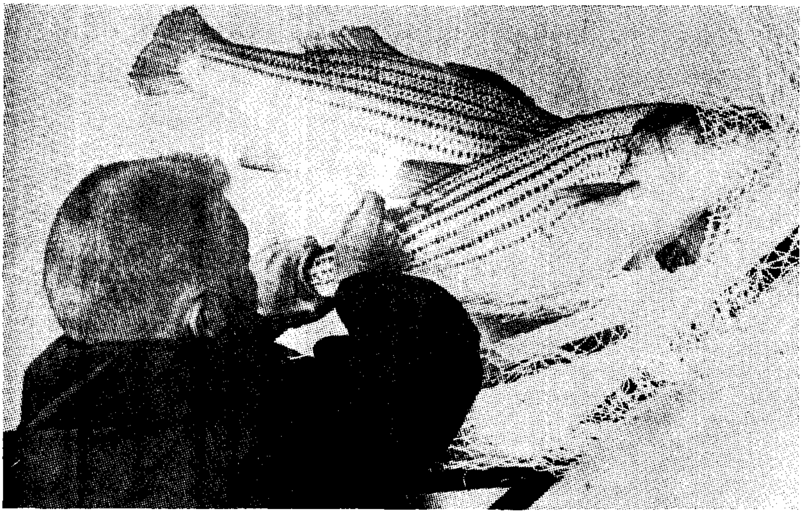

South Carolina Wildlife Resources Department,
Division of Game. James W. Webb, Director

A FINAL REPORT ON THE USE OF HORMONES TO OVULATE STRIPED BASS, Roccus saxatilis (Walbaum)

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ABSTRACT

During the 1964 striped bass spawning season, 383 female striped bass were treated with hormones at the Moncks Corner Striped Bass Hatchery and 337 (88%) were induced to ovulate as a result of the treatment.

Three hundred and seventeen of the successful females were spawned in the hatchery for a yied of 322 million eggs and a hatch of 100 million fry.

Chorionic gonadotropin was the most successful hormone used.

Approximately 60 million fry were stocked in the Wateree Reservoir, a 13,710 acre impoundment, and early seining results indicate a significant survival.

INTRODUCTION

Beginning around 1953, the national attention of both fishermen and fish biologists became focused upon the Santee-Cooper Reservoir because of the apparent self-establishment of striped bass in this huge, landlocked body of water. This interest heightened as the population expoded in subsequent years—proving that striped bass could indeed thrive in a freshwater, landlocked environment. (Stevens, 1957.)

In addition to its enthusiastic acceptance by fishermen as a superb game and table fish, its ability to control gizzard shad *(Dorosama cepedianum)* through predation made it additionally attractive to fish biologists working in the south where gizzard shad abundance is considered by most to be a serious problem in the management of southern reservoirs.

The demand thus created has resulted in the introduction of the striped bass into all the major reservoirs of South Carolina as well as several reservoirs in other states. The stocked fish apparently thrive and spawn but no young striped bass result. It has been tentatively concluded that the reservoirs of South Carolina, other than Santee-Cooper, are physically deficient as to the spawning requirements of striped bass and that reproduction is doomed for this reason.

The spawning requirement dictates that unless the freely spawned striped bass eggs remain suspended in a current until hatching, they will settle to the bottom and suffocate. A reservoir, therefore, must have many uninterrupted miles of stream above it for hatching purposes or at least the equivalent in terms of current within the reservoir or perhaps sandy or rocky areas where partially developed eggs and larvae may settle in highly oxygenated water and escape suffocation. Striped bass eggs require approximately 70 hours to hatch at 60°F.; 44 hours at 65° F. and 33 hours at 70° F.

When it became apparent that reproduction was probably not going to be successful in the other large impoundments of South Carolina, a hatchery was constructed in 1961 at Moncks Corner in order to circumvent this limiting factor to the successful establishment of striped bass throughout the state.

The hatchery was patterned after the Weldon Hatchery which has been operated successfully for many years by the North Caroina Wildlife Resources Commission. The Weldon Hatchery depends upon commercial fishermen who bring ripe males and females to the hatchery. The roe is removed surgically from the female and is fertilized by manually stripping sperm from a ripe male. The fertilized eggs are then placed into McDonald Hatching Jars which receive a constant supply of fresh water until the eggs hatch, whereupon, the fry swim into aquaria and are stocked soon thereafter.

Although commercial fishing for striped bass is illegal in South Carolina, it was felt that the large number of striped bass which frequent the fish sanctuary area below Pinopolis Dam would provide enough ripe female striped bass to substitute for the commercial catch which annually supplies the Weldon Hatchery.

During April and May of 1961, over 900 adult female striped bass between 8 and 25 pounds were examined without finding one with freely flowing eggs. The progress of the spawning season could be traced by the ratio of spent to unspawned females and all stages of ripeness were encountered except running ripeness. From this experience it was concluded that striped bass spawn their eggs shortly after ovulation and that the chance of finding a female ripe, but unspawned, is slight. It has subsequently been discovered that most of the actual spawning takes place over a wide area several miles below the sanctuary and that a large commercial effort would be required to obtain sufficient numbers of naturally ripe females for hatchery production.

Two other striped bass hatcheries have failed for the lack of ripe fish as related by Raney (1957:45) as follows: "According to Pearson (1938:829), attempts in the past to artificially propagate striped bass at Havre de Grace, Maryland failed because of the difficulty of getting ripe males and females simultaneously (see Snyder, 1918, 1919). Coleman and Scofield (1910) also ran experiments on the artificial propagation of striped bass in California; Scofield (1910) describes attempts to run a striped bass hatchery on the San Joaquin River, a project which was abandoned after three consecutive years of failure to collect ripe spawn.

For the 1962 season ,it was decided to construct a temporary pond in which striped bass could be held captive and, if possible, induced to ovulate with the use of hormones. Much precedence exists to recommend this technique beginning in 1934 when Brazilians found that the injection of dried fish pituitary glands into ripe fishes would cause most Brazilian fishes to spawn normal eggs in captivity. This and subsequent work until 1957 is well documented in *The Physiology of the Pituitary Gland of Fishes* by Grace E. Pickford and James W. Atz. In more recent years many other excellent papers on the subject have been published such as those by Sneed and Clemens (1959), Sneed and Dupree (1961) and Clemens and Sneed (1962).

PROCEDURES

During the 1962 and 1963 spawning seasons, the hatchery was operated on the basis of eggs received from striped bass which were induced to ovulate with hormones while being held captive in a temporary pond. Because of the high mortality of the adult striped bass as well as the high mortality of the eggs, success was limited only 16.5 million fry were produced during the two-year period (Stevens and Fuller, 1962—Stevens et al, 1963).

Much experience was gained ,however, and renovations to the holding pond were recommended which have proven to be essential to the large scale production of striped bass fry.

The temporary pond was watered with turbid water and visibility was nil. The females could not be observed and capture within the pond was very difficult. The renovations resulted in two permanent ponds which are watered with well water and include viewing windows lights and movable deviders. (Figue 1.) Capture is reduced to the simple chore of cornering the fish by closing the dividers and, as a

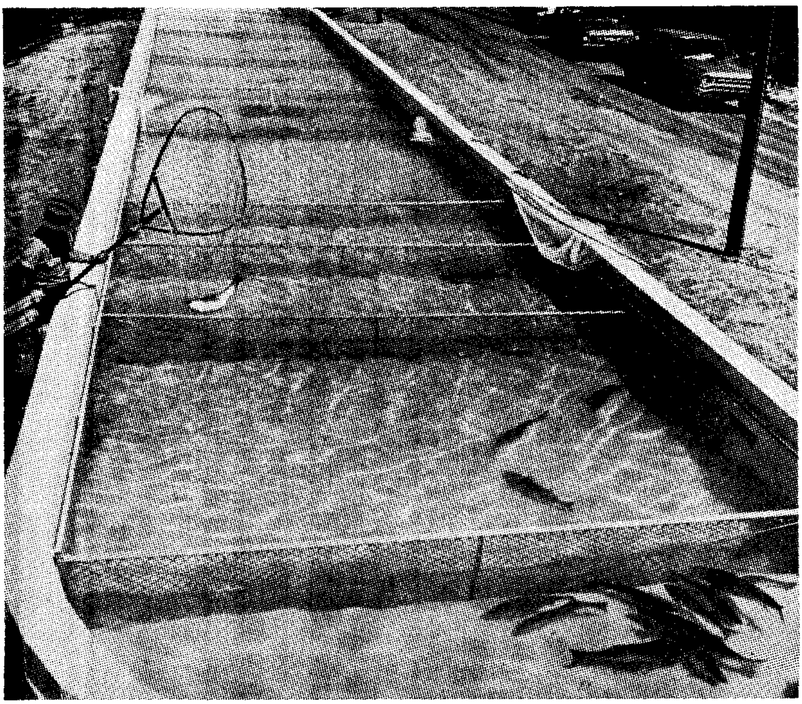


Fig. 1. Clear water, movable dividers, windows and lights greatly facilitate observation and capture of females. Ease of capture is essential to the avoidance of over-ripeness of the eggs.

result, the females in 1964 could be observed and captured with ease. Since egg mortality has proven to be due mainly to the phenomenon of over-ripeness, ease of capture within the pond is absolutely essential for large scale fry production.

Over-ripeness:

A very serious problem in the production of viable striped bass eggs is the phenomenon of over-ripeness. This has been by far the major source of egg mortality and, until fully recognized, has served to frustrate the best efforts to produce large numbers of striped bass fry. Ignorance of this phenomenon has resulted in many, more or less fruitless, tangental experiments in an attempt to mitigate egg mortality.

Over-ripeness is evidently a symptom of hypoxia within the ovary after ovulation. In the process of ovulation, the ova become detached from the ovarian tissues as the tissues withdraw from the lumen of the ovary. Since the ovarian tissue is the source of oxygen to the ova, the deleterious effects of hypoxia upon the freed ova become operative within a short, but heretofore unknown, period of time.

During the 1964 season, the lethal effects of over-ripeness were fully demonstrated by taking periodic egg samples from ripe females with a suction catheter and then by observing the hatchability of each sample in relation to the length of time that the eggs remained in the ovary after ovulation. (Figue 2.) From these observations it appears that a maximum grace period of about 60 minutes exists between ovulation and over-ripeness. Ideally, it is advisable to take the eggs immediately after ovulation. This requires the almost constant handling of the females in the terminal hours of the latent period and it is frequently difficult to ascertain whether a female is fully or only

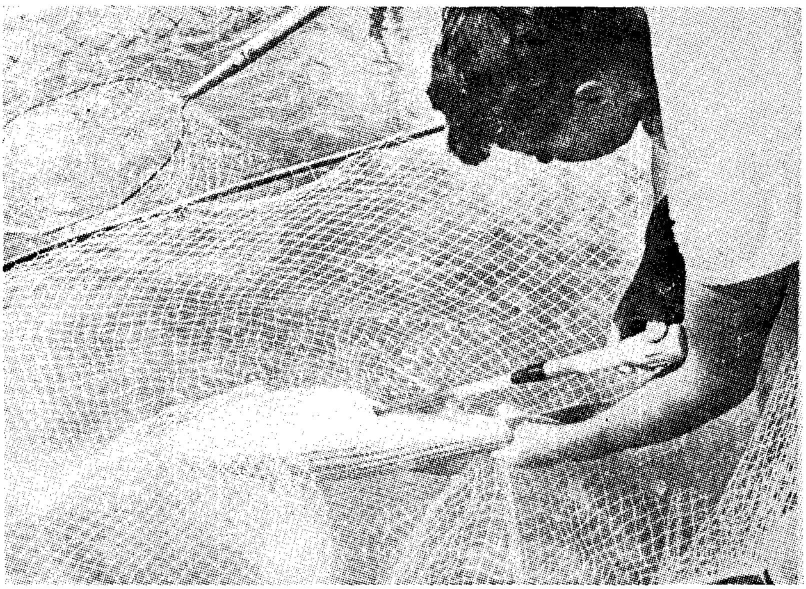


Fig. 2. A suction catheter (0.5 in. in diameter) was used to take periodic egg samples for the demonstration of the inimical effect of over-ripeness on the eggs which remain in the ovary too long after ovulation.

partially ovuated. In such cases it is prudent to allow her to ripen a little longer.

Because of the relatively great variation in latent periods among a given batch of females ,the avoidance of over-ripeness was very difficult until a suitable method of predicting the approximate time of ovulation was found. It can be appreciated that an hourly check on say 20 large females for a period of 24 hours or more requires a great amount of labor and it is also harmful to the fish.

It was found that with the use of a small glass catheter, a sample of eggs could be taken from a female and that, by examining the ova microscopically, the approximate spawning time for females could be predicted. (Figures 3 and 4.)

Egg Production and Fry Production:

During April and May of 1964, 383 adult, gravid, female striped bass averaging about 17 pounds in weight were captured in the sanctuary below Pinopolis Dam with the use of an electric seine. These females were then treated with hormones and placed in two holding ponds until ovulation occurred. Of this number 337 (88%)

Table 1 The outcome of 383 female striped bass used at the Moncks Corner Striped Bass Hatchery in April and May of 1964.

	Number	Per Cent
Spawned in hatchery	316	82.5
Spawned in pond	19	5.0
Dead and ripe	2	0.5
Negative	46	12.0
	383	100.0

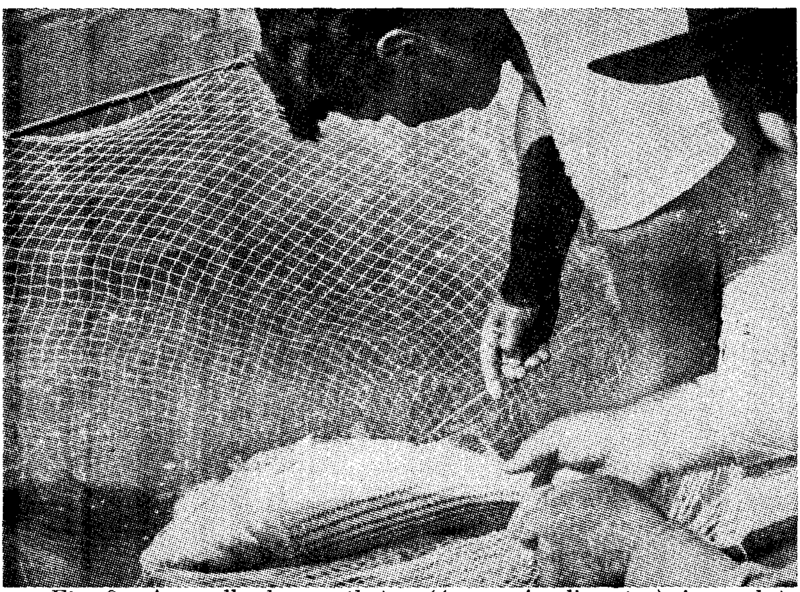
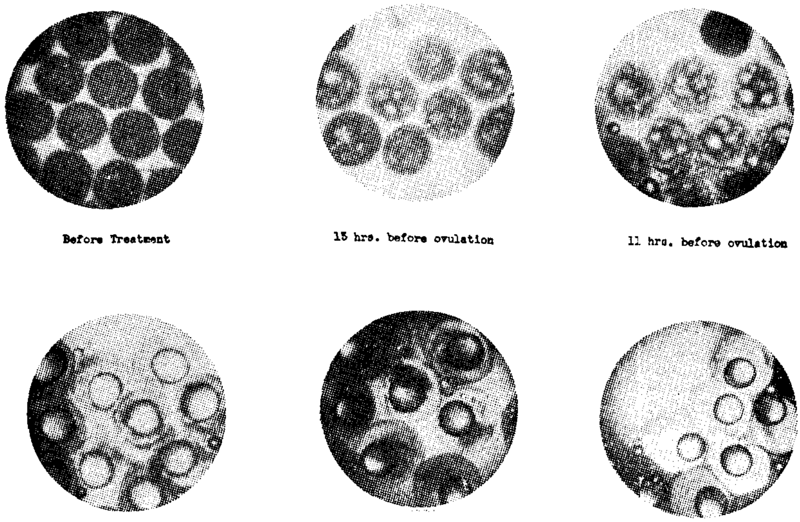


Fig. 3. A small glass catheter (4 mm. in diameter) is used to take periodic samples of eggs which are examined in order to predict the approximate time of ovulation for each female.

were successfully ovulated although 21 either spawned in the pond or were found dead and ripe. (Table 1.)

The number of eggs produced from 316 females which were spawned in the hatchery in 1964 was estimated at 322 million or roughly one million eggs per female. This estimate was achieved by weighing the eggs of each female and by calculating the number of eggs on the basis of 25,000 eggs per ounce.



Before Treatment — 15 hrs. before ovulation — 11 hrs. before ovulation

10 hrs. before ovulation — 7 hrs. before ovulation — 2 hrs. before ovulation

Fig. 4. The photographs above illustrate the appearance of typical eggs taken at several stages during the latent period. The examples should be considered as approximate only since individual variation renders the precise prediction of the time of ovulation difficult in most cases. Polarization of the oil globule and the yolk and an increase in the diameter and transparency of the egg represent the gross changes inherent in the process of ripening.

This indext was found by estimating the number of eggs in six-ounce samples from four different females. The eggs in the sample were estimated volumetrically on the basis of *Von Bayer's (1910) Table For Finding The Number of Fish Eggs of Given Diameter Per Liquid Quart. (Lagler, 1952.)*

Table 2 presents the hatching success for each year of operation. The improvement between years reflects the full recognition in 1964 of the role of over-ripeness in egg mortality as well as the ability to avoid over-ripeness through easy capture of females and through internal egg sampling with the use of a catheter.

Table 2 Striped bass eggs entering the Moncks Corner Hatchery in 1962, 1963 and 1964.

Year	Number of Fish	Eggs	Hatch	Per Cent Hatch
1962	24	36,000,000	2,640,000	7.3
1963	54	81,000,000	13,804,000	17.0
1964	316	322,000,000	100,000,000	31.0

Of the 316 females spawned in the hatchery in 1964, 49 produced no viable eggs and 9 others produced less than 100,000 fry per female.

Table 3 The response of 12 female striped bass to the single treatment with follicle stimulating hormone at several dose levels in April and May of 1964.

Milligrams of fish per fish	Number of fish treated	Per Cent successfully ovulated	Average latent period (hrs.)	Average number of eggs per fish	Average number of fry per fish*	Average hatching success*	Average mg. per pound
50	6	83.3	31.5	1,175,000	587,000	50.0	3.4
100	3	100.0	41.0	1,233,333	704,478	57.1	5.6
200	3	100.0	30.5	1,080,000	513,333	47.5	11.4

* Fry estimates were based on egg estimates 12-24 hours after spawning. Mortality after this age was ignored.

Table 5 The response of 240 female striped bass to the single treatment with chorionic gonadotropin at several dose levels in April and May of 1964.

International units of CG per fish	Number of fish treated	Per Cent successfully ovulated	Average latent period (hrs.)	Average number of eggs per fish	Average number of fry per fish*	Average hatching success*	Average I.U. of CG per pound
250	7	28.6	38.0	1,100,000	510,000	46.4	14
500	11	90.9	35.5	1,360,000	552,000	40.6	31
1,000	64	85.9	36.5	974,476	527,680	54.1	66
2,000	69	95.7	32.5	1,005,593	639,948	63.6	127
3,000	78	89.7	35.5	969,687	576,000	59.4	188
3,333	6	100.0	37.0	1,313,333	803,167	61.2	208
4,000	1	100.0	56.0	1,900,000	1,122,000	59.0	195
5,000	4	100.0	32.5	670,000	245,000	36.6	403
Total	240		35.5	994,863	584,488	58.8	

* Fry estimates were based on egg estimates 12-24 hours after spawning. Mortality after this age was ignored.

Over-ripeness was mainly responsible for the mortality and occurred before its role was fully recognized.

In the past, fry production has been based upon an egg count made when the eggs were 12-24 hours old. On this basis 152 million fry were produced in 1964. The estimate was reduced to 100 million fry on the basis of several fry-counts after hatching. It was found that an additional 33 per cent mortality obtains between eggs 12-24 hours old and actual hatching.

The whole subject of the hatching of striped bass eggs will receive much greater emphasis in the future now that the major problems of producing viable eggs have been solved .Hatching success will undoubtedly be improved much beyond the 31 per cent achieved in 1964.

Hormones:

During the three-year period, several preparations have been used, including chorionic gonadotropin (CG), follicle stimulating hormone, pituitary (FSH-P), pituitary lutenizing hormone (PLH), thyroid stimulating hormone (TSH), estrogen preparations, testosterone and fish pituitary glands. Of these, only CG and FSH were capable of inducing ovulation when used alone. FSH is much more expensive and demonstrates no advantage over CG. (Table 3). Combinations of two or more hormones were also successful in inducing ovulation but also increased the cost of treatment much above CG alone. (Table 4.)

Dose Levels:

Table 5 presents the average results of treatment with CG only of 240 female striped bass in 1964 at 8 dose levels between 250 and 5,000 International Units (I.U.) of CG per fish. The data can be interpreted as follows:

1. Fourteen I.U. of CG per pound represents a sub-liminal dose level.
2. Adequate dose levels begin at 31 I.U. of CG per pound and obtain through 403 I.U. of CG per pound.
3. From the standpoint of hatching success, a dose level of 127 I.U. of CG per pound appears desirable. Dose levels in excess of this produce no evident advantage.

Latency:

The latent period between injection and ovulation depends upon the nearness of the individual female to natural ovulation and possibly to individual variation in response to induced ovulation and captivity. Quite naturally, the majority of the females are much closer to natural ovulation during the middle and latter part of the spawning season than during the early part. Average latency is about 45 hours at the beginning of the season and about 30 hours during the latter part. A typical batch of females, after around April 15, will ovulate between 22 and 48 hours after treatment. It would be greatly advantageous if this variation could be reduced not only from the standpoint of the labor involved but also in order that the phenomenon of overripeness can be better guarded against.

Fish Salvage:

Toward the end of the 1964 season, experiments were conducted in an attempt to take ripe eggs without sacrificing the females. This would be very desirable from the practical standpoint because in 1964 alone females weighing some 6500 pounds were permanently removed from the sanctuary area. Also, the destruction of so many large fish is not well received by the public.

Compressed air was introduced into the peritoneal cavity and into the ovary and suction catheters were experimented with at length but

Table 4 The response of 68 female striped bass to one or more treatments with one or more hormones in April and May of 1964.

Hormones per fish [1]	Number of fish treated	% successfully ovulated	Avg. latent period per successful fish (hrs.)	Avg. No. of eggs per successful fish	Avg. No. of fry per successful fish [3]	Average hatching success	Hormones per pound [2]
250 CG 1,000 CG	1	0					78 CG
1,000 CG 500 CG	2	0					94 CG
1,000 CG 1,000 CG	7	71.4	42.0	956,000	741,400	77.6	118 CG
250 CG 2,000 CG	4	0					126 CG
1,000 CG 2,000 CG	10	80.0	42.5	1,316,666	819,714	66.2	184 CG
2,000 CG 2,000 CG	10	80.0	45.5	1,017,429	874,125	81.6	223 CG
2,000 CG 1,000 CG 2,000 CG	2	50.0	60.0	1,110,000	588,000	53.0	345 CG
50 FSH 25 FSH	1	100.0	60.0	1,100,000	720,000	65.5	5.3 FSH
50 FSH 50 FSH	3	100.0	46.0	1,200,000	674,667	56.2	6.5 FSH
25 FSH 25 FSH 50 FSH	1	0					3.8 FSH

Table 4—(Continued)

100 FSH 50 FSH	1	0					13.2 FSH
1,000 CG 25 PLH 1,000 CG	2	100.0	39.5	1,100,000	858,000	78.0	128 CG 1.6 PLH
1,000 CG 12.5 PLH 12.5 FSH	1	100.0	35.5	900,000	60,000	6.7	74 CG 0.9 PLH 0.9 FSH
2,000 CG 25 FSH 25 PLH	1	100.0	69.5	600,000	270,000	45.0	190 CG 2.4 FSH 2.4 PLH
2,000 CG 12.5 PLH 25.0 FSH	1	100.0	75.5	1,680,000	820,000	48.8	156 CG 0.7 PLH 1.4 FSH
12.5 FSH 12.5 FSH 500 CG	2	0					1.6 FSH 31 CG
25.0 FSH 1,000 CG 1,000 CG	1	0					1.9 FSH 155 CG
50 FSH 1,000 CG	4	0					2.5 FSH 50 CG
50 FSH 1,000 CG 1,000 CG	1	0					1.9 FSH 75 CG
50 FSH 2,000 CG				948,000	655,600	65.6	3.5 FSH 140 CG

Table 4—(Continued)

Hormones per fish [1]	Number of fish treated	% successfully ovulated	Avg. latent period per successful fish (hrs.)	Avg. No. of eggs per successful fish	Avg. No. of fry per successful fish [3]	Average hatching success	Hormones per pound [2]
50 FSH 25 PLH 2,000 CG	1	100.0	41.0	1,200,000	771,000	64.3	2.7 FSH 0.7 PLH 108 CG
50 FSH 50 FSH 2,000 CG	1	100.0	60.0	1,200,000	1,021,000	85.0	6.7 FSH 133 CG
100 FSH 50 PLH	5	100.0	27.0	1,504,000	846,000	56.3	5.1 FSH 2.6 PLH
100 FSH 2,000 CG	1	0					5.1 FSH 101 CG

[1] Females which were treated alike are grouped. Each entry denotes a separate treatment in the amount, kind and sequence listed.
[2] Measurements are in I.U. for CG and mg. for FSH and PLH.
[3] Estimates were made 12-24 hours after spawning. Mortality after this age was ignored.

neither method was satisfactory in taking the majority of the eggs from the ovary without injuring the eggs or the females or both.

Subsequently, however, it was found that by anesthetizing the female with M.S. 222, she would become sufficiently relaxed so that the eggs could be manually stripped. (Figure 5). If an overdose of M.S. 222 was avoided and the female was "walked" until she revived, the majority could be released in a relatively good condition. Good survival of the females after release was presumed and the method will receive further evaluation in the future.

The M.S. 222 was administered by spraying a water solution of the chemical onto the gills of the fish with the use of a 2½ cc. syringe. Anesthesia usually resulted within 60-90 seconds after application.

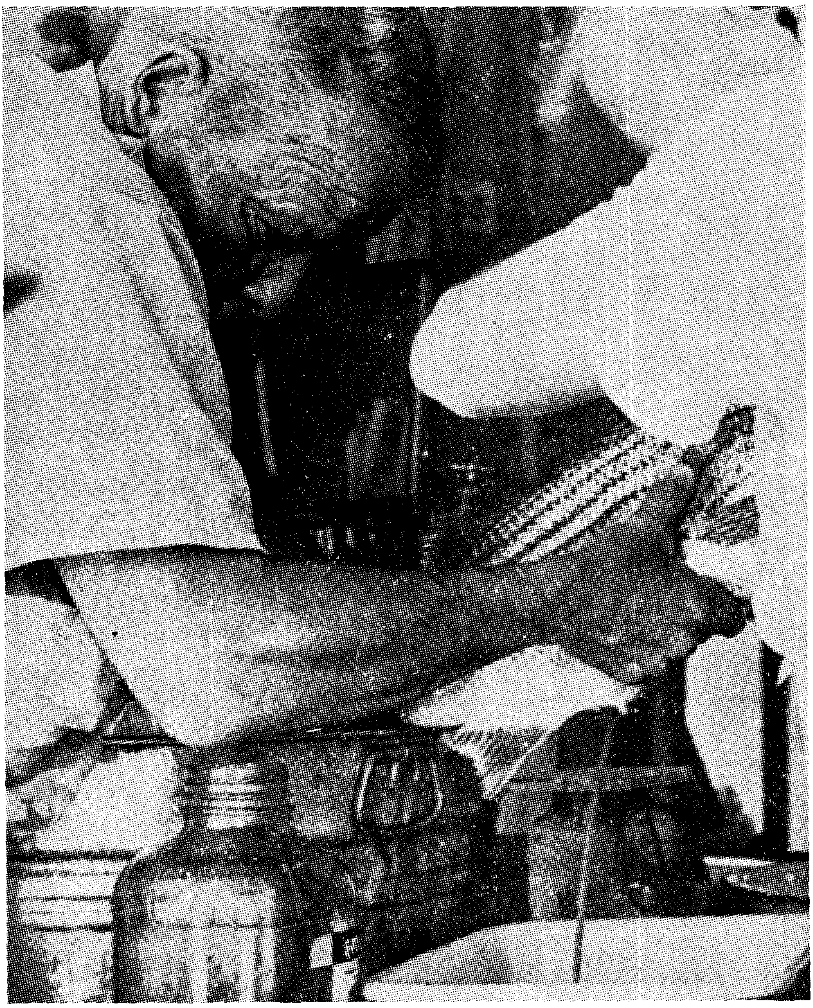


Fig. 5. By anesthetizing the female with M.S. 222 the eggs can be stripped from a ripe female and she can subsequently be released in relatively good condition.

Miscellaneous Observations:

1. Extremely over-ripe eggs will not water-harden.

2. Striped bass can be taken in the sanctuary with hook and line, bow nets, gill nets and electricity. Electricity is ,by far, the most efficient and least harmful method of capture. Most of the time the females remain in shock until released in the holding pond. A small per cent experience vertebral fracture but can be ovulated anyway if they are able to remain submerged and upright.

3. Intramuscular injection is preferable to interperitioneal injections because needling injury is reduced.

Stocking Procedure and Fry Survival:

It has been the practice to stock the fry on the day they hatch but at this stage of development the fry are unable to swim continuously and are probably vulnerable to anerobic bottom conditions as well as to predation by even the most lethargic forms. After three days, however, striped bass fry exhibit a strong continuous swimming ability which undoubtedly increases survivability. In the future, an attempt will be made to hold the fry for several days in aquaria in order to improve their quality.

An estimated 60 million fry from the 1964 hatchery operation were stocked in the Wateree Reservoir. Wateree is located in the piedmont of South Carolina and contains 13,710 acres of water and a shoreline of 242 miles. It was felt that by heavily stocking a relatively small impoundment, fry survival could be more easily evaluated.

As of this writing, marginal seining with a 35-foot common sense seine has been accomplished on five different dates between July 28 and September 17. Different seining areas were used in each instance although seining sites are very limited and no standard haul procedure was possible. In a total of 39 hauls, however, 37 fingerling striped bass were captured. The catch ranged from 0 to 8 fingerlings per haul. The extent to which fishing will be improved by this effort remains to be seen but at least a significant survival of fry is apparent.

CONCLUSIONS

Until additional experience is gained ,the following procedure will be used in the future operation of the hatchery.

1. Adult fish will be captured each morning with the use of an electric seine. The number captured will depend upon the number available, the hatchery capacity and the production schedule.

2. Each female will receive 2,000 I. U. of CG at the time of release into the holding pond. Different sized females will receive the same dose in order to avoid confusion as to dose level.

3. At about 22 hours, an egg sample from each female will be taken with a catheter in order to estimate the time of ovulation for each female.

4. The females will then be segregated on the basis of their nearness to ovulation.

5. Those nearest ovulation will receive hourly checks for ripeness by palpating the abdominal area.

6. Over-ripeness will be avoided, as far as possible, by subsequent egg samples with a catheter.

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