SPAWNING FREQUENCY AND SEX RATIO IN THE PERUVIAN ANCHOVY, *ENGRAULIS RINGENS*[1]

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ABSTRACT

Spawning frequency was determined for the first time for the Peruvian anchovy, *Engraulis ringens*, using the incidence of postovulatory follicles. The agreement between two independent frequency estimates, one for females taken one day after spawning and another for females taken two days after spawning, demonstrated the reliability of this method. In August-September, the main period of annual spawning, 16.04% of the female population of the central and northern anchovy stock off Peru spawned per day: i.e., every 6.23 days the average female spawned a new batch of eggs. Hydration of ovaries began as early as 0700 hours in the morning. Spawning occurred at night between 1800 and 0200 hours, reaching a maximum at about 2200 hours. Sex ratio was 57.9% females by weight. The vulnerability of females to the purse seine changed with their reproductive state. Females with hydrated ovaries who were ready to spawn seemed to attract males and to form male-dominated "spawning schools" by segregating from "normal" schools at night.

RESUMEN

La frecuencia de puesta en la anchoveta peruana *Engraulis ringens* ha sido determinada por primera vez, tomando como base la incidencia de folículos post-ovulatorios. La concordancia entre dos estimaciones independientes de esta frecuencia, obtenidas de hembras un y dos días después de la puesta, demuestran la fiabilidad de esta método. Anualmente, el período principal de la puesta abarca de Agosto a Septiembre, cuando la puesta diaria comprendió el 16.04% de las hembras de las poblaciones del norte y centro de la región peruana; es decir, cada 6.23 días una hembra promedio pone una nueva remesa de huevos. La hidratación de los ovarios se inició temprano, a eso de las 0700 de la mañana. La puesta se produjo entre las 1800 y 0200 horas, alcanzando un máximo a las 2200 horas aproximadamente.

La proporción de sexos calculada en peso fue de 57.9% de hembras. La vulnerabilidad de las hembras a la pesca con redes de jareta varía con la fase reproductora enque se encuentran. Hembras con ovarios hidratados, que estaban dispuestas para la puesta parecian atraer a los machos y formar cardúmenes de puesta dominados por machos separándose de los cardúmenes normales durante la noche.

INTRODUCTION

In the past, estimating the spawning biomass of multiple-spawning pelagic fish species like anchovies and sardines posed serious problems because no adequate methods were available to determine the spawning frequency, i.e., the fraction of the female population spawning per unit time. Recently, Hunter and Goldberg (1980) solved this problem for the northern anchovy, *Engraulis mordax*, by an ingenious technique. They followed up the suggestion of Yamamoto and Yoshioka (1964) that spawning frequency could be determined by incidence of postovulatory follicles—the remnants of ovulated follicles.

Follicles are the layers of cells surrounding developing oocytes in the ovaries. After ovulation, they immediately begin to deteriorate and are then called postovulatory follicles (Hunter and Goldberg 1980). Hunter and Goldberg identified postovulatory follicles in histological sections of northern anchovy ovaries and classified the females into three groups: those spawning on the night of capture (new, or day-0 postovulatory follicles), those spawning on the night previous to capture (day-1 postovulatory follicles), and those showing no evidence of recent spawning. Hunter and Goldberg then used the frequency of day-1 postovulatory follicles as a measure of spawning frequency. They used anchovy spawned in the laboratory to develop this technique (Leong 1971). Because the time of induced spawning was known, they could de-

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scribe histological criteria for aging postovulatory follicles. These criteria were then applied to field populations.

Hunter and Goldberg collected their field samples with a small trawler, which could catch adult anchovy only at night. They were therefore not able to obtain complete 24-hour time series of females with postovulatory follicles. This could have resulted in a bias in the estimation of spawning frequency, since Hunter and Goldberg (1980) had already shown that females captured on the night of spawning were oversampled.

It is well known that prior to ovulation the ovaries of teleost fish take up fluid, a process called hydration (Fulton 1898). Hunter and Macewicz (1980) suggested that if anchovies could be sampled during the day, it might be possible to use the incidence of females with hydrated ovaries ("hydrated females") to determine spawning frequency. The advantage would be that time and money could be saved because histological examination would no longer be required.

In this paper we estimate the spawning frequency of the Peruvian anchovy, *Engraulis ringens*, using Hunter and Goldberg's (1980) method. We can answer some of the questions posed by Hunter and Goldberg and Hunter and Macewicz (1980) because we used a different technique to sample anchovy. In Peru, anchovy were collected with a purse seiner and could therefore be sampled in the day as well as at night. Thus, females with postovulatory follicles and females in the hydrated, prespawning stage could be obtained for an entire 24-hour cycle. Any time-related bias in the incidence of females with postovulatory follicles would become obvious when a series of samples taken at regular intervals over 24 hours was examined. Thus, the use of a purse seine for the Peruvian anchovy gave new insights into bias in estimating spawning frequency. Because hydrated females were sampled during the day, the alternative approach of using incidence of hydrated females to estimate spawning frequency could also be evaluated. Thus the objectives of this paper are to evaluate Hunter and Goldberg's (1980) method for estimating spawning frequency by using a 24-hour sampling scheme and to determine the effect of different sampling gear (trawl versus purse seine) on the estimates of spawning frequency and sex ratio of Peruvian anchovy schools.

The findings of Hunter and Goldberg (1980) enabled the Southwest Fisheries Center, La Jolla, to develop the "egg production method" (Parker 1980; Stauffer and Picquelle 1980) for estimating spawning biomass of the northern anchovy off California. Two of the five parameters required for this method are spawning frequency and sex ratio. The data presented here were used to estimate the spawning biomass of the Peruvian anchovy using the egg production method (Santander et al., in press).

METHODS

Adult anchovies were collected with a purse seiner. The cruise was run from August 25 to September 17, 1981. It began in the south and proceeded northwards. The purse seine stations were usually located within 20 miles of the shore because of adverse weather conditions and the scarcity of anchovy schools farther offshore. Most of the collections came from the southern part of the investigation area because weather conditions in the north prohibited use of the purse seine (Figure 1). The total number of collections was 49.

Immediately after capture, the anchovy's body cavity was opened from the anus to the ventral fins. Only live specimens were processed because of the rapid degeneration of the postovulatory follicles. The fish were preserved in a 4% buffered formaldehyde solution. Seawater was not used to dilute the formaldehyde solution because it causes a white precipitate that makes reading the histological sections difficult.

Twenty mature females were collected at random

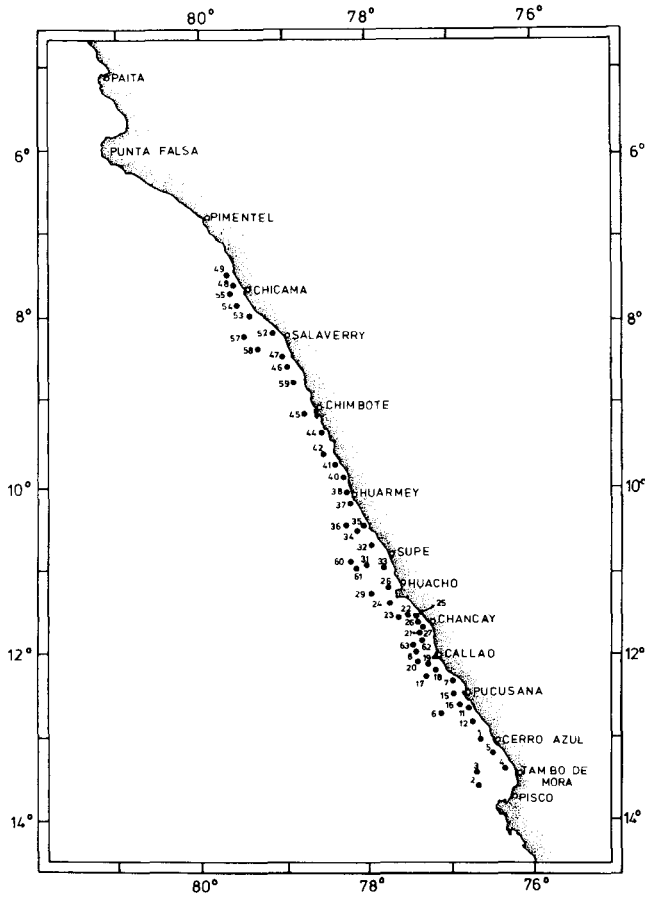

Figure 1. Map of surveyed area and location of samples of anchovies.

for histological analysis. Standard methods were used to process the ovaries. A small cube of about 0.125 cm^3 was cut from the center of the ovary. It was dehydrated in a series of alcohol solutions and embedded in paraplast. The histological sections were cut at 6 μm and stained with hematoxylin/eosin. A detailed account of the procedure is given in Alarcón et al. (in press). The criteria developed by Hunter and Goldberg (1980) for aging postovulatory follicles are based on the stages of degeneration through which they pass. Because postovulatory follicles degenerate rapidly, their age can only be determined up to 50 hours after spawning. The age classes for postovulatory follicles used here are somewhat different from those described by Hunter and Goldberg (1980).

The fraction of females (sex ratio) was estimated for each collection from a subsample consisting of the first 800 g of fish. The total body weight was used, because the gonad-free weight of males was not measured. The total body weight of hydrated females was adjusted (Santander et al., in press) for the excess weight of the hydrated ovary (Stauffer and Picquelle 1980). Immature fish were included because it was not possible to distinguish between mature and immature males. In order to attain exactly 800 g of fish it was necessary to use only that fraction of the weight of the last fish in the collection that completed the 800 g. Sample mean and variance were estimated according to Stauffer and Picquelle (1980):

$$\bar{\bar{R}} = \frac{\sum \bar{R}_i}{n}$$

and

$$V(\bar{\bar{R}}) = \frac{\sum (\bar{R}_i - \bar{\bar{R}})^2}{n(n-1)}$$

where

$\bar{R}_i$ = fraction of females by weight in percent in collection i

$\bar{\bar{R}}$ = average fraction of females by weight in percent from all collections.

For estimating the spawning frequency (fraction of mature females spawning per day) we used only postovulatory females taken at least 9 hours after peak spawning (2200 hours), to prevent bias arising from sampling females during the time of day when they are actually spawning. Two independent 24-hour sets of postovulatory follicles could be separated: one set of postovulatory follicles with an age between 9 and 32 hours after spawning and another set of postovulatory follicles with an age between 33 and 56 hours. Henceforth females with ovaries containing postovulatory follicles of 9-32 hours will be called day-1 females; those having ovaries containing postovulatory follicles of 33-56 hours will be called day-2 females.

Assuming that sampling of females with hydrated ovaries, day-1 females, or day-2 females is unbiased, then the spawning fraction for collection i is estimated by:

$$F_i = \frac{m_{hi}}{m_i} \text{ or } \frac{m_{1i}}{m_i} \text{ or } \frac{m_{2i}}{m_i}$$

where

$$m_i = m_{hi} + m_{1i} + m_{2i} + m_{ai}$$

and where

m_{hi} = number of hydrated females in collection i

m_{1i} = number of day-1 females in collection i

m_{2i} = number of day-2 females in collection i

m_{ai} = number of females that have not spawned within the past 9 to 56 hours (includes females with postovulatory follicles of an age of less than 9 hours)

m_i = number of mature females in collection i

F_i = spawning fraction in collection i

The results for the Peruvian anchovy were similar to results for the northern anchovy (Stauffer and Picquelle 1980); they indicated that hydrated females were oversampled. To correct for this apparent oversampling, under the assumption that the true fraction of hydrated females is the same as the fraction of day-1 or day-2 females, m_{hi} is replaced by $\frac{m_{1i} + m_{2i}}{2}$ such that

$$\hat{F}_i = \frac{m_{1i}}{\frac{m_{1i} + m_{2i}}{2} + m_{1i} + m_{2i} + m_{ai}}$$

or

$$\frac{m_{2i}}{\frac{m_{1i} + m_{2i}}{2} + m_{1i} + m_{2i} + m_{ai}}$$

where

$\hat{F}_i$ = corrected fraction of day-1 or day-2 females in collection i.

The estimates for mean and variance are given by:

$$\bar{F} = \frac{\sum m_{1i} + m_{2i}}{2\sum \frac{m_{1i} + m_{2i}}{2} + m_{1i} + m_{2i} + m_{ai}} = \frac{\sum m_{1i} + m_{2i}}{2 \sum m_{yi}}$$

and

$$V_{(\bar{F})} = \frac{1}{n(n-1)} \sum \left(\frac{m_{yi}}{\bar{m}}\right)^2 (\hat{F}_i - \bar{F})^2$$

where

$\bar{F}$ = average fraction of females spawning per day from all collections

$m_{yi} = \frac{m_{1i} + m_{2i}}{2} + m_{1i} + m_{2i} + m_{ai}$ = corrected number of mature females in collection i

$\bar{m}$ = average number of mature females corrected per collection i

$= \frac{\sum m_{yi}}{n}$

n = number of collections.

RESULTS

Peak Spawning Time

To age postovulatory follicles, one must determine the duration of the daily spawning period and its midpoint. This goal can be reached in three ways: (1) by recording time of incidence and frequencies of new postovulatory follicles from samples of adult anchovy females; (2) by recording the decline of occurrence of hydrated females; and (3) by recording time of occurrence and frequencies of newly spawned eggs from ichthyoplankton samples. Hunter and Macewicz (1980) demonstrated clearly that the percentage of northern anchovy females with hydrated oocytes declined steadily from 10% to 14% at 1800 hours to 0% at 2400 hours, and concluded that 2200 to 2300 hours was the period of maximum spawning.

The data from the Peruvian anchovy did not provide as clear a picture, for two reasons. Hunter and Macewicz (1980) collected all their samples at night between 1800 and 0500 hours, whereas only 40% of the Peruvian samples were collected between 1800 and 2300 hours, and only one sample was obtained between 2300 and 0700 hours. In the Peruvian samples, females with new postovulatory follicles were recorded for the first time at 1800 hours, but their numbers were very low (Table 1). The numbers of females with hydrated oocytes ranged between 7.5% and 51.7% from 0700 to 2030 hours and declined sharply to 3.3% and 5% at 2130 to 2230 hours, respectively (Figure 2). The best data for estimating the midpoint of the nightly spawning period was that provided by the ichthyoplankton survey. The occurrence of newly spawned eggs in the water column demonstrated that peak spawning time of the Peruvian anchovy is between 2200 and 2300 hours (Santander et al., in press).

Combining all these data leads to the conclusion that Peruvian anchovy spawning starts at sunset, around 1800 hours, and that the period of maximum spawning is between 2200 and 2300 hours. It was not possible to estimate the time when nightly spawning ceases, but by analogy to the northern anchovy, spawning probably ceases around 0200 hours. For convenience, 2200 hours is taken as the midpoint of the daily spawning period in the following analysis.

TABLE 1
Collections Containing Female Peruvian Anchovy with New Postovulatory Follicles

Collection no.	Time of day	No. of new postovulatory follicles
46	1800	1
4	1930	1
12	1930	2
41	2015	1
25	2100	1
21	2230	2
1	0315	1

Spawning Frequency

The spawning frequency is the fraction of mature females that spawns per day. In theory, one should get three independent estimates of this parameter: (1) the percentage of females with hydrated oocytes, (2) the percentage of females with day-1 postovulatory follicles, and (3) the percentage of females with day-2 postovulatory follicles. Because females with hydrated oocytes tend to be oversampled, they cannot be used for this purpose. The age of postovulatory follicles can only be determined up to about 50 hours after spawning, because older postovulatory follicles may be confused with other structures, such as atretic follicles (Hunter and Goldberg 1980; Hunter and Macewicz 1980).

Because the northern anchovy were sampled only at night (Hunter and Goldberg 1980; Hunter and Mace-

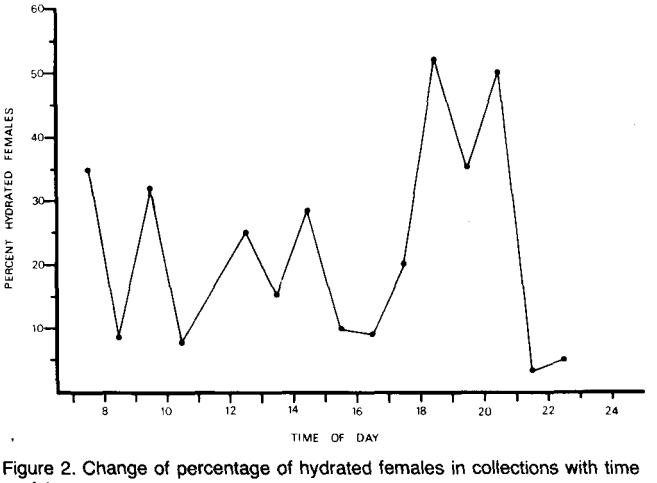

Figure 2. Change of percentage of hydrated females in collections with time of day.

wicz 1980; Stauffer and Picquelle 1980), investigators could use only day-1 postovulatory follicles to estimate spawning frequency. Our use of a purse seiner for sampling the Peruvian anchovy permitted collection of samples at any time of day. Consequently, we obtained two independent sets of data for estimating spawning frequency (day-1 and day-2 females) and compared them.

The way in which these two sets of data were obtained is demonstrated in Table 2. It was assumed that all anchovy females spawn at 2200 hours. Because the earliest sample was collected at 0700 hours, we considered that time the beginning of the day-1 and the day-2 periods. Postovulatory follicles found at this time are, according to their structure, assigned to one of the following three groups: day-1, day-2, or older postovulatory follicles. They are given ages of 9, 33, or more than 56 hours, respectively. Table 3 contains the summary of all data: (1) date and time of each collection, (2) numbers of hydrated, day-1, and day-2 females, (3) hours past spawning of each collection, (4) values corrected for oversampling hydrated females, and (5) percentage of hydrated, day-1, and day-2 females per collection.

Stauffer and Picquelle (1980) suggested that females with new postovulatory follicles may also be oversampled, but lacked data with which to test this hypothesis. To test it, we calculated the mean spawning frequencies for consecutive 24-hour periods. First, we calculated the mean spawning frequency for the first 24-hour period starting at 0700 hours—9 hours after mean spawning time. Only postovulatory follicles within the age range from 9 to 32 hours were included (Figure 3). Then we calculated the spawning frequency for the second 24-hour period, beginning at 0800 hours and including postovulatory follicles with

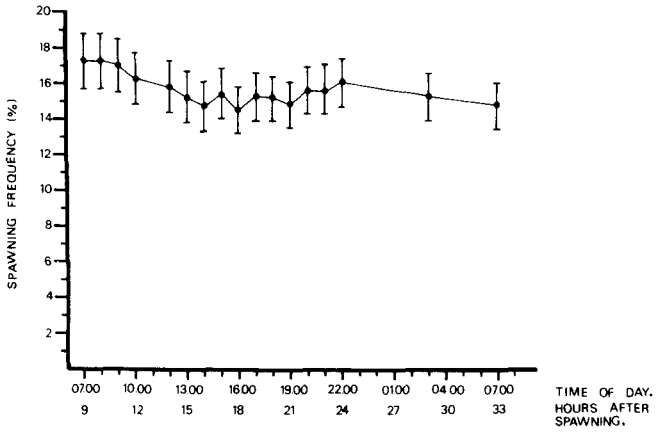

Figure 3. Spawning frequencies calculated from consecutive 24-hour periods. The time of day indicates at what time each 24-hour period begins. The corresponding hours after mean spawning time (2200 hours) are also indicated. Standard deviations are added.

TABLE 2
Time of Collection of Females and the Age of Postovulatory Follicles

	Time of day	Age of postovulatory follicles (hours past spawning)	
		Day-1	Day-2
Night of spawning	22.00 (Peak spawning)		
1st day following spawning	07.00-07.59	9	33
	08.00-08.59	10	34
	09.00-09.59	11	35
	10.00-10.59	12	36
	11.00-11.59	13	37
	12.00-12.59	14	38
	13.00-13.59	15	39
	14.00-14.59	16	40
	15.00-15.59	17	41
	16.00-16.59	18	42
	17.00-17.59	19	43
	18.00-18.59	20	44
	19.00-19.59	21	45
	20.00-20.59	22	46
	21.00-21.59	23	47
	22.00-22.59	24	48
	23.00-23.59	25	49
2nd day following spawning	00.00-00.59	26	50
	01.00-01.59	27	51
	02.00-02.59	28	52
	03.00-03.59	29	53
	04.00-04.59	30	54
	05.00-05.59	31	55
	06.00-06.59	32	56

an age from 10 to 33 hours. We repeated these calculations for a total interval of 48 hours, always advancing by one hour, so that the last 24-hour period starts again at 0700 hours—33 hours after mean spawning time and includes postovulatory follicles with an age from 33 to 56 hours. Theoretically, there should have been 25 different 24-hour periods. However, because no collections were taken at 1100 hours and only one between midnight and 0700 hours (Table 3), only 17 consecutive 24-hour periods are included in Figure 3. The spawning frequencies calculated from these 17 consecutive 24-hour periods showed no strong trend over the observed 48-hour interval, and ranged from 14.70% to 17.26%. We conclude that females with postovulatory follicles of 9 hours and older are equally available to the purse seine.

If spawning frequency data for day-1 females and for day-2 females are independent of each other and identically distributed, they could be combined. Their combined total would double the sample size and

TABLE 3
Data Summary

Time of day	Collection No.	Date	Sex ratio (% females)	Hydrated females No. (m_{hi})	Hydrated females %	Day-1 females Hours past spawning	Day-1 females No. (m_{1i})	Day-2 females Hours past spawning	Day-2 females No. (m_{2i})	$\frac{m_{1i}+m_{2i}}{2}$	m_{ai}	m_{yi}	$\hat{F}_1$ Day-1 (m_{1i}/m_{yi})	$\hat{F}_2$ Day-2 (m_{2i}/m_{yi})
07.15	28	5.9.	52.76	11	55	9	0	33	0	0	9	9.0	0	0
07.25	38	8.9.	55.30	3	15	9	3	33	3	3.0	11	20.0	.1500	.1500
08.00	33	6.9.	83.01	0	0	10	2	34	1	1.5	17	21.5	.0930	.0465
08.30	26	2.9.	68.74	0	0	10	2	34	4	3.0	14	23.0	.0870	.1739
08.45	48	12.9.	45.32	5	25	10	6	34	3	4.5	6	19.5	.3077	.1538
09.00	5	26.8.	44.96	11	55	11	2	35	1	1.5	6	10.5	.1905	.0952
09.00	10	28.8.	31.51	7	35	11	6	35	1	3.5	6	16.5	.3636	.0606
09.00	42	9.9.	33.38	2	10	11	0	35	0	0	18	18.0	0	0
09.15	29	5.9.	90.50	2	10	11	2	35	3	2.5	13	20.5	.0976	.1463
09.30	17	30.8.	54.44	10	50	11	2	35	0	1.0	8	11.0	.1818	0
10.00	22	1.9.	67.33	0	0	12	2	36	1	1.5	17	21.5	.0930	.0465
10.10	52	16.9.	45.80	3	15	12	6	36	3	4.5	8	21.5	.2791	.1395
12.00	2	25.8.	68.95	2	10	14	5	38	3	4.0	10	22.0	.2273	.1364
12.00	57	17.9.	63.62	2	10	14	6	38	5	5.5	7	23.5	.2553	.2128
12.10	34	7.9.	70.96	10	50	14	3	38	0	1.5	7	11.5	.2609	0
12.30	27	2.9.	49.89	6	30	14	1	38	2	1.5	11	15.5	.0645	.1290
13.00	18	30.8.	55.32	3	15	15	2	39	2	2.0	13	19.0	.1053	.1053
13.10	53	16.9.	62.04	4	20	15	6	39	3	4.5	7	20.5	.2927	.1463
13.15	49	12.9.	59.29	2	10	15	4	39	2	3.0	12	21.0	.1905	.0952
14.15	11	28.8.	47.43	3	15	16	4	40	4	4.0	9	21.0	.1905	.1905
14.15	58	17.9.	28.18	11	55	16	1	40	1	1.0	7	10.0	.1000	.1000
14.45	35	7.9.	70.51	3	15	16	0	40	6	3.0	11	20.0	0	.3000
15.30	6	28.8.	81.27	2	10	17	9	41	1	5.0	8	23.0	.3913	.0435
16.00	15	29.8.	32.57	5	25	18	1	42	3	2.0	11	17.0	.0588	.1765
16.10	40	8.9.	60.38	1	5	18	3	42	5	4.0	11	23.0	.1304	.2174
16.15	20	31.8.	26.36	3	15	18	4	42	6	5.0	7	22.0	.1818	.2727
16.30	3	25.8.	74.42	0	0	18	4	42	7	5.5	9	25.5	.1569	.2745
16.45	23	1.9.	78.73	0	0	18	6	42	4	5.0	10	25.0	.2400	.1600
17.10	44	9.9.	62.12	2	10	19	1	43	3	2.0	14	20.0	.0500	.1500
17.35	54	16.9.	71.94	6	30	19	7	43	4	5.5	3	19.5	.3590	.2051
18.00	8	27.8.	45.21	1	5	20	1	44	1	1.0	17	20.0	.0500	.0500
18.00	36	7.9.	36.11	13	65	20	2	44	4	3.0	1	10.0	.2000	.4000
18.00	46	11.9.	29.90	16	80	20	1	44	0	0.5	3	4.5	.2222	0
18.15	31	5.9.	67.15	2	10	20	5	44	1	3.0	12	21.0	.2381	.0476
19.00	24	1.9.	60.38	3	15	21	1	45	2	1.5	14	18.5	.0541	.1081
19.15	19	30.8.	46.13	4	20	21	4	45	7	5.5	5	21.5	.1860	.3256
19.30	4	25.8.	55.33	13	65	21	3	45	0	1.5	4	8.5	.3529	0
19.30	7	26.8.	58.28	2	10	21	2	45	4	3.0	12	21.0	.0952	.1905
19.30	12	28.8.	12.62	17	85	21	0	45	0	0	3	3.0	0	0
19.30	16	29.8.	43.93	8	40	21	3	45	3	3.0	6	15.0	.2000	.2000
19.40	55	16.9.	65.52	2	10	21	1	45	5	3.0	12	21.0	.0476	.2381
20.10	45	9.9.	73.93	1	5	22	5	46	6	5.5	8	24.5	.2041	.2449
20.15	41	8.9.	15.86	19	95	22	0	46	0	0	1	1.0	0	0
21.00	25	2.9.	78.68	2	10	23	4	47	7	5.5	7	23.5	.1702	.2979
21.00	47	11.9.	79.20	0	0	23	4	47	4	4.0	12	24.0	.1667	.1667
21.15	32	5.9.	64.18	0	0	23	2	47	2	2.0	16	22.0	.0909	.0909
22.30	21	31.8.	79.68	1	5	24	3	48	4	3.5	12	22.5	.1333	.1778
22.55	37	7.9.	64.36	1	5	24	9	48	1	5.0	9	24.0	.3750	.0417
03.15	1	25.8.	51.42	2	10	29	5	53	1	3.0	12	21.0	.2381	.0476

TABLE 4
Arithmetic Means and Probability Values p and q of the Binomial Distributions of Hydrated, Day-1, and Day-2 Females

Reproductive stage of females	$\bar{x}$	p	q
Hydrated	4.6122	.2306	.7694
Day-1	3.1633	.1726	.8274
Day-2	2.7143	.1481	.8519

thereby reduce the variance of the estimate of spawning frequency. If their occurrence were random, the observed frequencies of day-1 and day-2 females should follow a positive binomial distribution. To test this assumption, we applied the Kolmogorov/Smirnov test (Siegel 1956). The frequencies of all three groups of females in different reproductive stages are listed in Table 3. Table 4 gives arithmetic means and the probability values p and q of the binomial distribution. The term k of the positive binomial distribution, the maximum number of individuals in a collection, was 20; in other words, 20 females per collection were included in the estimate of the frequency of hydrated, day-1, and day-2 females. The 5% significance level (D) for the Kolmogorov/Smirnov test is 0.1943, and the estimated maximum differences ($\hat{D}$) were 0.3628 (hydrated), 0.1656 (day-1), and 0.1859 (day-2). These results demonstrate that the frequencies of day-1 and day-2 females were not significantly different from the positive binomial distribution at the 5% level, whereas the hydrated females do not correspond to the positive binomial distribution. This becomes more obvious when the cumulative percentages of the observed and expected class frequencies of all three groups of females are plotted (Figure 4).

If the spawning frequencies of day-1 and day-2 females are to be combined, they must not be statistically different. To establish this we used a test of the difference between paired independent samples

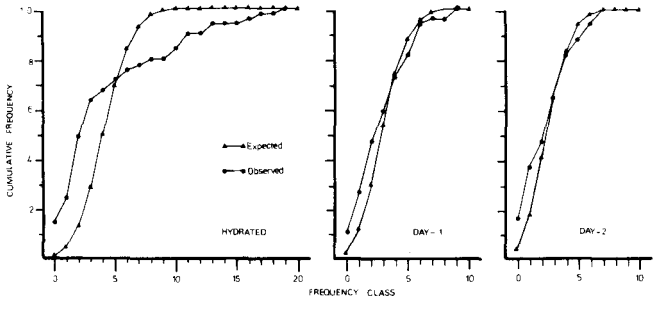

Figure 4. Cumulative frequencies of observed and expected (positive binomial distribution) class frequencies of females in different reproductive stages. The 49 collections of mature female anchovies are distributed into frequency classes according to the number of females they have in a particular reproductive stage (hydrated, day-1, or day-2); for example, in the left panel, frequency class 0 contains all collections having 0 hydrated females.

TABLE 5
Mean Percentage of Hydrated, Day-1, Day-2, and Day-1/Day-2 Females with Variance (V), Standard Deviation (SD), and Coefficient of Variation (CV)

Reproductive stage of females	Mean	V	SD	CV
Hydrated	.2306	1.2198×10^{-3}	.0349	.1515
Day-1	.1726	2.3248×10^{-4}	.0152	.0883
Day-2	.1481	1.7224×10^{-4}	.0131	.0886
Day-1 & day-2 (combined)	.1604	1.0175×10^{-4}	.0101	.0629

(Snedecor and Cochran 1967). The paired samples were the spawning frequency values of day-1 ($\hat{F}_1$) and day-2 ($\hat{F}_2$) females from each collection (Table 3). The null hypothesis was that the mean difference ($\bar{D}$) between $\hat{F}_1$ and $\hat{F}_2$ equals zero. $\bar{D}$ was 0.032. The t-test gave a value of 1.526 with n-1 = 48 degrees of freedom. This shows that the null hypothesis cannot be rejected at the 5% level of significance. Therefore, the two estimates can be combined, thus doubling the sample size.

The spawning frequency of the day-1 females was 0.1726 with a variance of 2.3248×10^{-4}, a standard deviation of 0.0152, and a coefficient of variation of 0.0883 (Table 5). The spawning frequency of the day-2 females was 0.1481 with a variance of 1.7224×10^{-4}, a standard deviation of 0.0131, and a coefficient of variation of 0.0886. When these two data sets were combined, the estimate of spawning frequency was 0.1604 with a variance of 1.0175×10^{-4}, a standard deviation of 0.0101, and a coefficient of variation of 0.0629 (Table 5). By combining the data we have reduced the coefficient of variation by nearly a third. This would be important when the estimate of spawning frequency is used to calculate spawning biomass using the egg production method (Parker 1980; Stauffer and Picquelle 1980), because by reducing the coefficient of variation for spawning frequency one reduces the coefficient of variation for the biomass estimate (Santander et al., in press).

A spawning frequency of 16.04% (Table 5) means that in August/September 1981 the average mature Peruvian anchovy female spawned a new batch of eggs every 6.23 days. The high daily incidence of hydrated females (23.06%; Table 5) clearly indicates that hydrated females were oversampled. Comparing the coefficient of variation for the incidence of hydrated females with that of the day-1 and day-2 females indicates that the number of hydrated females per sample was much more variable than were the numbers of day-1 or day-2 females per sample.

Hydrated females also seem to be more vulnerable to the purse seine than other females. In order to test

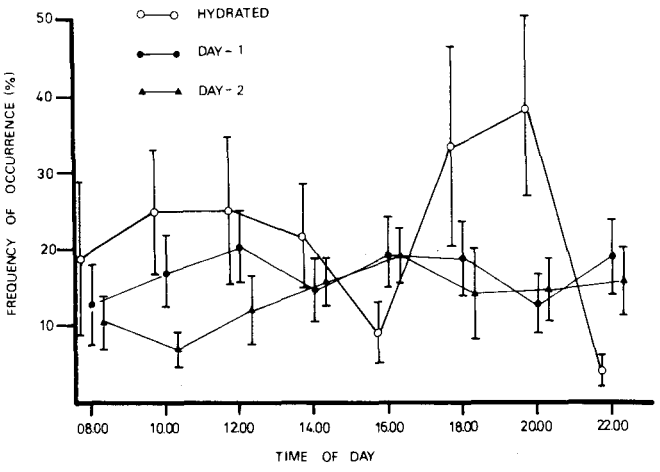

Figure 5. Change of percentage of frequency of occurrence of hydrated, day-1, and day-2 females in collections with time of day.

whether the three groups of females (hydrated, day-1, and day-2) show a diel change in their vulnerability to purse seining, the percentage of females in each group was calculated for 2-hour intervals and plotted at the midpoints of the 2-hour interval (Figure 5). The hydrated females had the highest percentage except for collections taken at about 1600 and at 2200 hours. The value at 2200 hours (4%) is low because most of the fish had completed spawning at that time. The very high values at 1800 hours (33%) and at 2000 hours (38%) indicate that hydrated females are particularly vulnerable at that time of day. At these hours, hydration of the ovaries has reached its maximum, and the ovaries are extremely heavy—in some cases up to 25% to 30% of the female's gonad-free body weight. The percentages of day-1 and day-2 females, on the other hand, remain relatively constant throughout the period, indicating that their vulnerability to capture does not change significantly with time of day.

Sex Ratio

The sex ratios (percentage of females on a weight basis) of all 49 collections showed large variations ranging from 12.62% to 90.50% females (Table 3). The average sex ratio was 56.43%, with a variance of 0.0007, a standard deviation of 0.0259, and a coefficient of variation of 0.0459. Similar variability in sex ratio of the northern anchovy has been reported by Klingbeil (1978), Hunter and Goldberg (1980), and Stauffer and Picquelle (1980). Klingbeil demonstrated that the greatest variability in the sex ratio occurs in the months of peak spawning. Hunter and Goldberg showed that sex ratio varied with spawning activity. We carried out the same analysis for the Peruvian anchovy.

We grouped all 49 collections into three classes based on their sex ratio. For each of these three classes we calculated the percentage of females in the following four spawning groups: (1) spawning on the night of capture (includes hydrated females and females with new postovulatory follicles), (2) day-1 females, (3) day-2 females, (4) females with no evidence of recent or imminent spawning (Table 6). In the male-dominated collections, which contained only 10% to 39% females, 54% of the females had spawned on the night of capture. On the other hand, in the female-dominated collections, where 70% to 99% of the fish were females, only 13% of the females had spawned on the night of capture. Correlation coefficients for sex ratio and (1) number of hydrated females, (2) number of day-1 females, and (3) number of day-2 females were -0.66, 0.35, and 0.34, respectively. Only the correlation coefficient of -0.66 for the hydrated females was significant at the 5% level. This shows that as the proportion of males in a collection increases, so does the proportion of hydrated females.

In addition, sex ratio also seemed to change with time of day. Most samples taken between 0700 and 1800 hours had 50% to 70% females, whereas after sunset (1800-2100 hours) the average ratio of females in the collections dropped below 50%. A similar pattern is described for the northern anchovy by Stauffer and Picquelle (1980). Average sex ratios (including all collections) computed for morning, afternoon, and night periods were 56.80%, 59.11%, and 54.25%, respectively (Table 7). These data show that the average sex ratio (56.43% females, $N = 49$) is probably biased by including those collections that have more than 30% hydrated females and were sampled at night. A more realistic sex ratio is computed by excluding all night collections sampled between 1800 and 2300

TABLE 6
Sex Ratio and Percentage of Females in Different Reproductive Stages

Sex ratio class (% females)	Number of collections	% Females				No. of females classified
		Spawning on day of capture	Day-1	Day-2	No evidence of spawning	
10-39	9	54	8	8	29	180
40-69	28	19	16	13	52	560
70-99	12	13	20	20	48	240

TABLE 7
Sex Ratios at Different Times of Day

Time of day	All collections		Excluding collections with >50% hydrated females		Excluding collections with >30% hydrated females	
	No. of collections	% of females	No. of collections	% of females	No. of collections	% of females
07.00-12.00	12	56.80	9	56.44	8	59.56
12.00-18.00	18	59.11	16	60.30	14	60.22
18.00-23.00	18	54.25	13	63.59	12	65.23

hours, the time of peak variability in sex ratio. From the remaining 30 collections we calculated a sex ratio of 57.90% with a variance of 0.0010, a standard deviation of 0.0311, and a coefficient of variation of 0.0536.

DISCUSSION

The spawning frequency of the Peruvian anchovy was found to be 16.04% in August-September 1981. This means that during the peak spawning period, Peruvian female anchovy spawn a new batch of eggs about once every six days. The spawning frequencies reported for the northern anchovy off California are somewhat lower: 14.5% in March-April 1980 (Stauffer and Picquelle 1980), 10.6% in February 1981 and 12.5% in April 1981 (Stauffer and Picquelle[2]), and 12.0% in January-March 1982 (Picquelle and Hewitt 1982). Hunter and Leong (1981) estimated that the northern anchovy spawns about 20 times per year; however, this has yet to be proven by an annual sampling program. These data on northern and Peruvian anchovy clearly contradict earlier assumptions that these two and other multiple-spawning pelagic species spawn only two or three times a year. Consequently, spawning biomass of such fishes has been considerably overestimated because of a severe underestimate of total fecundity (batch fecundity times spawning frequency).

Sex ratio is an important parameter for the application of the egg production method (Parker 1980), because of possible biases. The midwater trawl is reported to be a biased sampler with respect to sex ratio and hydrated females (Hunter and Goldberg 1980; Stauffer and Picquelle 1980). A higher-than-expected number of trawl samples had a high or a low number of females. In addition, hydrated females were twice as numerous as day-1 females. Stauffer and Picquelle[3] suggest that males and hydrated females segregate from other females at the hours of peak spawning at a depth where they are more vulnerable to the trawl.

The purse seine samples also appear to be biased in respect to sex ratio and numbers of hydrated females. In our study, oversampling of hydrated females occurred in the early morning hours, when the onset of hydration could be determined only by recording the migration of the oocyte nucleus to the pole. At this time, the oocytes have not increased perceptibly in size, and the ovary is only a small fraction of the total female weight. Three different explanations are possible for the oversampling of hydrated females in purse seines:

1. Hydration decreases the ability of female anchovies to avoid nets.
2. Females segregate vertically (by depth), and those with hydrated oocytes are more accessible to the purse seine than other females.
3. Females segregate horizontally (by area), and those with hydrated oocytes occur in different areas than those without hydrated oocytes.

In any case, using the incidence of hydrated females to estimate spawning frequency for anchovy (suggested by Hunter and Macewicz 1980) seems an inaccurate procedure, even if day samples of anchovies can be obtained.

The sex ratio in the 49 collections ranges from 12.62% to 90.50% females, with an average of 56.43% (Table 3). Night purse seine collections with a high percentage of hydrated females (with respect to the female fraction) also contain a high percentage of males. Hydrated females are oversampled both day and night, but the co-occurrence of high percentages of hydrated females and of males is recorded only at night. It might be hypothesized that the high male ratio in these night collections is because hydrated females, which are about to spawn, are attractive to and surrounded by a high number of males (Hunter and Goldberg 1980). If the hypothesis is correct that hydrated females are caught more often (than expected) because their vulnerability to the net increases, then only the hydrated females should be

[2]Stauffer, G.D., and S.J. Picquelle. The 1981 egg production estimates of anchovy spawning biomass. Unpublished manuscript, 29 p. NMFS, Southwest Fisheries Center, P.O. Box 271, La Jolla, California 92038.

[3]Ibid.

oversampled and not the males as well. Thus it seems more likely that the hydrated females segregate, either by depth or by area, from the "normal" school, taking a high percentage of males with them and forming "spawning schools" dominated by males.

The average sex ratio is biased because the night collections contain a high percentage of hydrated females. When these collections are omitted, the average sex ratio rises to 57.90%. If the true sex ratio of the population is 50% female, the purse seine clearly oversamples females, whereas the trawl seems to undersample them slightly (Stauffer and Picquelle 1980; Picquelle and Hewitt 1982). Klingbeil (1978) reported similar findings when he compared the sex ratios of anchovy obtained from commercial purse seiners (females:males = 1.60:1) and from research trawlers (females:males = 1.09:1). He suggested that the male-dominated schools may not form the large dense aggregations required for effective purse seining. This might also explain the difference in sex ratios between the northern and the Peruvian anchovy collections. The large Peruvian purse seiner (270 tons) may have shot the seine when the echo sounder indicated a relatively large, dense, female-dominated school, whereas each California trawl sample collected fish from a much larger area. Thus the trawl samples included more male-dominated spawning schools, which presumably are less dense and extend over a much larger area. Therefore, if spawning schools are segregated horizontally from normal schools, the trawl may be a more suitable tool for determining the sex ratio of anchovies than a purse seine.

Interpreting the different effects of trawling and seining on sampling of hydrated females is difficult. They were oversampled by the Peruvian purse seiner in 1981 and by the California trawler in 1980 and 1981 (Stauffer and Picquelle 1980; Stauffer and Picquelle[4]). However, hydrated females were not oversampled by the California trawler in 1982, possibly because the trawl may have fished a shallower depth (Picquelle and Hewitt 1982). The oversampling of hydrated females might be solved by a comparative study between a purse seiner and a larger midwater trawler, which allows fishing in depths not reached by a purse seine.

[4]See footnote 2 on page 51.

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