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Management Data Series Number 144
1988

Texas Parks and Wildlife Department
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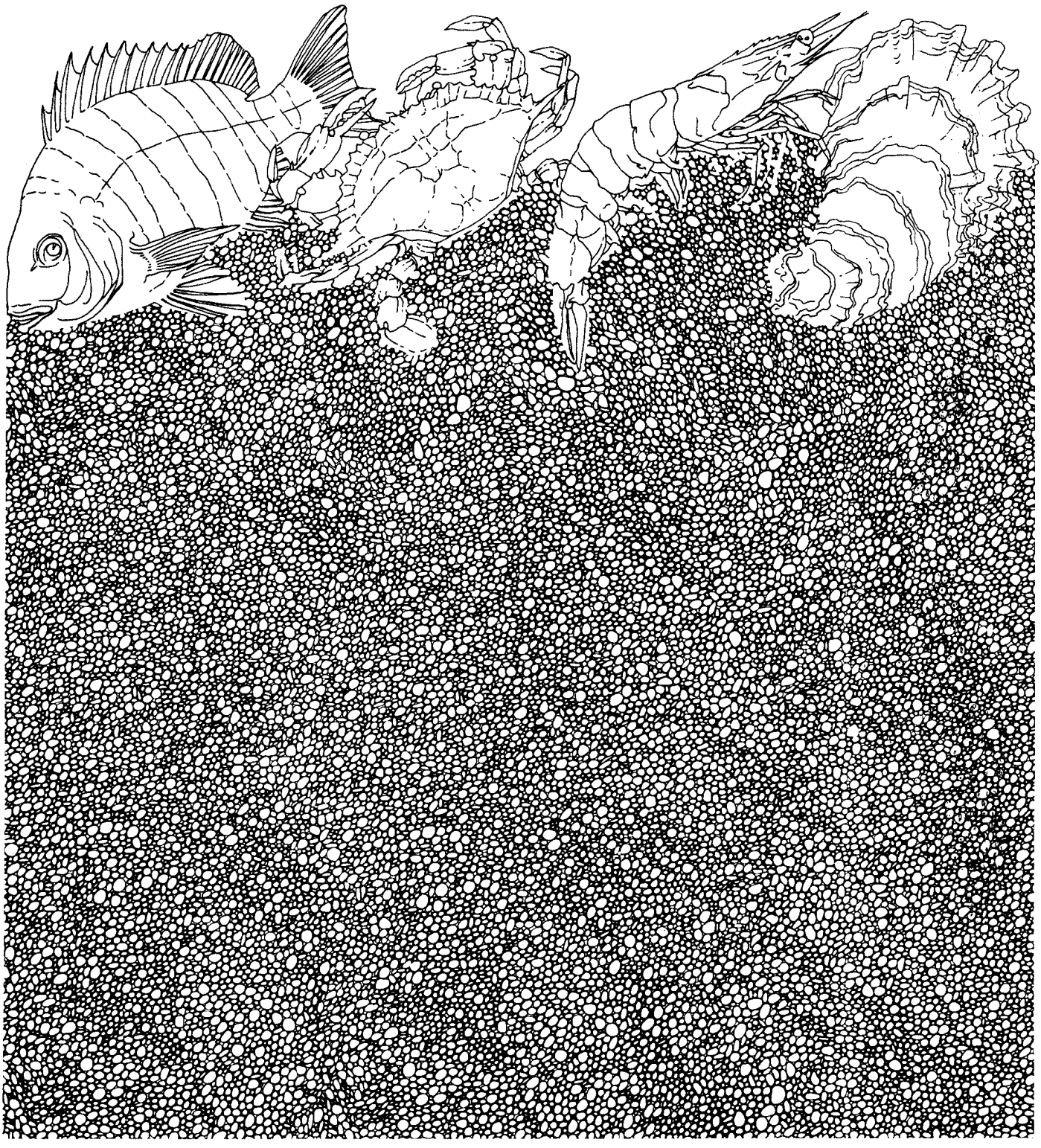

AN EVALUATION OF THE COLLECTION OF PREOVULATORY FEMALES AND HORMONE INDUCED TANK-SPAWNING OF SPOTTED SEATROUT

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ACKNOWLEDGMENTS

This study was conducted with partial funding from the U. S. Department of the Interior, Fish and Wildlife Service under DJ 15.605 (Project F-36-R).

ABSTRACT

Collection of preovulatory females for strip-spawning, and hormone induced-tank spawning of wild caught fish were examined as techniques to improve spawning success of spotted seatrout (Cynoscion nebulosus) for hatchery operations. Only 1 of 75 females collected 18 June 1986 was nearing ovulation. Five of 8 females collected for strip-spawning after noon 23 July 1987 were nearing ovulation. However, neither ovulation nor additional maturation occurred.

Ten female spotted seatrout collected on 3 occasions during 1986, but separately from those above, were injected with either luteinizing hormone-releasing hormone analog (LHRHa) and pimozide or with human chorionic gonadotropin (HCG), and placed in laboratory tank systems with spermiating male spotted seatrout. None of the females receiving LHRHa-pimozide injections released eggs, whereas, 9 of 10 females receiving HCG spawned within 30 hours, but the eggs were not fertilized. Males may have failed to fertilize the eggs because of capture stress or courtship behavior was inhibited by small (2200 l) tank size. Neither collection of preovulatory females nor hormone induced tank-spawning of wild caught spotted seatrout offer advantages over routine hormone induced strip-spawning methods.

INTRODUCTION

Temperature and photoperiod induced tank-spawning of captive spotted seatrout (Cynoscion nebulosus) has produced high quality spawns for research (Arnold et al. 1976, Lasswell et al. 1977). However, spawn frequency averaged only once per week producing approximately 36,000 eggs per spawn, which is considered insufficient for routine hatchery operations. As such, hormone induced strip-spawning procedures remain the current method of choice for spotted seatrout hatchery operations (Colura 1974; Colura et al. 1986) although the vagaries of strip-spawning, coupled with the degree of expertise necessary to ensure that manual fertilization coincides with ovulation continues to drive refinement of alternative spawning methods.

Collection of spawns by hand-stripping naturally ovulating females represents one of the earliest methods used to propagate fishes (Piper et al. 1982). The method works well with cold- and cool-water species but hormones are more commonly used to induce ovulation for strip-spawning of warm water fishes (Piper et al. 1982). However, recent spawning studies with snook (Centropomus undecimalis), a tropical to subtropical species, suggest percent fertilization and fry survival can be significantly improved by stripping naturally ovulating females rather than inducing ovulation with HCG (Roberts 1986). The method requires field identification of preovulatory females, return of these fish to the laboratory where natural ovulation occurs, and subsequent collection and fertilization of the ova using standard strip-spawning techniques. Procedures for identifying preovulatory spotted seatrout are available (Brown et al. in review) and as such, collection of preovulatory wild spotted seatrout is considered a potentially useful spawning method for hatchery operations.

The development of reliable hormone induced tank-spawning methods for wild caught spotted seatrout is also viewed as potentially useful for routine hatchery production. Developed for striped bass (Morone saxatilis), the method requires that gravid females be captured, brought to the laboratory, injected with hormones to induce ovulation, and placed in a tank with spermiating males to spawn (Bishop 1975). Hormone induced tank-spawning of wild caught spotted seatrout using human chorionic gonadotropin (HCG) has occasionally produced large numbers of eggs and fry for pond culture studies (Porter and Maciorowski 1984), but results have been inconsistent (Texas Parks and Wildlife Department, unpublished data). Recent studies suggest luteinizing hormone-releasing hormone analog (LHRHa) and pimozide injections are more successful than HCG in inducing ovulation and tank-spawning in laboratory reared orangemouth corvina, Cynoscion xanthulus, (Prentice and Thomas 1987). Accordingly, interest in the use of LHRHa-pimozide injections to tank-spawn wild caught spotted seatrout was generated.

The present study was undertaken to determine if the spawning methods described above could be applied to the culture of spotted seatrout. Specific objectives were: 1) a preliminary study to determine what portion of females collected were preovulatory and if these fish would ovulate after capture; and 2) to ascertain if LHRHa-pimozide injections would prove more reliable than HCG for hormone induced tank-spawning of

wild caught spotted seatrout.

MATERIALS AND METHODS

Spotted seatrout (≈350-550 mm total length) used for both study objectives were collected by hook and line from Matagorda Bay, Texas. Captured fish were immediately placed into approximately 60 cm diameter x 60 cm deep holding pens constructed of 2.5 cm stretch-mesh net material. Upon completion of collecting operations, a plastic bag (122 l) was placed around the outside of each holding pen to prevent water circulation and a commercial fish calmer (Hypno^{R}, Jungle Laboratories, Cibolo, TX or Trance^{R}, Argent Chemical Laboratories, Redmond, WA) was added until fish were anesthetized. Anesthetized fish were then placed in 140-l transport tanks supplied with compressed oxygen and transported without anesthetic. Handling procedures, following placement in the transport tanks, differed somewhat for collection of preovulatory females and fish to be used in hormone induced tank-spawning studies.

Attempts to capture preovulatory females were made 18 June 1986 and 23 July 1987. Females (75) captured on 18 June 1986 were collected between 0700 and 1030 h and transported approximately 3 km to a boat dock. Upon arrival at the boat dock, fish were placed in a 1.0 x 1.5 x 1.0 m holding pen. The fish were individually removed, anesthetized in a 140-l tank with Hypno^{R}, and an intraovarian biopsy performed using the methods of Hoff et al. (1972). Sampled ova were placed in 5-ml vials containing a 6:3:1 ethanol, formalin, glacial acetic acid solution to clear ooplasm. Individual samples were vigorously shaken for approximately 10 seconds. Ova were subsequently removed and microscopically examined at 40X magnification for evidence of lipid coalescence, germinal vesicle migration, or hydration indicative of impending ovulation (Brown et al. in review).

Females captured on 23 July 1987 were collected from 0830 to 1030 h (16 fish) and 1300 to 1530 h (8 fish). All fish were transported approximately 50 km to the Perry R. Bass Marine Fisheries Research Station (MFRS) where the fish were anesthetized again and ova examined as described above. Fish were then separated according to time of collection and placed into two 3.0 x 0.6 x 0.6 m tanks with continuous water exchange until 0800 h 24 July 1987 when intraovarian samples were again collected.

Separate collections of fish were made for hormone induced tank-spawning trials. Collections were made on 21 May, 18 June, and 7 July 1986. Sexes of anesthetized fish were determined in the field. Fifteen females and 15 males were then randomly selected and transported approximately 50 km to the MFRS on each sample date. Upon arrival at the laboratory each fish received an intramuscular injection of 50 mg oxytetracycline hydrochloride to reduce bacterial infection and transferred to a 9500-l recirculating seawater system until trial initiation.

Hormone induced tank-spawning trials were initiated 22 and 27 May 1986 using fish collected 21 May 1986; 20 and 24 June 1986 using fish collected 18 June 1986; and 8 July using fish collected 7 July 1986. Fish were removed from the tank system and again anesthetized in a 370-l tank with Hypno^{R} or Trance^{R}. Males were examined for spermiogenesis by abdominal massage. Females were weighed (nearest 10 g) and biopsied (Hoff et al. 1972) to obtain an intraovarian sample. Unpreserved samples were microscopically examined (100X magnification) and vitellogenic ova measured with a disc micrometer to the nearest 0.01 mm. On trial initiation date four females eligible for hormone induced strip-spawning (vitellogenic ovum diameter $\geq$ 0.45 mm, Colura et al. 1986) were randomly selected to receive hormone injection.

Two females received single intramuscular injections of 1100 IU/kg of body weight HCG, whereas the remaining two females received intraperitoneal injections of 0.1 mg/kg LHRHa and 10 mg/kg pimozide followed by a second injection of 0.1 mg/kg LHRHa 24 hours later. Each female was placed in a separate 2200-l tank with two sperimiating males and allowed to spawn in the tank. Males used for trials initiated on 22 May and 20 June were also used for trials initiated 27 May and 24 June, respectively.

Released eggs were recovered from the tank by dip net and examined by stereomicroscopy for mitotic division. If egg release did not occur five days after hormone injection the trial was terminated.

RESULTS

The attempt to collect preovulatory spotted seatrout in 1986 was virtually unsuccessful. All fish contained vitellogenic ova, but only one fish was identified as preovulatory. Five of 8 females collected in the afternoon of 23 July 1987 exhibited lipid coalescence whereas none of the fish collected in the morning exhibited signs of oocyte maturation. None of these fish ovulated during the evening hours nor did any additional maturation occur in either afternoon or morning collected fish.

Hormone induced tank-spawning trials with LHRHa and pimozide were also unsuccessful. None of the females injected with LHRHa-pimozide ovulated or released eggs. In contrast to the LHRHa-pimozide treated fish, nine of ten females receiving HCG ovulated and released eggs. None of the eggs, however, were fertilized.

DISCUSSION

Collection of preovulatory spotted seatrout and hormone induced tank-spawning methods used in the present study offered no advantages over routine hormone induced strip-spawning methods (Colura 1974, Colura et al. 1986). Afternoon rather than morning collection proved to be better for identification of preovulatory females. However, regardless of collection time ovarian maturation ceased in all fish collected in 1987. This

presumably was the result of capture stress which induces corticosteroid release and delays final oocyte maturation and ovulation (Billard et al. 1981). As such, collecting preovulatory spotted seatrout and allowing them to ovulate naturally before strip-spawning would appear to be impossible.

In contrast to tank-spawning experiments with orangemouth corvina (Prentice and Thomas 1987), LHRHa-pimozide injection did not induce ovulation or spawning in spotted seatrout. However, the orangemouth corvina had been tank reared for several years, while the spotted seatrout were administered hormones within one week of capture. As such, the LHRHa-pimozide dose successfully used to spawn tank reared orangemouth corvina may be inadequate to override stress-related repression of final egg maturation in wild caught spotted seatrout.

Although 90% of spotted seatrout females injected with HCG ovulated and released eggs, no fertilized eggs were recovered. Reasons for the lack of fertilization are unknown. Females may have produced infertile eggs or males may have failed to perform. All female spotted seatrout exhibited mean ovum diameters $\geq$0.45 mm which is adequate for fertile egg production in HCG induced strip-spawning (Colura et al. 1986). Further, eggs examined immediately after spawning on 23 May 1986 developed blastodiscs suggesting eggs were capable of being fertilized. As such, it is likely males failed to fertilize eggs. Males may have failed to spawn as a result of capture stress or small tank size (2200-l) may have inhibited courtship behavior.

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PWD-RP-3400-267-7/88