Temperature And Photoperiod Induced Maturation Of Southern Flounder

by Anne Henderson-Arzapalo[1], Robert L. Colura[2], and Anthony F. Maciorowski[3]

Management Data Series Number 154
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Texas Parks and Wildlife Department
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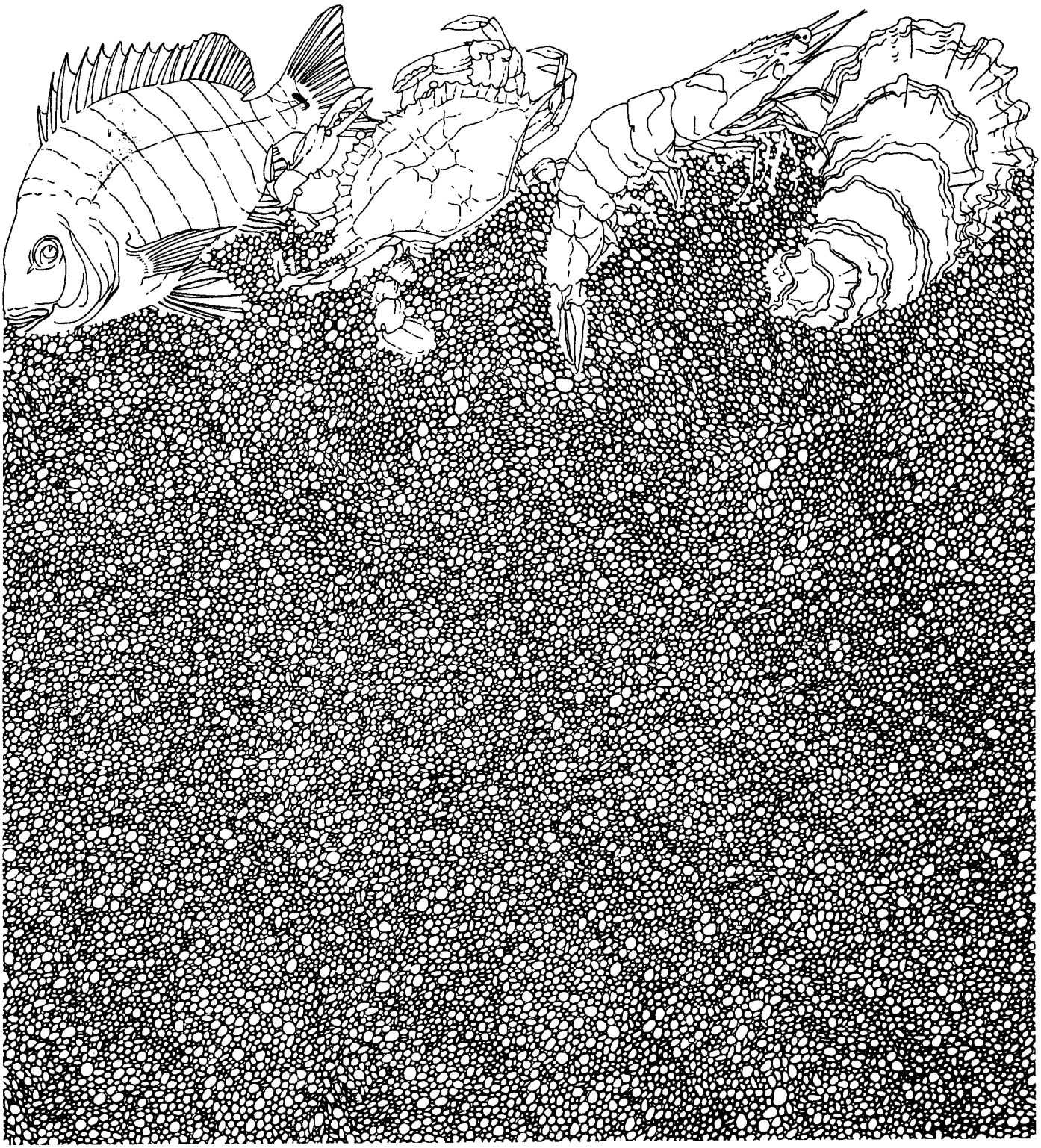

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ACKNOWLEDGEMENTS

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

Southern flounder (Paralichthys lethostigma) were maintained either in a 9500-l or 2300-l recirculating tank system from October 1983-March 1986 and subjected to controlled photoperiod and temperature conditioning to induce spawning. The fish were first subjected to a 6-month regime to delay natural spawning until spring (1984) followed by 6-month and 4-month regimes to induce either early fall (1984) or late summer (1985) spawning. Regardless of the conditioning regime, female gonadal development and egg release occurred only during December-February when water temperature was 18C and photoperiod maintained at 9-h light: 15-h dark. Male spermatogenesis coincided with female ovarian development but they did not fertilize eggs. The number of eggs released ranged from 66 to 28,900. Injection of 125 g LHRHa/fish failed to induce male courtship and fertilization. Strip-spawning attempts were also unsuccessful because eggs could not be manually extruded despite the presence of hydrated ova within the ovaries.

INTRODUCTION

The southern flounder (Paralichthys lethostigma) is the largest bothid flounder of the Gulf coast and has been viewed as an attractive candidate for mariculture and estuarine and freshwater stocking programs (White and Stickney 1973; Lasswell et al. 1978). Southern flounder have been spawned in the laboratory during the normal spawning season of December and January (Stokes 1977) by temperature and photoperiod conditioning (Arnold et al. 1977), and strip-spawned following injection of carp pituitary extract (Lasswell et al. 1978). Neither method, however, has been used to produce the large number of fry necessary to realize large scale pond production. Furthermore, pond culture of larval and juvenile southern flounder during the normal winter spawning season would be difficult due to slow growth and the possibility of winter kill. The present study was performed to determine if southern flounder could be induced to spawn outside the natural spawning season using temperature and photoperiod conditioning, thus providing a reliable source of fry for pond culture during the spring and summer.

MATERIALS AND METHODS

During October 1983, 70 southern flounder were collected by trammel net from Matagorda Bay, Texas. Fish were initially held in a 0.2-ha saltwater pond. On 27 October 1983, five females and five males were transferred to an indoor 9500-l (3.7-m diameter x 0.9-m deep) recirculating seawater system consisting of a fiberglass circular tank equipped with an external sand and gravel filter and rotating biodisc. Water temperature was controlled (±2 C) with 4500-W quartz immersion heaters and a 1.0-HP water chiller. A 100-W

incandescent light bulb and a pin-set timer regulated daily photoperiod. The tank was covered with layered styrofoam to exclude extraneous light. Ninety kilograms of clean sand were added to the tank to accommodate normal burrowing behavior of flounder. The fish were periodically treated with 0.25 mg/l Cu^{++} (Cutrine, Applied Biochemists, Mequon, WI) to control Amyloodinium sp. Burrowing activity of broodfish resulted in a patchy distribution of sand and caused continual abrasion against the tank floor. As a result, broodfish developed severe hemorrhagic lesions on the ventral portion of the lower jaw. Accordingly, fish were transferred to a second tank system on 13 February 1984.

The second recirculating tank system consisted of a 2300-l (2.44-m diameter by 0.61-m deep) circular fiberglass tank equipped with an undergravel filter system. The filter media consisted of either 1.25-cm foam rubber or polyester air conditioning filter material covered with 5 cm of blasting sand. The soft underlayer prevented excessive abrasion and eliminated the formation of mandibular lesions on broodfish. Water temperature and photoperiod were controlled as previously described. A 25-W or 7.5-W incandescent light bulb mounted in the styrofoam tank cover served as the light source. Water was continuously circulated through an ultraviolet sterilizer (Aquafine Corp., Valencia, CA) except during spawning. An external egg collector was connected to the tank via a 3.8-cm bulkhead mounted at the water surface. Eggs were skimmed from the water surface, passed down a 3.8-cm PVC pipe, and upwelled into a 9.14-l polyethylene container fitted with 0.57-mm mesh stainless steel screen sides. Water passed through the screen into a 138-l outer tank and was airlift-returned to the main tank.

Three conditioning cycles were used: a delaying regime (October 1983-March 1984) to postpone normal spawning until early spring (Table 1); a compressed 6-month regime (April 1984-March 1985) to induce out-of-season spawning beginning September 1984 (Table 2); and a compressed 4-month regime (March 1985-March 1986) to induce out-of-season spawning by July 1985 (Table 3). Tank temperature and photoperiod are shown graphically in Figure 1. Water temperatures simulated seasonal ambient conditions in the Gulf of Mexico between 95-90°W and 30-25°N (Leipper 1954). Seasonal photoperiod approximated those for Galveston, Texas rounded back to the hour (Texas Almanac 1983). If gonadal maturation did not occur by the end of the scheduled cycles, tank conditions were held at normal winter spawning conditions of 18 C and a 9-h light:15-h dark photoperiod until gonadal development occurred. During the 6-month conditioning cycle (Table 2), photoperiod was shortened to 4-h light:20-h dark between 7 January to 25 March 1985 to encourage male participation (Leong 1971). Fish were fed approximately 115 g of frozen bait shrimp daily, and periodically offered live shrimp or killifish (Fundulus sp. or Cyprinodon sp.).

Flounder were observed daily to monitor general condition and for external signs of female gonadal development (a bulge on the left ventral surface). The stage of gonadal maturation for all fish was determined on: 26 March and 20 November 1984; 20 February 1985; 4 September, and 6 and 20 December 1985; 14 and 28 February, and 14 and 31 March 1986. Flounder were removed from the tank, anesthetized with Hypno^{R} (Jungle Laboratories, San Antonio, TX) or Trance^{R} (Argent Chemical Laboratories, Redmond, WA), and examined. Sperm production was determined by abdominal massage to extrude milt. Ovarian tissue samples were removed using a 1.0-mm ID polyethylene

tube attached to an 18-gauge needle and 10-ml syringe, and microscopically examined to determine the stage of development (Hoff et al. 1972). Twenty ova were measured with an ocular micrometer and the mean ovum diameter calculated.

Initial unfertilized egg releases in the 1984-85 spawning regime were not quantified. Eggs released later were dip-netted from the egg collector on the day of spawning and transferred to a water-filled beaker. The total volume of water and eggs was determined, three 5-ml or five 1-ml subsamples were removed with a Hensen-Stemple pipet, and the eggs counted using a Ward plankton wheel and stereomicroscopy. The mean number of eggs per ml was used to determine the number of eggs in the total volume. Eggs were microscopically examined for mitotic division over several hours to determine percent fertilization.

Strip-spawning was attempted 28 January 1986 to determine if eggs and sperm were viable. Females were catheterized the preceding day, ovarian samples examined, and time to ovulation estimated. Sperm was collected from two males in 1.1-mm ID capillary tubes and microscopically examined for motility. The females were manually checked for egg release at approximately 2 h intervals from 0600 to 1600 h. All fish were injected intraperitoneally with 125 g of LHRHa (D-Ala^{6} des Gly^{10} ethylamide) to stimulate male courtship and fertilization on 28 January 1986 at 1600 h and returned to the tank (Lee et al. 1986).

Selected water quality characteristics were determined daily and adjusted to predetermined concentrations. Temperature was measured with a glass thermometer and salinity with a refractometer or SCT meter (Model 33, Yellow Springs Instruments, Yellow Springs, OH). Ammonia concentrations

were determined by nesslerization or a specific ion electrode (APHA et al. 1985). A gel-filled combination electrode was used to determine pH. Water was exchanged as needed to maintain pH between 7.0 to 8.5 and total ammonia <0.90 mg/l. Salinity was maintained between 28 to 34 o/oo by adding freshwater or a commercial synthetic sea salt mixture as necessary.

RESULTS

Temperature and photoperiod manipulation failed to induce viable spawns of southern flounder. Gonadal maturation and release of eggs occurred only when temperature and photoperiod simulated the natural (winter) spawning season. However, eggs were not released in any months except December-February regardless of the temperature and photoperiod. Ovarian maturation was readily apparent by visual inspection of broodfish and samples were easily obtained from developed fish. However, ovarian penetration could not be accomplished for females lacking apparent gonadal swelling.

The delayed spawning regime (Table 1) resulted in apparent female gonadal development and males were flowing when transferred to the second tank system (13 February 1984). No egg release occurred, however, and intraovarian samples could not be obtained from females on 26 March 1984.

The 6-month compressed cycle failed to result in gonadal development of females during September 1984. However, ovarian swelling was observed in November 1984. On 20 November 1984, only four of five males were found in the tank system. No remains of the fifth male were observed and the cause of death could not be determined. Three of the four remaining males were flowing, and samples were obtained from three of five females which had mean

ovum diameters of 0.12 to 0.53 mm (Table 4). Egg releases during the 6-month compressed cycle (Table 5) began 18 December 1985, approximately 2.5 months after instituting winter spawning conditions, and continued through 18 January 1985. Released eggs appeared normal, and mean egg diameter was 0.92mm. Five subsequent egg releases occurred prior to photoperiod reduction (7 January 1985) and five more after photoperiod reduction, but no embryos were found.

Attempts to observe egg releases were disruptive, and fish immediately burrowed into the sand substrate precluding observation of spawning fish. Feeding activity increased immediately before and during the spawning period. Flounder usually had distended stomachs November through February. Food consumption was erratic during the post-spawning phase. Occasionally, females were observed swimming at the water surface around the tank edges for several hours. Similar behavior was not observed for males. Egg releases generally occurred between 0500-0900 h.

Tank salinity was discovered to be 45 o/oo on 11 February 1985 due to a malfunctioning salinity meter. Salinity was immediately reduced to 35 o/oo. On 20 February 1985, two males were flowing, and three females had enlarged ovaries with mean ovum diameters between 0.50 to 0.58 mm. However, no new egg releases occurred.

The 4-month compressed cycle resulted in ovarian development in early December (Table 6) approximately 5 months after instituting winter spawning conditions. Egg releases (24) occurred from 8 December 1985 to 13 February 1986 and ranged from 66 to 28,900 eggs (Table 5). Again, no eggs were fertilized. Attempts to strip-spawn females containing hydrated ova on 28 January 1986 were unsuccessful. Injection of LHRHa also failed to induce

male courtship and egg fertilization, although eggs were released on 29 January 1986. By 31 March 1986 all females were refractory.

DISCUSSION

Although compressed conditioning cycles have been used to trigger spawning outside the normal spawning season for various marine fishes (Roberts et al. 1978; Hoff et al. 1972), temperature and photoperiod manipulation was not effective with southern flounder in the study. Regardless of the conditioning regime, southern flounder ovarian maturation and egg releases only occurred from December-February, the natural spawning season. Male gonadal development coincided with female development but males did not fertilize eggs. Similarly, other investigators have stimulated southern flounder gonadal development only during the natural spawning season. Female southern flounder maintained in outdoor tanks were reported to have matured in December, but the lack of males precluded spawning (White and Stickney 1973). Arnold et al. (1977) successfully matured and spawned southern flounder in the laboratory, but spawning occurred only under winter conditions (17 C, 9-h light) during the months of December-January.

Egg production obtained in this study (66-28,900 eggs/spawn) was similar to that reported by other researchers. Arnold et al. (1977) recovered 120,000 eggs from 13 separate photoperiod and temperature induced spawns. Lasswell et al. (1978) obtained an average 5,000 eggs per spawn using hormone-induced strip spawning methods. These data indicate that southern flounder batch fecundity is inherently small, when compared to most

cultured flatfish species (Liewes 1984) and may preclude large scale culture of the species.

Courtship behavior of southern flounder observed by Arnold et al. (1977) consisted of surface swimming by both sexes near midday. Only females were observed surface swimming during the present study, suggesting males did not participate. Elevated salinity (45 o/oo) in February 1985 should not have prevented males from spawning. Hypo- and hypersaline conditions typically do not inhibit maturation and spawning of marine or estuarine fishes, although abnormal salinities may result in death of gametes (Yashouv 1969, Harvey and Kelley 1984, Zanuy and Carrillo 1984). The failure to obtain fertilized eggs during 1986 when salinities were 28-34 o/oo supports the observation that males did not spawn. Reasons for the lack of male participation are unknown. However, the tank system used in the present study was approximately one-tenth the size used by Arnold et al. (1977), and may have inhibited courtship behavior. Similar courtship behavior has also been reported for turbot (Scophthalmus maximus), with natural tank spawning inhibited by small tank size (Bromley et al. 1986). Larger and deeper tank systems would probably allow normal courtship although a large number of broodfish would be needed to produce sufficient fry for hatchery pond production of southern flounder.

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Table 1. Six-month conditioning regime designed to delay southern flounder spawning until early spring 1984, Perry R. Bass Marine Fisheries Research Station, Palacios, Texas.

Month	Temperature (C)	Photoperiod (h light/day)
Oct 1983	25	12
Nov 1983	24	12
Dec 1983	22	11
Jan 1984	20	10
Feb 1984	18	9[a]
Mar 1984	18	9

[a]Fish transferred on 13 Feb to the second recirculating tank system.

Table 2. Six-month compressed conditioning regime designed to induce out-of-season southern flounder spawning in early fall 1984, Perry R. Bass Marine Fisheries Research Station, Palacios, Texas.

Month	Temperature (C)	Photoperiod (h light/day)
Apr 1984	26	11
May 1984	28	12
Jun 1984	30	13
Jul 1984	25	11
Aug 1984	20	10
Sep 1984	18	9
Oct 1984 through Jan 1985[a]	18	9
Jan 1985 through Mar 1985	18	4

[a]Tank conditions were maintained at 18 C and 9-h light:15-h dark photoperiod until gonadal development occurred. Egg releases occurred between 18 Dec to 18 Jan.

Table 3. Four-month compressed conditioning regime designed to induce out-of-season southern flounder spawning in late summer 1985, Perry R. Bass Marine Fisheries Research Station, Palacios, Texas.

Initiated	Week	Temperature (C)	Photoperiod (h light/day)
20 Mar 1985	1	18	9.0
	2	20	9.0
	3	21	10.0
	4	23	10.5
	5	25	11.0
	6	26	12.0
	7	27	13.0
	8	28	14.0
	9	30	13.5
	10	30	13.0
	11	30	13.0
	12	28	12.5
	13	27	12.0
	14	26	12.0
	15	25	11.0
	16	22	10.0
	17	20	9.5
	18	18	9.0
29 Jul 1985	19	18	9.0
	Aug 1985 through Mar 1986[a]	18	9.0

[a]Tank conditions were maintained at 18 C and 9-h light:15-h dark photoperiod until gonadal development occurred. Egg releases occurred 8 Dec 1985 through 13 Feb 1986.

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Initiated	Week	Temperature (C)	Photoperiod (h light/day)
20 Mar 1985	1	18	9.0
	2	20	9.0
	3	21	10.0
	4	23	10.5
	5	25	11.0
	6	26	12.0
	7	27	13.0
	8	28	14.0
	9	30	13.5
	10	30	13.0
	11	30	13.0
	12	28	12.5
	13	27	12.0
	14	26	12.0
	15	25	11.0
	16	22	10.0
	17	20	9.5
	18	18	9.0
29 Jul 1985	19	18	9.0
	Aug 1985 through Mar 1986[a]	18	9.0

[a]Tank conditions were maintained at 18 C and 9-h light:15-h dark photoperiod until gonadal development occurred. Egg releases occurred 8 Dec 1985 through 13 Feb 1986.

Table 5. Number of eggs released by captive southern flounder, Perry R. Bass Marine Fisheries Research Station, Palacios, Texas. Tank conditions were 18 C and 9-h light:15-h dark photoperiod except for the period from 7 Jan- 25 Mar 1985 when photoperiod was reduced to 4-h light daily.

1984-85 Spawning season		1985-86 Spawning season	
Date	No. eggs	Date	No. eggs
18 Dec 1984	ND	08 Dec 1985	5,000
19 Dec 1984	ND	13 Dec 1985	3,200
26 Dec 1984	ND	17 Dec 1985	2,900
31 Dec 1984	ND	18 Dec 1985	2,400
02 Jan 1985	ND	24 Dec 1985	1,400
03 Jan 1985	ND	30 Dec 1985	66
08 Jan 1985	1,900	31 Dec 1985	6,900
09 Jan 1985	6,200	01 Jan 1986	4,000
10 Jan 1985	3,100	02 Jan 1986	1,000
17 Jan 1985	3,100	06 Jan 1986	18,800
18 Jan 1985	18,100	07 Jan 1986	28,900
		10 Jan 1986	1,500
		11 Jan 1986	4,800
		13 Jan 1986	9,500
		17 Jan 1986	6,100
		24 Jan 1986	6,100
		26 Jan 1986	1,600
		29 Jan 1986	4,700
		30 Jan 1986	2,800
		31 Jan 1986	20,500
		01 Feb 1986	1,900
		07 Feb 1986	3,200
		09 Feb 1986	3,500
		13 Feb 1986	28,400

ND = Not Determined

Table 5. Number of eggs released by captive southern flounder, Perry R. Bass Marine Fisheries Research Station, Palacios, Texas. Tank conditions were 18 C and 9-h light:15-h dark photoperiod except for the period from 7 Jan- 25 Mar 1985 when photoperiod was reduced to 4-h light daily.

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Date	No. eggs	Date	No. eggs
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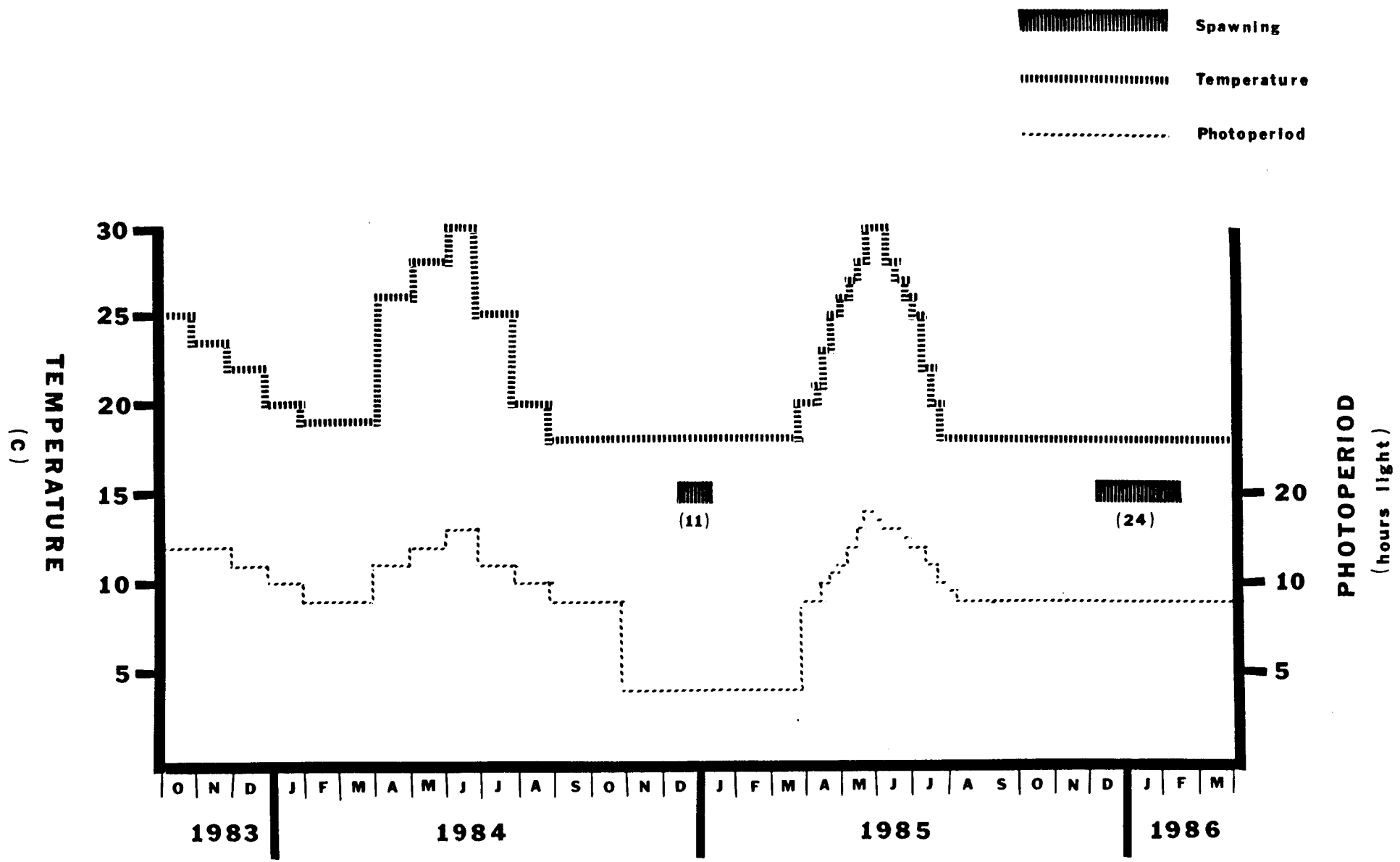
26 Dec 1984	ND	17 Dec 1985	2,900
31 Dec 1984	ND	18 Dec 1985	2,400
02 Jan 1985	ND	24 Dec 1985	1,400
03 Jan 1985	ND	30 Dec 1985	66
08 Jan 1985	1,900	31 Dec 1985	6,900
09 Jan 1985	6,200	01 Jan 1986	4,000
10 Jan 1985	3,100	02 Jan 1986	1,000
17 Jan 1985	3,100	06 Jan 1986	18,800
18 Jan 1985	18,100	07 Jan 1986	28,900
		10 Jan 1986	1,500
		11 Jan 1986	4,800
		13 Jan 1986	9,500
		17 Jan 1986	6,100
		24 Jan 1986	6,100
		26 Jan 1986	1,600
		29 Jan 1986	4,700
		30 Jan 1986	2,800
		31 Jan 1986	20,500
		01 Feb 1986	1,900
		07 Feb 1986	3,200
		09 Feb 1986	3,500
		13 Feb 1986	28,400

ND = Not Determined

Table 6. Gonadal condition of southern flounder exposed to a 4-month compressed conditioning cycle, Perry R. Bass Marine Fisheries Research Station, Palacios, Texas, 1985-86. Spawning occurred from 8 Dec 1985 through 13 Feb 1986.

Date	Sex	TL (mm)	Mean ovum[a] diameter (± SD) (mm)	Tank conditions: Temperature (C)	Tank conditions: Photoperiod (h light/day)	No. Individuals with flowing milt: Flowing	No. Individuals with flowing milt: Not flowing
04 Sep 1985[b]	Female	430[c]	no sample	18	9		
		435	fluid only				
		452	no sample				
		522	fluid only				
	Male			18	9	0	4
06 Dec 1985	Female	415	no sample	18	9		
		435	0.56 ± 0.12				
		440	fluid only				
		457	no sample				
		532	tissue and fluid				
	Male			18	9	0	4
20 Dec 1985	Female	410	1.05 ± 0.04	18	9		
		437	0.52 ± 0.80				
		445	0.60 ± 0.08				
		468	0.56 ± 0.08				
		533	0.50 ± 0.05				
	Male			18	9	3	1
14 Feb 1986	Female	415	0.75 ± 0.30	18	9		
		430	0.45 ± 0.24				
		445	0.69 ± 0.12				
		460	0.87 ± 0.28				
		535	0.60 ± 0.09				
	Male			18	9	3	1

Figure 1. Temperature and photoperiod regimes used for maturation of southern flounder, October 1983-March 1986. Numbers in parentheses denote the number of spawns.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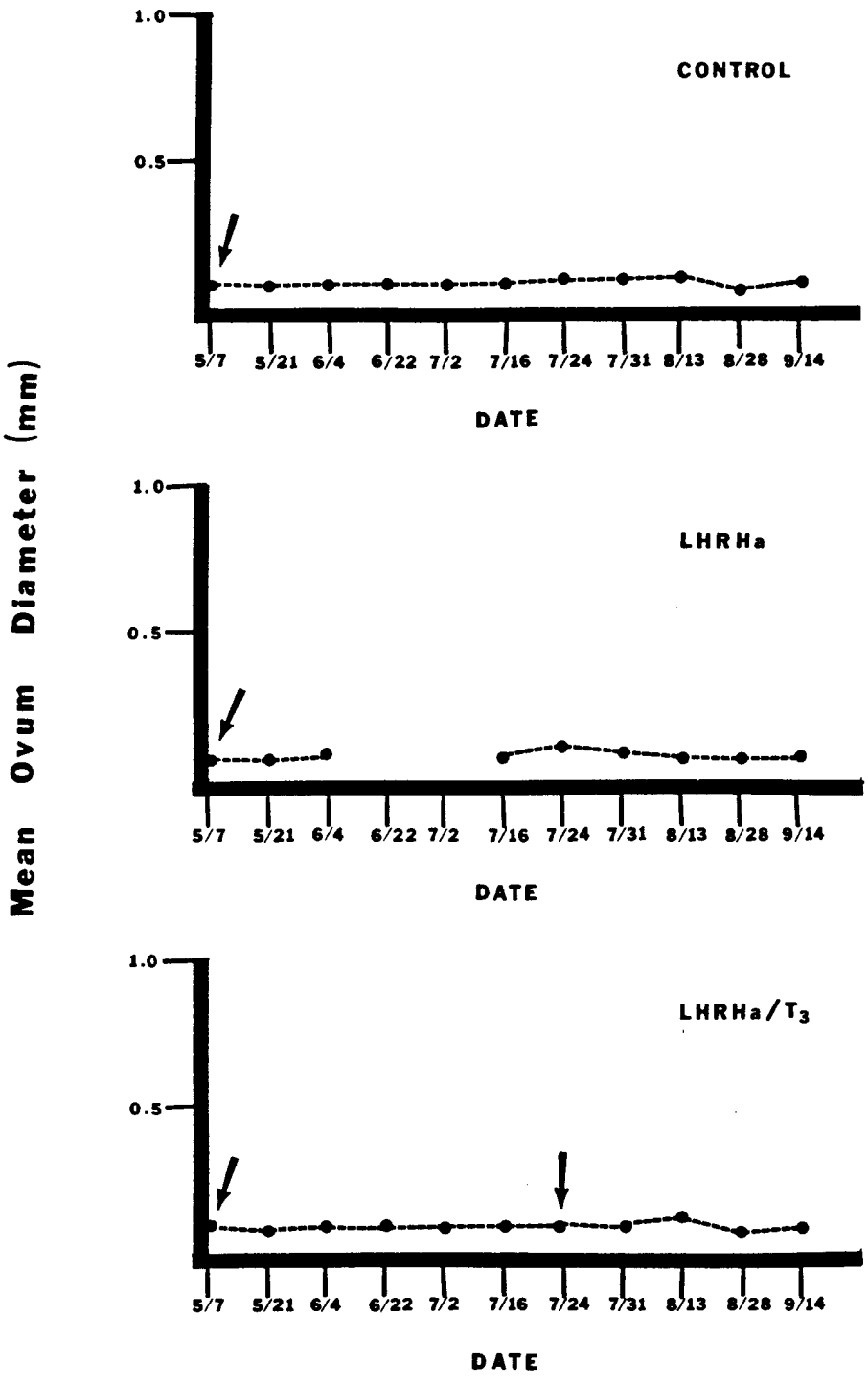
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