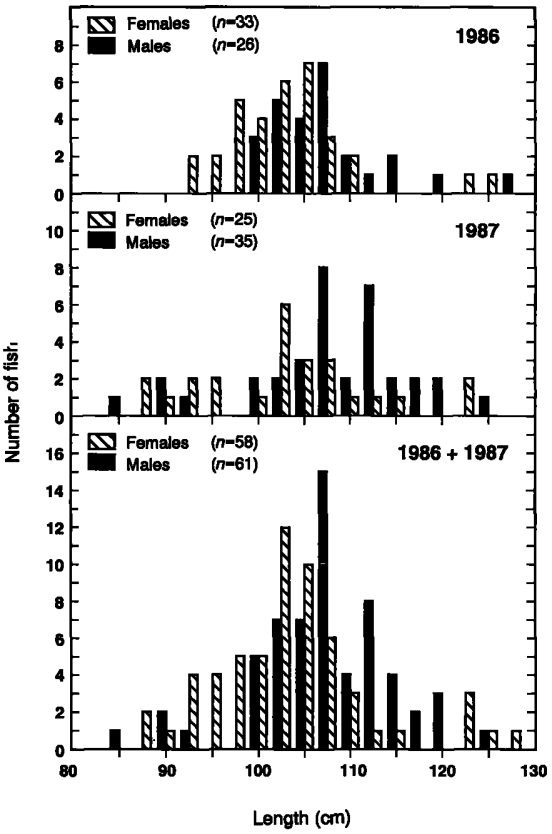

Figure 2

Length frequencies of yellowfin tuna, *Thunnus albacares*, sampled for reproductive data during May of 1986 and 1987 from around Clipperton Atoll in the eastern Pacific Ocean.

atresia (none, <50%, and >50%), i.e. atretic classes 1, 2, and 3, based on visual estimates of the proportion of atretic oocytes and presence or absence of beta (β) atresia, atretic class 4.

Postovulatory follicles were staged according to their appearance and were assigned ages on the basis of the time of capture relative to the estimated time of spawning. The degeneration and resorption of yellowfin tuna postovulatory follicles were described and classified into a series of distinct stages, each with an assigned time after spawning, in 6-hour intervals. An estimate of the spawning time and frequency for females was obtained from the evaluation of this series of yellowfin tuna ovarian tissues.

The classification of yellowfin tuna testes was based on the size of the duct, the thickness of the myoid tissue (Grier et al., 1989) surrounding the duct, the amount of spermatozoa within the duct, the degree to which the duct was convoluted, whether the tissue adjacent to the duct appeared to be heavily nucleated, and the staining characteristic of the tissue adjacent to the duct.

Slides were scored by stage number and specimen identification was not revealed. The original variables were subjected to discriminant analysis operating on the covariance matrix by using BMDP statistical software (Dixon et al., 1990). This procedure separates individuals into two or more groups based on characteristics of variables by which the groups differ (Johnson and Wichern, 1982). The two groups evaluated with this technique were those individuals sampled between 0001 and 1200 h (group 1) and those sampled from 1201 to 2400 h (group 2). These two time intervals were chosen on the basis of the approximate modal time of spawning for females. An estimate of the spawning frequency for males was obtained from the evaluation of this series of yellowfin tuna testicular tissues.

Oocyte size distributions and estimation of batch fecundity

Oocytes from a 1-g tissue sample were measured to the nearest 0.03 mm at 30× magnification with an ocular micrometer in a dissecting microscope. For each fish, mean diameter (random axis) was determined from 20 oocytes present in the most advanced mode of oocytes.

Following the procedures of Hunter et al. (1985), batch fecundity (number of oocytes released per spawning) was estimated for 12 females. For each female, counts of migratory-nucleus or hydrated oocytes in three subsamples of about 0.05 g each were weighed to the nearest 0.1 mg. Migratory-nucleus and hydrated oocytes can be easily distinguished from other oocytes by their larger size and appearance. Those with migratory nuclei are less opaque than yolked oocytes, whereas hydrated oocytes are translucent. No new postovulatory follicles were present in the ovaries used for estimation of batch fecundity.

Each of the three subsamples yielded an estimate of batch fecundity for each female, calculated from the product of the number of migratory-nucleus or hydrated oocytes per unit weight of the subsample and the total weight of the ovaries. The mean of these three estimates provided the spawning batch fecundity estimate for each fish. The weight of each fish used to estimate batch fecundity was estimated from the length-weight relationship of Chatwin (1959) in order to calculate relative fecundity (oocytes per gram of body weight). To describe the relationship between batch fecundity and FL for this set of data, a linear regression between the natural logarithms of the

batch fecundity estimates and natural logarithms of FL for each fish was calculated. The result of the regression analysis is given in the antilog form as a power curve. Analysis of covariance was applied to the log-transformed batch fecundity and length data from this study and the data of Joseph (1963) (whose data were truncated so that the length ranges of the two data sets coincided) to compare the two sets of data.

In order to estimate the cost of spawning a single batch of eggs, 12 preserved oocytes (10% neutral-buffered formalin) in the migratory-nucleus stage from each of two fish, were individually measured, rinsed in distilled water, placed on a nucleopore filter for about 10 seconds, and then weighed to the nearest 0.001 mg. The average weight of an oocyte in the migratory-nucleus stage multiplied by the mean relative fecundity provided an estimate of the cost of spawning a single batch of eggs in percentage of body weight (Hunter et al., 1986).

The costs of a single spawning for both females and males were also estimated by subtracting the percentage of calculated body weight of the gonads just after spawning from the percentage of body weight of the gonads just before spawning occurred.

Results

Classification of ovaries and spawning incidence

The estimate of spawning time and rates of resorption of postovulatory follicles for yellowfin tuna was based on the classification of ovaries taken from 58 fish sampled at different times of the day (Table 1). All females had reached sexual maturity. Sexually mature females were those whose ovaries contained advanced yolked oocytes (oocyte classes 3, 4, or 5) in the most advanced modal group, or had partially yolked oocytes (oocyte class 2) along with α or β atresia, or both (atretic classes 1, 2, 3, or 4). Of the 58 females sampled, 52 were classified as reproductively active. Reproductively active females are those whose ovaries contain advanced yolked oocytes and may or may not contain postovulatory follicles and those in whom α atresia is either absent or less than 50% (atretic states 0 or 1).

Analyses of the ovaries sampled in 1986 and 1987 indicated that yellowfin tuna spawning occurred between approximately 2230 and 0330 h. This estimate was based on the following observations: 1) ovaries sampled during the afternoon (1300–1800 h) had migratory-nucleus stage oocytes in the most advanced mode and those sampled between 1800 and 2000 h had hydrated oocytes; 2) ovaries collected between 2230 and 0330 h had new postovulatory follicles (Tables 1 and 2). The earliest spawning appears to have occurred around 2230 h, as evident in an 1145-mm FL female; this fish had new postovulatory follicles with no evidence of cellular degeneration and had large numbers of ovulated eggs within the lumina of both ovaries.

The mean diameter of the most developed group of oocytes within ovaries, plotted by time of day (Fig. 3), provided further evidence of the spawning time for yellowfin tuna. From 0001 to 1200 h, the mean oocyte diameter increased from about 0.55 mm to 0.64 mm. From 1201 to 2000 h, the oocytes began the migratory-nucleus stage, during which the mean diameter increased to about 0.80 mm by 1601 to 2000 h. This advanced batch of oocytes became hydrated, was then ovulated, and was spawned sometime after 2000 h. The mean diameter of the remaining most-developed modal group of oocytes was about 0.50 mm.

Postovulatory follicles with no signs of degeneration were classified as 0 to 6 hours old (Fig. 4, A and B). These postovulatory follicles had an involuted shape with numerous folds and a relatively open follicular cavity. The granulosa cell layer lining the lumen (epithelium) showed regularly aligned cuboidal cells with prominent basal nuclei. The thecal connective tissue layer was distinct, in only minimal contact with the granulosa, and appeared to be of about the same thickness as the latter.

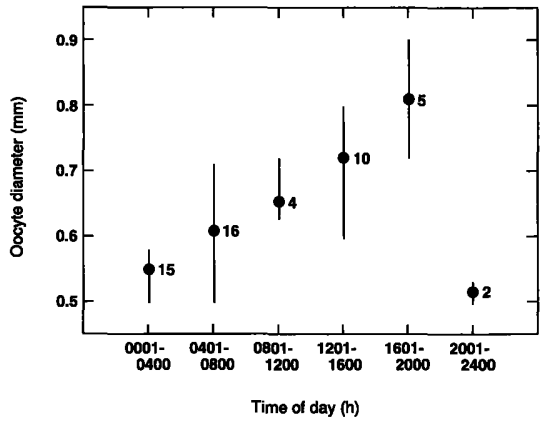

Figure 3

Mean diameters (mm) of the most advanced group of oocytes of yellowfin tuna, *Thunnus albacares*, by time of day. Means are represented by solid circles, ranges by vertical lines, and the numbers of females are noted.

Table 1

Reproductive status of female yellowfin tuna, *Thunnus albacares* (n = 58).

Year and day of month	Time of day (h)	Length (mm)	Oocyte class[1]	Postovulatory follicle class[2]	Atretic state[3]
1986					
5	0531	975	3	2	0
5	0534	1,025	3	2	1
5	0547	980	3	2	1
5	0550	982	3	2	0
5	0553	1,060	3	2	0
6	0220	985	3	1	0
6	0230	1,060	3	1	0
6	0230	949	2	0	4
6	0230	995	3	1	0
6	0240	915	3	1	1
6	0537	1,070	3	2	1
7	0235	1,057	3	0	2
7	0310	966	3	1	0
7	1310	997	3	3	0
7	1320	1,034	4	3	0
7	1335	1,044	4	3	0
7	1340	1,088	4	3	1
7	1700	1,240	4	4	0
7	1700	1,220	4	4	0
8	0300	990	3	1	0
8	0310	1,034	3	1	0
9	0410	1,066	3	2	0
9	0410	942	3	2	0
9	0415	1,045	3	2	1
9	0420	1,075	3	2	1
9	0420	1,015	3	0	1
9	1507	1,052	4	3	0
10	1121	1,048	2	0	4
12	0330	935	2	0	4
12	0330	1,022	3	2	1
12	0438	1,093	3	2	1
12	0505	1,005	3	0	2
12	0530	1,018	2	0	4

Postovulatory follicles estimated to be 6 to 12 hours old showed distinct signs of cellular degeneration (Fig. 4, C and D). They were smaller and less convoluted, and there were some irregular cells and pycnotic nuclei in the granulosa and thecal layers. The granulosa no longer showed a well-organized alignment of cell walls or nuclei and was in obvious contact with the theca. At this stage the thecal layer appeared thicker.

Postovulatory follicles estimated to be 12 to 18 hours old showed advanced cellular degeneration (Fig. 4, E and F). They were considerably smaller and lacked the distinctly convoluted appearance of previous stages, but still had follicular cavities. A thicker thecal layer surrounded the much thinner granulosa cell layer, and the two were in close contact. The granulosa layer was still distinguishable, although it now consisted of a few irregular cells with pycnotic nuclei.

Postovulatory follicles estimated to be 18 to 24 hours old showed signs of the final stages of degeneration (Fig. 4, G and H). The follicle was smaller still, not convoluted but elongate, and had a follicular cavity. The granulosa layer consisted of few cells, was very thin, and not clearly separable from the thicker thecal layer. Prominent, sharply staining pycnotic nuclei were apparent in the degenerating thecal layer.

Several observations indicated that postovulatory follicles are histologically detectable for no more than 24 hours after spawning. Postovulatory follicles of only a single degenerative stage were found in ovaries sampled throughout the day, and the stage of degeneration was consistent among ovaries sampled

Table 1 (continued)

Year and day of month	Time of day (h)	Length (mm)	Oocyte class[1]	Postovulatory follicle class[2]	Atretic state[3]
1987					
3	0310	1,030	3	2	0
3	0315	1,025	3	1	1
3	0445	1,034	3	2	0
3	0510	953	3	2	0
3	0520	1,035	3	2	1
3	1850	925	5	4	0
3	2300	1,115	3	1	1
4	0330	1,046	2	0	4
4	1510	880	4	3	1
4	1515	945	4	3	1
4	1515	900	3	0	4
4	1525	1,090	4	3	1
4	1525	1,056	4	3	0
5	0330	1,008	3	2	0
5	0330	1,029	3	1	0
5	0330	1,080	3	1	0
5	0345	1,042	3	0	2
5	1900	1,023	5	4	0
6	0810	1,073	3	2	0
6	0810	1,220	3	2	1
6	0912	1,054	3	2	0
6	1125	1,062	3	0	2
6	1729	880	4	4	0
6	2230	1,145	3	1	0
7	0610	921	2	0	4

[1] Most advanced oocytes are 1 = unyolked; 2 = partially yolked; 3 = fully yolked; 4 = migratory nucleus stage; 5 = hydrated stage.
[2] Postovulatory follicles are 0 = absent; 1 = age 0–6 h; 2 = age 6–12 h; 3 = age 12–18 h; 4 = age 18–24 h.
[3] Atretic classes are 0 = absent; 1 = <50% α; 2 = >50% α; 3 = β; 4 = $\alpha + \beta$.

at similar times over several different days and during the two different years. Postovulatory follicles estimated to be 12 to 24 hours old occurred in ovaries which also contain migratory-nucleus or hydrated oocytes (Table 1).

The incidence of both postovulatory follicles and late-stage oocytes indicated daily spawning in yellowfin tuna (Fig. 5). Five of the six fish collected between 1300 and 1700 h in 1986 and all seven fish collected between 1500 and 1900 h in 1987 that had oocytes in the migratory-nucleus or hydrated stages also had postovulatory follicles present (Table 1). Assuming that the postovulatory follicles are not detectable for longer than 24 hours, this observation indicates that these fish had spawned the previous night and would spawn again the next night. For the combined samples of 1986 and 1987, the fraction of mature females with postovulatory follicles was 0.79, equivalent to a mean spawning interval of about 1.27 d. If only females that were classified as reproductively active are considered, the fraction of fish spawning (those with postovulatory follicles) was 0.88, a mean interval of 1.14 d. The mean spawning intervals for each of the years were similar, 1.27 d in 1986 and 1.25 d in 1987.

Table 2

Spawning state of female yellowfin tuna, *Thunnus albacares*, by time of day.

	Advanced oocytes		Postovulatory follicles		
Time (h)	Migratory nucleus	Hydrated	New[1]	Old[2]	Total
0001–0400	0	0	10	3	18
0401–0800	0	0	0	14	18
0801–1200	0	0	0	3	5
1201–1600	7	0	0	9	10
1601–2000	3	2	0	5	5
2001–2400	0	0	2	0	2
Total					58

[1] No histological evidence of cellular degeneration.
[2] Histological evidence of cellular degeneration.

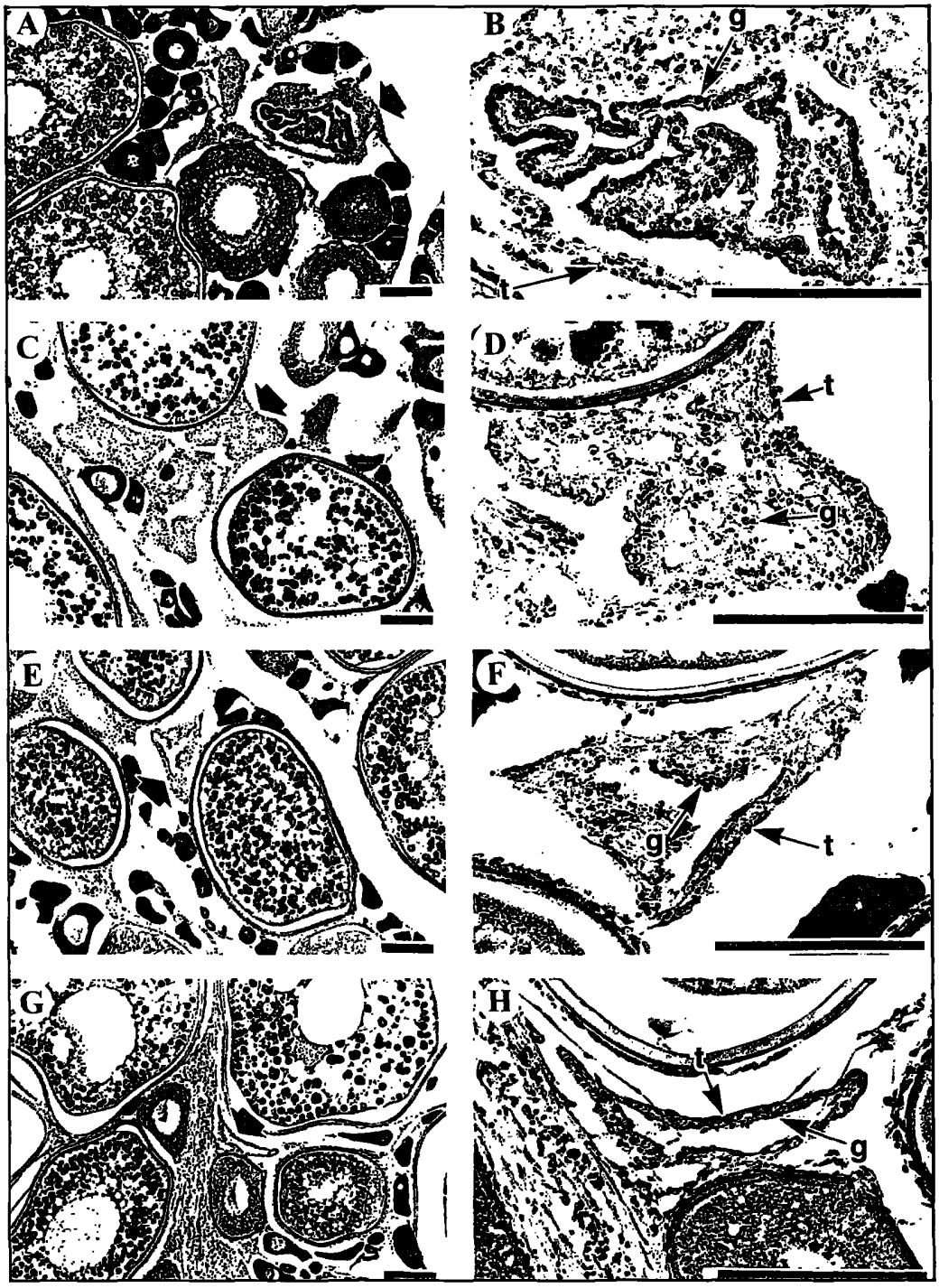

Figure 4

Degeneration of postovulatory follicles of yellowfin tuna, *Thunnus albacares*, at various times after spawning. Arrows in left panels indicate the postovulatory follicles shown under higher magnifications in right panels. (**A** and **B**) 0–6 h after spawning (no signs of degeneration); (**C** and **D**) 6–12 h after spawning (distinct signs of degeneration); (**E** and **F**) 12–18 h after spawning (advanced stages of degeneration); (**G** and **H**) 18–24 h after spawning (final stages of degeneration). Bar = 0.1 mm; g = granulosa epithelial cell layer; t = thecal cell layer.

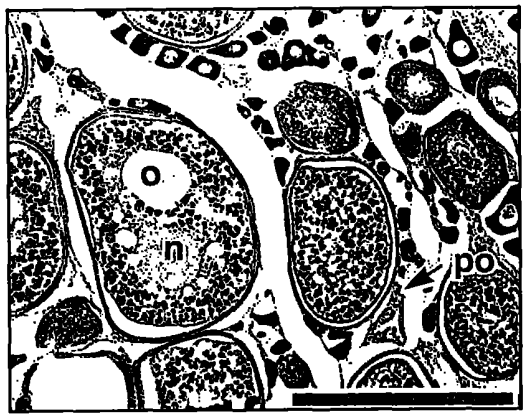

Figure 5

Migratory-nucleus-stage oocyte and a postovulatory follicle in advanced stage of degeneration in yellowfin tuna, *Thunnus albacares*. Bar = 0.5 mm; n = nucleus; o = oil droplet; po = postovulatory follicle.

Classification of testes and spawning incidence

The yellowfin tuna testis is bilobed and each lobe consists of interstitial and germinal compartments. The germinal compartments are situated within lobules that radiate perpendicularly from the central longitudinal sperm duct (vas deferens) or efferent duct and terminate at the periphery of the lobe, the tunica albuginea. Primary spermatogonia are distributed along the lobule lengths, and this typical lobular-type testis is classified as an unrestricted spermatogonial testis type (Grier, 1981, 1993). The lobules of reproductively active yellowfin tuna are filled with sperm that are released after completion of spermiogenesis into the lobular lumen, which is continuous with the vas deferens (Nagahama, 1983). The vasa deferentia are straight tubes with thick, muscular walls, which merge caudally and exit through the genital orifice. The lumen of the vas deferens is lined along its length with cuboidal to columnar epithelium and varies in general appearance from smooth to convoluted.

Structural characteristics of the vas deferens, subjected to discriminant analyses, indicated significant differences ($F_{0.05(3,57)}$=19.96, P<0.001) among yellowfin tuna sampled between 0001 and 1200 h (group 1) and those sampled between 1201 and 2400 h (group 2) for the pooled 1986 and 1987 data (Table 3). The results are similar for the years examined separately. Although the second group, on the basis of spawning time of 2230 to 0330 h, may contain males that had spawned within 12 h, there were no males sampled during that time interval in 1986 and only four in 1987. The characteristics that best distinguish between fish of the two groups are the amount of sperm in the duct, shape of the duct, and staining characteristics of the vas deferens epithelium (Table 3). The fish from group 2 are characterized by a relatively sperm-filled vas deferens, an open duct that is smooth along its border, and no conspicuous dark staining of the vas deferens epithelium (Fig. 6). The spaces apparent in Figure 6 (C–F) between the wall of the duct and the sperm clumped in the center of the duct are most likely artifacts of the preparation method caused by embedding the tissue sample in paraffin

Table 3

Summary of stepwise discriminant analyses between yellowfin tuna, *Thunnus albacares*, groups 1 (0001–1200 h) and 2 (1201–2400 h) for the pooled 1986 and 1987 data, using the six sperm duct characteristics.

Step number	Variable entered	Number of variables	Approximate *F*-statistic	Degrees of freedom
1	Fullness	1	41.57	59
2	Shape	2	26.32	58
3	Stain	3	19.96	57

Classification matrix

			Number of fish	
Group	*n*	Percent correct	Group 1	Group 2
1	33	81.8	27	6
2	28	82.1	5	23
Total	61	82.0	32	29

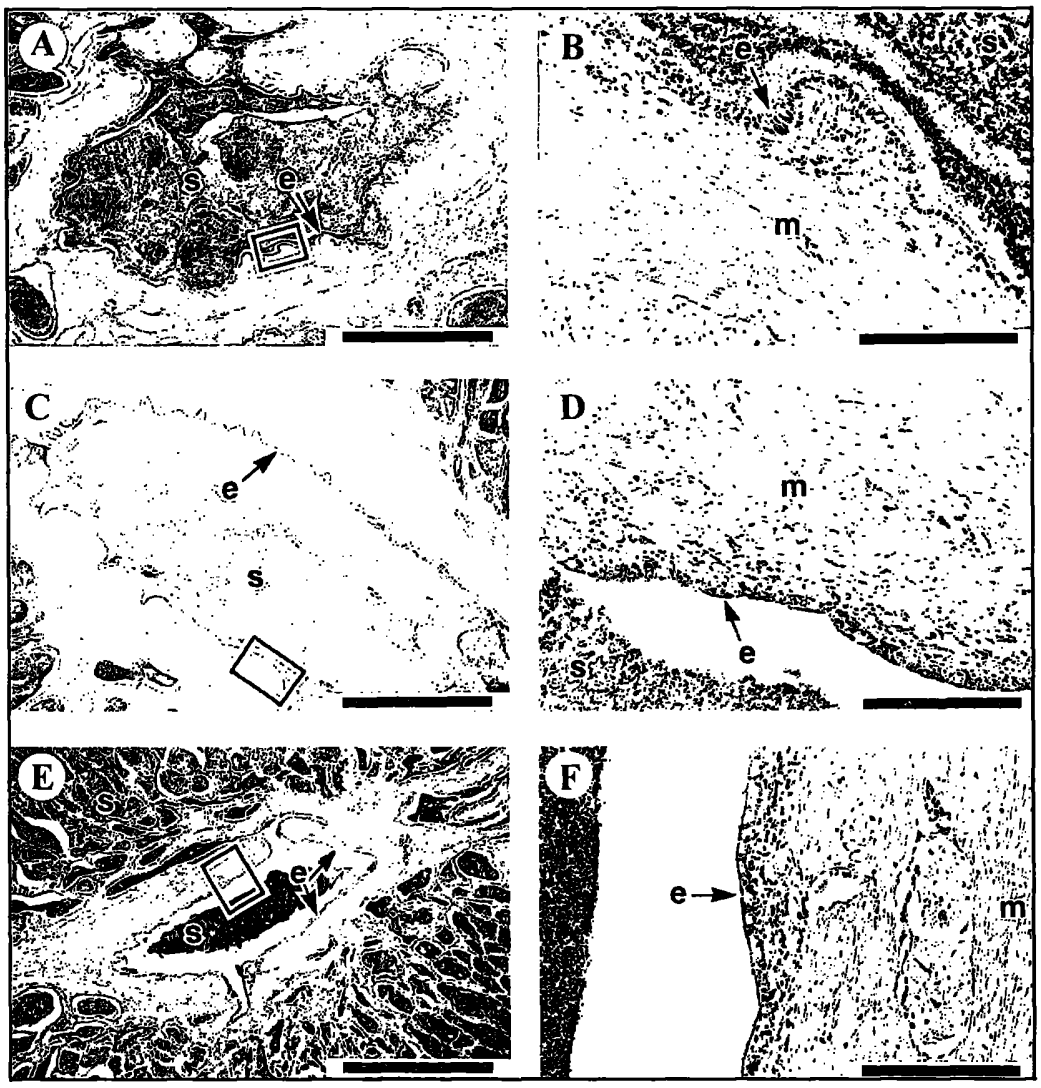

Figure 6

Vas deferens filled with sperm from three yellowfin tuna, *Thunnus albacares* (**A** and **B**; **C** and **D**; **E** and **F**) sampled during 1201–2400 h. The rectangles in left panels indicate the regions shown under higher magnifications in right panels. Bar = 1 mm in A, C, and E; 0.1 mm in B, D, and F. e = epithelium; m = myoid tissue; s = sperm.

(Quintero-Hunter et al., 1991). The fish from group 1 are characterized by a vas deferens relatively devoid of sperm, an extremely convoluted duct, and a darkly stained vas deferens epithelium (Fig. 7).

The percent-correct classification for the pooled 1986 and 1987 data was a total of 82.0%, with 81.8% for group 1 and 82.1% for group 2 (Table 3). The histogram of the canonical variable (Fig. 8) from this analysis for the two groups shows the two distributions. The six fish sampled during the 0001 to 1200 h period that were not classified into group 1 showed no characteristics of spawning within 12 hours and were thus misclassified into group 2. Of the five fish misclassified into group 1, three fish were sampled during the spawning period, two at 2245 and one at 2300 h, and showed characteristics of the other individuals in group 1 that had spawned within 12 hours. Another fish sampled at 1300 h showed characteristics more similar to those of group 1, and the other fish showed intermediate characteristics between the two groups but were more similar to those of group 1.

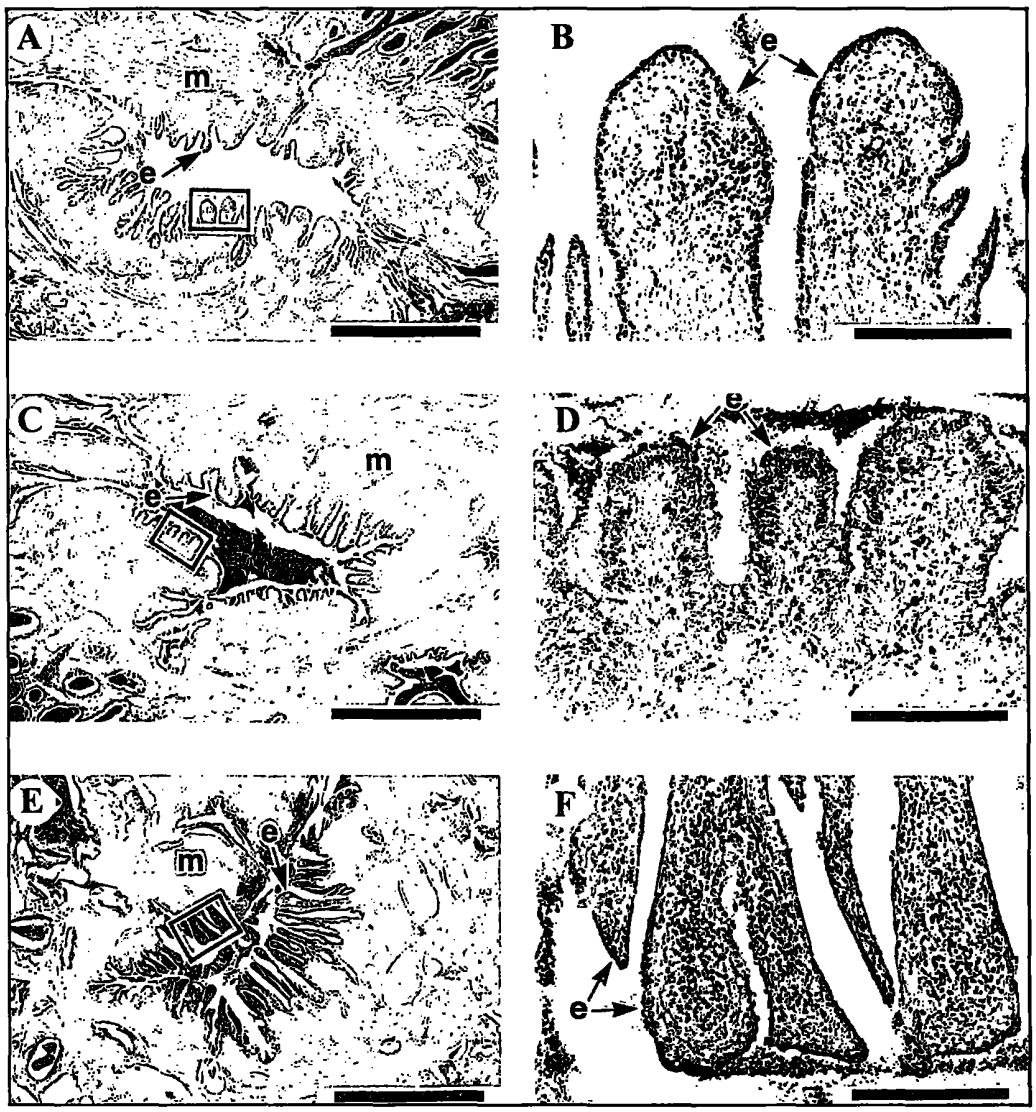

Figure 7

Vas deferens relatively devoid of sperm from three yellowfin tuna, *Thunnus albacares* (**A** and **B**; **C** and **D**; **E** and **F**), sampled during 0001–1200 h. The rectangles in left panels indicate the regions shown under higher magnifications in right panels. Bar = 1 mm in A, C, and E; 0.1 mm in B, D, and F. e = epithelium; m = myoid tissue; s = sperm.

These data indicate that evidence of recent spawning in males is detectable for only about 12 hours after the spawning event. Apparently the lobules, which are full of sperm in mature fish, refill the main sperm duct during this time, and the other characteristics, such as the convoluted shape of the duct and the conspicuous dark staining of the vas deferens epithelium return to the state observed in prespawning individuals. Assuming that spawning males are detectable between 0001 and 1200 h, an estimate of the daily spawning frequency of males during the six days of sampling in 1986 and the four days in 1987 at Clipperton Atoll can be derived. Ten of the 12 fish in 1986 and 17 of the 21 fish in 1987 that were sampled between 0001 and 1200 h were classified as having spawned in the past 12 hours. Thus about 83.3% and 81.0% of the males had spawned within the past 24 hours in 1986 and 1987, respectively. This indicates that the mean interval between spawnings was about 1.20 days in 1986 and

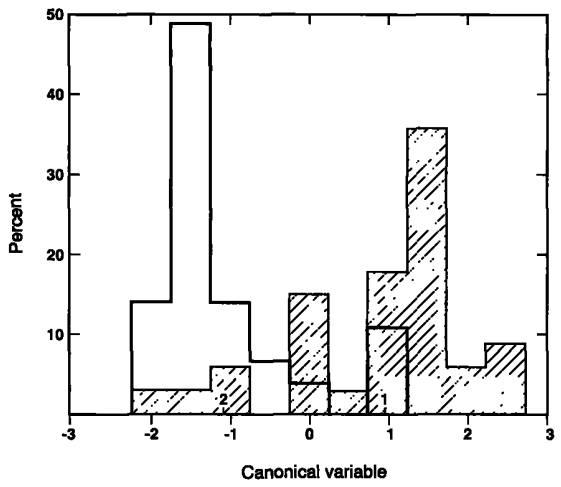

Figure 8

Histogram of the canonical variable from discriminant analysis of the data for the two groups and six characteristics of the vas deferens. The shaded portion represents group 1 (sampled between 0001 and 1200 h) and the white area outlined in bold represents group 2 (sampled between 1201 and 2400 h). The group centroids are also plotted.

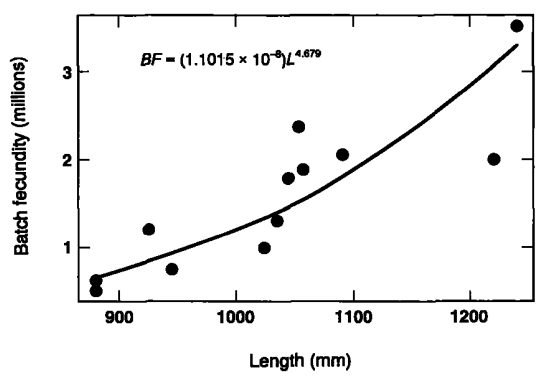

Figure 9

Batch fecundity as a function of length for yellowfin tuna, *Thunnus albacares*, collected around Clipperton Atoll in the eastern Pacific Ocean during May of 1986 and 1987. BF = the batch fecundity in number of oocytes and L = the length in millimeters in the predictive regression equation.

1.24 days in 1987 (1.22 days for the pooled data). In other words, spawning occurred nearly every day for males, as was the case for females.

Batch fecundity

The estimated mean relative fecundity and standard deviation for 12 fish was 68.0 ±20.7 oocytes per gram of body weight (Table 4). The relationship between batch fecundity and length (Fig. 9) can be described by the following equation:

$$BF = (1.1015 \times 10^{-8})L^{4.679}, \qquad [r^2 = 0.76]$$

where BF = batch fecundity in number of oocytes; and

L = length in millimeters.

The predicted batch fecundities and 95% prediction intervals from this equation for yellowfin tuna of 1,000 mm and 1,200 mm FL are 1,199,841 (991,129; 1,452,359) and 2,815,639 (2,025,445; 3,913,724), respectively.

Cost of spawning

Yellowfin tuna oocytes in the most advanced group enter the migratory-nucleus phase about 6 to 8 h before spawning and begin to hydrate about 4 to 6 h before spawning (Fig. 3; Table 1). The hydration process results in a rapid increase in size of the oocytes and in wet weight of the ovary but there is no increase in dry weight (LeClus, 1979). The mean size of oocytes in the migratory-nucleus stage just before the hydration process begins is 0.75 to 0.80 mm (Fig. 3; Table 1). The mean wet weight of a yellowfin tuna oocyte in the migratory-nucleus stage was estimated to be 0.157 mg. This estimate was based on two females of 945 and 1056 mm FL whose oocyte diameters were $\bar{x}$ = 0.78 mm [SD=0.01 mm] and $\bar{x}$ = 0.79 mm [SD=0.01 mm] and whose oocyte weights were $\bar{x}$ = 0.156 mg [SD=0.015 mg] and $\bar{x}$ = 0.158 mg [SD=0.011 mg], respectively. The mean relative fecundity of yellowfin tuna, from this study, is 68.0 oocytes per gram of body weight. The cost of a single spawning (excluding behavioral activities) is thus about 1.1% of body weight. If a female spawns every 1.14 d, the average daily cost of spawning a batch of eggs is 0.97% of the body weight per day.

The costs of a single spawning (excluding behavioral activities) for both females and males were also estimated by subtracting the percentage of calculated body weight of the gonads just after spawning (0001–0400 h) from the percentage of body weight of the gonads just before spawning occurs (1600–2000 h). For females, the average weight of the ovaries with oocytes in the migratory-nucleus stage (preceding spawning) was 2.12% of body weight, and just after spawning was 0.78%, which yields an estimate for the cost of spawning of 1.34% of body weight. For the males, the average weight of the testes preceding spawning was 1.43%, and just after spawning

Table 4

Batch fecundities of 12 female yellowfin tuna, *Thunnus albacares*, with the most advanced group of oocytes in the migratory nucleus or hydrated stage.

Length (mm)	Estimated body weight (kg)	Ovary weight (g)	Batch fecundity	
			Number of oocytes	Oocytes per gram of body weight
880	13.7949	183.85	495,984	36.0
880	13.7949	207.50	620,679	45.0
925	16.0256	368.32	1,194,253	74.5
945	17.0950	246.71	738,153	43.2
1023	21.7215	462.31	984,394	45.3
1034	22.4346	562.91	1,296,181	57.8
1044	23.0963	667.20	1,776,735	76.9
1052	23.6349	793.42	2,356,848	99.7
1056	23.9103	766.20	1,876,038	78.5
1090	26.3084	927.76	2,047,401	77.8
1220	36.9719	986.41	1,984,357	53.7
1240	38.8329	1,485.34	3,496,706	90.0

was 1.09%, which yields an estimate for the cost of spawning of 0.34% of body weight. Based on the estimated mean interval between spawnings of 1.14 days for females and 1.22 days for males, estimates of the average daily costs of spawning are 1.18% and 0.28% of the body weight per day for females and males, respectively.

Discussion

Spawning time

The estimated time of spawning for yellowfin tuna at Clipperton Atoll was between 2230 and 0330 h. This is similar to the spawning time of 2100 to 2400 h by a group of 3- and 6-year-old yellowfin tuna reported by Masuma et al. (1993). The spawning of yellowfin tuna at night was reported earlier by Harada et al. (1980) on the basis of running-ripe and fully hydrated oocytes. Based on oocyte developmental stages and new postovulatory follicles, the assumption was made that yellowfin tuna spawn after 2200 h in the Coral Sea (McPherson, 1991) and bigeye tuna from about 1900 to 2400 h off Java and southwest of Hawaii (Nikaido et al., 1991). Captive skipjack tuna typically spawn at 2400 h, which appears to be close to the time of natural spawning on the basis of histological examinations of skipjack ovaries (Hunter et al., 1986). Although there are some reported observations of courting behavior in scombrids during daylight hours (Iverson et al., 1970), they are primarily anecdotal and appear to be exceptions to the normal behavior of night spawning. Nocturnal spawning also appears to be a common pattern among clupeoids (Blaxter and Hunter, 1982), coral reef species that have pelagic larvae (Helfman, 1986), and other groups of fishes (Ferraro, 1980). Spawning at night may be an adaptation to minimize predation by planktivores or damage by ultraviolet light, or both.

Spawning frequency

The 1986 and 1987 yellowfin tuna samples from Clipperton Atoll indicated that the average interval between spawnings was 1.27 d. Based on the frequency of ovaries containing postovulatory follicles of known age, the average interval between spawnings was 1.18 d for skipjack tuna from the South Pacific (Hunter et al., 1986), 1.54 d for yellowfin tuna in the Coral Sea (McPherson, 1991), and 1.11 d for bigeye tuna off Java and southwest of Hawaii (Nikaido et al., 1991). Spawning occurred during 127 of 155 days for a group of 3- and 6-year-old yellowfin held in a sea pen, but the spawning frequency of individuals was not determined (Masuma et al., 1993). This implies that reproductively active yellowfin tuna, as well as skipjack and bigeye tunas, in tropical waters, spawn almost daily. This high frequency of spawning implies that in a reproductively active female there is continuous maturation of oocytes, which are recruited from the reservoir of primary oocytes. Deviations from the apparently normal daily spawning pattern could be related to recent feeding success (Wootton, 1990) or physiologi-

cal stress, as shown for captive skipjack tuna (Hunter et al., 1986).

The yellowfin tuna postovulatory follicle is similar to that described for most other marine fishes. The rate of postovulatory follicle degeneration in yellowfin tuna, however, is more rapid than that for fish inhabiting cooler waters. The degeneration process of the postovulatory follicle, along with the oocyte developmental rate, is apparently correlated with temperature. Clarke (1987) showed that the Hawaiian anchovy (*Encrasicholina purpurea*) spawns at intervals of about 2 d, as compared with about 7 d for northern anchovy (*Engraulis mordax*) (Hunter and Goldberg, 1980) and about 6 d for the Peruvian anchovy (*Engraulis ringens*) (Alheit et al., 1984), both cooler-water species. The postovulatory follicle can be detected in the northern anchovy for just under 48 hours (Hunter and Goldberg, 1980), whereas for yellowfin and Hawaiian anchovy that period is no more than 24 hours.

This paper provides a method for direct estimation of the frequency of spawning of males by histological examination of testicular tissues. Structural characteristics of the vas deferens, specifically the amount of sperm present, the shape of the duct, and the staining of the epithelium are useful for detecting whether a male yellowfin tuna has recently spawned. Specific cellular characteristics have not been utilized in the separation of the testis into prespawning and postspawning groups. Although positive identification of cell types along with nuclear and cytoplasmic features may be of additional value in detection of reproductive activity, this would have required an ultrastructural investigation of cell types, which was beyond the scope of the present investigation. The main limitation to the technique is that spawning cannot be detected more than 12 h after it occurs. Because yellowfin tuna are normally captured during daylight hours in the purse-seine fishery in the eastern Pacific, spawning-frequency estimates from males could be determined only for fish caught during the relatively short time between about 0600 and 1200 h. Spawning-frequency estimates for yellowfin tuna from throughout the eastern Pacific are thus better estimated from the presence of postovulatory follicles that are detectable in ovaries from fish captured throughout the entire day, with the assumption that the males spawn at similar times.

Batch fecundity

There is only a short period from the late afternoon until about 2200 h (previous to spawning) when ovaries with migratory-nucleus or hydrated oocytes are found in yellowfin tuna. McPherson (1991) reported hydrated oocytes from yellowfin tuna collected between 1200 h and 2000 h in Australian waters, and Hunter et al. (1986) reported the presence of migratory-nucleus oocytes from skipjack tuna collected at 1955 h in the South Pacific. The use of migratory-nucleus or hydrated oocytes for batch-fecundity determinations is crucial because only oocytes in these stages can be distinguished from the less-developed subsequent batch of oocytes.

The estimate of mean relative fecundity for yellowfin tuna obtained from this study (68.0 oocytes per gram of body weight) is considerably less than that of Joseph (1963) (106 oocytes per gram of body weight). Analysis of covariance applied to the log-transformed batch fecundity and length data from the present study and the truncated data set from Joseph (1963) indicated a significant difference in the test for equality of slopes ($F_{0.05(1,65)}$=10.74, P<0.005). Furthermore, the adjusted mean batch fecundity of 1,461,465 oocytes for the data from the present study is less than the adjusted mean batch fecundity of 2,454,049 oocytes from the data set of Joseph (1963). The differences in these estimates are probably due to the methods used, rather than to spatial or temporal variation. The biases associated with the method used by Joseph created an overestimate of the number of oocytes in the spawning batch. The batch fecundities for numerous species of fish have been shown to vary geographically and temporally. This may be a function of inter- and intra-population variation influenced by both genetic and environmental components (Wootton, 1979).

Cost of spawning

The best estimates of the average daily cost of spawning in yellowfin tuna are 0.97% and 0.28% of the body weight per day in females and males, respectively. Although the two methods employed for estimating the costs of spawning for females are both appropriate and produce close estimates, the method based on oocyte weight and relative fecundity is more precise.

An estimate of total daily energy costs for reproductively active yellowfin tuna can be obtained from a bioenergetics approach. Olson and Boggs (1986) estimated a mean daily ration of 5.2% body weight from a bioenergetics model incorporating energy expenditures for swimming, standard metabolism, growth, excretion, egestion, and food assimilation. The estimated mean energy expenditure for growth was about 0.41% of body weight per day. If the estimated daily costs of spawning for females and males of 0.97% and 0.28% of the body weight per day are added to the overall bioenergetics estimate, daily

ration estimates of about 6.2% and 5.5% of body weight for females and males, respectively, is obtained for total energy expenditures.

There are two implicit assumptions in these calculations of reproductive costs: 1) the energy content of oocytes and milt is equivalent on a weight basis and 2) dry-weight–wet-weight relationships are similar for somatic and gonadal tissues. Furthermore, these estimates should be considered conservative because there are other physiological and behavioral costs associated with spawning that are not included in these calculations.

For skipjack tuna, Hunter et al. (1986), estimated the cost of a single spawning to be about 2% of the body weight. Based on an estimated spawning frequency of 1.18 days, the daily cost of spawning a single batch of eggs would be 1.7% of body weight per day. However, reproductive costs were probably overestimated because a value of 100 eggs per gram of body weight (Matsumoto et al., 1984) was used. This relative fecundity estimate was not based upon counts of hydrated oocytes. In addition, the calculation included the weight of a spawned egg, rather than the weight of a late-migratory–nucleus-stage oocyte immediately before hydration. An estimate of the cost of a single spawning in black skipjack tuna (Schaefer, 1987) was reported to be 1.77% of the body weight. Based on an estimated spawning frequency of 2.1 to 5.7 days, the daily cost of spawning a single batch of eggs would be 0.31 to 0.84% of body weight per day. These estimates may also be biased because of the inherent problems in an estimation of spawning frequency by the occurrence of fish with hydrated ovaries versus by the postovulatory follicle method (Hunter and Macewicz, 1985).

Estimates of the average daily cost of spawning for female and male yellowfin tuna from the present study appear to be reasonable in terms of energy allocation. The annual investment of energy in reproduction as a proportion of total energy is estimated to be about 16% for females and 5% for males. These values are within the range of those reported for other fishes (Wootton, 1990). Given that yellowfin tuna probably spawn year round in the tropical regions of the eastern Pacific Ocean (Orange, 1961) and that the mean interval between spawnings is around 1.2 days, a female (90 to 100 cm FL) would spawn the equivalent of about 3.5 times its body weight per year, whereas a male (90 to 100 cm FL) would spawn about the equivalent of its body weight per year. For a female, this is almost 2.5 times the energy allocated for somatic growth, whereas for a male it is only about 0.75 times (Olson and Boggs, 1986).

These preliminary results provide the necessary histological criteria for further assessment of the spawning dynamics of yellowfin tuna. Previous batch-fecundity estimates for yellowfin tuna and other scombrids not based on counts of migratory-nucleus or hydrated oocytes are probably overestimates. A more comprehensive investigation of the reproductive biology of yellowfin in the eastern Pacific (Bayliff, 1991) will provide size-specific estimates of batch fecundity and spawning frequency.

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