INDUCED SPAWNING OF THE NORTHERN ANCHOVY, *Engraulis mordax* GIRARD

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ABSTRACT

Anchovies were induced to mature their gonads by an artificial photoperiod of 4 hr light and 20 hr darkness at 15° C. Single injections of suspensions of salmon pituitary, carp pituitary, or a solution of human chorionic gonadotropin (HCG) promoted increase in egg diameters but did not induce spawning. Two injections, a first of HCG and the second 2 days later of either salmon pituitary or carp pituitary, induced spawning. At each spawning 6,000 to 16,000 eggs were collected, and 25% to 80% of the eggs hatched. Larvae grown from these eggs were morphologically similar to those caught in the sea.

An investigation was started at the National Marine Fisheries Service Fishery-Oceanography Center, La Jolla, Calif., in 1969, to examine methods for spawning the northern anchovy, *Engraulis mordax* Girard, under controlled laboratory conditions in order to supply eggs and larvae for experimental studies. The strategy for spawning anchovies in captivity was to provide an environment in which the fish would mature their gonads and subsequently to induce spawning through hormone treatment. The role of the environment and the use of hormone injections for inducing spawning in other species of fish has been well documented by Pickford and Atz (1957). This report describes a method for bringing the anchovy to ripeness and the effectiveness of various hormone treatments in inducing spawning. As far as is known this was the first successful attempt to artificially mature and spawn this pelagic fish in the laboratory.

MATERIALS AND METHODS

Anchovies, averaging 90 mm in length, were purchased from a San Diego bait dealer in March of 1969, transported to the laboratory, and held in circular plastic lined wading pools 4.6 m in diameter with 0.9 m of water. By the beginning of the injection trials in August of 1970, the fish had grown to an average length of 125 mm, at which length half of the fish should have been mature (Clark and Phillips, 1952). The fish were subjected to a photoperiod of 4 hr light (32 ft-c at the brightest spot on the surface of the water) and 20 hr dark (1 ft-c) for 3 months prior to the trials. Observations in the preceding year revealed that anchovies tend to mature more readily under relatively prolonged dark conditions. The tanks were constantly supplied with fresh seawater and the temperature was maintained at 15° C (Lasker and Vlymen, 1969). Much of the spawning of anchovies in nature occurs at or near this temperature (Ahlstrom, 1956). The fish were fed twice a day. In the first feeding, given at the beginning of the 4-hr light period, the fish were fed 6% of their live body weight in rotating daily rations of ground squid, ground anchovies, and brine shrimp. In the second feeding, given near the end of the light period, the fish were fed 1% of their body weight in trout chow.[2] Under this light, temperature, and food regimen approximately one-fourth of the fish developed gonads which weighed more than 6% of their body weight.

Three series of injection trials were conducted. In the first series the following dosages and types of injections were tested: 2.5, 5.0, and 10.0 mg of salmon (*Oncorhynchus tshawytscha*) pituitary[3] prepared essentially by the method of

[1] National Marine Fisheries Service Fishery-Oceanography Center, La Jolla, Calif. 92037.

[2] Ralston Purina trout chow, size 2. Reference to commercial products does not imply endorsement.

[3] Obtained through the courtesy of Dr. Irwin Haydock and the California Department of Fish and Game, Nimbus Fish Hatchery, Rancho Cordova, Calif.

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Haydock (1971), 2.5, 5.0, and 10.0 mg of commercial carp pituitary,[4] 1.0, 2.5, and 5.0 mg of deoxycorticosterone acetate (DOCA),[5] 1.0 mg of luteinizing hormone (PLH),[6] and 25, 50, and 100 international units (IU) of human chorionic gonadotropin (HCG).[5] The treatments were given in single 0.1-ml injections using a carrier of Holtfreter's solution (Emmel and Cowdry, 1964) in all cases except for DOCA where sesame oil was used. The suspensions of pituitary were prepared by triturating a weighed quantity in a small tissue grinder with enough liquid to form the proper concentration. The suspension was then pipetted into a small serum bottle where it could be withdrawn with an injection syringe. The injections were administered intraperitoneally with a 24-gauge needle between the pelvic fin and vent. Prior to injection the fish were anesthetized in 50 liters of water with 7 ppm quinaldine (Vrooman and Paloma, 1966).

Under each treatment 12 to 15 fish were injected. The sex and level of ripeness of living anchovies are difficult to distinguish and a portion of the fish in these trials were immature. Therefore it was necessary to inject this relatively large number of fish to increase the probability that some would be sufficiently developed to respond to hormone injections. Injected fish were placed in small holding tanks 1.2×1.2 m with 0.9 m of water. These tanks had running seawater and the temperature was also maintained at 15° C. Nets with 202-μ mesh were placed at the outflows of these tanks to collect eggs in the event of spawning. If no spawning occurred after 48 hr, the fish were stripped and fertilization attempted by the dry method (Davis, 1961). In this method the eggs and sperm are expressed, mixed, and left to stand in a dry container for 5 to 10 min before being placed in water. It was noticed in all trials that at least some males produced motile sperm.

The fish were killed after stripping and ovaries were removed from the females. Each ovary was teased apart and the major diameters of the most advanced eggs measured. The maximum diameters of injected fish were compared with the diameters of more than 500 captive uninjected females sampled during the previous 16 months to determine which of the treatments were effective in producing growth.

The effect of two injections of different hormones were examined in the second series of trials. The first injection was 50 IU of HCG and the second, given 48 hr later, was one of the following: 2.5 mg of salmon pituitary, 10.0 mg of carp pituitary, 5.0 mg of DOCA, 1.0 mg of PLH and 250 IU of gonadotropin from pregnant mare serum (PMS).[6] The methods of anesthetizing, injecting, and holding of fish were the same. If spawning did not occur within 48 hr after the second injection, the fish were stripped and fertilization attempted. In this and the final series of trials the fish were not killed after stripping and measurements of egg diameters were not made. Here the only criterion for success was spawning or production of viable eggs through stripping.

The effect of three injections of the same hormone was tested in the third and final series of trials. One group of fish was given three injections of 2.5 mg of salmon pituitary and another group three injections of 50 IU of HCG. The injections were spaced a day apart and the procedures were the same as described earlier. If no spawning occurred within 24 hr after the third injection, the fish were stripped and fertilization attempted.

RESULTS

None of the fish that were given a single injection spawned or produced viable eggs through stripping. Most of the stripped eggs were clumped, opaque, and apparently had not ovulated. Some of the treatment, however, produced noticeable increases in egg diameters. Table 1 shows the number of females under the various single-injection treatments and the number with and without eggs larger than 1.0 mm. The number of females was small in some

[4] Purchased from Stoller Fisheries, Spirit Lake, Iowa.
[5] DOCA and HCG purchased from Sigma Chemical Co., St. Louis, Mo.
[6] PLH and PMS purchased from Calbiochem, Los Angeles, Calif.

TABLE 1.—Female anchovies after various hormone injections having eggs larger than 1.0 mm in diameter. The occurrence of females with eggs larger than 1.0 mm is considered to be an indication of induced egg maturation. Of more than 500 captive uninjected fish sampled during the previous 16 months none had eggs larger than 1.0 mm. The average injected fish weighed about 25.0 g and measured 125 mm in length.

Type of injection	Dose/fish	No. females injected	No. females with eggs >1.0 mm
Salmon pituitary (*Oncorhynchus tshawytscha*)	2.5 mg	8	2
	5.0 mg	7	4
	10.0 mg	8	1
Commercial carp pituitary	2.5 mg	5	0
	5.0 mg	3	0
	10.0 mg	4	1
Human chorionic gonadotropin (HCG)	25 IU	4	1
	50 IU	8	1
	100 IU	6	0
Deoxycorticosterone acetate (DOCA)	1.0 mg	3	0
	2.5 mg	6	0
	5.0 mg	8	0
Luteinizing hormone (PLH)	1.0 mg	4	0

cases because most of the injected fish were males or had died through handling. Of the more than 500 females examined during the previous 16 months, none had eggs larger than 1.0 mm. The occurrence of females, Table 1, with eggs larger than 1.0 mm indicates that salmon pituitary, carp pituitary, and HCG are capable of promoting overnight growth of eggs. Salmon pituitary appeared to be the most potent for producing growth. The largest eggs observed were over 1.3 mm in diameter and within the size range, 1.23 to 1.5 mm, of naturally spawned eggs (Bolin, 1936). DOCA and PLH at the dosages tested were not effective in stimulating egg growth. The preponderance of fish with smaller eggs may be due to a low state of ovarian development at the time of injection.

In the second series of trials, HCG followed by DOCA, PLH, or PMS did not induce spawning and subsequent stripping produced only unovulated eggs which were not successfully fertilized. The combinations of HCG followed by salmon pituitary and HCG followed by carp pituitary induced spawning within 18 hr after the second injection. The fish spawned and fertilized the eggs themselves and large numbers of eggs were caught in the nets at the outflows of the tanks. Spawning was repeated several times with each of these two combinations of injections. The spawnings produced from 6,000 to 16,000 eggs with the percentage hatching varying from 25 to 80%. The larvae from these induced spawnings appeared morphologically normal and many were reared past 25 days by the methods of Lasker et al. (1970). The differences in the hatching percentage may be attributed to variation in the state of gonad development of parent fish at the time of injections.

The number of eggs collected suggests that only one or two females from any of the spawning groups contributed eggs. According to estimates by MacGregor (1968) female anchovies spawn almost 600 eggs per gram of fish. The average female in these trials weighed approximately 25 g and should have produced nearly 15,000 eggs. Only one or two females from any of the twice-injected groups extruded ovulated eggs upon stripping. Although the eggs were translucent and measured about 1.5 mm in diameter less than 10% hatched after being mixed with motile sperm.

In the final series of trials, three injections of salmon pituitary or three injections of HCG over a 3-day period failed to induce spawning. The stripped eggs were opaque and fertilization was not successful. These limited results suggest that the combination of HCG followed by salmon pituitary is more effective for induction of spawning than when these hormones are administered alone.

The results of these injection trials demonstrate that the northern anchovy can be induced to spawn in captivity and two effective treatments are HCG followed by salmon pituitary or HCG followed by carp pituitary after gonads are matured by a specific light-dark treatment of the fish. The induction of spawning of anchovies in the laboratory provides a practical way for supplying viable eggs for studies on larvae. In this study the time of spawning was controlled and eggs can probably be produced the year around if a large stock of fish is maintained. This was emphasized by the fact that the fish in this study were induced to

spawn during the late summer and fall months when anchovy eggs are virtually absent from the sea off San Diego. As far as is known, these are the first reported spawnings of *Engraulis mordax* in captivity and the first hormone-induced spawnings of engraulids.

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