Sustained, Natural Spawning of Southern Flounder  
*Paralichthys lethostigma* Under an  
Extended Photothermal Regime  

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**Abstract**  
Hormone-induced spawning of southern flounder *Paralichthys lethostigma* has produced substantial numbers of viable eggs, but wide variations in fertilization and hatch rates have been reported. Recently, sustained natural spawning of southern flounder broodstock, without hormone induction, has been achieved in our laboratory. Adults (average weight = 1.12 kg; *N* = 25), including 6 captured as juveniles in 1993 and 19 captured as adults during September 1998, were stocked in two 4.8-m³ controlled-environment tanks in October 1998 and held under natural photothermal conditions until January 1999, when an artificial winter photoperiod of 10 L:14 D was initiated and then maintained through April 1999. Sex ratio was approximately 13 females:8 males:7 unknown. Natural spawning was observed in early December 1998 and increased in frequency to a peak in March 1999, before declining in late April. Water temperature ranged from 13.9 to 24.5 °C during the spawning period. Natural spawnings over 142 d produced a total of 18.3 × 10⁶ eggs, with a mean fertilization rate of 28.0% (range = 0–100%), yielding 4.94 × 10⁶ fertilized eggs. The mean percentage of eggs that remained buoyant in full-strength seawater (34 ppt) was 41.3% (0–98%), while hatching rate of buoyant eggs was 37.3% (0–99%) and survival of yolksac larvae to the first-feeding stage was 30.2% (0–100%). Gonadal biopsies in late April identified six females from both tanks as probable spawners. A preliminary comparison suggests that natural spawning produced much larger numbers of viable eggs per female, with higher egg quality (i.e., fertilization and hatching success) than hormone-induced spawning. In contrast to natural spawning, hormone-induced strip-spawning enabled timing of spawnings to be more precisely controlled. These results suggest that a combination of both natural and hormone-induced spawning of photothermally conditioned fish will help produce the large numbers of eggs required to support commercial production.

The southern flounder *Paralichthys lethostigma* is a high-valued recreationally and commercially harvested flatfish found in estuarine and shelf waters of the Atlantic and Gulf coasts of the United States from North Carolina to Mexico (Gilbert 1986). With the implementation of fishery quotas for the summer flounder (*P. dentatus*) in the early 1990s, landings of southern flounder have increased. Today, the southern flounder is the number one flatfish species landed in North Carolina, and management plans are being developed to protect this fishery, which is also threatened by overfishing (Copeland et al. 1999).

Interest in the southern flounder as an aquaculture candidate in the southeastern United States is related to its wide temperature and salinity tolerances; 50-d old juveniles can tolerate salinities as low as 5 ppt, while older juveniles can tolerate freshwater (Smith et al. 1999a). This suggests that this species could potentially be cultivated in inland fresh and brackishwater ponds or recirculating tanks, as well as in coastal areas (Berlinsky et al. 1996; Daniels et al. 1996; Jenkins and Smith 1999; Smith et al. 1999a). At present, reliable methods for controlled breeding and production of
high quality eggs are needed for development of commercial growout systems.

In Japan, where commercial culture of the congeneric flounder species *P. olivaceus* (Japanese flounder) is well established, commercial hatcheries rely mainly on photothermal conditioning and natural spawning of Japanese flounder broodstock to supply the large numbers of eggs needed to support growout operations and re-stocking programs (Iijima et al. 1986; Tsujigado et al. 1989; Mihelakakis et al. 1995). In the United States, natural spawnings of paralichthid species (including the southern flounder) have been rare, and culturists have therefore focused on hormone-induced spawning to produce embryos for research and commercial applications. Intramuscular implantation of a cholesterol-cellulose pellet containing gonadotropin releasing hormone-analogue (GnRHa) has produced repetitive spawning and substantial numbers of viable eggs in southern flounder, but wide variations in fertilization and hatch rates have been reported (Berlinsky et al. 1996).

Recently, sustained, natural spawning, without hormone induction, of captive Southern flounder broodstock was achieved in photothermally-conditioned tanks. The objectives of this paper are to describe the environmental and culture conditions and the reproductive performance associated with natural spawning. A preliminary comparison of natural spawning and hormone-induced spawning is also made.

**Materials and Methods**

**Natural Spawnings**

This study was conducted at the Center for Marine Science, University of North Carolina at Wilmington (CMS-UNCW), between December 1998 and April 1999. Adult broodfish southern flounder used in this study were obtained from two sources. One group (“laboratory-reared”) was collected as juveniles at 100–105 mm standard length in the summer of 1993 near Beaufort, North Carolina and raised to the adult stage in flow-through seawater tanks. A second group (“wild-caught”) was captured as adults by commercial poundnet fishermen during September 1998 in Pamlico Sound, North Carolina. Wild-caught fish were held in 20-m diameter outdoor concrete tanks supplied with flow through brackish water (12–20 ppt salinity) for 6 wk before transport to CMS-UNCW. All fish were individually tagged with internal anchor tags and held for 3 wk in flow-through seawater (34 ppt) tanks under ambient conditions before stocking into a controlled-environment broodfish tank system.

The controlled-environment broodfish tank system consisted of two outdoor circular fiberglass tanks (diameter = 2.46 m; depth = 1 m; volume = 4.76 m³). Brood tanks were insulated and provided with a conical fiberglass cover fitted with a timer-controlled, fluorescent fixture, containing two 20-watt daylight bulbs. Average light intensity at the water surface was 234 lux. Both tanks were 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 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% per d. Immersion heaters placed in the reservoir tank controlled water temperature.

In October 1998, one broodtank (tank 1) was stocked with 13 fish (2.73/m³) consisting of six “laboratory-reared” and seven “wild-caught” adults with an average weight of 0.948 kg (2.59 kg/m³). At stocking, sex ratio was 7 female: 3 males: 3 unknown. In November 1998, the second broodtank (tank 2) was stocked with twelve fish (2.52/m³), consisting of only wild-
caught adults, with an average weight of 1.28 kg (3.23 kg/m³). Sex ratio was unknown at the time of stocking.

Gonadal maturity of individual brooders was assessed periodically (23 November 1998, 11 January 1999 and 27 April 1999) by biopsy of anesthetized (0.3 g/L 2-phenoxethanol) fish, using a polyethylene cannula (1.57 mm o.d. × 1.14 mm i.d.) (Shehadeh et al. 1973; Watanabe et al. 1998). Ovarian samples were preserved in a solution of 10% formalin in seawater. General stage of oocyte development (i.e., pre-vitellogenic, cortical vesicle, vitellogenic, and atretic) was determined from the microscopic appearance, and males were identified by the presence of milt when pressure was applied to the gonadal area.

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 of broodstock averaged about 1% body weight per d.

The natural reproductive period of Southern flounder in North Carolina waters is presumed to be January (Berlinsky et al. 1996). Fish in the outdoor tanks were exposed to ambient light and temperature conditions until 9 January 1999. At this time, timers were used to maintain a constant winter photoperiod of 10L: 14D and heaters maintained water temperature above approximately 14.5 °C (Fig. 1).

Egg collectors were checked daily for spawned eggs. Once daily, when spawning had occurred, eggs were siphoned from the collector, transferred to a 15-L separatory funnel in seawater (32–37 ppt) and buoyant eggs ("floaters") separated from sinking eggs ("sinkers"). The numbers of eggs in each fraction were estimated using volumetric methods.

Floaters were transferred to 15-L airlift "in-tank" incubators placed inside the reservoir tank or situated in an indoor laboratory. In-tank incubators were stocked at a
density of 1,000 eggs/L, while indoor incubators were stocked at densities of 300–600 eggs/L. Indoor incubators were provided with 1-μm-filtered seawater (sterilized by ultraviolet light) at 16–19°C and supplied with diffused aeration. Using volumetric methods, survival of embryos was estimated at time of hatching (d2–d3 post-fertilization) and at the first-feeding stage (d6–d7 post-hatching), when 100% of the larvae possessed functional (fully pigmented) eyes, mouth, and alimentary tract.

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. Fertilization rates and hatching rates were expressed as percentages of total eggs and of buoyant eggs. Survival to the first-feeding stage was expressed as a percentage of total and of buoyant eggs. Data from each tank were maintained separately.

**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) ppt; dissolved oxygen, 7.64 (6.01–9.01) mg/L; pH 8.12 (7.8–8.4); total ammonia-nitrogen, 0.029 (0–0.08) mg/L; nitrite-nitrogen, 0.022 (0.003–0.051) mg/L; nitrate-nitrogen, 2.75 (0.6–9.4) mg/L. Temperature, salinity, dissolved oxygen and pH in the incubators were also monitored once at the end of the incubation period. Average values (and ranges) were as follows: salinity, 35.5 (34–38) ppt; dissolved oxygen, 8.50 (7.59–9.27) mg/L; pH 8.36 (8.2–8.5); temperature 17.4 (16–18.6) C.

**Hormone-Induced Spawning**

Hormone-induced spawning trials were conducted at the Tidewater Research Station (North Carolina State University) in Plymouth, North Carolina over two seasons (1999 and 2000). Adult southern flounder (average weight 1.2 kg) were captured by commercial poundnet fishermen during September 1998 in Pamlico Sound, North Carolina. Fish were stocked in September 1998 into tanks (diameter = 3.1 m, depth = 1.0 m; volume = 7.4 m³) supplied with recirculating seawater (34 ppt). Broodfish were exposed to artificial photoperiod and temperature conditions simulating ambient, reaching 9 h L:15 h D and 16°C by 15 December and gradually increasing to 15 h L:9 h D and 25°C by 21 June. Beginning in January 1999, females with maximum oocyte diameters of 500 μm were selected for hormone-induced spawning. To induce spawning, females were implanted with a 95% cholesterol and 5% cellulose pellet (Sherwood et al. 1988) containing [D-Ala⁶ Des-Gly⁸] LHRH ethylamide (GnRH-a, Sigma Chemical Co., St. Louis, Missouri, USA) at a dose of 100 μg/kg (Berlinsky et al. 1996).

Following hormone treatment, females were either allowed to spawn volitionally in the tanks ("tank spawning"), or spawned by applying manual pressure to the abdomen of ovulated females ("strip-spawning"). Before strip-spawning, ovulation was first determined by the release of small amounts of eggs upon gentle abdominal pressure ("squeeze check"). Eggs from a single female were collected in a glass beaker and mixed with the sperm from two males (Berlinsky et al. 1996), then left undisturbed in at least 100 mL of seawater for 1 h. The floating eggs were separated from the sinking eggs in a 1-L separatory funnel. Embryos were incubated in a 70-L round fiberglass tank containing 34 ppt filtered seawater at 16°C and at a maximum density of 500 eggs/L. Fertilization and hatching rates were determined as described above.

In 2000, an alternative method was used to monitor ovulation. Under this "light table" method, developed by commercial aquaculturists (George Nardi, Great Bay Aquafarms, personal communication), the potential spawner was placed on a clear
plexiglass table illuminated from below by an incandescent bulb, allowing light to be transmitted through the fish. Because of the flattened body conformation of flounder, size and location of the gonads were visualized through the body wall. Females in which portions of the large ovarian mass turned from dark to translucent, due to intra-ovarian oocyte hydration and ovulation, were selected for strip spawning as described above.

Results

Natural Spawnings

Gonadal biopsy on 23 November 1998 revealed a few individuals with late yolk globule stage eggs with mean oocyte diameters ranging from 437–445 μm and running ripe males. On 3–5 December 1998, fully hydrated ova were first observed in the egg collector from tank 1, consisting of both laboratory-reared and wild-caught adults. Since none of these individuals had been treated with hormones, natural, volitional spawning had occurred. From 99,600 to 253,700 unfertilized eggs were released daily from 3–5 December, but spawning ceased through the remainder of December. On 4 January 1999 spawning resumed in tank 1 and increased in frequency to a peak in March 1999 before declining in mid-April (Fig. 2). Fertilized eggs were first observed on 12 January in tank 1 (Fig. 2).

In tank 2, which consisted entirely of wild-caught adults, onset of natural spawning was delayed, with unfertilized eggs first observed in the egg collector on 5 February 1999 (Fig. 3). Natural spawning in tank 2 increased in frequency to a peak in late March and early April before declining in late April. In tank 2, fertilized eggs were first observed on 2 March.

When data from both tanks 1 and 2 are considered (Fig. 4), consistent spawning began in early January, increased in frequency during the month of February to a peak in March before declining to a low level by late April. During a 142-d spawning period from 3 December 1998 to 23 April 1999, eggs were collected on 70 d in tank 1 (Fig. 2) and on 53 d in tank 2 (Fig. 3; Table 1). Total numbers of eggs collected per d from each tank ranged from 5,490 to 601,250, averaging 159,596 in tank 1 and 133,104 in
Total eggs production per day during natural spawning of wild-caught broodstock southern flounder broodstock in a 4.76-m$^3$ tank. Height of each bar represents numbers of fertilized and unfertilized eggs, while black portions of each bar represent numbers of fertilized eggs.

For the duration of the 142-d spawning period, a total of 11,331,319 eggs were collected from tank 1 and 7,054,489 were collected from tank 2, for a total of 18,385,808 eggs from both tanks and for an average of 129,477 eggs per d (Table 1). As evidenced by the various stages of embryonic development at the time of collection,
spawning occurred at all times of day, with no diurnal pattern evident.

**Egg Buoyancy, Fertilization, and Hatching**

Fertilization rates, expressed as a percentage of total naturally spawned eggs, varied from day to day from 0 to 97.2% (Fig. 4), averaging 30.6% in tank 1 and 24.9% in tank 2, with an overall average of 28.0% for both tanks (Fig. 2; Table 1). A total of 3,470,516 fertilized eggs were produced in tank 1, while 1,472,577 were produced in tank 2, for a total of 4,943,092 for both tanks combined. Availability of fertilized eggs from both tanks, however, generally increased to a maximum in March and early April (Fig. 4). On days that spawning was observed, an average of 34,811 fertilized eggs were collected from each tank.

During incubation of eggs in full-strength seawater (32–36 ppt), the percentage of total eggs spawned that remained buoyant (i.e., “floaters”) varied among spawnings from 0 to 99.2%, with an average of 41.3% for both tanks (Table 1). When all egg batches for both tanks were considered, a significant ($P < 0.01$) linear relationship between percentage egg buoyancy and overall fertilization rate was evident (Fig. 5a), with 61.3% of the variance in buoyancy rate accounted for by the variation in the fertilization rate of the eggs. However, when only eggs for which rate of buoyancy was determined in the later gastrula and neurula stages were considered (Fig. 5b), a higher percentage (81.5%) of the variance of buoyancy rate was accounted for by the variation of the fertilization rate of the eggs (Fig. 5b). This was related in part to the fact that, in some egg batches in which fertilization was determined during the early cleavage stages, development was arrested prior to the blastula stage. These eggs were characterized by irregular cleavage during early cell division (Figs. 6b, d) and by a darkening of the germ cells during the pre-blastula stage (Fig. 6f), followed a short time later by a brownish discoloration of the egg cytoplasm around the central region (Fig. 6f). This necrotic process was associated with a loss of buoyancy.

For both tanks 1 and 2, fertilization rates of floaters averaged 61.8% (Table 1). Hatching rate averaged 37.3% of floaters (15.4% overall). Survival of yolk sac larvae to the first-feeding stage averaged 30.2% of floaters (12.5% overall) for both tanks, producing a total of 2,397,206 first-feeding stage larvae.

**Temperature Considerations**

At the time of the first spawnings from 3–5 December 1998, water temperature averaged 20.1–21.2 C, although no fertilized eggs were obtained during this period. No spawnings were observed as water temperatures declined sharply during the month of December, reaching a minimum of 10.9 C on 27 December. From 5 January to 23 April, the period during which the majority of spawnings occurred, water temperatures varied over a wide range of 13.9 C to 24.5 C (Fig. 1). Fertilized eggs were also obtained under this temperature range, although availability of fertilized eggs increased to a peak during March and early April, while water temperature averaged around 17.5 C. Numbers of eggs spawned decreased as water temperatures increased steadily in April. Spawning appeared to be stimulated by a change in weather conditions; a warming or cooling trend was followed by an increase in egg release.

**Growth and Sex Ratios of Broodstock**

A gonadal examination of all broodstock made on 11 January 1999 revealed the following sex ratios: 8 females: 3 males: 2 unknown in tank 1 and 5 females: 2 males: 5 unknown in tank 2. Males were identified by the presence of running milt. A gonadal examination of all broodstock made on 27 April 1999 revealed four individuals in tank 1 and two individuals in tank 2 with atretic, hydrated eggs, suggesting probable spawners. Assuming that these six individuals
participated in spawning, an average of 3,064,301 eggs was released per female (approximately 2,760,632/kg) during the study period.

During the period 11 January to 27 April, average weight and biomass density of fish in both tanks increased slightly from 1.09 kg and 1.86 kg/m$^3$ to 1.12 kg and 2.94 kg/m$^3$, while average TL remained unchanged at 45.3 cm. On 27 April, males averaged 43.9 cm and 1,009 g, not significantly different from females, which averaged 45.1 cm and 1,134 g.

**Hormone-Induced Spawnings**

In 1999, 14 tank spawnings following hormone induction produced 345,000 eggs, of which 55% were floaters, but no fertilization was obtained under this method (Table 2). Seventeen strip-spawning trials using the “squeeze check method” to monitor ovulation yielded 756,000 eggs, of which 68% were floaters, with 40% of these floaters fertilized (27% overall). Hatching rate of floaters averaged 30%. Of the 17 strip-spawning trials, only five produced fertilization rates of >70%. These spawnings were associated with relatively high rates of buoyancy (78%), fertilization (80% of floaters, 62.4% overall) and hatching rate (77% of floaters).

In 2000, 26 hormone-induced strip-spawning trials using the “light table method” to monitor ovulation produced 1,658,000 eggs, of which 466,000 (28%) were floaters, with 61% of these floaters fertilized (19% overall). Hatching rate of floaters averaged 38%.

For both seasons (1999–2000) combined, 57 hormone-induced spawning trials produced 2,759,000 eggs, of which 1,170,000 (42%) were floaters, with 40% of these floaters fertilized (17% overall). Hatching rate of floaters averaged 26%.

**Discussion**

In this study, commercially significant quantities (i.e., 4.94 × 10$^6$) of viable embryos of southern flounder were produced through sustained, natural spawning of captive broodstock. Previous attempts to obtain natural spawning and fertilization of this species had limited success. Using photoperiods and temperatures which simulated natural seasonal changes, Arnold et al. (1977) obtained natural spawning from 3 of 6 females for 13 consecutive days in 30-m$^3$ tanks producing 120,000 eggs with a fertilization rate of 30–50% and a hatching rate of 6–35% of fertilized eggs. In a later study (Henderson-Arzapalo et al. 1988), controlled photoperiod and temperature were also effective in stimulating release of 200,000 eggs from 5 females over 2 spawning seasons, but no fertilization was ob-

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**Table 1.** Summarized data on natural spawning of southern flounder broodstock in two 4.76-m$^3$ tanks (3 Dec. 1998 to 23 April 2000). Each tank was stocked with 12–13 adults. Data are presented for each tank and for both tanks combined.

<table>
<thead>
<tr>
<th>Tank no.</th>
<th>No. of days observed</th>
<th>Total eggs spawned (no. spawned per day)</th>
<th>No. of floaters (no. per day)</th>
<th>Floaters (%) (range)</th>
<th>Fertilization rate (%) overall</th>
<th>Fertilization rate (%) floaters (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70</td>
<td>11,331,319 (5,490–601,250)</td>
<td>5,737,902 (0–412,750)</td>
<td>46.6</td>
<td>30.6</td>
<td>61.3</td>
</tr>
<tr>
<td>2</td>
<td>53</td>
<td>7,054,489 (22,750–419,000)</td>
<td>2,199,867 (0–244,000)</td>
<td>34.2</td>
<td>24.9</td>
<td>62.3</td>
</tr>
<tr>
<td>1 ± 2</td>
<td>123</td>
<td>18,385,808</td>
<td>7,937,769</td>
<td>41.3</td>
<td>28.0</td>
<td>61.8</td>
</tr>
</tbody>
</table>

1. Estimated as hatching rate (% overall) = floaters (%) × hatching rate (% of floaters).

2. Estimated as survival to first-feeding (% overall) = floaters (%) × survival to first-feeding (% of floaters).
tained due to lack of male participation in spawning.

In this study, an average of 3,064,301 eggs was released per female (2,760,632/kg) during the study period, comparable to what Smith et al. (1999b) reported for the southern flounder under hormone-induced tank spawning (5,927,333/female; 1,924,459/kg). This is also similar to what was reported for the Chilean flounder P. microps, which produced an average of 2,400,000 eggs per kg female during natural spawning in tanks. These fecundity levels are low when compared to the Japanese flounder, which produce up to 25 million eggs per female over a 6-mo period (Smith et al. 1999b). Relatively large numbers of southern flounder broodstock may be required by commercial hatcheries to achieve equivalent levels of production.

The results of this study demonstrate that wild-caught southern flounder adults, conditioned through photothermal manipulation for only 6 mo, can be spawned successfully without hormone induction during their first season in captivity. This is important to avoid a prolonged period of acclimation to captivity. In wild-caught turbot Scophthalmus maximus, efficient spawning occurred only after 2 yr of habituation to captivity (Devauchelle et al. 1988). In southern flounder, only females >2 kg spawned naturally in captivity (Arnold et al. 1977), and successful hormone-induced tank spawning was attained with broodstock held in captivity for at least 1.5 yr and receiving photothermal conditioning for a minimum of 12 wk prior to spawning (Smith et al. 1999b).

The natural spawning period for southern flounder is believed to be December and January (Henderson-Arzapalo 1988; Berlinksy et al. 1996; Smith et al. 1999b). In this study, viable embryos were produced from January through late April 1999, indicating that the extended winter photoperiod regime was effective in prolonging the spawning season by at least 2 mo beyond the natural spawning period for this species. Using a combination of photothermal conditioning and GnRHa implants, southern flounder broodstock spawned volitionally in tanks over an extended period of 99 d from January to late April (Smith et al. 1999b).

**TABLE 1. Extended.**

<table>
<thead>
<tr>
<th>No. of fertilized eggs (range)</th>
<th>Hatching rate (% of Hatching floats) (range)</th>
<th>Survival to first-feeding stage (% of floats) (range)</th>
<th>Survival to first-feeding stage (% overall)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,470,516  (0–177,300)</td>
<td>41.9  (0–87.1)</td>
<td>19.5  (0–100)</td>
<td>34.2  (0–100)</td>
</tr>
<tr>
<td>1,472,577  (0–233,996)</td>
<td>31.9  (0–99.1)</td>
<td>10.9  (0–84.1)</td>
<td>9.1</td>
</tr>
<tr>
<td>4,943,092  (0–243,996)</td>
<td>37.3  (0–100)</td>
<td>15.4  (0–100)</td>
<td>12.5</td>
</tr>
</tbody>
</table>

**FIGURE 5.** Relationship between overall fertilization rate (y, %) and egg buoyancy (x, %) for naturally-spawned eggs of southern flounder. When data for all egg batches (N = 116) were used (Fig. 5A), regression analysis defined this relationship as follows: y = 0.746 x – 3.2314 (R² = 0.6127; P < 0.01). When only data for which buoyancy was determined during the gastrula through neurula stages (N = 32) were used (Fig. 5B), this relationship was defined as follows: y = 1.0727 x – 14.396 (R² = 0.815, P < 0.001).
Figure 6. Normal (left side) and abnormal embryos (right side) of southern flounder during the 8-cell stage (A,B), 32-cell stage (B,C), and multi-cell stage (D,E). Necrotic eggs at the multi-cell stage were characterized by a darkening of the germ cells (E, bottom left) followed by a gradual brownish discoloring of the cytoplasm (E, top).

Sustained, natural spawning and fertilization of southern flounder in this study was probably promoted by a number of interacting factors. Fish were received from fishermen in good health, and water quality parameters in broodtanks were maintained within optimal ranges throughout the study. Fish fed well and showed little or no evidence of disease throughout the study period. In addition to the simulated winter photothermal conditions, which maintained fish in a reproductive state from December through April, other factors such as tank size, color, and low illumination levels apparently minimized stress and were conducive to natural spawning. The results dem-
Table 2. Summarized data on hormone-induced spawnings of southern flounder (Paralichthys lethostigma) during the 1999 and 2000 seasons, including eggs collected following tank and strip spawnings. Average percent hatch (range) was determined 12–24 h after first observed hatch for floating eggs only.

<table>
<thead>
<tr>
<th>Yr</th>
<th>Method of spawning</th>
<th>No. trials</th>
<th>Total eggs spawned</th>
<th>Floaters</th>
<th>Fertilization rate (% overall)</th>
<th>Fertilization rate (% of floaters)</th>
<th>No. fertilized eggs</th>
<th>Hatching rate (% of floaters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>99</td>
<td>Tank</td>
<td>14</td>
<td>345,000</td>
<td>190,000</td>
<td>55</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Strip¹</td>
<td>17</td>
<td>756,000</td>
<td>514,000</td>
<td>68</td>
<td>27</td>
<td>40</td>
<td>206,000</td>
</tr>
<tr>
<td>00</td>
<td>Strip²</td>
<td>26</td>
<td>1,658,000</td>
<td>466,000</td>
<td>28</td>
<td>19</td>
<td>61</td>
<td>284,000</td>
</tr>
<tr>
<td>99–00</td>
<td>Tank or strip</td>
<td>57</td>
<td>2,759,000</td>
<td>1,170,000</td>
<td>42</td>
<td>17</td>
<td>40</td>
<td>490,000</td>
</tr>
</tbody>
</table>

¹ Ovulation determined by “squeeze check”.
² Ovulation determined using a light table.

Demonstrated that, under proper conditions, wild-caught southern flounder adults can be spawned successfully, without hormone induction, during their first season in captivity.

In this study, a temporary cessation of spawning throughout the latter part of December was likely related to the inhibitory effects of low water temperature, which fell to 10.9 C in late December. This is consistent with an earlier report that a decrease in water temperature from 17 to 14 C inhibited spawning in southern flounder, which resumed when temperature were returned to 17 C (Smith et al. 1999b). Based on the observation in this study that egg release increased following a change in weather conditions, we hypothesize that pulsatile temperature conditions from January through April may have been an important stimulus for sustained natural spawning. Gentle raising or lowering of water temperature stimulates spawning in red drum (Roberts 1990). No clear diurnal pattern of spawning periodicity could be discerned. In turbot, ovulatory spawning rhythms showed a distinct diurnal rhythmicity which was constant for any one female, but varied among females (McEvoy 1984).

Sustained-release GnRH-a pellet implants are highly effective in inducing ovulation of successive batches of eggs in the southern flounder, allowing repetitive strip-spawning (Berlinksy et al. 1996). These workers produced substantial numbers (1.6 × 10⁶) of eggs from multiple strip-spawnings of 12 females over a 2-wk period, but fertilization rates varied considerably (7–95%) between females and between spawns from individual fish. As an alternative to strip-spawning, photothermal conditioning coupled with GnRHa implants have also resulted in successful tank-spawning of southern flounder (Smith et al. 1999b). From a broodstock consisting of 3 females, these workers collected eggs on 64 d during a 99-d period. On days that spawning occurred, mean number of eggs collected was 277,844 for a total of 17,782,000 of which 32.8% were fertilized (range = 0–82%), although survival through hatching was not reported. A combination of photothermal conditioning and GnRHa implants apparently reduced stress and resulted in higher egg production and spawning over an extended period (Smith et al. 1999b).

Results of this study demonstrate that photothermal conditioning of southern flounder can produce natural spawning, without the use of hormones, resulting in relatively high spawning success in terms of total egg production, egg quality and the duration of the spawning period. Natural spawning of southern flounder broodstock produced a total of 18.4 million eggs, from 6 females, with an overall fertilization rate of 28% and a hatching rate of 37.3% of floaters. In comparison, 57 hormone-induced spawning trials produced 2,759,000 eggs, with lower fertilization and hatching rates of 17% overall and 26% of floaters, respectively. While spawning success var-
ied widely under both natural and hormone-induced spawning, the data suggest that natural spawnings generally resulted in higher egg quality. Natural spawning was also much less labor intensive and time consuming than hormone-induced spawning, producing large numbers of viable embryos with little or no handling of broodstock.

Higher egg quality under natural spawning (as opposed to hand-stripping) has also been reported in turbot (Bromley et al. 1986) and was attributed to reduced stress and risk of physical damage to brooders when allowed to spawn naturally. In addition, proper timing of strip-spawning in relation to ovulation markedly influences egg yield, fertilization and hatching rates in flatfish and other marine batch spawners (Kjorsvik and Holmefjord 1995). Strip-spawning can disturb the ovulation process when performed prematurely, cause a rapid loss of egg viability inside ovary after ovulation if stripping is delayed (overripening), or be subject to low milt production and quality in males (McEvoy 1984; Devauchelle et al. 1988; Bromage 1995; Berlinksy et al. 1996).

Even under natural spawning, considerable variability in egg quality was observed in this study, with an average of 62% of eggs being non-viable. Production of relatively large proportions of non-viable eggs has been observed during natural spawning of other flatfish. In turbot, 53–66% of naturally spawned eggs were non-viable (Bromley et al. 1986) and in sole spawning in tanks, 49% of the eggs produced were not fertilized (Houghton et al. 1985). In Chilean flounder, 67–92% of naturally spawned eggs were non-fertile (Silva 1994). In Japanese flounder, proportions of non-viable eggs collected during the spawning season varied from 47–93% (Tsujigado et al. 1989; Mihelakakis et al. 1995).

In this study, temperature regime during the 142-d period of natural spawning varied over a relatively broad range of approximately 15 to 24.5 C. In turbot, vitellogenesis occurred over a wide temperature range (6.5–15.7 C), but highest spawning success was attained at 10 C during the end of gametogenesis, with no fertilization obtained at high temperatures within this range (Devauchelle et al. 1988). In Japanese flounder, spawning was obtained over a 113-d period from February to June during which water temperature showed a general increase from 9.1 to 19.8 C and was correlated with a decline in egg size (Mihelakakis et al. 1995). Studies are needed to determine the effects of thermal regime on vitellogenesis, spawning, and egg quality in southern flounder.

In this study, egg batches with high fertilization and hatching rates were characterized by transparent, spherical eggs with a single oil droplet, regular cell cleavages and high buoyancy. Non-viable (albeit fertilized) eggs showed irregular cell cleavages with development stopping by the blastula stage, and with necrotic eggs showing a discoloring of the cytoplasm and a loss of buoyancy. These characteristics are identical to those reported in non-viable turbot eggs (Devauchelle et al. 1988). Additionally, under both natural and hormone-induced spawning, some batches of eggs were buoyant, but non-fertile. For example, of 31 hormone-induced spawning trials in 1999, 14 tank spawnings yielded 345,000 eggs, 55% of which were buoyant, but unfertilized (Table 2). Buoyancy has been used by some workers as an indirect measure of fertilization success. Present results show that, since high rates of buoyancy can occur in unfertilized eggs, as well as eggs of poor viability, buoyancy may not necessarily provide a good indication of egg viability if determined prior to the blastula stage of development. In halibut, fertilization rates were not reduced, but hatching rates were significantly lowered when eggs were stripped and fertilized prematurely (Kjorsvik and Holmefjord 1995).

While natural spawning minimizes labor, handling stress and may improve egg quality, reliability and control are of great concern to the commercial culturist. Hormone-induced spawning, on the other hand, en-
ables spawnings to be timed to suit the needs of the culturist. Culturists may therefore opt to compensate for lower egg quality by hormone-induced strip-spawning of multiple females concurrently. A combination of both GnRHa-induced and natural spawnings of photothermally-conditioned fish may help supply the large numbers of eggs necessary to support commercial hatcheries. To improve and minimize variability in egg quality, additional research is needed on hormone-induced spermiation in males, and on hormonal, nutritional and environmental effects on egg quality.

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Literature Cited


