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Management Data Series Number 140
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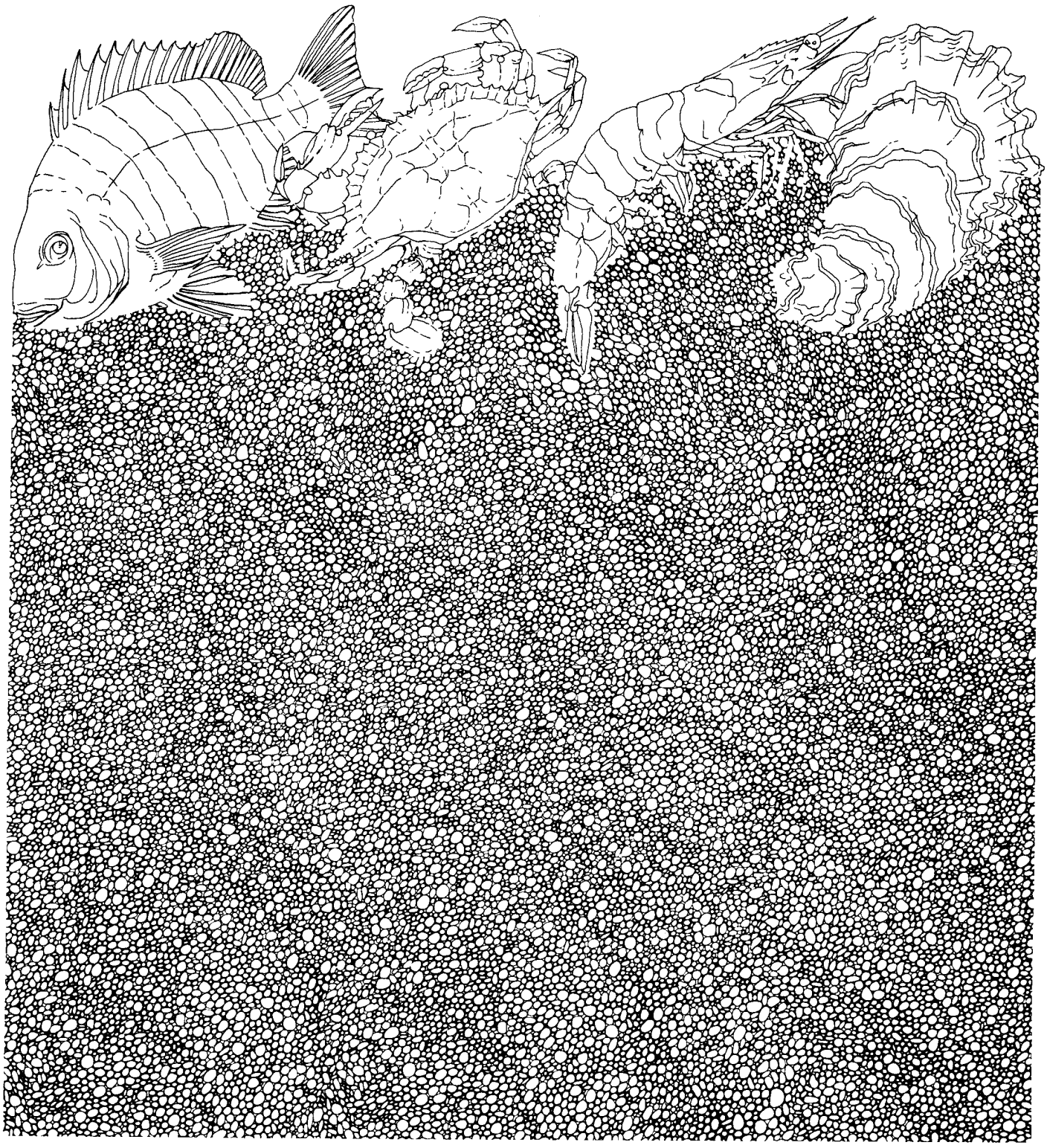

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ACKNOWLEDGEMENTS

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

ABSTRACT

Snook (Centropomus undecimalis) maintained in recirculating tank systems and ponds at the Perry R. Bass Marine Fisheries Research Station (MFRS) and the Gulf Coast Conservation Association/Central Power and Light Company/Texas Parks and Wildlife Department Marine Development Center (MDC) were subjected to hormone treatments to induce gonadal maturation. Four females at the MFRS and two females at the MDC were initially treated 6 and 7 May 1987 respectively by surgically implanting into the abdomen of each fish a 2.16 mm OD X 1.8 cm long sealed silastic tube containing 125 μg LHRHa. Simultaneously, two males at both locations received implants containing 7 mg crystalline testosterone. A male and female at each facility received empty implants (controls). Fish were maintained in tanks until 2 July 1987 when two hormone-treated females and one treated male at the MFRS were transferred to a saltwater pond. On 24 July 1987 a hormone treated tank maintained male and female at the MFRS received 250 μg testosterone and 25 μg LHRHa respectively. On the same day a treated male and female at the MDC each received 0.5 mg/kg body weight 3,3,5-triiodothyronine sodium salt and 0.1 μg/kg body weight LHRHa injection followed by a second injection 7 hours later of 0.4 μg/kg body weight LHRHa. Hormone therapies did not advance gonadal maturation of female snook. Two males at the MDC were spermiating at implantation and intermittently throughout the study. The pond held male at the MFRS produced motile sperm when sampled on 13 August 1987. Handling stress is thought to have inhibited gonadal maturation. Further investigation into the basic maintenance of snook and supplemental hormone treatments are required to spawn this species under controlled conditions.

INTRODUCTION

Snook (Centropomus undecimalis) are an increasingly important fish in both aquaculture and stocking programs (Maciorowski et al. 1986, Tucker 1987). Lack of predictable spawning techniques has hindered research and hatchery production goals for this species. Current spawning methodology involves capturing wild ovulating females immediately prior to spawning followed by strip-spawning (Maciorowski et al. 1986). Unfortunately, this technique often results in the death of broodfish. As snook populations diminish, controlled spawning of captive broodfish is considered extremely important.

Previous attempts at controlled maturation and spawning of snook utilized simulated annual temperature and photoperiod changes in recirculated tank systems (Henderson-Arzapalo et al. 1988). Limited gonadal maturation was observed, but no spawning. Inhibition of gonadal maturation may have been due to stress from frequent handling, configuration of the tank environment, or other unknown factors.

Luteinizing hormone-releasing hormone (LHRH) and its high-potency analogs (LHRHa) have been used to induce maturation and spawning in many fish (Crim and Glebe 1984, Fitzpatrick et al. 1984a, Rottman and Shireman 1985). Chronic or slow release application of LHRHa singly or in conjugation with other hormones has proved very effective (Billard et al. 1984a, Lee et al. 1986a, Kreiberg et al. 1987). Administration may be through repeated injections or by slow-release capsules such as cholesterol pellets or silastic implants (Weil and Crim 1983, Lee et al. 1986b, Marte et al. 1987). This study examined the effect of LHRHa and selected hormone conjugates on the maturation and spawning of snook.

MATERIALS AND METHODS

Adult snook (645-1000 mm TL) were captured by rod-and-reel during 1985, and transported to either the Texas Parks and Wildlife Department (TPWD) Perry R. Bass Marine Fisheries Research Station (MFRS), Palacios, Texas, or the Gulf Coast Conservation Association/Central Power and Light Company/TPWD Marine Development Center (MDC) in Corpus Christi, Texas. Snook at the MFRS were prophylactically treated for diseases, transferred to a recirculating tank system, and subjected to temperature and photoperiod conditioning as described by Henderson-Arzapalo et al. (1988). Snook at the MDC were placed in a 13,200-liter recirculating tank system and subjected to temperature and photoperiod conditioning regimes designed to stimulate red drum spawning (McCarty et al. 1986). Tank systems at both locations were maintained independently. Temperature-photoperiod conditioning regimes used prior to and during the experimental period are presented in tables 1 and 2. Snook are serial spawners, with an extended spawning season lasting from May through November in south Florida (Marshall 1958, Gilmore et al. 1983, Tucker and Campbell 1987). As such, the regimes were designed to simulate summer spawning conditions from June through August 1987.

Snook were fed a mixture of dead bait shrimp, chopped fish, squid, and live forage ad. libitum. Water temperature and salinity was determined daily with an SCT meter (Model 33, Yellow Springs Instruments, Yellow Springs, OH),

or with a glass thermometer and refractometer (American Optical Company, Garden City, NY). Ammonia-nitrogen (mg/l) was determined by specific ion electrode or by nesslerization (APHA et al. 1985), and a specific ion electrode used to measure pH three times weekly. Water was exchanged as needed to maintain ammonia $\leq$ 0.9 mg/l and pH $\geq$ 7.5. Salinity ranged 10-25 o/oo at the MFRS, and 27-40 o/oo at the MDC.

Initial hormone treatments were performed 6 and 7 May 1987 at the MFRS and MDC, respectively when tank environmental conditions were 15 h light:9 h dark and 28 C (Tables 3 and 4). Salinities at the MDC and MFRS were 29 and 23 o/oo, respectively. Implants were constructed of 1.02 mm ID x 2.16 mm OD x 1.8 cm long silastic tubing sealed with silicone medical adhesive (Dow Corning, Midland, MI) as described by Moore (1981) and Lee et al. (1986b). Implants contained either 125 μg LHRHa dissolved in 9% sterile saline (D-Ala^6 des Gly^{10} ethylamide; Argent Chemical Laboratories, Redmond, WA) or 7 mg crystalline testosterone (4-androsten-17 B-ol-3-one; Sigma Chemical Company, St. Louis, MO). Four females at the MFRS and two females at the MDC received LHRHa implants, two males at both locations received testosterone implants, and a male and female at both facilities received empty silastic implants (controls). MDC snook were tagged for identification with a streamer tag through the dorsal fin and maintained together in one tank. MFRS fish were divided into three replicate tank systems; two LHRHa implant females and a testosterone implant male in each of two tanks and the two control fish in another. Two hormone-treated females and one treated male were transferred to a 0.13-ha saltwater pond on 2 July.

A hormone-treated male and female at both facilities received additional hormone therapy on 24 July. At the MFRS, each fish received implants containing 250 μg LHRHa and 250 μg testosterone. At MDC, the fish received 0.5 mg/kg body weight 3,3',5-triiodothyronine sodium salt dissolved in 9% saline (T_3) and 0.1 μg/kg body weight LHRHa intramuscularly injected at 1400 h, followed by a second injection of 0.4 μg/kg body weight LHRHa at 2100 h.

Implantation procedures were as follows: fish were individually removed from the tanks, anesthetized in a smaller tank containing 122 mg/l MS-222 (Argent Chemical Laboratories, Redmond, WA), and then moved to a padded support box. A tygon tube connected to a small submersible pump was placed in the fish's mouth, and water containing 88 mg/l MS-222 was flushed over the gills (20 l/minute) to maintain respiration. The fish was placed with the abdomen upwards and the area swabbed with antiseptic solution. An incision approximately 1 cm long was made through the skin and abdominal wall about 3 cm anterior to the anus and slightly to the left. The implant was inserted into the abdominal cavity and an antiseptic cream applied to the wound. Each fish also received an intramuscular injection of 50 mg oxytetracycline hydrochloride. The fish was moved to a recovery tank containing aerated saltwater and a small amount of furacin, moved gently through the water until opercular movement resumed, and transferred back to the tank system.

Gonadal condition of fish was determined at implantation and every 2 weeks until 14 September. MFRS snook were also sampled 25 April due to additional surgery needed by one female. Fish were either handled and anesthetized as previously described or the tank volume reduced to about 1000 liters and a commercial fish calmer such as Hypno (Jungle Laboratories,

Cibolo, TX) or Trance (Argent Chemical Laboratories, Redmond, WA) added. Male spermatogenesis was determined by abdominal massage. Milt was collected in a capillary tube and microscopically examined for motility. Ovarian tissue was removed by catheterization (Hoff et al. 1972), and microscopically examined. Diameters of 30 randomly selected ova were measured with an ocular micrometer and an mean (± SD) calculated. In addition, the percentage of ova in various stages of development (Kuo et al. 1973) was visually estimated.

RESULTS

The hormonal therapies used in the present study did not consistently advance gonadal maturation in tank-maintained snook (Figures 1 and 2). Two MFRS females, one LHRHa implant fish and one control, exhibited very limited maturation. Ovarian tissue samples collected 17 June and 2 July contained mostly primary oocytes with a few (< 5%) more advanced stage II and III vitellogenic ova. Other females had only primary oocytes present on all sampling dates. The female which received a second LHRHa and testosterone implant died 30 July, 7 days after the second implantation and before any additional maturation occurred (the fish jumped from the tank during refilling after the 30 July sampling). Transfer to a saltwater pond also did not advance ovarian maturation; one female did not yield any ova during the entire experimental period (samples contained only tissue fragments and fluid) (Figure 1).

Maturation of male snook was more successful (Tables 5 and 6). Two males at the MDC were flowing at implantation (7 May), however, only one produced motile sperm. The male producing motile sperm (a control fish) remained fertile through 28 August. The other male was subjected to both testosterone implantation and T_3 and LHRHa injection, and produced milt intermittently during the study period although sperm were non-motile. The remaining male had received a testosterone implant on 7 May, and produced non-motile milt on 31 July. MFRS males were not flowing on any sampling date prior to 13 August, when the male transferred to the saltwater pond began producing motile sperm. All flowing males had begun to regress by 14 September.

Fish recovered well after implantation surgery, and incisions were healed by the first sampling date. The snook did not respond well to the repeated sampling, taking 4-5 days to resume feeding, and all fish gradually lost weight over the study period. Recovery time after anesthesia increased as the study progressed. Snook transferred to the saltwater pond rapidly gained weight and appeared healthier than tank-maintained fish. The pond contained a dense forage population of small fish and shrimp.

One female required removal of a tumor-like growth from the lower jaw on 25 April. The fish was removed, tranquilized, and a small (approximately 1 cm diameter by 0.5 cm high) cartilaginous tumor excised from the lower jaw. The wound was cauterized, and the fish returned to the tank. The tumor continued to grow, and required removal on 2 July and 13 August. The area was frozen with liquid nitrogen after the growth was removed 13 August. Neither cauterization nor freezing with liquid nitrogen prevented tumor regrowth. This individual did not yield any ova during the experimental period.

DISCUSSION

Factors inhibiting maturation in tank-maintained snook are complex and probably act synergistically. Although not effective in the present study, LHRHa has been used successfully with many fish species to advance maturation and induce spawning (Crim and Glebe 1984, Barnabe and Barnabe-Quet 1985, Ramos 1986). LHRHa effectiveness is significantly enhanced when maturation is already advanced and environmental conditions are appropriate (Crim and Glebe 1984, Billard et al. 1984b, Harvey et al. 1985). Additionally, snook are difficult to acclimate to tank systems, and are often excitable and erratic feeders (Henderson-Arzapalo et al. 1988, Daniel Roberts, Jr., Florida Department of Natural Resources (FDNR), unpublished data). The fish struggle violently during handling, and are slow to recover and resume feeding. Snook in the present study lost weight and were in poor condition by study termination due in part to the bimonthly handling. Handling stress and resultant corticosteriod release may have inhibited gonadal maturation (Billard et al. 1981). The female subjected to additional surgery for tumor removal did not yield any ova during the experimental period, presumably due to stress.

Gonadal maturation and vitellogenesis require substantial energy, protein, and lipid input to support gamete production. Snook are easily disturbed when maintained in tank systems, and erratic feeding behavior could reduce nutrient reserves available for gonadal maturation. Inadequate nutrition, either in terms of quantity or quality of food, can retard maturation, reduce spawn or egg size, and result in poor hatchability and low fry viability (Watanabe et al. 1984, Springate et al. 1985, Kanazawa 1985). Food provided in the tank systems may be deficient in some essential vitamins. Snook at MFRS refused to eat a vitamin supplemented chopped fish-shrimp mixture intended to fortify their diet.

Live forage was only added in limited amounts due to the risk of introducing diseases and parasites. The possibility of inadequate nutrition is further supported by the improved condition of pond-maintained snook allowed to feed on natural forage.

Two female snook exhibited some vitellogenic ova in the 17 June and 2 July samples, and some males were flowing during most of the experimental period, indicating that environmental conditions were somewhat conducive to spawning. Snook are serial or batch spawning fish, and normally have oocytes in all stages of development during spawning season (Rodrigues Couto and de Souza Guedes 1981, Tucker and Campbell 1987). As normal vitellogenesis and maturation advances, more mature ova increase in cell volume and mask the more numerous but small immature oocytes. Normal ovarian tissue samples prior to spawning appear to contain mostly stage III vitellogenic ova, with mean ovum diameters approximately 0.4 mm. Vitellogenetic (Stage III) ova appear gold-yellow and contain numerous oil globules giving it a granular appearance. Although the few more advanced ova present in the 17 June and 2 July ovarian tissue samples appeared normal, the total number present was abnormally low for successful spawning. Lee et al. (1986c) demonstrated a positive correlation between the percentage of ova in various stages of maturation and successful induced spawning of milkfish, Chanos chanos.

Previous maturation and spawning experiments with snook in controlled tank systems had similar results. Temperature and photoperiod conditioning resulted in similar abnormal ovarian maturation (Henderson-Arzapalo et al. 1988), and normal spermatogenesis (MDC unpublished data, Daniel Roberts, Jr. FDNR unpublished data). Successful maturation and spawning of captive snook may require a combination of improved routine maintenance techniques, improved diet, and hormone therapy. Further investigation into the basic maintenance of snook and supplemental hormone therapy is needed to successfully mature and spawn this species under controlled conditions.

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Table 1. Temperature and photoperiod regime utilized with experimental snook at the Marine Development Center, October 1986-September 1987.

Date	Temperature (C)	Photoperiod (h light)
01 Oct 1986	27	13
29 Oct	25	12
08 Nov	25	11
28 Nov	17	10
05 Dec	14	9
17 Dec	16	10
28 Dec	16	11
07 Jan 1987	19	12
20 Jan	21	12.5
02 Feb	24	12.5
05 Mar	25	12.5
30 Mar	24	12.5
13 Apr	25	13
27 Apr	27	14
11 May	28	15
01 Jun	28	15
01 Jul	28	15
29 Jul	29	14
04 Aug	28	14
07 Aug[a]	27	13
17 Aug[a]	26	12
24 Aug[a]	26	11
11 Sep	24	11

a Temperature fluctuations of 2-3 C occurred on 13, 20, and 26 Aug. Temperature was reduced then returned to the original temperature within 24 hours.

Table 2. Temperature and photoperiod regime utilized with experimental snook at the Perry R. Bass Marine Fisheries Research Station, October 1986-September 1987.

Date	Temperature (C)	Photoperiod (h light)
01 Oct 1986	30	15
01 Nov	28	15
01 Dec	28	15
22 Dec	26	14
05 Jan 1987	22	13
19 Jan	20	11
02 Feb	20	10
16 Feb	20	10
02 Mar	22	11
16 Mar	22	12
30 Mar	24	12
13 Apr	28	14
27 Apr	28	14
11 May	28	15
25 May	28	15
01 Jun	30	15
01 Jun-04 Aug	30	15
04 Aug	28	14
07 Aug	27	13
10 Aug[a]	27	13
17 Aug[a]	27	12
24 Aug[a]	27	12
31 Aug	24	11
07 Sep	24	11
11 Sep	24	11

a Temperature fluctuations of 2-3 C occurred on 13, 20, and 26 Aug. Temperature was reduced then returned to the original temperature within 24 hours.

Table 3. Summary of experimental hormone treatments used with snook at the Perry R. Bass Marine Fisheries Research Station.

Tank	Sex	Total length (mm)	Treatment	
			Initial	Secondary
1	F	Not determined	125 μg LHRHa[b]	none
1	F	715	125 μg LHRHa	250 μg LHRHa[b] 250 μg testosterone[d]
1	M	655	7 mg testosterone[c]	250 μg LHRHa 250 μg testosterone
2	F	750	125 μg LHRHa	transferred to pond
2	F	780	125 μg LHRHa	transferred to pond
2	M	655	7 mg testosterone	transferred to pond
3	F	760	blank	none
3	M	645	blank	none

[a]Fish lower jaw damaged, total length not determined
[b]Dissolved in 9% sterile saline
[c]Dissolved in alcohol and allowed to crystallize
[d]Dissolved in castor oil

Table 4. Summary of experimental hormone treatments used with snook at the Marine Development Center.

Fish tag number	Sex	Total length (mm)	Treatment	
			Initial	Secondary
35843	F	935	125 μg LHRHa[a]	0.5 mg/kg T_3[a] 0.5 μg/kg LHRHa[c]
No tag	F	1000	125 μg LHRHa	
35844	F	915	125 μg LHRHa	
35847	M	910	7 mg testosterone[b]	0.5 mg/kg T_3[d] 0.5 μg/kg LHRHa
No tag	M	910	7 mg testosterone	
35899	M	855	7 mg testosterone	

[a]Dissolved in 9% saline
[b]Dissolved in alcohol and allowed to crystallize
[c]Dissolved in 7% saline
[d]T_3: 3, 3', 5-tri-iodothyronine sodium salt dissolved in 7% saline

Table 5. Summary of male snook treatment and milt production at the Perry R. Bass Marine Fisheries Research Station, 25 April - 10 September 1987. "F" denotes flowing milt, "NF" signifies not flowing, "M" and "NM" denote motile and non-motile sperm in milt sample.

Sample date	LHRHa Testosterone	Testosterone	Control
25 Apr	NF	NF	NF
06 May	NF	NF	NF
20 May	NF	NF	NF
03 Jun	NF	NF	NF
17 Jun	NF	NF	NF
02 Jul	NF	NF	NF
15 Jul	NF	NF	NF
24 Jul (second implant)	NF	NF	NF
30 Jul	NF	NF	NF
13 Aug	NF	FM	NF
27 Aug	NF	FM	NF
10 Sep	NF	F(NM)	NF

Table 6. Summary of male snook treatment and milt production at the Marine Development Center, 7 May-14 September 1987. "F" denotes flowing milt, "NF" signifies not flowing. "M" and "NM" denote motile and non-motile sperm in milt sample.

Sample date	Control	LHRHa + T_3 Testosterone	LHRHa Testosterone
07 May	FM	FNM	NF
21 May	F[a]	NF	NF
04 Jun	FM	FNM	NF
22 Jun	FM	NF	NF
02 Jul	FM	NF	NF
16 Jul	FM	NF	NF
24 Jul	FM	NF	NF
31 Jul	FM	FNM	FNM
13 Aug	FM	NF	NF
28 Aug	FM	NF	NF
14 Sep	NF	NF	NF

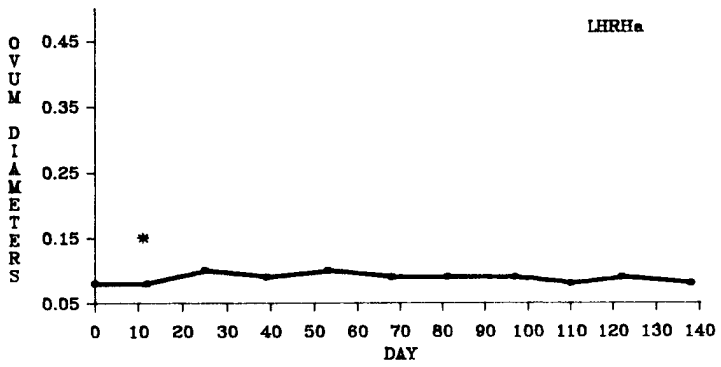
[a]Motility not determined

Figure 1. Mean ovum diameters (mm) of snook at the Perry R. Bass Marine Fisheries Research Station, 25 April-September 1987. Arrows indicate implantation dates. Means were determined separately for ova at different stages of maturation.
Asterisks indicate implantation date.

LHRHa: Single implant, 125 μg LHRHa
LHRHa/test: Two implants, 125 μg
LHRHa followed by 250 μg
LHRHa and 250 μg testosterone

Control: blank

LHRHa/Pond: Single implant, 125 μg LHRHa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0.05
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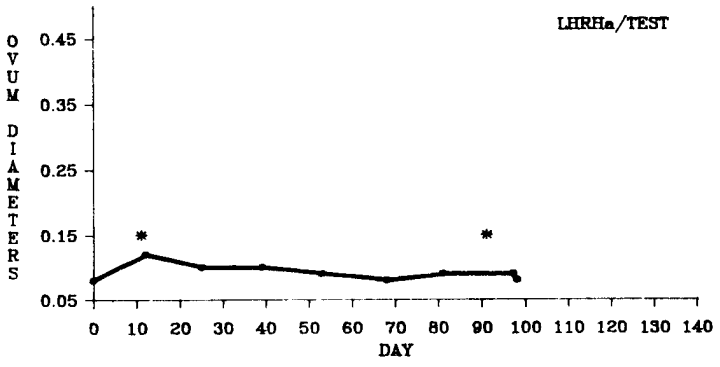
DAY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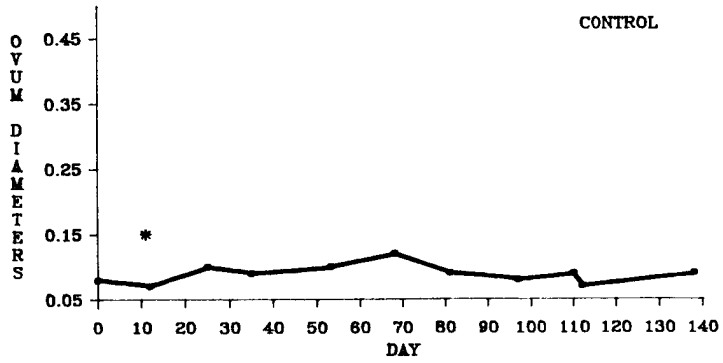
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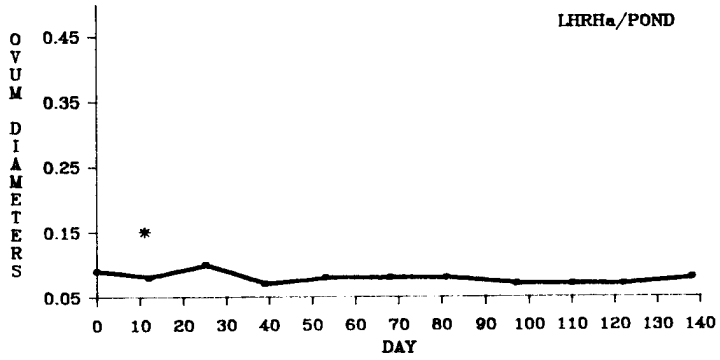
DAY


LHRHa/POND
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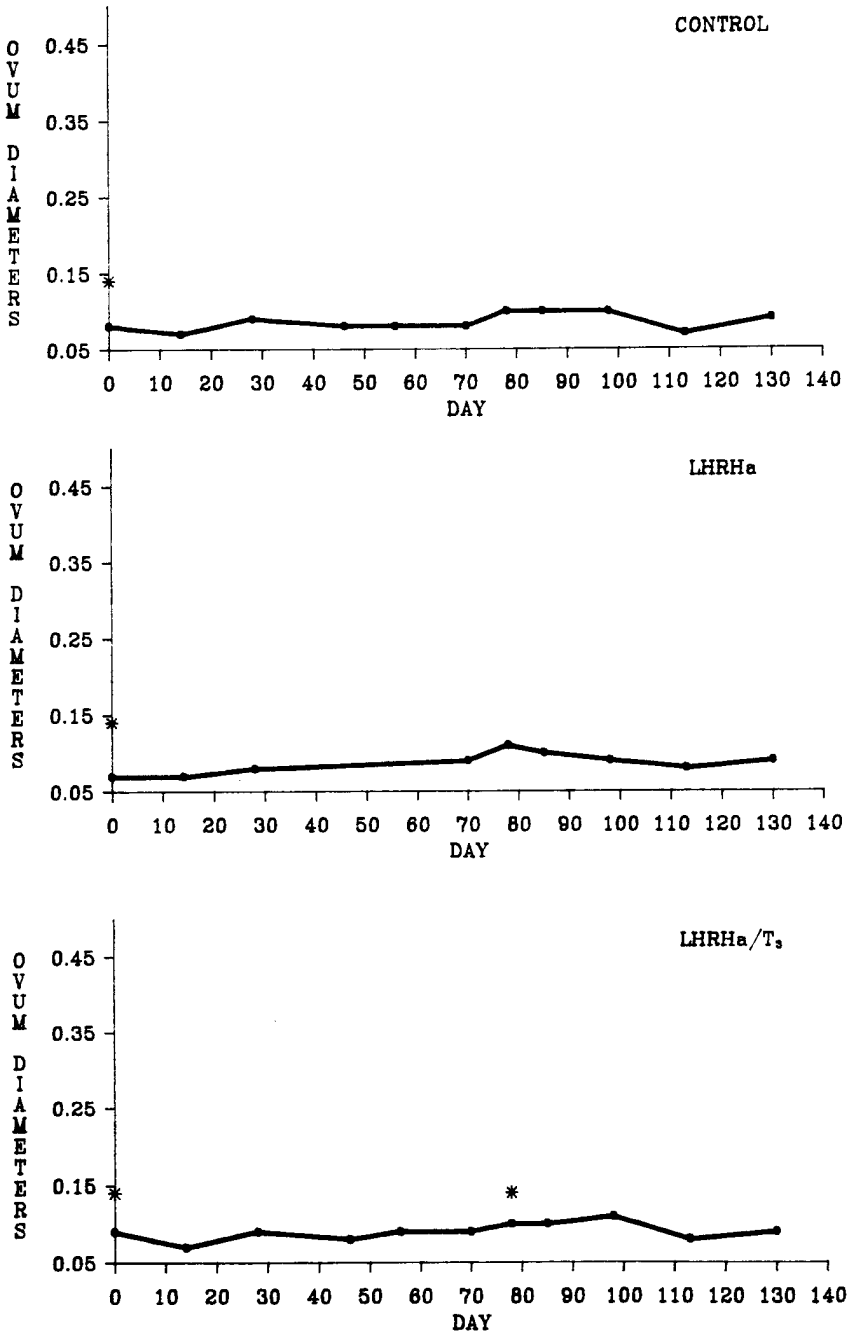
0.35
0.25
0.15
0.05
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140
DAY

Figure 2. Mean ovum diameters (mm) of snook at the Marine Development Center, 7 May-14 September 1987. Asterisks indicate implantation or injection date.


CONTROL
OVUM DIAMETERS
0.45
0.35
0.25
0.15
0.05
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140
DAY
LHRHa
OVUM DIAMETERS
0.45
0.35
0.25
0.15
0.05
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140
DAY
LHRHa/T3
OVUM DIAMETERS
0.45
0.35
0.25
0.15
0.05
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140
DAY

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PWD-RP-3400-293-5/89