SPERM CONCENTRATIONS AND EGG FERTILIZATION RATES DURING SPAWNING OF CAPTIVE ANCHOVY, *ENGRAULIS MORDAX*

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ABSTRACT

The minimum sperm density needed for maximal fertilization success was determined for northern anchovy. On the average, 90% of anchovy eggs were fertilized in a concentration of 100 or more sperm per ml. These results were obtained by varying the number of hormone-treated male and female northern anchovy spawned in 3-m^3 tanks and examining the egg fertilization rate. These results were compared with two other studies.

RESUMEN

Se determinó la densidad mínima de esperma necesaria para maximizar el éxito de fecundación de la anchoa del Pacífico norte. En promedio, 90% de los huevos de anchoa fueron fertilizados con una concentración de 100 ó más espermas por mililitro. Estos resultados fueron obtenidos variando el número de machos y hembras de anchoa, desovados en tanques de 3 m^3 y previamente tratados con hormonas, y examinando posteriormente la tasa de fecundación de los huevos. Estos resultados fueron comparados con dos estudios previos.

INTRODUCTION

Only a small percentage of the anchovy eggs collected during the CalCOFI cruises have been identified as being undeveloped. Thus the northern anchovy may have evolved a mode of spawning in which nearly every egg contacts sperm in sufficient numbers for fertilization. The concentrations of sperm required for high rates of fertilization, and the role of the male in establishing these concentrations have been observed in only a few fishes and never in pelagic spawners. This report presents laboratory observations on the concentration of sperm in the water at the time of anchovy spawning, and on how that concentration relates to the fertilization rate.

MATERIALS AND METHODS

The concentration of sperm in the spawning tank, and the percentage of eggs fertilized were estimated for 19 spawning trials. Each trial consisted of a hormone-induced spawning and the collection of sperm and egg samples immediately after detection of eggs in the water. Collected anchovy were acclimated to the holding tanks for several months before hormone treatments, and the trials began only when the majority of fish had well-developed gonads.

The fish were induced to spawn with a procedure modified from Leong 1971; the females received 50 I.U. of human chorionic gonadotropin and 5 mg of carp pituitary, while the males received only 50 I.U. of human chorionic gonadotropin. The injection on the first day, 50 I.U. of human chorionic gonadotropin, was given to all fish. On the following day, all fish were squeezed, and males were identified by the secretion of milt. Fish that did not secrete milt when squeezed were assumed to be females and given the second injection. The number of males was reduced in some trials to lower sperm density.

Shedding of eggs began spontaneously 35 to 42 hours after the first injection. A strainer was passed through the water at half-hour intervals to determine when eggs were released. The spawning tank was slightly more than 3 m in diameter and contained 2.96 m^3 of water with a depth of .41 m. The flow-through tank had a water flow rate of 15 liters per minute, and the spawning temperature was about 17°C. The salinity averaged 32‰.

Three water samples provided an estimate of the average sperm concentration in the spawning tank during a trial. The samples, 100 ml in volume, were taken at mid-depth from different areas of the pool. Two drops of rose bengal stain and two ml of concentrated Formalin were added to each sample to stain and preserve the spermatozoa. Immediately after collection, each sample was passed through a millipore filter (0.25-μm pore size and 25-mm diameter) under slight vacuum to separate and concentrate the spermatozoa, which have a head 4 μm × 1.3 μm and a tail about 55 μm long (figure 1). The filter was placed on a microscope slide with immersion oil and warmed to clarify the filter and make the spermatozoa visible.[1] I estimated the

[Manuscript received February 2, 1989.]

[1] A brochure by the Millipore Corporation gives further details on the clarification and use of millipore filters.

Figure 1. Anchovy sperm (photo by Bev Macewicz). Head 4 μm × 1.3 μm; tail ~ 55μm.

number of spermatozoa on the filter by counting the number in several transects of measured lengths and widths with an inverted microscope at 400X magnification. I counted at least 300 sperm per filter except at the lowest concentration. Estimates made by the millipore filter method were compared to estimates made with a hemacytometer for five milt samples to test the technique (table 1). The milt were diluted 1:20,000 for counting with the hemacytometer and 1:20,000,000 for the millipore filter. The sperm densities estimated by the two methods did not differ statistically ($t = 4.54; p < .05$).

All fish in a trial were used only once and were killed at the end of the trial. Routine measurements on males included length, weight, testes weight, and gonadosomatic index (percentage testes weight of body weight).

The egg fertilization rate was determined by examining 200–300 eggs for signs of development. The eggs were collected soon after the detection of spawning and incubated in a beaker for an hour before examination. Developing eggs were easy to distinguish during the early stages of cell division, with pictures from Moser and Ahlstrom (1985) and Bolin (1936) as guides. Eggs that did not develop after the one-hour waiting period were assumed to be unfertilized.

RESULTS AND DISCUSSION

The estimated density of sperm in the water of the spawning tank, percentage of eggs fertilized, and other pertinent data from each of the 19 trials are given in table 2. The relation between the egg fertilization rate and density of sperm appears in figure 2. The figure shows the fertilization rate increasing with higher sperm density for densities up to 100 ml^{-1}; maximum rates begin to appear near that level. The regression equation for observations with less than 100 sperm ml^{-1}, $Y = 1.00$, indicates that the rise in egg fertilization was proportional to the density of sperm for the trials, and indicates a 50% level of fertilization for a sperm density of 50 ml^{-1}. The regression equation for observations with sperm density greater than 100 ml^{-1}, $Y = 88.1 + 0.0007$, suggests a slight increase in fertilization rate with higher sperm density, but the slope was not significantly different from zero. The average fertilization rate for sperm densities above 100 ml^{-1} was 88.9%, with a range of 70.1% to 99.0%. Some of the lower rates were probably due to imperfec-

TABLE 1
Comparison of Two Methods Used to Count Sperm

Milt sample	Hemacytometer estimate (10^9)	Millipore filter estimate (10^9)	Ratio of millipore to hemacytometer estimate
1	6.76	4.00	.59
2	6.28	5.92	.94
3	7.88	6.74	.86
4	8.04	8.78	1.09
5	8.64	9.66	1.12
$\bar{x}$	7.52	7.02	.92

TABLE 2
Estimated Density of Sperm and Percentage of Eggs Fertilized When Anchovy Were Induced to Spawn in the Laboratory

Trial	Estimated sperm density in water of spawning tank (n/ml^{-1})[a]	Percentage of eggs fertilized	Number of fish		Average male gonosomatic index (%)[c]
			Male[b]	Female	
1	9013	93.0	17	19	7.8
2	1435	98.0	4	19	6.4
3	862	98.0	18	—	4.7
4	744	80.1	14	11	6.1
5	337	99.0	14	18	10.9
6	268	90.0	25	35	8.7
7	217	70.1	6	20	10.1
8	165	80.0	1	16	8.6
9	141	84.6	17	14	7.3
10	122	96.4	18	17	8.3
11	97	97.0	19	16	3.7
12	87	84.0	11	22	6.9
13	86	89.2	27	14	7.9
14	78	70.0	8	10	8.0
15	66	60.2	13	—	9.4
16	55	80.0	8	16	8.3
17	50	54.2	2	14	4.8
18	25	18.5	11	16	5.1
19	4	4.4	2	15	13.0

[a]Estimated from three 100-ml water samples from spawning tank containing 2.96 m^3 water.
[b]Average male length was 116.6 mm; average weight was 21.0 g.
[c]Postspawning testes weight ÷ body weight × 100.

tions in the induced spawning procedure, which in some cases may have stimulated the release of immature gametes.

The rough relationships between sperm density and the number of males suggest that the number of sperm released per male was highly variable. The average concentration of sperm in the water may not be the same as the concentration surrounding newly extruded ova. In many species the male deposits sperm directly over the newly spawned eggs (Breder and Rosen 1966), and in those situations the eggs are surrounded by sperm at a much higher concentration than that surrounding the average egg in the water column. However, I did not see any close interaction between the sexes during the trials. Members in the spawning school usually milled about slowly and did not display any obvious behavior patterns such as pairing or posturing to indicate that spawning was in progress; yet my samples indicated that spawning had occurred. The sexes may have simply released gametes into the water, perhaps with some synchrony but without apparent change in behavior. If the spawning anchovy simply released gametes into the water without pairing and body contact, then the eggs were fertilized in water containing the observed sperm concentrations. Whether the simple mode of spawning I observed in the laboratory also occurs in the sea is unknown. Anchovy spawn only at night (Smith and Hewitt 1985; Bolin 1936), and the dark conditions could possibly limit the use of visual cues in their spawning behavior.

The minimum sperm density needed for maximal fertilization success has been studied in only a few teleosts, and the results have been varied. Ginzburg (1972) studied the problem by placing roe with

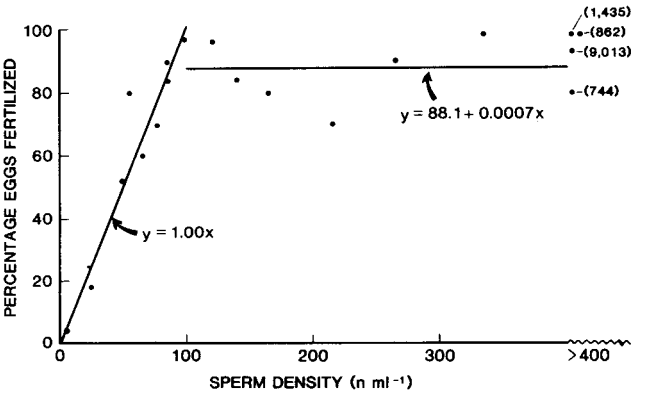

Figure 2. Percentage of eggs fertilized and sperm density during induced spawning of northern anchovy. Density is number of sperm ml^{-1} water of spawning tank. Densities greater than 400 ml^{-1} are in parenthesis.

stripped milt in various dilutions and found that concentrations of 10^5–10^6 sperm ml^{-1} were necessary for maximum fertilization in certain salmonids and sturgeon. In a different setting Hourston and Rosenthal (1976) sampled surface water of milky discoloration above a school of spawning herring *Clupea harengus palasii* and found concentrations of 148 and 129 sperm ml^{-1}, which they theorized to be more than ample for high fertilization success.[2] The sperm densities at which maximum fertilization occurred in the present study are close to those observed by Hourston and Rosenthal, but magnitudes lower than those observed by Ginzburg. Additional studies would be required to resolve these differences.

In any future work, other factors such as swimming speed and longevity, length of time ova are receptive to sperm, properties in the egg that may attract or activate sperm, and temperature should be considered as well as sperm density; only sperm density was considered in this study.

[2]Hourston and Rosenthal calculated that a herring egg would attract 24 sperm when the density is 150 ml^{-1}, but P. E. Smith, NMFS, La Jolla, personal communication, found an error in their calculation; the number attracted should be corrected to 0.024.

ACKNOWLEDGMENTS

I thank John Hunter, Paul Smith, and Gail Theilacker for reviewing the manuscript and making useful suggestions, and Eric Lynn for his help during the trials.

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