

Progress Toward Year-round Spawning of Southern Flounder Broodstock by Manipulation of Photoperiod and Temperature

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Abstract

Reliable methods have been developed for controlled spawning of captive southern flounder, *Paralichthys lethostigma*, broodstock during their natural winter (December–February) spawning season. From 1999 to 2004, we evaluated the effects of manipulation of photoperiod and temperature on both advance and delay spawning to produce viable embryos throughout the year. Wild-caught adult broodstock were held in 4.8- to 7.0-m³ controlled-environment tanks at a sex ratio of approximately 12 females to 4 males. Broodstock were subjected to different artificial photothermal conditioning regimes: extended winter (EW), accelerated (A-10-, A-6-, A-4.5-, and A-3.8-mo regimes), and delayed (D-16- and D-14-mo regimes), with gradual and abrupt transitions, respectively, from long to short daylengths. Under an EW cycle, fish were exposed to constant short daylengths (10 L: 14 D) after the winter solstice in January. Eighty-seven natural spawnings from December to April produced 18.3×10^6 eggs, with 20.9% hatching successfully (i.e., overall egg viability). Under an A-10-mo cycle, rate of decrease in daylength was accelerated after the summer solstice in July, to reach winter conditions in October. Seven induced spawning trials from October to November produced 897×10^3 eggs, with 40.4% viability. Under an A-6-mo cycle, rate of change of photoperiod was accelerated after the winter solstice in January, to reach winter conditions in July. Three induced spawning trials in July produced 550×10^3 eggs, with 14.7% viability. Under an A-4.5-mo cycle, broodstock exposed to EW from January through April were exposed to an accelerated cycle to reach winter conditions by October. Four induced spawning trials from September to November produced 729×10^3 eggs, with 28.7% viability. Under an A-3.8-mo cycle, broodstock exposed to EW conditions from January through April were exposed to an accelerated cycle to reach winter conditions by September. Five induced spawning trials from September to November produced 510×10^3 eggs, with 45.9% viability. Under a D-16-mo cycle, fish were exposed to a decelerated decline in photoperiod after the summer solstice in July, to reach winter conditions in May, when atretic females were observed. Under a D-14-mo cycle, fish were exposed to constant summer conditions from December through mid-June and then to an abrupt decline in photoperiod to winter conditions in late June. Six induced spawning trials from September to November produced 763×10^3 eggs, with 13.0% viability. Production of viable embryos was greatest during the extended winter because of abundant natural spawnings. While successful natural spawnings were rare during the fall or summer, viable embryos were produced through induced spawnings during all seasons of the year, with no significant ($P > 0.05$) differences in egg viability. Extended winter conditions prolonged spawning from 3 to 5 mo. Accelerated (3.8–10 mo) regimes were effective in producing viable embryos from summer through fall, but a minimum of 5 mo was required to complete gonadal recrudescence. While constant long daylengths after the summer solstice delayed gonadal recrudescence, with spawning obtained 2.5 mo after an abrupt reduction to short daylengths, a decelerated decline in photoperiod did not. Artificial control of daylength enabled precise control of gonadal recrudescence and year-round spawning in southern flounder without adverse effects on the quality of eggs and larvae and will improve availability of seedstock for commercial aquaculturists.

The southern flounder, *Paralichthys lethostigma*, is a high-valued recreationally and com-

mercially harvested marine flatfish that inhabits estuarine and shelf waters of the south Atlantic and Gulf coasts of the USA from North Carolina to Mexico (Gilbert 1986; Wenner et al. 1990). Today, the southern flounder is the number one

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flatfish species landed in North Carolina. From 1995 to 2004, commercial landings of southern flounder in North Carolina averaged 1534 mt valued at \$5,911,311 (NCDMF 2005). In 2004, commercial landings was 1115 mt valued at \$3,878,115. Recreational angler landings from 1995 to 2004 averaged 89.5 mt, but increased markedly to 196 mt in 2004. Based on the 2004 stock assessment, the southern flounder is overfished, and a management plan was implemented in 2005, including closed seasons, size and creel limits, and gear restrictions (NCDMF 2005). Declining natural populations and wide temperature and salinity tolerances of juveniles and adults make this species a versatile candidate for intensive culture in inland as well as in coastal areas of the southeastern USA (Berlinsky et al. 1996; Daniels and Borski 1998; Jenkins and Smith 1999; Smith et al. 1999a; Daniels and Watanabe 2003). Recent studies in our laboratories have demonstrated that hatchery-reared fingerlings can be grown to full marketable sizes in intensive recirculating tank systems in both freshwater and seawater with similar growth performance (H. V. Daniels, R. Murashige, and W. O. Watanabe unpublished data).

The natural spawning period for southern flounder in the Mid-Atlantic as well as in the Gulf of Mexico is late fall to winter (Powell and Schwartz 1977; Henderson-Arzapalo 1988; Berlinsky et al. 1996; Smith et al. 1999b) when adults migrate from estuaries to spawn in offshore areas (Reagen and Wingo 1985). Southern flounder have group synchronous ovarian development, and each female produces successive clutches within a spawning season (Berlinsky et al. 1996; Watanabe and Carroll 2001). Seasonal spawning limits seedstock availability to only 2–3 mo each year and represents an important constraint to commercial aquaculture.

Current evidence indicates that the seasonally changing pattern of daylength (i.e., photoperiod) is the primary determinant of seasonal spawning in intensively farmed fish species (Lam 1983; Bromage et al. 1993; Nagahama et al. 1993; see review by Bromage et al. 2001), while water temperature plays a second-

ary role in controlling the specific timing of final oocyte maturation and ovulation (Bye 1990; Carrillo et al. 1991; Davies and Bromage 1991; Tveiten and Johnsen 1999). Artificial manipulation of photoperiod is indispensable to commercial production of seedstock of a number of marine fish species (Bye 1990; Bromage et al. 2001) such as gilthead sea bream, *Sparus aurata* (Zohar et al. 1995); sea bass, *Dicentrarchus labrax* (Coves et al. 1991; Carrillo et al. 1993, 1995); Atlantic cod, *Gadus morhua* (Buckley et al. 2000); and marine flatfishes such as the Atlantic halibut, *Hippoglossus hippoglossus* (Smith et al. 1991; Bjornsson et al. 1998); turbot *Scophthalmus maximus* (Person-Le Ruyet et al. 1991); Japanese flounder, *P. olivaceus* (Kikuchi 2000); and summer flounder *P. dentatus* (Watanabe et al. 1998; Watanabe and Carroll 2001).

Techniques have been developed in recent years for hormone-induced (Berlinsky et al. 1996; Smith et al. 1999b) and natural spawning (Watanabe et al. 2001) of captive southern flounder broodstock during their natural winter spawning season. The objectives of this study were to evaluate methods for manipulation of photoperiod and temperature to both advance and delay spawning to produce viable embryos during all seasons of the year.

Methods

This study was conducted at the University of North Carolina Wilmington, Center for Marine Science (UNCW-CMS) Aquaculture Facility (Wrightsville Beach, NC, USA), and at North Carolina State University (NCSU) Tidewater Research Station (Plymouth, NC, USA), between December 2000 and 2004.

Experimental Animals

Adult southern flounder broodstock were collected in pound nets by commercial fishermen from Pamlico Sound, North Carolina, USA, from 1998 to 2003. Fish were individually tagged with internal anchor tags and held for 3 wk in flow-through seawater (34 g/L) tanks under ambient conditions before stocking into controlled-environment broodfish tank systems for photothermal conditioning and spawning

experiments. Females averaged 1.23 kg (range = 1.03–2.89 kg), while males averaged 0.80 kg (range = 0.46–1.07 kg).

Experimental System

The controlled-environment brood tank system at UNCW consisted of six outdoor circular fiberglass tanks (diameter = 2.46 m, depth = 1 m, volume = 4.76 m³) supplied with seawater of 32–37 g/L salinity pumped from the Atlantic Intracoastal Waterway. Brood tanks were insulated, had black interiors, and were provided with a conical fiberglass cover and sliding door. The cover was fitted with a timer-controlled fluorescent fixture, containing two 20-watt daylight bulbs, providing an average light intensity of 234 lx at the water surface.

Brood tanks were arranged in three groups of two, each group supported by a common water-recirculating system, consisting of a high-rate sandfilter, fluidized bed biofilter, foam fractionator, and ultraviolet sterilizer. Water from each tank drained through an egg collector (diameter = 0.76 m, depth = 0.76 m, volume = 0.24 m³) before entering a reservoir tank (diameter = 1.54 m, depth = 1 m, volume = 1.86 m³), from which water was pumped to the biofilter system. Water temperature in each system was controlled by recirculating water through a 3-hp heat pump. Water flow to each tank was approximately 38 L/min, and makeup water was added continuously to the reservoir tank to provide an exchange rate of approximately 10%/d.

At NCSU, an indoor controlled-environment brood tank system was used, consisting of two circular fiberglass tanks (diameter = 3.0 m, depth = 1.0 m, volume = 7.4 m³) supplied with artificial seawater (Crystal Seas Marinemix, Marine Enterprises International, Inc., Baltimore, MD, USA) of 32–37 g/L salinity. Water recirculation, photoperiod, and temperature control systems were similar to those described above.

Experimental Design

From 1999 to 2004, a series of trials were conducted to manipulate spawning time in southern flounder broodstock using different artificial photothermal regimes: (1) extended

winter, (2) accelerated (A-10-, A-6-, A-4.5-, and A-3.8-mo regimes), and (3) delayed (D-16- and D-14-mo regimes), with gradual and abrupt transitions, respectively, from long to short days. These different regimes (Figs. 1, 2) are illustrated in relation to a simulated natural photothermal regime (Figs. 1a, 2a) designed to simulate ambient conditions in southeastern North Carolina coastal waters. Under the natural regime, photoperiod is at a minimum (10 h) in December, then gradually increases to a maximum of 15 h in midsummer (July), declining to a minimum the next winter. Temperature changes are in phase with the photoperiod cycle, increasing from a minimum of 15 C in December to a maximum of 25 C in July and then declining to 15 C the following winter. Under these simulated natural conditions, southern flounder spawn during the winter months of December–February (Watanabe et al. 2001), consistent with the natural spawning season in North Carolina waters.

Extended Winter Regime

Under the extended winter regime (Fig. 1b), fish were exposed to simulated natural photothermal conditions from January 2003 until winter spawning conditions (10 L: 14 D; 16 C) were reached in late December 2003 and then held under constant winter conditions through May 2004.

Accelerated Regimes (A-10-, A-6-, A-4.5-, and A-3.8-Mo)

Under the A-10-mo regime (Fig. 1c), broodstock were exposed to simulated natural photoperiod conditions from January until July 2003, when maximum photoperiod conditions (15 L: 9 D) were reached. Subsequently, the rate of decrease in daylength was accelerated, so that winter photoperiod conditions (10 L: 14 D) were reached in fall (October 2003), rather than in winter (January 2004). Broodstock were exposed to constant winter temperature conditions from January to May 2003 (Fig. 1c) and then to a rapid increase to a maximum (26 C) in August 2003, followed by a rapid decline to a minimum (16 C) in November 2003.

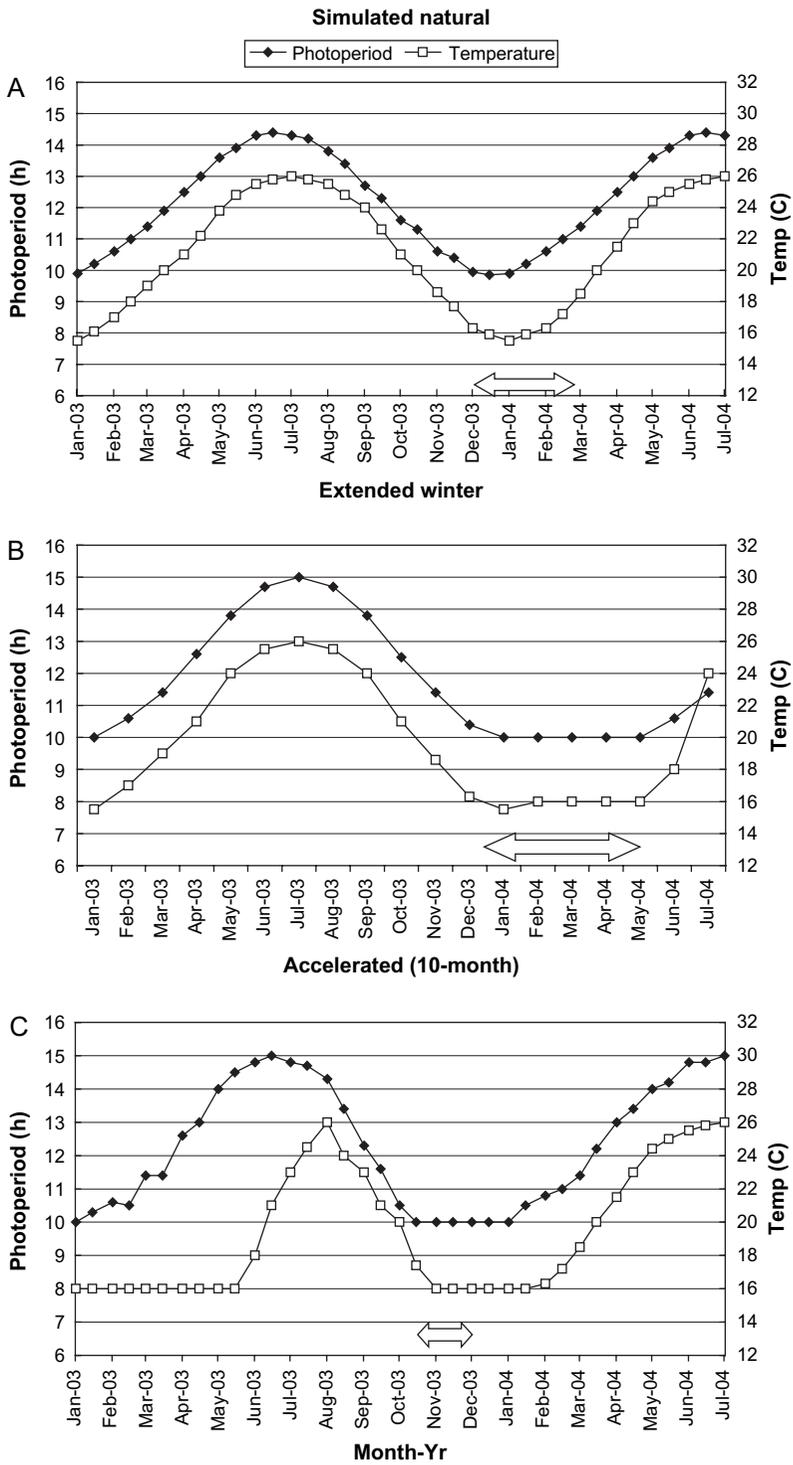


FIGURE 1. *Continued.*

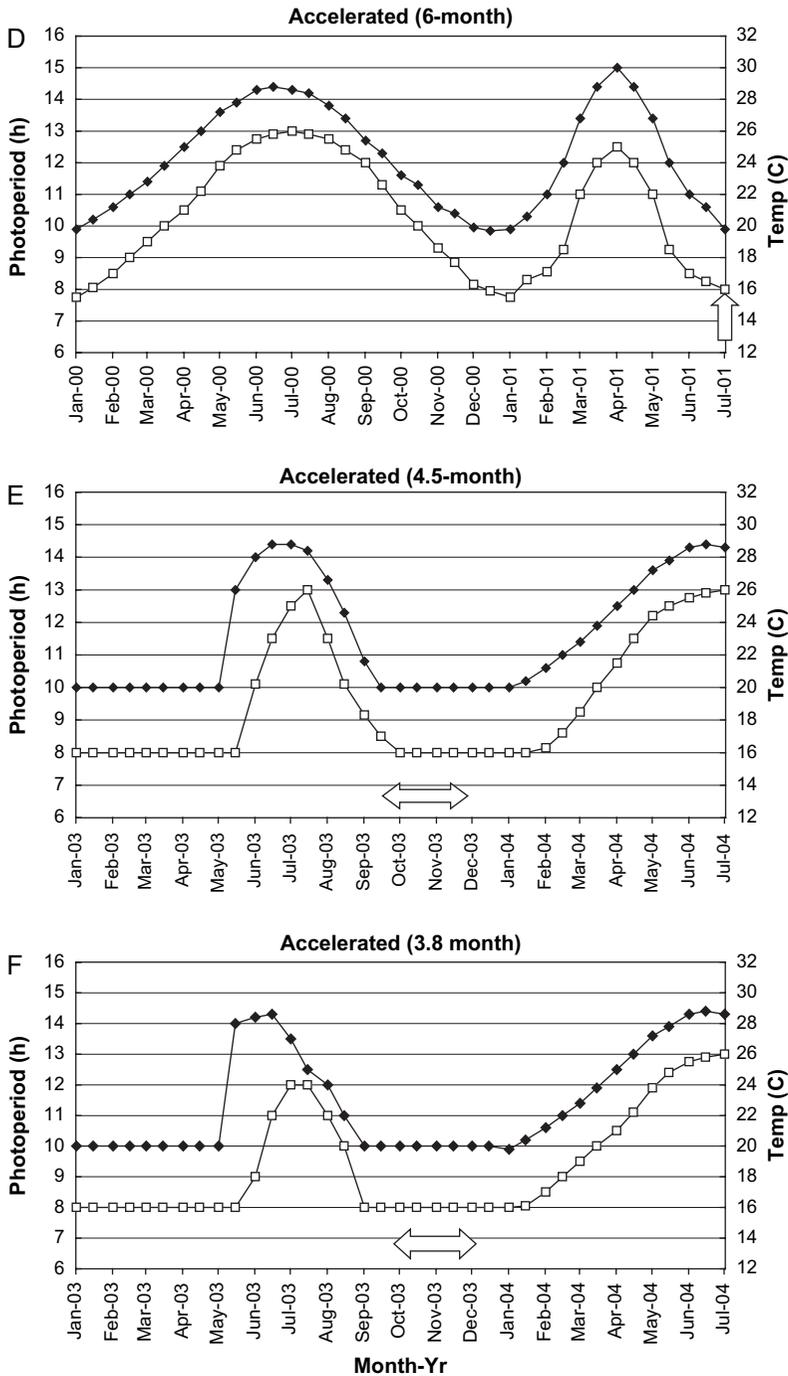


FIGURE 1. Artificial photoperiod and temperature regimes used to manipulate spawning time in southern flounder, *Paralichthys lethostigma*: simulated natural cycle (A), extended winter cycle (B), accelerated 10-mo cycle (C), accelerated 6-mo cycle (D), accelerated 4.5-mo cycle (E), and accelerated 3.8-mo cycle (F). Arrows above x-axis indicate spawning period.

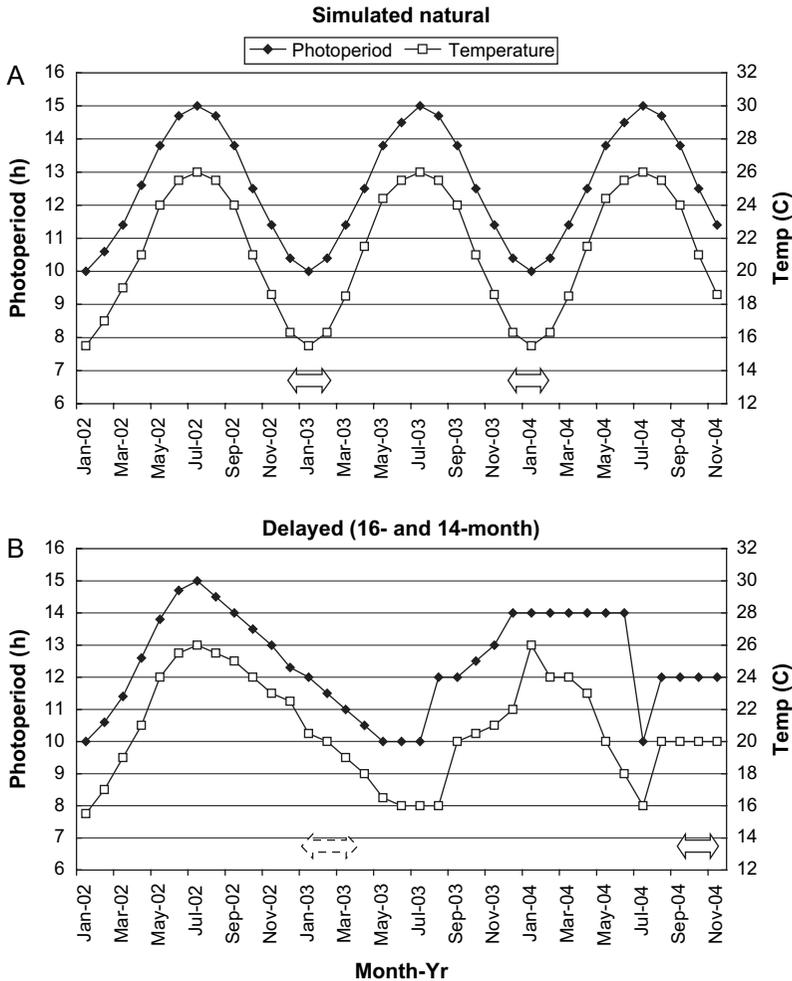


FIGURE 2. Artificial photoperiod and temperature regimes used to manipulate spawning time in southern flounder, *Paralichthys lethostigma*: simulated natural cycle (A), and delayed 16- and 14-mo cycles with gradual and abrupt transitions, respectively, from long to short daylengths (B). Arrows above x-axis indicate spawning period.

Under the A-6-mo cycle (Fig. 1d), broodstock were exposed to simulated natural photothermal conditions from January 2000 to January 2001, when the rate of change of photoperiod was accelerated, so that maximum summer photothermal conditions (15 L: 9 D; 25 C) were reached in spring (April 2001) and minimum winter conditions (10 L: 14 D; 16 C) in summer (July 2001).

Under the A-4.5-mo cycle (Fig. 1e), broodstock were exposed to constant winter conditions (10 L: 14 D; 16 C) from January through April 2002 (i.e., extended winter cycle) to prolong spawning. To condition these fish to spawn

again in midfall, broodstock were exposed to an A-4.5-mo photothermal cycle beginning in May 2002, so that maximum summer conditions (14.5 L: 9.5 D; 26 C) were reached in July 2002 and minimum winter conditions by October 2002.

Under the A-3.8-mo cycle (Fig. 1f), broodstock were exposed to constant winter photothermal conditions (10 L: 14 D; 16 C) from January through April 2003 (i.e., extended winter cycle) to prolong spawning. To condition fish to spawn again in late summer (September 2003), fish were exposed to an A-3.8-mo photothermal cycle beginning in May 2003, so that

maximum summer conditions (14.5 L: 9.5 D; 26 C) were reached in June 2003 and minimum winter conditions by September 2003.

Delayed Regimes

Under the D-16- and D-14-mo photothermal regimes (Fig. 2b), seasonal changes were extended to a period greater than 1 yr to condition the fish to spawn later than they would under natural conditions. Two consecutive delayed regimes were tested using the same broodstock, but with gradual and abrupt transitions, respectively, from long to short days. Under the first D-16-mo cycle, fish were exposed to simulated natural photothermal conditions from January through July 2002, and then to a slower than normal rate of decline in photoperiod after the summer solstice, so that minimum winter conditions were not reached until the following spring (May 2003) (Fig. 2b).

These same broodstock were then exposed to a second D-14-mo photothermal regime (Fig. 2b) in which broodstock were subjected to gradually increasing photoperiod conditions from a minimum in May 2003 to maximum summer conditions in December 2003 and then maintaining these summer conditions through mid-June 2004. Broodstock were then exposed to an abrupt decrease in photoperiod, so that winter conditions (10 L: 14 D) were reached by late June 2004. The decline in temperature from December through June was more gradual. In September 2004, photoperiod and temperature were increased to 12 L: 12 D and 20 C, respectively.

Stocking Data

For each experiment, brood tanks were each stocked with approximately 16 fish (2.7 fish/m³ or 2.59 kg/m³). Sex ratio was approximately three females to one male. Fish in two brood tanks were exposed to each photothermal regime. Fish were fed to satiation once daily (approximately 0900 h), a diet that consisted primarily of Atlantic silversides, *Menidia menidia*, supplemented with small amounts of krill and commercially prepared diets containing 45% (INVE Aquaculture, Grantsville, Utah, USA) and 55% protein (Corey Feed Mills

Ltd., New Brunswick, Canada) and 16% fat. Feeding rates averaged about 1% body weight/day.

Natural Spawning

Detailed procedures for natural and induced spawning of southern flounder and for incubation of eggs and larvae were provided in an earlier report (Watanabe et al. 2001) and are briefly described here. Under simulated natural photothermal conditions, captive southern flounder broodstock undergo gonadal maturity and often spawn naturally under winter conditions without hormone induction (Watanabe et al. 2001). Therefore, external egg collectors were checked daily for naturally spawned eggs under all photothermal regimes. Once daily, when spawning had occurred, eggs were siphoned from the collector, transferred to a 15-L separatory funnel in seawater (32–37 g/L) and buoyant eggs (“floaters”) separated from sinking eggs (“sinkers”). The number of eggs in each fraction was quantified using volumetric methods. Floaters were transferred to 15-L incubators (300–600 eggs/L) supplied with flow-through seawater at 16–19 C. Using volumetric methods, survival of embryos was estimated at time of hatching (Days 2–3 postfertilization) and at the first-feeding stage (Days 6–7 posthatching). Fertilization rate was determined as the percentage of eggs undergoing normal embryonic development, while hatching rate was determined as the percentage of larvae hatched from fertilized eggs.

Hormone-induced Spawning

Hormone-induced spawning was conducted for better control of the timing of spawning and to obtain viable eggs when natural spawnings were infrequent. Gonadal maturity of individual brooders was assessed by biopsy, and mature (i.e., vitellogenic stage) females with a mean oocyte diameter of at least 385 μ m were selected for induced spawning trials (Berlinsky et al. 1996; Smith et al. 1999b; Watanabe et al. 2001). Males were identified by the presence of milt when pressure was applied to the gonadal area. To induce spawning, selected females were implanted with a 95% cholesterol and 5% cellulose pellet (Sherwood et al. 1988)

containing [D-Ala⁶ Des-Gly¹⁰] luteinizing hormone releasing hormone (LHRH) ethylamide (GnRH-a; Sigma Chemical Co., St. Louis, MO, USA) at a dose of 50 µg/kg (Berlinsky et al. 1996; Smith et al. 1999b; Watanabe et al. 2001).

Females were strip spawned approximately 48 h after implantation, using backlighting as an aid in monitoring ovulation (Daniels and Watanabe 2003). The potential spawner was placed on a clear plexiglass table illuminated from below, and ovulatory-stage females, identified by a translucent area near the genital pore of the otherwise dark ovarian mass, were deemed ready for stripping. Eggs were collected in a glass beaker and mixed with the sperm from two males (Berlinsky et al. 1996), then left undisturbed in at least 250 mL of seawater for 15 min. The floating eggs were separated from the sinking eggs and incubated through hatching and the first-feeding stage. Fertilization and hatching rates and survival to the first-feeding stage were determined as described above. Implanted females sometimes spawned volitionally into their brood tank ("tank spawning"). Tank-spawned eggs were collected and quantified as described above for naturally spawned eggs.

Water Quality

Temperature, salinity, and dissolved oxygen were monitored in brood tanks daily, while pH, total ammonia-nitrogen, nitrite, and nitrate were monitored weekly. Mean daily values (and ranges) were as follows: salinity, 35.2 (32–37) g/L; dissolved oxygen, 7.64 (5.16–10.8) mg/L; pH, 8.12 (7.8–8.4); total ammonia-nitrogen, 0.07 (0–0.21) mg/L; and nitrite-nitrogen, 0.039 (0.0–0.375) mg/L. Temperature, salinity, dissolved oxygen, and pH were also monitored once at the end of the incubation period. Average values (and ranges) were as follows: salinity, 35.5 (34–38) g/L; dissolved oxygen, 8.50 (7.59–9.27) mg/L; pH, 8.36 (7.7–8.4); and temperature, 17.4 (16–18.6) C.

Statistics

Fertilization and hatching success were expressed as percentages of total eggs spawned. Parameter values were expressed as means and mean values compared among spawning sea-

sons by ANOVA or Kruskal Wallis test (Sokal and Rohlf 1995). All analyses were performed using Systat 11 software (Systat Software, Inc., Richmond, CA, USA).

Results

Extended Winter Regime

Under the extended winter regime (Table 1; Fig. 1b), spawning began on December 2, 2003, and continued through April 22, 2004, extending the natural spawning season from 3 to 5 mo.

A total of 87 natural spawnings produced $18,301 \times 10^3$ eggs (210×10^3 eggs/spawn), with 24.7% overall fertilization success (72.3×10^3 fertilized eggs/spawn) and 20.9% hatching success (63.8×10^3 yolk sac larvae/spawn) (Table 1). Because of prolific natural spawning, no induced spawning trials were conducted during this period.

Accelerated Regimes

Under the A-10-mo cycle, spawning began on October 29, 2001 (Table 2; Fig. 1c), about a month earlier than normal, and continued through November 30, 2001. Seven induced spawning trials were conducted, producing 897×10^3 eggs (128.1×10^3 eggs/spawn), with 39.5% fertilization success (57×10^3 fertilized eggs/spawn) and 40.4% hatching success (63.5×10^3 yolk sac larvae/spawn) (Table 2). Twenty-two natural spawnings during this period produced 3162×10^3 eggs (143.7×10^3 eggs/spawn), with 1.16% overall fertilization success (2.71×10^3 fertilized eggs/spawn) and 0.34% hatching success (0.80×10^3 yolk sac larvae/spawn) (Table 1).

Under the A-6-mo cycle, induced spawnings began on July 12, 2001 (Table 2; Fig. 1d), and continued through July 17, 2001. Eleven induced spawning trials with three females produced 550×10^3 eggs (50×10^3 eggs/spawn), with 34.3% overall fertilization success (14.1×10^3 fertilized eggs/spawn) and 15.7% hatching success (7.85×10^3 yolk sac larvae/spawn) (Table 2). No natural spawnings were observed under the A-6-mo cycle (Table 1).

TABLE 1. Summarized data on natural^a or volitional^b spawning of southern flounder, *Paralichthys lethostigma*, broodstock under different artificial photothermal regimes.

Photothermal regime	No. of days eggs observed	Spawning period	Total eggs produced ($\times 10^3$)	No. of eggs per spawn, $\times 10^3$ (SE) [range]	Floaters, % overall (SE) [range]	Fertilization success, % overall (SE) [range]	Hatching success, % overall (SE) [range]	Fertilized eggs per trial, $\times 10^3$ (SE) [range]	Yolk sac larvae per trial, $\times 10^3$ (SE) [range]
EW	87 ^a	December 2, 2003, to April 22, 2004	18,301	210.4 (15.9) [10.0–7.00]	39.9 (2.84) [0–97.6]	24.7 (2.91) [0–80.0]	20.9 (2.67) [0–74.4]	72.3 (11.6) [0–493]	63.8 (10.9) [0–411]
A-10	22 ^a	October 29, 2001, to November 30, 2001	3162	143.7 (19.6) [27.0–315]	20.8 (4.28) [0–81.7]	1.16 (0.90) [0–18.9]	0.34 (0.30) [0–6.56]	2.71 (2.13) [0–45.3]	0.80 (0.717) [0–15.8]
A-6	0	N/A	0	0	0	0	0	0	0
A-4.5	31 ^b	September 25, 2002, to November 30, 2002	6426	207.0 (17.3) [68.0–440]	21.2 (3.09) [1.84–79.0]	0	0	0	0
A-3.8	5 ^b	September 29, 2003, to November 26, 2003	777	155.4 (35.1) [106–295]	26.2 (11.9) [2.00–55.0]	0	0	0	0
D-14	0	N/A	0	0	0	0	0	0	0

EW = extended winter; A-10 = accelerated 10-mo regime; A-6 = accelerated 6-mo regime; A-4.5 = accelerated 4.5-mo regime; A-3.8 = accelerated 3.8-mo regime; D-14 = delayed 14-mo regime; nd = no data; N/A = not applicable.

Values represent totals for all trials, or means with SE in parenthesis and range in brackets.

^a Natural spawning without hormone intervention.

^b Volitional spawning after hormone induction (“tank spawning”).

TABLE 2. Summarized data on induced spawning of southern flounder, *Paralichthys lethostigma*, broodstock under different artificial photothermal regimes.

Photothermal regime	No. of females (no. of trials) ^a	Spawning period	Total eggs produced ($\times 10^3$)	No. of eggs per spawn, $\times 10^3$ (SE) [range]	Floaters, % overall (SE) [range]	Fertilization success, % overall (SE) [range]	Hatching success, % overall (SE) [range]	Fertilized eggs per trial, $\times 10^3$ (SE) [range]	Yolk sac larvae per trial, $\times 10^3$ (SE) [range]
EW	N/A	N/A	0	0	0	0	0	0	0
A-10	7	October 29, 2001, to November 30, 2001	897	128.1 (34.5) [50–275]	68.8 (10.1) [14.6–98.0]	39.5 (8.76) [9.99–65.8]	40.4 (10.9) [1.48–75.0]	57 (21.2) [5.07–161]	63.5 (25.9) [0.741–164]
A-6	3 (11)	July 12, 2001, to July 17, 2001	550	50.0 (16.2) [35.0–85.0]	62.5 (10.0) [38.1–69.8]	34.3 (8.69) [0–77.5]	15.7 (7.84) [0–24]	14.1 (21.9) [0–28.8]	7.85 (4.13) [0–14.2]
A-4.5	4 (5)	September 25, 2002, to November 30, 2002	729.0	145.8 (25.5) [98.0–241]	55.0 (13.5) [22.4–99.6]	37.7 (14.9) [13.5–95.2]	28.7 (10.5) [8.1–67.5]	69.9 (40.6) [15.0–229]	52.4 (28.5) [8.96–163]
A-3.8	5 (6)	September 29, 2003, to November 26, 2003	510.0	85.0 (20.9) [21–167]	91.5 (3.26) [78.2–99.0]	57.1 (15.1) [26.7–94.1]	45.9 (13.2) [20.5–88.1]	54.6 (18.6) [5.60–97.5]	43.3 (16.2) [5.88–92.5]
D-14	6	September 17, 2004, to November 4, 2004	763.00	127.2 (55.2) [0–350]	26.5 (18.8) [0–100]	20.9 (19.5) [0–98.9]	13.0 (14.5) [0–65.0]	10.9 (9.24) [0–47.5]	6.24 (6.24) [0–31.2]

EW = extended winter; A-10 = accelerated 10-mo regime; A-6 = accelerated 6-mo regime; A-4.5 = accelerated 4.5-mo regime; A-3.8 = accelerated 3.8-mo regime; D-14 = delayed 14-mo regime; nd = no data; N/A = not applicable.

Values represent totals for all trials, or means with SE in parenthesis and range in brackets. No significant ($P > 0.05$) differences were observed for egg production (no. of eggs per spawn), fertilization success, or hatching success among the accelerated and delayed regimes.

^a For strip spawning. An individual female was stripped one or more times on separate days.

Under the A-4.5-mo cycle (Table 2; Fig. 1e), spawning began on September 25, 2002, 5.5 mo after the previous spawning season, and continued through November 30, 2002.

Five induced spawning trials with four females produced 729×10^3 eggs (145.8×10^3 eggs/spawn) with 37.7% overall fertilization success (69.9×10^3 fertilized eggs/spawn) and 28.7% hatching success (52.4×10^3 yolk sac larvae/spawn). Under the A-4.5-mo cycle, a total of 31 tank spawnings were also observed, producing 6426×10^3 eggs, but with no fertilization success (Table 1).

Under the A-3.8-mo cycle (Table 2; Fig. 1f), spawning began on September 29, 2003, 6 mo after the previous spawning season, and continued through November 26, 2003. Six induced spawning trials with five females produced 510×10^3 eggs (85.0×10^3 eggs/spawn), with 57.1% overall fertilization success (54.6×10^3 fertilized eggs/spawn) and 45.9% hatching success (43.3×10^3 yolk sac larvae/spawn) (Table 2). A total of five tank spawnings were obtained under this A-3.8-mo cycle (Table 1), producing 777×10^3 eggs, but with no fertilization success.

Delayed Photothermal Regimes

Under the D-16-mo cycle (Fig. 2b), characterized by a gradual decline in photoperiod from summer to winter conditions, female brooders showed only atretic oocytes when winter conditions were reached in May 2003, indicating that gonad development had occurred during winter (January–February 2003) and that fish were in late stages of regression. No natural spawnings were obtained under this D-16-mo cycle.

Under the D-14-mo cycle (Fig. 2b), characterized by an abrupt decline in photoperiod from summer to winter conditions from June to July, only previtellogenic-stage oocytes were evident by late July 2004. Photoperiod and temperature were increased to 12 h and 20 C, respectively, on August 1, 2004. Mature females were observed by mid-September 2004, approximately 2 mo after the abrupt decline. Induced spawning was initiated on September 17, 2004 (Fig. 2b; Table 2), and continued through November 4, 2004. Six induced spawning trials

produced 763×10^3 eggs (127.2×10^3 eggs/spawn), with 20.9% fertilization success (10.9 fertilized eggs/spawn) and 13.0% hatching success (6.24×10^3 yolk sac larvae/spawn) (Table 2). No natural spawnings were obtained under this D-14-mo cycle.

Comparison of Reproductive Performance Among Seasons

Total fertilized egg production during the different seasonal and aseasonal spawnings is shown in Figure 3. In addition, data are shown for extended winter–spring spawnings during 1998–1999 (Watanabe et al. 2001) and 2001–2002 (W. O. Watanabe and C. A. Woolridge, unpublished data). Greatest production of fertilized eggs occurred during the extended winter–spring spawning seasons of 1998–1999, 2001–2002, and 2003–2004, ranging from 3395×10^3 eggs in 2001–2002 to 6288×10^3 eggs in 2003–2004. This was attributed to the large number of natural spawnings that were obtained during the extended winter–spring season. In contrast, natural spawnings were rare during the fall or summer out-of-season periods. Induced spawnings, on the other hand, were obtained during all seasons of the year.

Fertilization success of eggs produced by induced spawnings, ranged from 20.9% in fall 2004 to 57.1% in fall 2003, with no significant ($P > 0.05$) differences. Hatching success of eggs produced through induced spawnings ranged from 13.0% in fall 2004 to 45.9% in fall 2003, with no significant ($P > 0.05$) differences.

Discussion

Simulated Natural Photothermal Regime

The natural spawning period for southern flounder in the Mid-Atlantic as well as in the Gulf of Mexico is believed to be the winter months of December through February, when the photoperiod is 10 L: 14 D and the water temperature is between 14 and 18 C (Powell and Schwartz 1977; Henderson-Arzapalo 1988; Berlinsky et al. 1996; Smith et al. 1999b). In this study, the first appearance of mature, postvitellogenic-stage females under simulated natural photothermal regimes occurred in association with declining

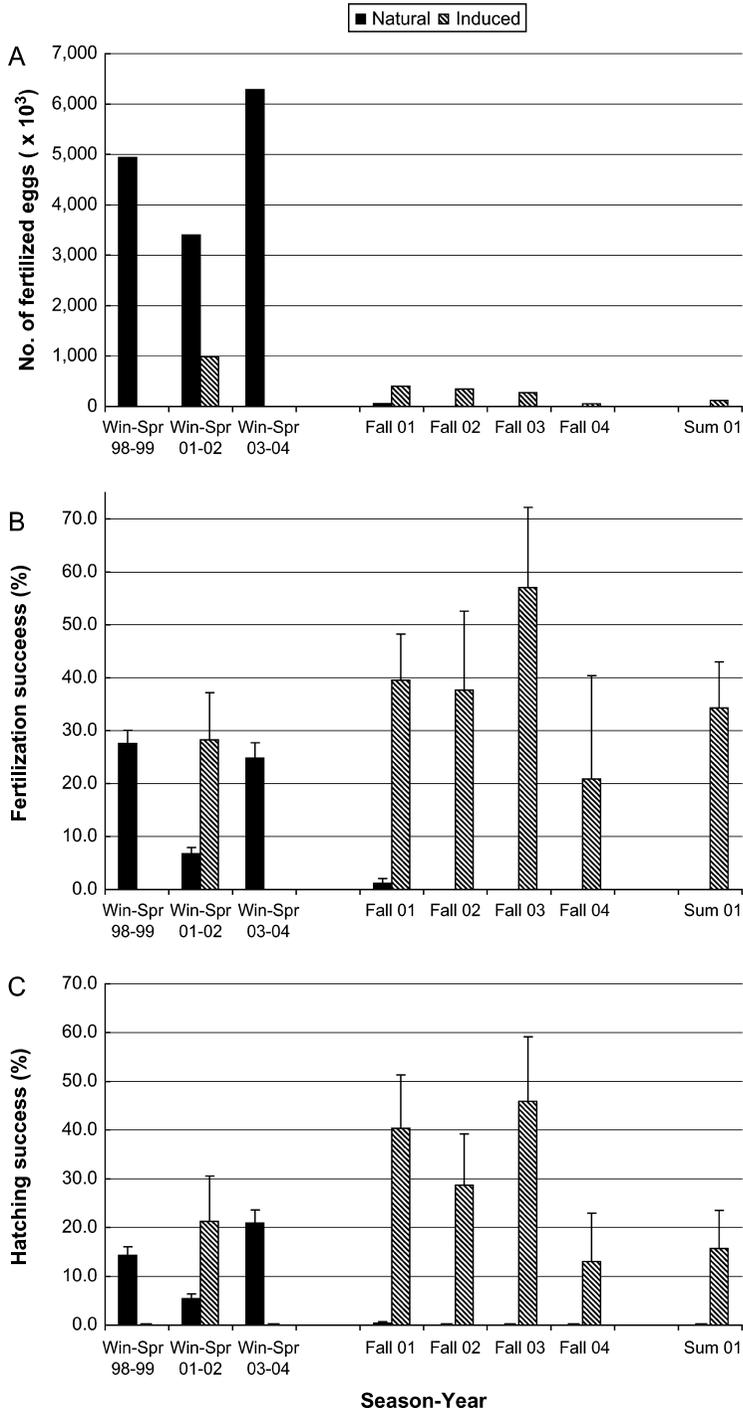


FIGURE 3. Reproductive performance of southern flounder, *Paralichthys lethostigma*, broodstock during different seasons (extended winter–spring, fall, and summer) and different years (1998–2001). Data are shown for number of fertilized eggs (A), fertilization success (B), and hatching success (C). Cumulative values are shown in A, while in B and C, values represent means \pm SE (n = 5–11 for induced spawnings and n = 5–254 for natural spawnings). For induced spawnings, fertilization and hatching success of eggs were not significant ($P > 0.05$) among seasons.

daylength and temperature just prior to the annual minima, which simulated habitat conditions during the premigratory period (Reagen and Wingo 1985). This is in accord with previous studies showing that short or decreasing photoperiod and/or low or decreasing temperature are stimulatory to gametogenesis in marine finfish species that spawn in autumn and winter, including grey mullet, *Mugil cephalus* (Kuo et al. 1974); red drum, *Sciaenops ocellatus* (Arnold 1988); California halibut, *P. californicus* (Caddell et al. 1990), and sea bass (Carrillo et al. 1995).

Extended Winter Regime

In this study, well-conditioned (>12 mo in captivity) southern flounder broodstock exposed to simulated natural photothermal conditions from January 2003 to December 2003, and then to constant winter conditions (10 L: 14 D; 16 C) through April 2004 (Fig. 1b), spawned naturally (without the use of hormones) over a prolonged period of 140 d from December 2003 through April 2004. This is consistent with earlier findings (Watanabe et al. 2001), where recently wild-caught (<4 mo in captivity) southern flounder broodstock exposed to ambient photothermal conditions from October 1998 to January 1999, and then to a constant winter photoperiod (10 L: 14 D) and temperature (14.0–24.5 C) through April 1999, spawned naturally over a period of 142 d from December 1998 through April 1999 (Watanabe et al. 2001). Wild-caught southern flounder broodstock exposed to an artificial photothermal regime simulating conditions typical of the continental shelf from November through January, and then to a constant winter regimen (10.5 L: 13.5 D; 17 C) from February through May, were induced to spawn using GnRH-a implants over a period of 99 d from January to late April (Smith et al. 1999b). The available data demonstrate that photothermal manipulation was very effective in prolonging the spawning period by at least 2 mo beyond the natural spawning season for well-conditioned as well as recently captured southern flounder broodstock. Spawning periods of Atlantic cod and haddock, *Melanogrammus aeglefinus*, have been significantly prolonged by maintaining fish on

short daylengths simulating winter spawning conditions (Buckley et al. 2000).

Accelerated Regimes

In this study, accelerated photothermal regimes were highly effective in advancing maturation and timing of spawning of southern flounder broodstock, so that rematuration and spawning were achieved in less than 12 mo. The shortening of the interspawning interval was proportional to the degree of compression (i.e., acceleration) of the photoperiod cycle. For example, beginning from winter photothermal conditions in January, fish exposed to an A-10-mo photoperiod cycle (Fig. 1c) were spawned successfully the following November, 1 mo earlier than under natural conditions. Likewise, beginning with winter photothermal conditions in January, fish exposed to an A-6-mo cycle regime (Fig. 1d) were spawned successfully in July 2000, 5 mo earlier than under natural conditions.

In this study, fish exposed to extended winter photothermal conditions to prolong spawning and then to an A-4.5-mo photothermal regime beginning in May rematured and were spawned successfully from late September through late November, only 5 mo after their previous spawning period and 2 mo earlier than under natural conditions (Fig. 1e). However, when fish were exposed to extended winter photothermal conditions till May and then exposed to an even more A-3.8-mo regime, they rematured and were spawned successfully from late September through late November, again 5 mo after their previous spawning period (Fig. 1f). Hence, compression of the photoperiod cycle from 4.5 to 3.8 mo did not shorten the interspawning period. This suggests that a minimum of 5 mo was required for rematuration and spawning under accelerated photothermal conditions, probably because of the time required for post-spawning fish to regain the requisite levels of energy and storage depots (e.g., lipid) and for deposition of yolk into the growing ovary (Rowe et al. 1991; Bromage et al. 2001).

In this study, frequency of successful natural spawnings declined with degree of acceleration or deceleration of the photoperiod cycle.

Highest numbers of viable naturally spawned eggs were produced during the extended winter–spring period following exposure to a simulated natural photothermal regime. A small number of naturally spawned eggs were produced under the A-10-mo regime, but none were produced under the A-6-, A-4.5-, or A-3.8-mo regimes or under the D-16- and D-14-mo regimes. This is also consistent with numerous reports indicating that advancing maturation reduces the percentage of spawning fish and with the idea that postspawning fish must regain energetic and nutritional status for rematuration and spawning to occur (Bromage et al. 2001).

Delayed Regimes

In rainbow trout (*Oncorhynchus mykiss*), Pacific salmon (*Oncorhynchus* spp.), and Atlantic halibut, spawning was delayed if the seasonal photoperiod was extended into periods of time longer than 1 yr (MacQuarrie et al. 1979; Bromage et al. 1984, 1993, 2001; Bjornsson et al. 1998). In this study, under the D-16-mo photothermal regime, fish were exposed to a gradual (i.e., decelerated) decline in photoperiod over a period of 12 mo, from maximum summer conditions in July 2003 to minimum winter conditions in July 2004 (Fig. 2b). However, only atretic oocytes were observed in July 2004, suggesting that females had developed and then regressed their gonads earlier that winter. This further suggested that, starting from the summer solstice (i.e., maximally regressed conditions), decreasing photoperiod and temperature, although at a rate much slower than under natural conditions, was sufficient to induce gonadal recrudescence the following winter (December–February), although artificial spring photothermal conditions prevailed. This is in agreement with a number of studies with commercially cultured marine finfish (e.g., turbot, sea bass, gilthead bream, and Atlantic halibut) showing that direction of photoperiod change is more important to gonadal development than rate of change or absolute length of the photoperiod and that spawning can occur at a different daylength, depending on degree of acceleration or delay of the light cycle (Bjornsson et al. 1998; Bromage et al. 2001).

To avoid gonadal maturation and spawning observed under declining, albeit decelerated, photothermal conditions, an alternative delayed regime was tested, in which photoperiod was held constant at 14 L: 10 D for 6 mo after summer conditions were reached in December 2003 (Fig. 2b). Then, in June 2004, photoperiod was abruptly decreased to minimal winter conditions in early July. In contrast, the decline in temperature from January to July was gradual. Because no gonadal development was observed by early September, photoperiod and temperature were increased slightly to 12 L: 12 D and 20 C, respectively, and mature females were obtained and successfully spawned in mid-September. Thus, gonadal recrudescence was effectively delayed by exposing fish to constant long daylengths after the summer solstice, as has been observed in a number of fish species, including rainbow trout (Whitehead and Bromage 1980), Atlantic salmon (Taranger et al. 1998), and gilt-head sea bream (Kissil et al. 2001). The results also suggested that, starting from the summer solstice (i.e., maximally regressed conditions), 2.5 mo were required to complete ovarian development following an abrupt transition of photoperiod/temperature to winter conditions. Onset of vitellogenesis in grey mullet occurred 2 mo after exposure to a short photoperiod (6 L: 18 D) regardless of preconditioning effects, at temperatures ranging from 17 to 26 C (Kuo et al. 1974).

Egg Production by Season

When fertilized egg production was compared among different seasons and different years (Tables 1, 2; Fig. 3), production of fertilized eggs was greatest during the winter and spring (i.e., extended winter regime), primarily because of abundant natural spawnings during this period. Hormone-induced spawnings and viable eggs, however, were obtained during all seasons of the year.

Based on fertilization and hatching success of eggs produced by induced spawnings during different seasons of the year, gamete quality during the out-of-season periods (fall and summer) was no different from that achieved during the winter–spring period. From 1998 to 2004, mean

egg viability (percent overall hatching success) during the fall (32.0%) and summer (14.7%) was not significantly ($P > 0.05$) different from that achieved during the winter–spring period (11.2%). In rainbow trout, quality of the gametes under advanced or delayed spawning was no different from that achieved under ambient conditions (Bromage et al. 1992).

Summary and Conclusions

The results of the present study demonstrated that photothermal manipulation enabled precise control of gonadal recrudescence and year-round spawning in southern flounder. Simulated natural conditions followed by extended winter conditions prolonged spawning from 3 to 5 mo. Accelerated (3.8–10 mo) photothermal regimes were effective in producing viable embryos from summer through fall, but a minimum of 5 mo was required to complete gonadal recrudescence, even under a radically accelerated photoperiod cycle. A D-16-mo regime with decelerated decline in photoperiod did not delay gonadal recrudescence. On the other hand, constant long daylengths after the summer solstice delayed gonadal recrudescence and an abrupt transition to short daylengths stimulated gonadal recrudescence after 2.5 mo. Under altered photoperiod regimes, gonadal maturation and spawning occurred at different daylengths, consistent with the idea that direction of change is more important than absolute length of the photoperiod. Natural spawnings were commonly observed during winter and spring (i.e., extended winter), but hormone-induced spawnings were needed to produce viable eggs in fall and summer.

Artificial control of daylength enabled spawning and production of viable eggs in southern flounder during all four seasons of the year, without adverse effects on the quality of the eggs and larvae. These techniques will improve availability of seedstock for commercial aquaculturists.

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