

SPAWNING INCIDENCE AND BATCH FECUNDITY IN NORTHERN ANCHOVY, *ENGRAULIS MORDAX*¹

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ABSTRACT

Histological criteria were developed to age postovulatory follicles from examination of laboratory-spawned northern anchovy and used to estimate the frequency of spawning of natural populations. One-day-old, postovulatory follicles were the preferred estimator of spawning frequency; 48 hours after spawning postovulatory follicles could not be consistently identified because of their rapid degeneration; and the occurrence of postovulatory follicles less than 24 hours old was affected by hour of sampling and sexual composition of the school. The nightly spawning incidence was estimated to be $16 \pm 4\%$ of the northern anchovy population in February and 12% in March. Thus, females spawned about every 6-8 days during the peak of the breeding season. The rate of oocyte development corresponded with this reproductive rate, indicating that a new mode of yolked oocytes matured about once a week. Nearly all eggs in the most advanced mode in the ovary were spawned in one night and the number of eggs spawned was estimated to be 389 ± 59 eggs/g of female (ovary-free wet weight). The high spawning frequency and the prolonged breeding season of the northern anchovy indicate that total fecundity may be limited by food availability and energy reserves.

Ichthyoplankton surveys have become one of the standard methods for estimating biomass of marine fish populations. At high latitudes many fishes produce a single spawning batch per year (Qasim 1956) and spawning biomass may be directly estimated from total fecundity and production of eggs and larvae. Multiple (fractional) spawning fishes are characteristic of subtropical and tropical seas (Nikolsky 1963) and estimation of spawning biomass from egg and larval production is dependent upon the number of spawnings per year and the number of eggs per spawning. At present, no adequate method exists for estimating these two parameters for pelagic, multiple spawning fishes.

Past methods have employed measurements of size-frequency distribution of yolked oocytes (usually eggs >0.2 mm in diameter). Eggs are often distributed in one to three modes and the number of eggs in the most advanced mode has been assumed to be equal to the number of eggs produced per spawning (Clark 1934; MacGregor 1968). Another approach has been to count all yolked oocytes in reproductively active females and to

assume that these are equal to the number of eggs spawned in a season (Macer 1974). Neither approach provides conclusive evidence for the number of spawnings nor total egg production. All eggs in the most advanced mode may not ovulate (Clark 1934; Yamamoto and Yamazaki 1961) and atresia may reduce the number of eggs per spawning (Macer 1974; Ivankov 1976). Further, the total number of yolked oocytes may not provide an estimate of total fecundity because some of the small un yolked oocytes, not included in such counts, could mature later during the same breeding season.

It has long been known in teleost fishes (Cunningham 1898) that at ovulation a remnant of the ovulated follicle (empty or postovulatory follicle) remains in the ovary. Postovulatory follicles are believed to be transitory because of their rarity in field-collected material (Wheeler 1924; Yamamoto 1956; Gokhale 1957; DeVlaming 1972; Goldberg 1977; Andrews⁴), but actual measurements of their longevity are rare because the time of spawning must be known. Yamamoto and Yoshioka (1964), using *Oryzias latipes* which spawns every 3 days, reported postovulatory follicles were barely distinguishable on the third day after spawning. They suggested that the frequency of spawning

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⁴Andrews, C. B. 1931. The development of the ova of the California sardine (*Sardina caerulea*). Unpubl. manusc., 88 p. Stanford University, Stanford, CA 94305.

METHODS

could be determined by the presence of postovulatory follicles but the estimate would have to be made soon after spawning. Through techniques of Leong (1971) it was possible to induce spawning in the northern anchovy, *Engraulis mordax*, in the laboratory, making it possible to characterize the histological degeneration of postovulatory follicles on a time basis. Thus, it seemed feasible that spawning frequency of natural populations of *E. mordax* could be estimated from incidence of postovulatory follicles in females once the period in which they could be detected was established. Moreover, once recently spawned fish were identified, the rate of maturation of subsequent egg batches as well as the number of eggs produced per batch could be estimated.

The objectives of this study were to establish the detection period for postovulatory follicles in northern anchovy and to estimate the incidence of natural spawning through histological examination of these structures. In addition, by using this information to guide our selection of specimens, we provide a new estimate of the number of eggs released per spawning or batch fecundity, and the time required for subsequent spawnings. Previous estimates for anchovy based on frequency distributions of yolked oocytes include those of MacGregor (1968) and Norberg.⁵

The period over which postovulatory follicles can be detected in the ovary was determined from anchovy held in the laboratory. Groups of anchovy reared to sexual maturity were induced to spawn using the method of Leong (1971). A total of 119 females were sampled; fish were killed at the time of spawning and thereafter at 24-h intervals up to 9 days after spawning. Ovaries were fixed in Bouin's fixative or 10% neutral buffered Formalin⁶ and embedded in Paraplast. Histological sections were cut at 6 μ m and stained with Harris' hematoxylin followed by eosin counterstain, Masson's trichrome, periodic acid-Schiff reagent, or Heidenhain's iron hematoxylin. A classification system for postovulatory follicles was established and laboratory specimens were classified without prior knowledge of their age to estimate the accuracy of the technique. Field samples were then classified using the same criteria. Three field collections of anchovy females from the Southern California Bight were examined to determine the frequency of spawning in natural populations: 3 commercial purse seine samples of 38-65 females each from March 1977; 4 research trawl samples of 1-11 females from September 1977; and 29 research trawl samples of 10 or 11 females from February 1978 (Figure 1).

⁵Norberg, R. H. 1975. Investigations on the fecundity of northern anchovy, jack mackerel and Pacific mackerel. Unpubl. manuscript, 23 p. Calif. Dep. Fish Game, 350 Golden Shore, Long Beach, CA 90802.

⁶Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

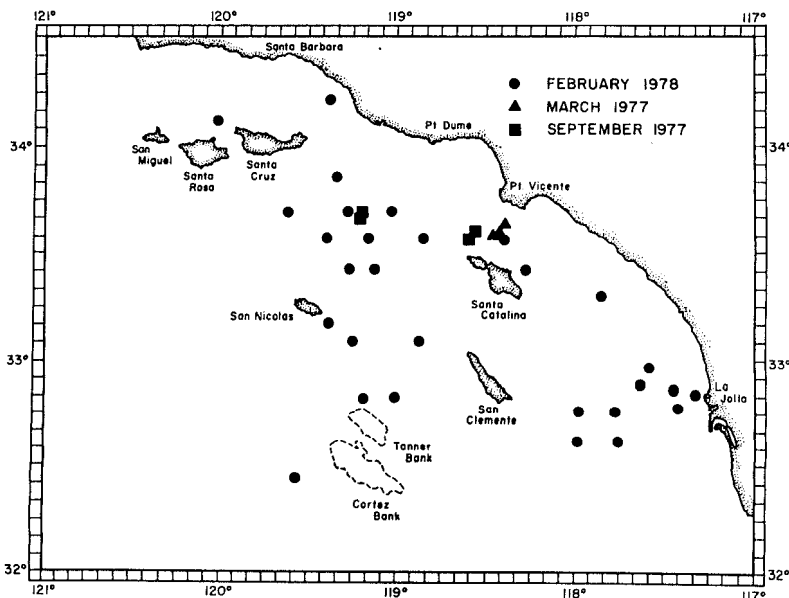


FIGURE 1.—Location of samples of female northern anchovy taken off southern California in March and September 1977 and February 1978.

The number of eggs per mode was estimated for 117 of the field-caught specimens by fixing a weighed sample in Gilson's fluid and determining the size-frequency distribution of yolked oocytes (MacGregor 1968; Macer 1974). One-hundred and fifty of the oocytes >0.20 mm on the major axis were measured to the nearest 0.05 mm from each sample and all the remaining oocytes (>0.20 mm) were counted. We shall use "diameter" to refer to these measurements but since anchovy eggs are longer than wide, it is not a diameter in a strict sense but the major axis of an oblate spheroid.

The form of the distribution of egg diameters within an ovary was similar to those illustrated for other multiple spawning fishes (Macer 1974). Distributions varied from ones composed of two to three distinct modal groups of eggs to ones with only a single mode. Even in those with very distinct modal groups the tails of adjacent modes often overlapped. We used the program NORMSEP (Abramson 1971) to separate modal groups, estimate the mean egg diameter within a mode, and estimate iteratively the number of eggs within a mode. Although one must arbitrarily assume egg diameters within a mode are normally distributed, the program does eliminate some of the subjectivity in judging the range of diameters to include within a mode and how the tails of adjacent modes should be proportioned.

Just prior to ovulation and spawning the modal group of eggs about to be spawned takes up fluid and swells to three or four times its former volume (Fulton 1898). These hydrated eggs greatly increase the ovary weight and increase the total weight of the female. To avoid this bias in female weight we used female weight less ovary weight (ovary-free wet weight) to express fecundity-weight relations. We also provide fecundity estimates based on total weight in tabular form so that conversions can be made if desired.

CLASSIFICATION OF OVARIES

Ovaries of laboratory matured females that had spawned within 24 h in all cases contained postovulatory follicles. They were similar in appearance to those described for a variety of teleosts (Cunningham 1898; Wheeler 1924; Bowers and Holliday 1961; Yamamoto and Yoshioka 1964; Moser 1967; Scott 1974). In specimens killed 0-6 h after spawning, postovulatory follicles consisted of irregularly shaped structures composed of columnar follicle cells and an underlying connective tis-

sue theca (Figure 2A, B). In some cases the columnar cells had hypertrophied slightly. The lumen characteristically contained eosinophilic granules of uncertain origin.

Degeneration was pronounced in material examined 24 h after spawning. The postovulatory follicle (Figure 2C) had greatly shrunk or collapsed on itself, vacuoles had become common, and walls of the follicle cells were no longer distinguishable (Figure 2D). The granular material that was observed in postovulatory follicles taken at the time of spawning was still present but not as abundant. The prominent underlying connective tissue theca seen in new postovulatory follicles was no longer distinct. Degeneration had progressed further, 48 h after spawning. The follicle was one-half to one-fourth smaller than at 24 h, the lumen was very small or indistinguishable, eosinophilic granules were absent, and nuclear sizes were greatly reduced.

Owing to their rapid degeneration, postovulatory follicles were difficult to age in laboratory specimens sampled 48 h after spawning. At this time they may be confused with intermediate stages of atretic oocytes (Lambert 1970). On the other hand, classification of postovulatory follicles into age 0 day and age 1 day was done with an accuracy of 76 to 84% (Table 1). In view of this, the following system was established for classification of ovaries from field-caught specimens:

Hydrated: ovaries with many hydrated eggs (eggs enlarged by fluid uptake just prior to ovulation) and no postovulatory follicles. (Spawning considered to be imminent.)

Age 0 day: new postovulatory follicles, showing no sign of degeneration as described above (Figure 2A, B). Hydrated eggs may occasionally be present. Elapsed time from spawning <24 h.

Age 1 day: regressing postovulatory follicles, showing degeneration as described for specimens (Figure 2C, D) sampled 24 h after spawning. Elapsed time from spawning ≥ 24 h but <48 h.

Nonspawning (mature): ovaries with many yolked oocytes; may contain postovulatory follicles in advanced stages of degeneration which cannot be readily distinguished from other atretic structures. Elapsed time from spawning 48 or more hours.

Immature: few or no yolked oocytes.

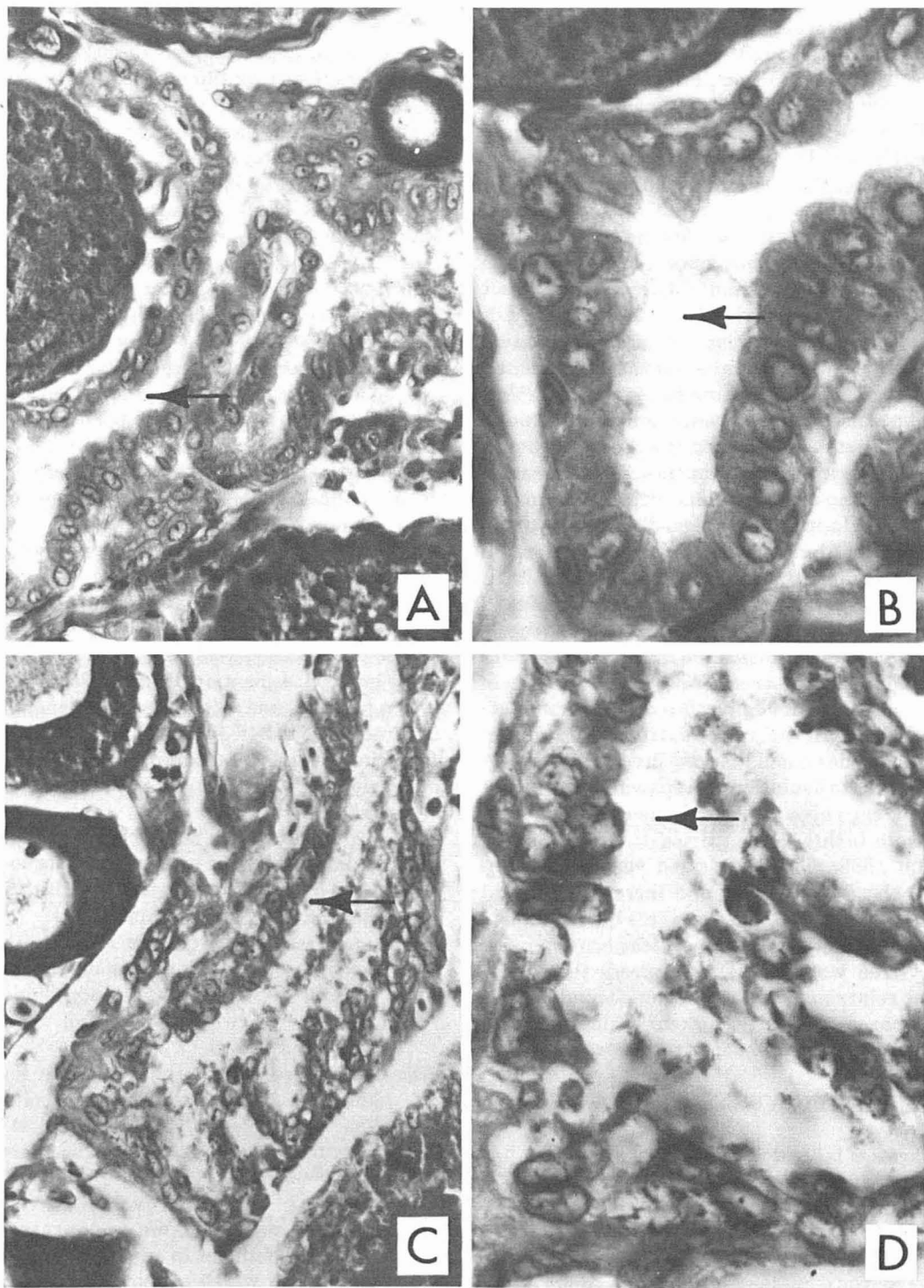


FIGURE 2.—Photomicrographs of northern anchovy ovaries from laboratory specimens: A (400 \times) and B (1,000 \times), postovulatory follicles (elapsed time from spawning 0-6 h); C (400 \times) and D (1,000 \times), postovulatory follicles (elapsed time from spawning 24 h). Arrow indicates lumen of postovulatory follicle.

TABLE 1.—Results of blind classification¹ (number of females) of postovulatory follicles of female northern anchovy spawned in the laboratory.

Elapsed time from spawning (days)	n	Classification			Percent correctly classified
		Postovulatory follicles		Postovulatory follicles older than 2 days or no evidence of spawning	
		0 day	1 day		
0	21	16	5	0	76
1	19	0	16	3	84
2	23	0	0	23	100
3	20	0	0	20	100
4-9	38	0	0	38	100

¹Elapsed time from spawning was unknown to classifier.

All sea samples were classed into the above categories. The mean incidence for each category was calculated without regard to the classification uncertainties reported in Table 1. Confidence intervals for mean incidence for each reproductive state were calculated for the February set of samples but not for those taken in March or September because these sets contained only three or four samples. The normal approximation of the binomial distribution was used to estimate the confidence interval (C.I.) for the mean incidence of

day 1 postovulatory follicles (95% C.I., Table 2). A normal-log negative binomial distribution (Johnson and Kotz 1970; Zweifel⁷) was used to estimate confidence intervals for the mean incidence of hydrated eggs and new postovulatory follicles because it gave a better fit to the data distribution than did the binomial distribution (Figure 3).

RESULTS

Sexual Maturity

All females taken in February and March were mature, but only 67% of those taken in September were mature. No relation existed between reproductive activity and length in the females examined. Twelve females taken in February in southern inshore stations (Figure 1) were <90 mm standard length (SL), which is smaller than any of

⁷Zweifel, J. R. 1978. Confidence intervals for the mean when sampling from natural environments. Unpubl. manuscript, 10 p. Southwest Fisheries Center, NMFS, NOAA, P.O. Box 271, La Jolla, CA 92038.

TABLE 2.—Reproductive state¹ of female northern anchovy collected off southern California in February 1978.

Station number	Day of month	Time of day	Mean length (mm)	Number of females in various reproductive states					Total females
				Hydrated eggs	Postovulatory follicles		Mature, no evidence of spawning		
					0 day	1 day			
2	15	1822	124	0	0	2	9	11	
3	15	2053	122	1	2	2	5	10	
4	15	2121	116	1	1	1	7	10	
5	15	2308	117	2	2	2	4	10	
7	16	1908	116	4	0	0	6	10	
8	16	2147	106	2	6	0	3	11	
9	16	2215	114	0	2	0	8	10	
10	16	2326	114	0	1	0	9	10	
11	17	0044	112	0	1	1	8	10	
13	17	1835	138	0	0	1	10	11	
15	17	2132	134	6	0	1	3	10	
21	18	2002	137	1	0	0	9	10	
27	19	2100	141	0	2	4	4	10	
28	19	2230	135	0	1	2	7	10	
34	20	2254	138	3	0	3	4	10	
37	21	2002	139	0	0	5	5	10	
38	21	2117	132	0	0	3	7	10	
42	22	1934	103	4	0	3	4	11	
45	22	2332	80	0	2	0	8	10	
48	23	1838	136	0	0	3	7	10	
49	23	2005	122	1	0	1	8	10	
50	23	2202	120	0	0	1	9	10	
52	24	0125	117	0	0	1	9	10	
55	24	1915	126	0	1	3	6	10	
57	24	2224	125	2	0	0	9	11	
58	25	0009	120	1	2	4	3	10	
62	25	1915	116	2	1	1	6	10	
72	27	0045	111	0	0	2	8	10	
75	27	2112	112	2	0	1	7	10	
ΣX				32	24	47	192	295	
%				11	8	16	65	100	
² 95% C.I.				6-19	5-14	12-20	—	—	

¹Immature state not included in table because all females were mature.²95% confidence intervals for mean percent of females in each reproductive class.

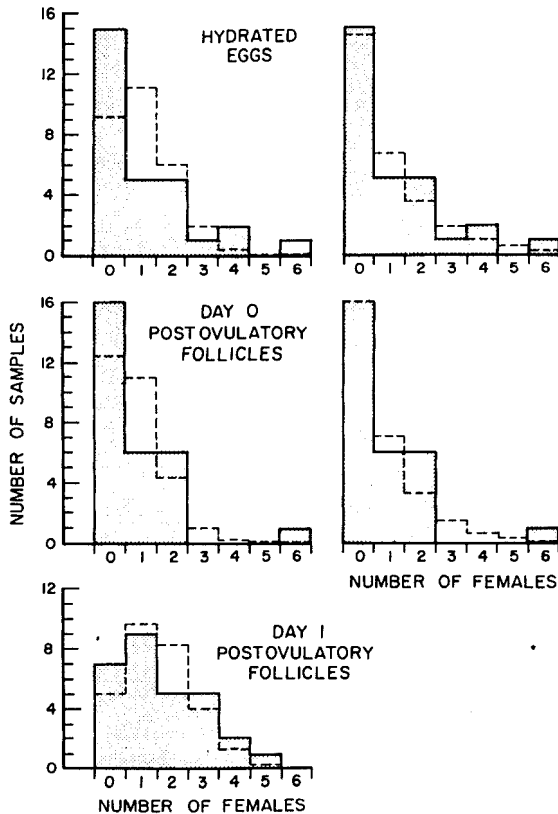


FIGURE 3.—Frequency distribution of the numbers of samples from February 1978 containing 0-6 females in each of three reproductive classes (solid bars). On left sides of the upper two panels are distributions for hydrated eggs and for day 0 postovulatory follicles, compared with that expected (dashed line) from the binomial distribution. On the right sides they are compared with that expected from the negative binomial; the negative binomial gave the better fit in both cases. Bottom panel: the frequency distribution for day 1 postovulatory follicles is compared with that expected (dashed line) from the binomial distribution, which gave an adequate fit to the data.

the reproductively active females found by Clark and Phillips (1952). They reported that only a few females mature at 90-100 mm SL, about 30% mature at 100-120 mm, and 50% at 130 mm, whereas all females we collected in February and March, regardless of size, were sexually mature. Similar to our observations, Brewer (1978) reported anchovy as small as 81 mm SL with well-developed ova from San Pedro Bay. The size of anchovy at first reproduction may have changed since 1946-52 when Clark and Phillips made their observations. On the other hand, all of our collections and those of Brewer (1978) were from southern California whereas those of Clark and Phillips

(1952) included collections from the north (Monterey). It is possible that specimens living at the northern end of the range of the central subpopulation could be larger at first maturity.

Incidence of Spawning

Variation existed in the percentage of females occurring in the three reproductive states that were indicative of imminent or recent spawning. Of the females taken in February, 11% had only hydrated eggs, 8% had new postovulatory follicles, and 16% had 1-day-old postovulatory follicles (Table 2). It is critical to our objective of estimating spawning frequency to consider which of these states provided the better estimates. The distribution of the number of samples containing females with hydrated eggs and that for females with 0 day postovulatory follicles gave a poor fit to the binomial distribution indicating possible bias from contagion within samples, whereas no such problem existed for 1-day-old postovulatory follicles (Figure 3). Furthermore, evidence existed for a bias in sampling females with hydrated eggs and those with new postovulatory follicles that was related to time of day (discussed below) and with sexual composition of the school (to be discussed in a separate section). For these reasons, we believe the 1-day-old postovulatory follicles are the preferred estimator of spawning frequency.

Anchovy spawn only at night, and estimates made by Smith⁸ from staged eggs indicated that most spawning occurs between 2000 and 0400 h. Since hydration precedes ovulation and spawning, one would expect the females with hydrated eggs to be the most common before 2000. In February, females with new postovulatory follicles were probably undersampled and females with hydrated eggs possibly oversampled, because most samples were taken before midnight. No females with hydrated eggs occurred in the February collections at night before 1900 h; six March females taken in the morning showed the first signs of hydration but the eggs were not sufficiently developed to be classified as hydrated. In February the number of females with hydrated eggs increased sharply after 1900 and generally remained high until 2400 (Table 3). Females with new postovulatory follicles occurred for the first

⁸Smith, P. E. 1978. A field study of anchovy spawning time. Southwest Fish. Cent. Admin. Rep. No. LJ-78-8, 1 p. Southwest Fisheries Center, NMFS, NOAA, P.O. Box 271, La Jolla, CA 92038.

TABLE 3.—Percent of female northern anchovy sampled off southern California in February 1978 with hydrated eggs, and postovulatory follicles (age 0 day and age 1 day) by time of day.

Time	Percent of females			Total no. of females
	Hydrated eggs ¹	Postovulatory follicles		
		0 day ²	1 day	
1801-1900	0	0	19	32
1901-2000	24	5	17	41
2001-2100	6	8	24	50
2101-2200	22	14	12	51
2201-2300	10	6	12	51
2301-2400	7	17	7	30
0001-0100	3	10	23	30
0101-0200	0	0	10	10
Total				295

¹Only hydrated eggs, no postovulatory follicles.²Includes some females with hydrated eggs and new postovulatory follicles.

time between 1900 and 2000, but were more common later in the night. As expected, the occurrence of females with 1-day-old postovulatory follicles showed no pattern with time of sampling.

Despite our failure to obtain fish over the entire night of spawning, the proportion of females in February with hydrated eggs, combined with those with new postovulatory follicles, was 19% and within the confidence interval for the estimate based on 1-day-old postovulatory follicles (Table 2).

February data indicate that spawning over a 2-wk period occurred at a rate of about $16 \pm 4\%$ of the population per night. This means that mature females spawned every 6-7 days. The three purse seine samples taken in March 1977 also indicated a high frequency of spawning; 14% of the females had 1-day-old postovulatory follicles and 9% had new postovulatory follicles (Table 4). The March collections were taken in the early morning after spawning had ended, hence the new and 1-day-old postovulatory follicles can be considered as sepa-

rate estimates of spawning frequency for different days. This line of reasoning leads to the conclusion that the proportion of females spawning in March may have been about 12%, equivalent to spawning every 8 days.

Of the 24 females taken in September, only 1 had hydrated eggs, 2 had new postovulatory follicles, and none had 1-day-old postovulatory follicles. Lower spawning activity would be expected in September because egg and larval survey data indicate peak spawning usually occurs in February and March and has declined greatly by September although some spawning occurs throughout the year (Lasker and Smith 1977).

Sex Ratio and Incidence of Spawning

The sex ratio of northern anchovy schools is known to vary markedly from schools composed of nearly all females to ones composed of nearly all males (Collins 1969; Klingbeil 1978). It seemed useful to determine if spawning activity varied with sex ratio because the greatest variability in sex ratio occurs during the peak months of spawning (Klingbeil 1978). Twenty-five fish from each trawl sample taken in February 1978 were sexed. We grouped these samples into three classes on the basis of sex ratio (number of females/(males + females)) and calculated the proportion of females in each of the three sex ratio classes that fell within the following reproductive classes: spawning on the night of capture (females with hydrated eggs or new postovulatory follicles); spawning on the night before capture (females with 1-day-old postovulatory follicles); and no evidence of spawning (none of the above categories).

TABLE 4.—Reproductive state of female northern anchovy collected in March 1977 and September 1977 off southern California.

Station number	Day of month	Time of day	Mean SL (mm)	Hydrated eggs	Number of females in various reproductive states			Total females	
					Postovulatory follicles		Mature, no evidence of spawning		
					0 day	1 day	Immature		
March 1977									
1	20	0400	120	0	2	11	32	0	45
2	21	0330	119	0	7	6	54	0	67
3	21	0630	113	0	5	4	29	0	38
ΣX				0	14	21	115	0	150
%				0	9	14	78	0	
September 1977									
1	9	2108	124	1	0	0	4	0	5
2	9	2300	129	0	2	0	9	0	11
3	10	2043	113	0	0	0	0	1	1
4	10	2236	112	0	0	0	1	6	7
ΣX				1	2	0	14	7	24
%				4	8	0	58	29	

In samples containing mostly males (mean sex ratio = 0.25), nearly 40% of the females spawned on the night of capture, whereas in samples containing mostly females (mean sex ratio = 0.84), only 10% of the females spawned on the night of capture (Table 5). A chi-square test indicated that the proportion of females spawning on the night of capture differed among the three classes of sex ratios ($P < 0.001$) but no difference existed for females with 1-day-old postovulatory follicles. Therefore, most spawning occurred in male dominated schools but females that spawned on the previous night occurred in about equal proportions in all schools.

That groups of pelagic spawners could be male dominated is indicated by the reproductive behavior of another pelagic spawner, the Pacific bonito, *Sarda chiliensis*, described by Magnuson and Prescott (1966). They reported that during courtship, groups of males closely follow a single female. If male anchovy show similar behavior, then trawl collections taken at the time of spawning might be male dominated. To explain the dominance of females in commercial catches, Klingbeil (1978) suggested that male-dominated groups may not form the large dense schools necessary for effective purse seining. It seems reasonable that actively spawning (male-dominated) groups, would not be as likely to preserve the density or the integrity of the school as well as nonspawning groups. Thus, the variability in sex ratio of anchovy schools may be attributable in part to reproductive behavior, that is, the formation of male-dominated spawning groups which may be smaller and less dense than commercial schools. The female component of such groups would be expected to change from day to day because after 24 h, spawned out females occurred with equal frequency in all samples regardless of sex ratio.

Growth of Oocytes

Incidence of postovulatory follicles indicated

that in February 16% of the females spawned daily or an individual female spawned on the average once every 6.25 days. In the fecundity section that follows, we show that nearly all of the eggs in the most advanced mode are spawned in one night. Thus, a new mode of eggs must mature every 6-7 days to maintain a spawning frequency of 16%. In the laboratory, spawning begins when the average diameter of the eggs in the most advanced mode is between 0.6 and 0.7 mm. Thus the eggs remaining in the ovary after spawning must attain this size in 6-7 days.

To determine if such rapid oocyte growth seemed reasonable, we estimated the mean diameter of eggs in the most advanced mode and in the second mode for some of the females taken in February and March. Females with hydrated eggs were placed in the same class as those with new postovulatory follicles because hydrated eggs were not included in this analysis. The number of females analyzed and the elapsed time from spawning were: elapsed time 0 day (hydrated eggs and new postovulatory follicles) $n = 43$; elapsed time 1 day (1-day-old postovulatory follicles) $n = 35$; and elapsed time 3.5 days (nonspawning mature females) $n = 38$. The time from spawning in the last class was unknown. We assigned the midpoint of the interval 2-5 days to this class because all fish classified as nonspawning would fall within this interval if the spawning cycle were 6 days. The mean diameter of oocytes in the second mode was estimated only for nonspawning females. In these more mature females, most of the oocytes in the second mode were >0.2 mm, whereas, in less mature fish a significant proportion of the oocytes in the second mode were <0.2 mm, and thus below the lower limit of our measurements.

The average diameter of eggs in the ovary in the most advanced mode immediately after spawning was 0.46 mm; 1 day after spawning it had increased to 0.51 mm and was 0.59 mm in nonspawning females. Figure 4 illustrates how growth in the diameter of eggs in the most advanced mode and

TABLE 5.—Sex ratio in samples (females/(males + females)) and percent of spawning northern anchovy taken in February 1978 off southern California.

Sex ratio class	Sex ratio		Percent of females			Number females classified
	Class mean	Number samples ¹	Spawning on night of capture ²	Spawned day before capture ³	No evidence of spawning	
0.10-0.39	0.25	7	39	15	46	72
0.40-0.69	0.54	10	16	12	75	101
0.70-0.99	0.84	12	10	20	70	122

¹Twenty-five fish per sample were used to calculate sex ratio and 10 or 11 females were examined histologically.

²Includes females with hydrated eggs and those with recent postovulatory follicles.

³Females with 1-day-old postovulatory follicles.

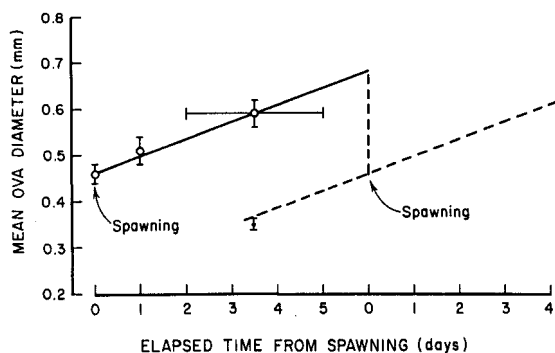


FIGURE 4.—Growth of oocytes in female northern anchovy during an assumed 6-day spawning cycle. Open circles are the mean diameter of eggs in the most advanced mode for: females with hydrated eggs (hydrated eggs excluded) combined with those with recent postovulatory follicles ("spawning"); females with day-old postovulatory follicles (second open circle); and for nonspawning females (third open circle). Horizontal bar indicates period in a 6-day cycle that females would be classified as nonspawning; and the point is plotted at the midpoint of that interval. Vertical bars are ± 2 SE of mean and solid circle indicates the mean diameter of eggs in the least advanced mode for the nonspawning class.

that in the second mode could produce a 6-day spawning cycle, the second mode becoming the most advanced mode at the time of spawning. The mean diameter of the eggs in the most advanced and the second mode for the nonspawning class, when plotted at 3.5 days, seems in a reasonable position relative to the other points, indicating that the cycle may be about 6 days. A linear trajectory of oocyte growth of 0.04 mm/day indicates spawning at a diameter between 0.6 and 0.7 mm, in keeping with laboratory findings. This analysis indicates that the mean diameter of yolked oocytes of females in various reproductive stages is consistent with a 6-7 day spawning cycle.

Batch Fecundity

MacGregor (1968) estimated the number of eggs in the most advanced mode in northern anchovy ovaries to be 574 eggs/g wet weight, from an analysis of frequency distribution of eggs in 19 females. Norberg (see footnote 5) concluded that northern anchovy fecundity was 556 eggs/g, from an examination of 119 females. The supposition underlying both estimates was that the number of eggs in the most advanced mode represents the number of eggs spawned. Owing to the importance of batch fecundity in any estimate of spawning biomass from egg and larval production, we de-

cidated to reexamine spawning batch fecundity in the northern anchovy.

The assumptions underlying batch fecundity estimates are: all eggs in the most advanced mode are spawned; fecundity is directly proportional to weight; and no bias exists in the estimation of the number of eggs within the most advanced mode nor in the selection of mature females for analysis. We consider these assumptions for females taken in February 1978 using fecundity estimates for each reproductive class.

Histological examination of females with postovulatory follicles indicated that very few hydrated eggs were retained after spawning. Thus, the number of hydrated eggs within ovaries prior to ovulation (females with no postovulatory follicles) should give the most accurate estimate of the number of eggs spawned. Another advantage of using hydrated eggs was that they stand out as an isolated class, distinct from all others; they differ in appearance and are as much as 2-3 times larger than yolked oocytes. Hence, they only need to be counted; neither statistical techniques nor one's judgment need be used to separate overlapping modes.

The mean number of hydrated eggs per gram of female (ovary free) was 389 ± 59 (± 2 SE) eggs and was only 7% less than that estimated for females with the most mature ovaries (nonspawner class) (Table 6). Thus, nearly all eggs in the most advanced mode were destined to be hydrated and spawned. Fecundity estimates were substantially higher and more variable in the other three reproductive classes. Many of the females in these classes had only one mode of yolked eggs whereas about 90% of those classified as nonspawners had two modes. Fecundity estimates for the less mature females tend to be higher because the eggs destined to form a second mode have not grown sufficiently to be separated from the rest of the yolked oocytes. More variability exists because of variation among females in the extent of the differentiation of the second modal group of eggs.

In summary, we believe our most accurate estimate of batch fecundity is 389 hydrated eggs/g ovary-free female weight. If an estimate based on total female weight is needed, we recommend the one for nonspawning fish (Table 6) reduced by the fraction of eggs which may not be hydrated (7%). The adjusted fecundity for nonspawners is 368 eggs/g female weight.

Fecundity as estimated above is a function of female weight, ovary weight, and the number of

TABLE 6.—Fecundity (eggs per gram female) estimates for northern anchovy females collected in February 1978 off southern California.

Sample	Number of females	Fecundity				Percent of females with two modal groups of nonhydrated eggs
		Ovary-free female weight		Total female weight		
		\bar{x}	SD	\bar{x}	SD	
Most advanced nonhydrated eggs of females with:						
Hydrated eggs ¹	23	530	360	462	309	39
Day 0, postovulatory follicles ²	13	693	387	497	201	69
Day 1, postovulatory follicles	19	619	313	592	294	69
None of the above (nonspawners)	33	418	186	396	171	91
Hydrated eggs	23	389	141	340	114	—
MacGregor (1968)	19	606	151	574	131	—

¹Only the nonhydrated eggs in most advanced mode are included.

²Females having both hydrated eggs and postovulatory follicles were included.

advanced eggs in a weighed sample of the gonad. The number of hydrated eggs per gram of ovary did not vary with fish weight and was $2,880 \pm 373$ (± 2 SE) eggs/g of ovary. If any weight-related bias existed, it probably was related to the gonad weight-female weight relation. To ascertain how ovary weight varied with female weight, we separated the data into three classes on the basis of mean diameter of eggs in the most advanced mode and plotted ovary weight as a function of female weight for each class (Figure 5). The relation was slightly curvilinear; the departure from linearity was most obvious in the 0.51-0.60 mm egg diameter class. Considering the variability in the number of mature eggs per gram of ovary, and the slight departure from linearity, no practical purpose is achieved in expressing fecundity as a function of female weight rather than as a direct proportion of weight, although direct proportionality is somewhat less accurate for extreme weight classes.

The relation between gonad weight and female weight differed somewhat among the diameter classes as can be seen in Figure 5. This might be expected because the weight of the ovary should increase somewhat with the average diameter of the eggs in the most advanced mode. We analyzed the data using multiple regression to determine if the $\ln(\log e)$ gonad weight (G) could be estimated from the diameter of eggs in the most advanced mode (D), \ln ovary-free female weight (W), and the interaction term ($D \ln G$). Both female weight and the interaction term had a significant effect on gonad weight, whereas diameter alone did not. The final multiple regression equation was:

$$\ln G = -4.213 + 1.069 \ln W + 0.555 D \ln W$$

where $r^2 = 0.92$. Solving for diameter we obtain

$$D = \frac{\ln G + 4.213 - 1.069 \ln W}{0.555 \ln W}$$

This equation may be useful for estimation of maturity stages for anchovy from weight relationships: 60% of the estimates of mean diameter were within ± 0.1 mm of the observed values and the residuals were distributed evenly. The equation is more useful than gonad index (ovary weight/female weight), which is commonly employed to assess maturity, because it produces a number that can be directly related to reproduction and avoids a weight bias for extreme weight classes. The weight bias in gonad index is apparent by examination of data in Figure 5 (lowest); a 30 g female with eggs of 0.65 mm in the most advanced mode has a gonad index of 0.064 whereas that of a 10 g female at the same stage of maturity has an index of 0.040. The equation also identifies females with hydrated eggs; the average diameter of eggs estimated by the equation for females with hydrated eggs was 1.20 ± 0.12 mm ($n = 22$) and is close to the mean of spawned eggs (1.34 mm). Obviously, such equations would be specific to populations having similar weight relations, but it does seem a useful approach for assessing maturity.

DISCUSSION

This paper provides a method for direct estimation of the frequency of spawning of a multiple spawning pelagic fish population. From such estimates it may be possible to directly estimate spawning biomass from the abundance of eggs and larvae over a short segment of the breeding season. One of the major assumptions underlying the estimate is that a representative sample of females is obtained. Spawning frequency would be overestimated if nonspawning females were in re-

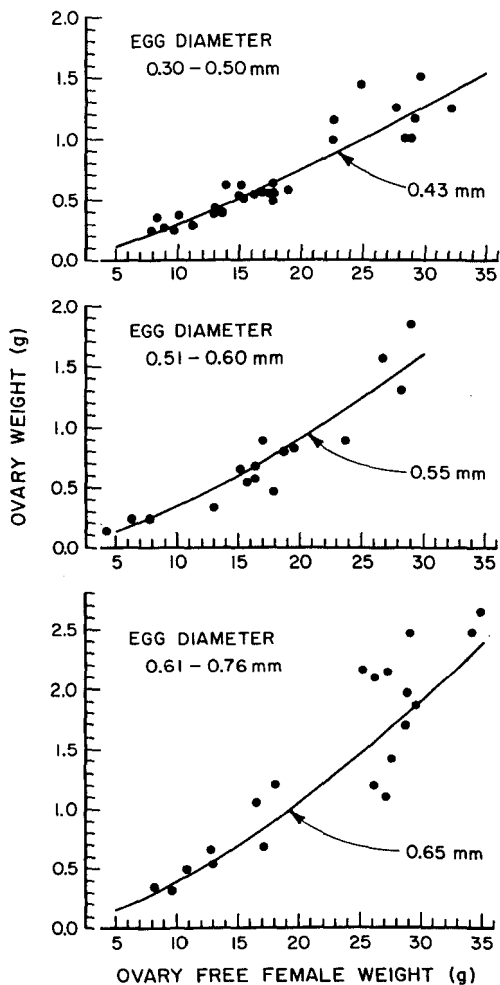


FIGURE 5.—Relation between wet weight of ovary and ovary-free wet weight of female northern anchovy classed into three groups, based on the mean diameter of eggs in the most advanced mode. Each point is a value for a single female. The lines represent multiple regressions, described in the text, for the mean egg diameters given by the arrows.

gions or at depths not sampled by the trawl or commercial purse seine. Studies need to be conducted at other times of the year and employing other sampling techniques to answer these questions.

This paper also describes a new method for estimation of batch fecundity in a multiple spawning fish. Our estimation for northern anchovy based on hydrated eggs (389 eggs/g female weight, less ovary), was substantially less than that of MacGregor (1968) (606 eggs). This difference could be attributed to annual variation in fecundity because variations of this size are known to

occur in fishes (Bagenal 1973). On the other hand, our selection of females on the basis of reproductive state also may have been responsible for at least part of the difference. Our estimates for females that had recently spawned (day 0 and day 1 postovulatory follicles, Table 6) were close to MacGregor's estimate. In recently spawned females, modal groups of eggs were less distinct, and often one mode was considered to exist when the eggs may have been destined to form two spawning batches. If MacGregor (1968) used such females, this could explain in part why his estimate was higher than our estimates based on hydrated eggs or on fish classed as nonspawning. In addition, our technique of partitioning eggs occurring between two modal groups according to an assumed normal distribution may have decreased our estimate somewhat relative to past methods. Use of hydrated eggs avoids these problems, but it does require histological examination to insure that none of the females used for the estimate have begun ovulation. Apparently, some of the females we captured were spawning because their ovaries contained many new postovulatory follicles as well as many hydrated eggs. We usually examined only one set of histological sections to determine if ovulation had occurred; histological examination of an entire ovary was impractical. We believe our examination was adequate because our estimate based on hydrated eggs was close to the one based on females with the most advanced ovaries (nonspawning).

In addition to the obvious application to the estimation of spawning biomass, this work provided insights into the reproductive biology of *E. mordax*. The high spawning frequency, the ability to rapidly mature new batches of yolked oocytes, and the long breeding season of the northern anchovy (Lasker and Smith 1977), indicate that energy reserves and the availability of food may set the limit to the number of spawnings and hence to total fecundity. The analysis has also provided a possible explanation for the variability in the sex ratio of catches of anchovy.

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