

CONTROLLED SPAWNING OF SOUTHERN FLOUNDER *PARALICHTHYS LETHOSTIGMA*: ISSUES AND PROGRESS

Theodore I. J. Smith* and Michael R. Denson
South Carolina Department of Natural Resources
P. O. Box 12559 Charleston, SC 29412-2559 USA

*Voice (843) 762-5047; Fax (843) 762-5110; e-mail: smitht@mrd.dnr.state.sc.us

ABSTRACT

The southern flounder *Paralichthys lethostigma* is a popular recreational and commercial species along the coasts of the southern United States. In recent years it has become one of the flatfishes of interest for aquaculture development. Ecological data and research results indicate that this species is both eurythermal and euryhaline, making it attractive for coastal and, possibly, inland culture. During the past several years, research has focused on developing controlled reproduction techniques for captive wild southern flounder. In South Carolina, post-metamorphosed juveniles are recruited to inshore waters from December-February with spawning believed to occur along the continental shelf. Collection of non-spawning adults can be accomplished during spring and summer and in fall prior to the offshore migration. Adult fish caught by trammel net sustain little damage during capture and can be readily adapted to tank conditions. Feeds consisting of live fishes and crustaceans (e.g., *Fundulus* spp., penaeid shrimps) are readily eaten as are chopped fish (e.g., mullet, *Mugil* spp.; mackerel, *Scombrus* spp.). Time in captivity affects reproductive results. The percent of GnRH-a spawnable females increased from 29% for fish in captivity only 1.5-3.5 mo to 70% for those held under captive conditions for 5.5-6.3 mo. All females held for more than 24 mo could be spawned, and due to their increased size, these fish produced about three times the number of eggs as the recently captured fish. However, there appeared to be a decrease in percent fertilization among fish held in captivity longer. Fertile eggs could be stripped from naturally ovulating females but timing of ovulation and frequency of success were substantially lower than that obtained from GnRH-a-treated females. Some previously hormone spawned females could be re-implanted with GnRH-a and re-spawned. However, number of eggs produced and apparent percent fertilization decreased. Availability of milt was a concern during strip spawning research in 1997. Recent work indicated males could be repeatedly stripped and produce high volumes of milt, if not stressed by handling and captive conditions. A series of studies was conducted to improve tank spawning techniques. When fish are subjected to spawning conditions (10-11 h light; 17-18 C) and left undisturbed in a 3.7 m diameter x 1 m deep tank, females often produce a large number of eggs but typically there is low or no fertilization in spite of the presence of ripe (spermatogenic) males. GnRH-a treatment of larger females (2 kg) often results in the production of fertilized eggs during some tank spawning events. Treatment of males with testosterone or methyltestosterone and GnRH-a did not induce male participation. GnRH-a treatment of both males and females in a tank offered no benefit over treating only the females.

In summary, southern flounder readily acclimate to captivity and will mature under photothermal conditioning. Use of GnRH-a implants in females improves the timing of strip spawning and currently appears necessary for predictable production of fertilized eggs from volitional tank spawning. Methods to improve male participation in tank spawning events need to be identified.

INTRODUCTION

The southern flounder *Paralichthys lethostigma* supports commercial and recreational fisheries along the Atlantic coast and Gulf of Mexico. Commercial landings during 1992-1996 of summer *P. dentatus* and southern flounder combined (species not distinguished) averaged 8,726 mt. However, in 1997 landings were less than half of this value (NMFS 1998). Southern flounder is also a target of recreational fisheries that occur along the south Atlantic coast and in the Gulf of Mexico (NMFS 1998).

Based on studies to date, this species appears to have desirable aquaculture characteristics. Reported growth rates of wild fish in South Carolina indicate that females attain a mean size of about 0.9 kg at 24-28 mo of age (Wenner et al. 1990). Recent work supported by South Carolina and North Carolina Sea Grant Programs showed that southern flounder is euryhaline and displays increased tolerance to low salinity and fresh water with increasing age (Daniels and Borski 1998; Smith et al. 1998, 1999a). Thus, there may be opportunity to grow this species in inland as well as coastal sites as is

done with the euryhaline hybrid striped bass (Smith et al. 1995). Although a detailed economic analysis of southern flounder aquaculture has not been conducted, market prices appear supportive for commercial development of this species. In 1997, ex-vessel price of summer and southern flounders was US\$4.10/kg (NMFS 1998). However, live east coast flounders are regularly sold to upper-scale Japanese restaurants in the northeastern United States, where they command a premium price (~US\$15/kg). They are also shipped live to Tokyo, Japan, where they are priced at US\$45-60/kg (Ackerman 1997).

A number of studies have focused on the spawning of southern flounder. Arnold et al. (1977) reported the first successful spawning of this species. They used photothermal conditioning to tank spawn 3 of 6 females and produced 120,000 eggs from 13 separate spawns with 30-50% fertility. The following year, Lasswell et al. (1978) reported the strip spawning of 25,000 eggs (average 5,000 eggs spawn⁻¹) from 14 females induced to ovulate using carp pituitary extract. Later, Henderson-Arzapalo et al. (1988) examined photothermal conditioning alone and in combination with LHRH-a implants. However, the females only spawned during simulated natural winter spawning conditions in December - February, and there was no fertilization, apparently due to lack of male participation (Henderson-Arzapalo et al. 1988).

Over the past several years, a number of collaborative spawning trials have been conducted with Dr. Craig Sullivan (North Carolina Sea Grant Program) and Dr. David Berlinsky (University of Rhode Island) at the South Carolina Department of Natural Resources (SCDNR) Waddell Mariculture Center (WMC) in Bluffton, and the Marine Resources Research Institute (MRRI) in Charleston. All studies utilized captive wild fish which were held under controlled photothermal conditions. Results indicated that females containing oocytes $\geq 500 \mu\text{m}$ in diameter could be induced to ovulate using GnRH-a implants inserted intramuscularly. Eleven of 12 fish were strip spawned in our initial tests, and they produced a total of 1.6 million eggs with batch fertility ranging from 7 to 95% (Berlinsky et al. 1996). In an effort to reduce broodstock handling

stress, increase egg production, and extend the spawning period, GnRH-a implants were also given to broodstock which were placed in spawning tanks. Initial results with three females were very impressive with a mean of 277,800 eggs/d being produced on 64 d during a 99-d spawning period. Total egg production was 17,782,000 and mean fertility was 32.8% (Smith et al. 1999b).

Other studies focused on aquaculture development have been conducted in South Carolina. Larval rearing trials during 1995-1997 indicated that small juveniles can be grown in tanks to a size of 25-30 mm TL using live foods but that pigmentation abnormalities occur (Denson and Smith 1997). This problem with pigmentation is similar to findings in North Carolina (Daniels et al. 1996). A recently completed study indicated that photoperiod may influence larval survival and that the presence of a sand substrate improved ocular side pigmentation (Carter and Smith unpub.). In addition, we have demonstrated production of small juveniles (25-125 mm TL) in fertilized earthen ponds (Jenkins et al. 1997; Jenkins and Smith 1999). This suggests that perhaps phase I juveniles (50+ mm TL) could be mass-produced using extensive pond systems similar to those used for red drum and hybrid striped bass culture. However, survival during these first attempts was poor (~5-6%) suggesting that pond management techniques need to be improved. Weaning of young pond-reared juveniles (2.5 mo old) to artificial diets was rapid (2 wk) and incidence of pigmentation abnormalities was low (Jenkins and Smith 1999). In North Carolina, it was shown that tank-reared juveniles could be rapidly converted to commercially produced rations (Daniels and Hodson 1999).

In summary, research results to date indicate that southern flounder have characteristics which make them attractive for culture. However, additional work is required to develop cultured broodstock, improve predictability of spawning, assure high quality gamete production, and to improve nursery and grow-out conditions.

This manuscript presents results from recent studies focused on initiation and control of

spawning. In particular, a variety of studies on strip spawning and tank spawning were conducted to improve the predictability of successful spawning of captive broodstock and to elucidate mechanisms which control tank spawning.

METHODS

General

For the various studies, wild adults were captured using trammel nets set in coastal waters (Smith et al. 1999b). For 1-4 wk after capture, fish were held in tanks under ambient estuarine conditions. During this time the fish were acclimated to captive conditions, treated for external parasites, and converted to chopped natural feeds. After this period, fish were typically moved to indoor tanks and maintained under controlled conditions.

Results reported herein are based on these wild fish held in indoor tanks located in separate rooms each of which had independent temperature, lighting, and water quality control. Each environmental room housed a holding/conditioning/spawning tank (3.7 m x 1.1 m deep), a biological bead filter (model PBF-6, Arman Aquaculture, Vacherie, Louisiana, USA); a UV filter (80W, Aqua Ultraviolet, Temecula, California, USA); a heat exchanger tank (1.2 x 0.6 x 1.2 m deep); and an external egg collector tank (110 cm high x 70 cm in diameter). The egg collector which contained a mesh bag, was connected to the spawning tank at the surface during the tank spawning trials. During the conditioning phase, fish were typically held at densities of 50-75 fish/tank (4.8-7.1 fish/m² bottom area; ~1.7-2.5 kg/m³). The flounder were fed to satiation (usually three times/wk) a diet consisting primarily of squid and mackerel. Sampling data indicated that most fish grew substantially while in captivity. Males used in the various studies ranged from ~330-400 mm TL and weighed ~500-700 g. Due to sexual dimorphism, mature females were much larger and ranged in size from ~410-670 mm TL and weighed ~900-4000 g.

Photothermal conditions were strictly controlled. Lighting (297 lux at the surface) was provided by overhead fluorescent lights (two

double 43 W T8 electronic bulbs). The lights were automatically controlled as either on or off without phasing (no dusk or dawn simulation). Temperature was controlled to ± 1 C using heat exchangers connected directly to the main heated and chill water systems for the building. During the strip and tank spawning studies, photothermal conditions were maintained at 10-11 h light and 17-18 C. Recirculated water was provided at a rate of 150 L/min to cause a circular rotation. During the various studies, typical water quality conditions were: 6-9 mg/L dissolved oxygen; salinity 32-34 g/L; pH 7-8; total ammonia nitrogen <1 mg/L; nitrite <0.1 mg/L; and nitrate 1-35 mg/L.

The number of eggs reported are based on counts of sub-samples of water hardened fertilized eggs. On six occasions, a 1 ml sample of eggs was obtained and counted under a microscope. Egg counts ranged from 836 to 1,104, and overall mean was 956 eggs/ml. For purpose of convenience, eggs are presented as 1,000 eggs/ml.

Strip Spawning Research

Fish were placed in the environmental control rooms and subjected to simulated natural photothermal conditions. However, the natural spawning period was extended for several months by maintaining the photothermal conditions during which flounder are believed to spawn. Females used in these studies were selected from the tank populations based on gonadal biopsy. Selected fish had oocytes ≥ 500 μ m in diameter. These fish received a 95% cholesterol, 5% cellulose pellet (Sherwood et al. 1988) containing 100 μ g of GnRH-a (Peninsula Labs, Belmont California, USA) as described in Smith et al. 1999b. Males received no hormone treatment during any of the studies. Females and males were held in 2-m diameter tanks (33-34 ppt salinity) during the strip spawning studies. Females were visually inspected several times during 48 h post hormone treatment until ovulation occurred. They were then checked at successive 24 h intervals until egg production ceased. Typically, ovulation occurred in 48 h and eggs were stripped 3-5 times during a 5-7-d period. To reduce handling stress, females were examined only when their

abdominal area was sufficiently swollen to cause a protrusion around the vent and when scales in the area were slightly raised. Females were anesthetized in a solution of sea water and tricaine methanesulfonate (MS-222) before stripping. Males were selected based on the expression of milt with slight abdominal compression. Motility of sperm was confirmed by activation with sea water and observation under a compound microscope. Milt was routinely collected from the males using a 5-ml syringe and then stored in an ice bath until use (within 1h). Eggs were manually expelled by providing slight abdominal pressure and collected in a ceramic bowl. Eggs were covered with milt from at least two males and the mixture stirred for 2 min. Next, sea water was added and the mixing continued for an additional 2 min. After fertilization, eggs were placed in a 20-L bucket and slightly aerated for 2 h. After this period, eggs were drained into a graduated cylinder and volume of floating and sinking measured. During the strip spawning studies in 1998, the volume of floating eggs relative to total egg production was used as an index of fertilization. However, in 1999, ~200 eggs were sub-sampled from the floating eggs and examined under a dissecting microscope for evidence of development. In 1999, percent fertilization was based on this sub-sample and calculated based on the total number of eggs taken (floating + sinking). Based on previous work (Smith et al. 1999a) it was determined that sinking eggs were dead (unfertilized, broken, deformed).

Study - SS1: Natural vs Hormonal Induction

Captive females will naturally mature and ovulate in tanks having photothermal control (Arnold et al. 1977). However, we sought to compare the efficacy of stripping naturally ovulated eggs to hormone induced ovulation. In 1998 two studies replicated two treatments: 1) controlled environmental conditioning coupled with strip spawning; and 2) controlled environmental conditioning coupled with GnRH-a implants and strip spawning. The studies were run sequentially using females (and males) from the same broodstock holding tank. Study duration was 26-30 d.

Study - SS2: Re-induction of Ovulation of Recently Spawmed Fish

At times it may be beneficial to re-use recently spawned fish. A study was conducted to determine whether southern flounder could be re-induced to produce additional clutches of eggs after completion of a spawning event (multiple days of stripping or tank spawning). After initial spawning, females were placed in 2-m diameter recovery tanks for a period of 1-3 wk. In this study 12 females were selected based on ovarian biopsy results that showed that oocyte diameters remained >500 μm after spawning trials were completed and recovery period was over. All fish were implanted with a hormone pellet. All techniques were as described above.

Study SS-3: Relationship of Captivity Time to Spawning Success

Over the years there appeared to be improved predictability in maturation, ovulation and fertilization of eggs from fish that had been in captivity for more than several mo. During 1998, adult flounder were collected on 30 June and 27 October. Spawning of these animals was attempted from December - February. The results of these strip spawning trials were compared to those from fish which had been held in captivity for > 2 yr. Males collected on 27 October were used in all spawning trials to minimize possible affects which could occur if different groups of males were used with the various time in captivity groups of females. Results are presented only for females which could be discriminated as mature females based on their larger size and ovarian biopsy samples. Smaller fish could be either males or immature females. Thus, the actual number of males was not known at time of the spawning attempts.

Study - SS4: Milt Production

Previous work had suggested that milt production could be a limiting factor in fertilization of strip spawned eggs (Berlinsky et al. 1996). In 1998, a study was conducted to measure milt volume and quality from a group of males that had been in captivity for at least 24 mo. Near the end of a strip spawning study, a group of 8 males was isolated in a 2-m holding

tank and feeding was continued three times a wk. During this 10-d study, males were sampled every other d by applying abdominal pressure and collecting all available milt.

TANK SPAWNING RESEARCH

To reduce stress associated with strip spawning and to increase egg production, a tank spawning study was conducted in 1997 which was highly successful (Smith et al. 1999b). However, attempts to duplicate this success have indicated that results can be variable. Thus, a series of studies were conducted to identify possible controlling factors in volitional spawning to improve the predictability of tank spawning techniques. All studies utilized fish which had been in captivity for 24-36 mo. Males and females used in the studies were selected using the same criteria as those in the strip spawning studies (e.g. $\geq 500 \mu\text{m}$ oocytes, running milt). Females were implanted with 100 μg of GnRH-a in studies indicating hormone treatment of females. Unless otherwise noted, males were not treated with hormones. In the 1998 studies, 3 females were placed in a tank with 6 males (1:3 sex ratio) while in the 1999 studies 3 females were placed in a tank with 4-6 males. Water salinity was maintained at ≥ 32 ppt so that the eggs would float (Smith et al. 1999a). Eggs were skimmed off the water surface and collected in the external egg collector tank which contained a 250- μm mesh bag, 58 cm diameter x 66 cm deep. The egg collectors were inspected at least daily and eggs were removed and volumetrically measured in a 1-L graduated cylinder containing a known volume of sea water. Percent fertility was based on observation of embryonic development in a sample of 200 eggs randomly taken from the floating eggs and expressed based on total egg production (floating vs. sinking eggs). The total number of floating eggs collected in 1 d may be from one or more spawns within a tank as well as from remnant groups of eggs left over from the spawn the previous day.

Study TS1: Size of Females and Hormone Treatment

Published information indicated that only larger females could be induced to spawn (Arnold et al. 1977). Thus, a study was conducted to examine the effect of size of females on spawning as well as the need for hormone inducement of ovulation. Three treatments were examined consisting of: 1) GnRH-a implanted females ≤ 1.5 kg (actual mean size 1.2 kg, range 1.0 - 1.5 kg); 2) GnRH-a implanted females ≥ 2.0 kg (actual mean size 2.9 kg, range 2.0 - 5.5 kg); and 3) no hormone treatment, females ≥ 2.0 kg (actual mean size 2.5, range 2.0 - 3.3 kg)(control). Due to limitations in the number of tanks, only treatment 2 could be replicated in two tanks. Due to their smaller size, 4 females (and 8 males) were used in treatment 1 while 3 females (and 6 males) were utilized in treatments 2 and 3.

Study TS2: Approaches to Improve Male Participation with Naturally Ovulating Females

Results of study TS1 suggested that lack of male participation was an issue which should be examined. Study TS2, was a non-replicated experiment with several components which attempted to address this issue. The fish used in this study were those from study TS1, treatment 3 (control, no hormone treatment females or males). After completion of study TS1, males were inspected and found to be running ripe. These males were returned to the tank with the non-hormone treated females and removed 1 wk later as no spawning had occurred. Six new spermiating males which were being used in a strip spawning study were placed in the tank with the 3 females. During the following week no spawning occurred. The males were removed and implanted with testosterone silastic elastomers and then returned to the tank for an additional wk. As no appreciable spawning had occurred, the females were removed and given 100 μg GnRH-a implants and the study continued for an additional 11 d.

Study TS3: Approaches to Improve Control of Volitional Spawning

Results of study TS2, conducted in 1998, suggested hormone-treated females stimulated male participation. In 1999, study TS3 was conducted to further examine the use of hormones to improve male participation in volitional spawning. In this study, three hormone treatments (and a control) were examined: 1) GnRH-a treatment of females, no hormone treatment of males; 2) no hormone treatment of females, 50 µg GnRH-a + 1 mg/cm fish TL methyltestosterone treatment of males; 3) GnRH-a treatment of females, 50 µg GnRH-a treatment of males; 4) control - no hormone treatment of females or males. Due to tank limitations, the treatments were replicated during sequential studies. However, treatment 2 was not replicated. Duration of study 1 was 40 d while study 2 was concluded after 21 d, as all spawning had ceased.

RESULTS AND DISCUSSION

Due to the number of studies conducted and their different objectives, results from each study will be presented and discussed separately.

Study SS1: Natural vs Hormonal Induction

Although fish would naturally mature and ovulate under controlled photothermal conditioning, timing of ovulation was difficult to predict. Consequentially, repeated handling was necessary to attempt to identify the appropriate time to strip eggs. In study 1, no eggs were obtained from the three environmentally conditioned eligible females over a 26-d period. In contrast, the three eligible females which had been treated with GnRH-a implants completed spawning within 5 d and produced a mean of 453,000 eggs/fish (Table 1). In study 2, the two environmentally conditioned fish both ovulated and could be repeatedly strip-spawned. One fish was stripped 9 times over 30 d and produced a total of 1 million eggs of which 74% floated (Table 1). The other fish was stripped 3 times and produced 230,000 eggs of which 54% floated. The GnRH-a treated females all ovulated and produced a mean of 488,000 eggs of which 86% were

Table 1. Data from strip spawning of natural and hormone induced ovulating southern flounder.

Treatment	Eggs (x10 ³)		Spawning Duration	
	Total	Floating (%)	(No.)	(Days)
<u>STUDY 1</u>				
Natural	0	-	-	26
Natural	0	-	-	26
Natural	0	-	-	26
GnRh-a ♀	660	18	3	5
GnRh-a ♀	360	56	4	5
GnRh-a ♀	340	65	4	5
<u>STUDY 2</u>				
Natural	230	54	3	30
Natural	1000	74	9	30
GnRh-a ♀	450	91	3	6
GnRh-a ♀	595	72	4	6
GnRh-a ♀	420	95	3	6

floating. These fish were stripped 3-4 times and stripping was completed in 6 d.

These results indicate that naturally ovulating females can be strip-spawned, however, timing of ovulation is difficult to predict. In contrast, hormone treatment of eligible females resulted in greater predictability in timing of ovulation, and spawning was completed in a shorter time period (within several d). Unfortunately, percent fertilization was not determined for the floating eggs so no conclusion was possible concerning relative fertility of natural vs. hormone induced production of eggs.

Study SS2: Re-induction of Ovulation of Recently Spawmed Fish

Results of implantation work showed that 11 of 12 females responded to the hormone treatment. Five of the 12 spawned only once. Egg production ranged from 50 to 270,000 eggs. Egg quality was also highly variable ranging from 0-100% floating eggs. Six of the fish were from a tank spawning trial and individual egg production from the first implantation was not known. However, previously strip-spawmed individuals were used to compare first and second

Table 2. Data from previously spawned fish re-implanted with GnRh-a. Fish were strip-spawned.

Fish No.	Initial Implantation			Re-Implantation		
	Spawnings (No.)	Total eggs (x10 ³)	Floating (%)	Spawnings (No.)	Total eggs (x10 ³)	Floating (%)
1	4	440	91	3	336	33
2	4	472	92	2	270	33
3	3	426	63	2	330	39
4	3	660	18	1	150	60
5	4	360	56	2	200	45
6	4	340	65	0	-	-

implantation egg production. One fish from this group produced no eggs after the second implantation, and egg production and percentage of floating eggs was generally lower amongst the other females (Table 2).

Study SS3: Relationship of Captivity Time to Spawning Success

There was a clear relationship between occurrence of females that could spawn and egg production with time in captivity (Table 3). The percent of females that could spawn increased from 29% for fish in captivity only 1.5-3.5 mo to 70% for those held under captive conditions for 5.5-6.3 mo. Females held for more than 24 mo were all eligible and could be spawned. Egg production per female was similar for those in

captivity ≤ 6.3 mo and over three times greater for fish held in captivity >24 mo (Table 3). Production of fertilized eggs per female was almost double for fish in captivity >24 mo but on a weight basis production of fertilized eggs was similar among all groups (mean 71,000/kg). Mean percent egg fertilization per fish was almost double for the newly acquired groups of fish as compared to those in captivity for > 24 mo and all groups of fish showed, substantial within group, variation in percent fertilization (Table 4).

The results showed that the fish in captivity grew larger with time. As a result, more fertilized eggs per female were produced and the predictability of spawning improved with captivity time. However, the number of fertilized eggs decreased, with the oldest fish in captivity

Table 3. Data on captivity time and spawning success of female southern flounder.

Captivity Time (mo)	Eligible/ Total	Mean Wt. (g)	Spawned (No.)	Total Eggs/ (%)	Total Fertility ¹ female (x10 ³)	(%)
1.5 - 3.5	5/14	944	4	29	232	30.8
5.5 - 6.3	7/10	1048	6	70	203	33.3
>24	7/7	1776	7	100	754	17.3

¹Total fertility = all fertilized eggs divided by all eggs produced.

Table 4. Data on captivity time, egg production, and fertilization for southern flounder.

Captivity time (mo)	Fertilized eggs (x10 ³) per female		Fertility per fish (%) ¹	
	kg		Mean	Range
1.5 - 3.5	72	76	37.7	8.0 - 79.9
5.5 - 6.3	68	65	32.9	10.4 - 64.6
>24	130	73	17.2	4.0 - 48.2

¹Based on batches of eggs which were fertilized.

providing the lowest fertility. The reason for this is not clear and may be related to a number of factors including physiological changes with age or perhaps nutritional factors associated with captivity, both of which can affect egg quality.

Although the number of potential males could not be determined due to the possible presence of immature females, running ripe males did readily occur with the fish in captivity for only 1.5-3.5 mo. In tanks where the >24 mo captive females were held, all smaller fish were running ripe males during spawning conditions.

Study SS4: Milt Production

Although milt volume was often measured in μl during the work of Berlinsky et al. (1996), this was not the case in the present study. Males were prolific in milt production and most could be stripped every other d over a 10-d period (Table 5). Milt was viable in all cases and

total volume per fish ranged 3.6 to 20.0 ml. Greatest production came from the two largest males (18.5, 20.0 ml). Maximum period for milt stripping was not determined as these fish had been previously stripped and were still running at the time the study was terminated. It appears that reduced stress associated with gentle stripping (only 1.0 to 1.5 ml per stripping event is routinely taken) and adequate holding conditions were responsible for the improved performance of the captive males. During this study, the fish actively fed indicating that stress levels were minimal.

Study TS1: Size of Females and Hormone Treatment

Size of females and hormone treatment influenced results (Table 6). The larger females which relied on natural photothermal conditioning alone, spawned 36 times over the 90-d study and produced a total of 5.3 million eggs. However,

Table 5. Milt production of male southern flounder stripped every other day during a 10-day period.

Wt (g)	Fish Size TL(mm)	Stripping No. (Milt ml)					Total (ml)
		1	2	3	4	5	
493	339	1.3	1.0	2.0	1.8	0.3	6.4
534	338	2.4	3.4	0	1.0	0.5	7.3
547	387	2.7	2.1	2.5	1.2	0.5	9.0
554	344	1.1	1.5	0	0	1.0	3.6
631	366	3.3	1.5	0.8	2.5	2.0	8.3
634	362	1.3	0.8	2.0	1.3	0.3	5.7
726	356	6.1	3.8	4.0	2.8	3.3	20.0
826	393	7.2	3.8	3.5	3.0	1.0	18.5

Table 6. Effect of size of females and hormone treatment on tank spawning success of environmentally conditioned (EC) fish.

Treatment	Duration (Days)	Spawns (No.)	Total Eggs ($\times 10^6$)	Overall Fertility (%)	Fertility (Days)	Batch Fertility (%)	
						Mean	Range
EC + GnRh-a <1.5kg ♀	60 ¹	13	1.5	0	-	-	-
EC >2.0 kg ♀	90	36	5.3	0	-	-	-
EC + GnRh-a >2.0 kg ♀							
Rep 1	90	40	6.3	12	23	32	5-69
Rep 2	90 ²	42	4.6	18	18	33	8-59

¹Spawning ceased on day 30

²Spawning continued for 150 days, data not presented.

there was no fertilization, apparently due to lack of male participation. In contrast, the hormone treated larger females produced an average of 41 spawns and an average of 5.5 million eggs (Table 6). Average overall fertility was 10%. Males on average participated 21 d and average fertility was 32.5%. The smaller females which also received the hormone treatment spawned 13 times during the initial 30 d and then ceased to spawn. Total production was 1.5 million eggs, but none were fertilized. These results suggest that hormone treatment and use of larger females are required to produce successful tank spawning events. However, even under these conditions, this study showed that males don't participate in all spawning events and that daily fertility levels vary considerably.

Study TS2: Approaches to Improve Male Participation

Although this study was not replicated, results strongly suggested that females control

male participation. Replacement of nonperforming males with other ripe males did not stimulate spawning nor did use of testosterone-treated males. However, treatment of females with GnRH-a resulted in ovulation and spawning on 10 or 11 d and production of 2.3 million eggs (Table 7). Further, males participated on 5 of the 10 d during which 87% of the eggs were spawned. Mean fertilization was 7.1% and ranged from 11-34%.

Study TS3: Approaches to Improve Control of Volitional Spawning

Results of this study clearly suggest female motivation causes male participation in the spawning event. In study 1, multiple spawning occurred in the tank containing the males which were hormone treated with GnRH-a + methyl testosterone. However, there was no fertilization. In the tank containing the GnRH-a treated females, spawning occurred on 29 days and 3.9 million eggs were produced (Table 8).

Table 7. Results of study to induce male participation in tank spawning.

Treatment	Time (Days)	Spawns (No.)	Egg Production (x10 ³)	Fertilization (%)
No hormones ♀ + ♂	7	0	-	-
No hormones ♀ + new ♂	7	0	-	-
No hormones ♀ + ♂ with testosterone	7	1	10	0
GnRh-a ♀ + ♂ with testosterone ¹	11	10	2,265	6.2

¹Fertilized eggs were produced during 5 spawns. Mean fertility was 7.1%, range 11-34%.

Table 8. Effect of hormonal treatment on tank spawning of southern flounder.

Treatment	Duration (Days)	Spawns (No)	Total Eggs (x10 ⁶)	Total	Fertility (%) Daily Max
STUDY 1					
♀ GnRh-a	40	29	3.9	4.8	59.2
♀ GnRh-a/MT ¹	40	11	0.5	0	0
Control ²	40	11	1.5	0.3	25.9
STUDY 2					
♀ + ♂ GnRh-a	21	14	1.6	6.3	6.0
♀ + ♂ GnRh-a	21	13	2.3	0	0
♀ GnRh-a	21	16	1.5	4.8	13.8
Control ²	21	7	0.4	0	0

¹MT = methyl testosterone

²Control = no hormone treatment of % and &.

Fertilization occurred on 11 d and percent fertilization ranged from 0.8 to 59.2%. Fish in the control group (no hormone treatment) spawned on 11 days. On one of these days natural fertilization occurred (25.9%).

In study 2, the tank with the GnRH-a treated females produced fertilized eggs while the control tank (no hormone treatment) produced only unfertilized eggs (Table 8). GnRH-a treatment of both males and females was not an improvement over treating just the females and in one replicate there was no fertilization.

The controlling mechanism for motivation of males is not clear but GnRH-a treatment of females alone does result in production of fertilized eggs. However, hormone treated males were not stimulated to participate in tank spawning.

CONCLUSIONS

Information obtained from these various studies will help improve the predictability of spawning southern flounder. As was shown with a number of other species including sea bass *Lates calcarifer* (Almendras et al. 1988), winter flounder *Pseudopleuronectes americanus* (Harmin and Crim 1992), and striped bass *Morone saxatilis* (Hodson and Sullivan 1993), GnRH-a was effective in inducing final maturation and ovulation in southern flounder (Berlinsky et al. 1996; Smith et al. 1999b). The use of GnRH-a implants to induce ovulation substantially narrowed the time frame for egg taking and improved the predictability of spawning success relative to strip spawning based on natural ovulation. Re-implantation of GnRH-a was also useful for production of additional batches of eggs from some previously spawned females. As observed during these and previous strip spawning studies, there was variability in fertilization success and this may be related to egg quality. Current assessment techniques used in selection of eligible females may not be adequate for evaluation of egg quality (C. Sullivan NCSU, personal communication). Work focused on this issue is underway in North Carolina and South Carolina. Additionally, correct timing of ovulation to maximize egg viability is very difficult to

achieve using strip spawning techniques and this no doubt accounts for variability in spawning success as well (Smith et al. 1995).

Volitional tank spawning has a number of advantages over strip-spawning. First, spawning can be controlled to occur over an extended period and tank spawning typically results in very high egg production. Second, labor requirements are minimized as the eggs are easily collected external to the spawning tank. Third, handling stress on broodstock is essentially eliminated and at termination of the spawning activity, fish are normally in good health and fitness. However, hormone induction coupled with strip spawning does result in more concise initiation and conclusion of the spawning event but overall production of eggs is lower.

There appear to be physiological and behavior issues associated with captive wild males which need to be addressed. Males naturally mature in captivity and can produce copious volumes of milt if not severely stressed. This was reflected in the study where the males were repeatedly stripped five times over 10 d and produced up to 20 ml of milt. However, there appears to be a controlling mechanism(s) which influences the involvement of the males in volitional tank spawning events that are not well known at present. Although running ripe, males did not normally participate in spawning with naturally ovulating females during our tank studies. Treatment of the females with GnRH-a had a mitigating influence and often resulted in male participation in tank spawning events. However, timing and predictability of spawning was not well controlled. Additional tank spawning research is needed to better simulate natural conditions including the use of larger and perhaps deeper spawning tanks.

In summary, a basic spawning technology is available to produce southern flounder. However, refinements are needed to improve efficiency and predictability. Besides the issues identified above, additional research is needed to identify suitable broodstock diets, and to develop and evaluate cultured broodstocks.

ACKNOWLEDGMENTS

The authors appreciate the laboratory assistance of staff during this research, especially Louis Heyward, Wallace Jenkins, Stephen Long and Lisa Carter. We also thank Craig Sullivan, North Carolina State University, and David Berlinsky, University of Rhode Island, for advice during the various studies. William Roumillat and Charles Bridgman assisted in the collection of the broodstock and the later individual also helped provide daily care to the fish. This research was supported in part by the United States Department of Commerce, National Oceanic and Atmospheric Administration, Office of Sea Grant under South Carolina Sea Grant Consortium contract number NA86RG0052. This is contribution number 437 from the South Carolina Marine Resources Center. Reference to trade names does not imply endorsement.

LITERATURE CITED

- Ackerman, J. 1997. Recreating the bounty of the sea. Boston Sunday Globe, March 6, 1997, section F, pp. 1,4. Boston, MA.
- Almendras, J. M., J. Duenas, J. Nacario, N.M. Sherwood and L.W. Crim. 1988. Sustained hormone release III. Use of gonadotropin-releasing hormone analogues to induce multiple spawning in sea bass, *Lates calcarifer*. *Aquaculture*. 74: 97-111.
- Arnold, C.R., W.H. Bailey, T.D. Williams, J. Johnson and J.L. Lasswell. 1977. Laboratory spawning and larval rearing of red drum and southern flounder. Southeastern Association Fish and Wildlife Agencies. 31: 437-440.
- Berlinsky, D.L., W. King, T.I.J. Smith, R.D. Hamilton, J. Holloway, Jr. and C.V. Sullivan. 1996. Induced ovulation of southern flounder *Paralichthys lethostigma* using gonadotropin releasing hormone analogue implants. *Journal of the World Aquaculture Society*. 27(2): 143-152.
- Daniels, H.V. and R. G. Hodson. 1999. Weaning success of southern flounder juveniles: Effect of changeover period and diet type on growth and survival. *North American Journal of Fisheries Management*. 61: 47-50.
- Daniels, H.V. and R. J. Borski. 1998. Effects of low salinity on growth and survival of southern flounder (*Paralichthys lethostigma*) larvae and juveniles, pp. 187-191. *In*: W.H. Howell, B.J. Keller, P.K. Park, J.P. McVey, K. Takayanagi and Y. Uekita (eds.), *Nutrition and Technical Development of Aquaculture*. UJNR Tech. Rep. No. 26, Univ. New Hampshire Sea Grant College Prog., Durham, NH.
- Daniels, H.V., D.L. Berlinsky, R.G. Hodson and C.V. Sullivan. 1996. Effects of stocking density, salinity, and light intensity on growth and survival of southern flounder (*Paralichthys lethostigma*) larvae. *Journal of the World Aquaculture Society*. 27(2): 153-159.
- Denson, M. R., and T.I.J. Smith. 1997. Effects of diet and light intensity on survival, growth and pigmentation of southern flounder (*Paralichthys lethostigma*). *Journal of the World Aquaculture Society*. 28: 366-373.
- Harmin, S.A., and L.W. Crim. 1992. Gonadotropic releasing hormone analog (GnRH-a) induced ovulation and spawning in female winter flounder, *Pseudopleuronectes americanus*. *Aquaculture*. 104: 375-390.
- Henderson-Arzapalo, A., R. L. Colura and A. F. Maciorowski. 1988. Temperature and photoperiod induced maturation of southern flounder. Management Data Series Number 154, Texas Parks and Wildlife Department, Austin, TX.
- Hodson, R. G., and C. V. Sullivan. 1993. Induced spawning of domestic and wild striped bass, *Morone saxatilis*, broodstock implanted with GnRH analogue and injected hCG. *Aquaculture and Fisheries Management*. 24: 389-398.
- Jenkins, W.E., T.I.J. Smith, C.V. Sullivan and D.L. Berlinsky. 1997. Production of southern flounder (*Paralichthys lethostigma*) in an

- outdoor nursery pond. *Journal of the World Aquaculture Society*. 28: 211-214.
- Jenkins, W.E., and T.I.J. Smith. 1999. Pond nursery production of southern flounder (*Paralichthys lethostigma*) and weaning to commercial diets. *Aquaculture*. 176: 173-180.
- Lasswell, J.L., G. Garza and W.H. Bailey. 1978. Hormone-induced spawning of southern flounder. *Progressive Fish-Culturist*. 40: 154.
- National Marine Fisheries Service. 1998. Fisheries of the United States, 1997. Current Fishery Statistics No. 9700. Washington, D.C. 156 pp.
- Smith, T.I.J., W.E. Jenkins and M.R. Denson. 1998. Tank and pond nursery production of juvenile southern flounder, *Paralichthys lethostigma*, pp. 21-32. In: W.H. Howell, B.J. Keller, P.K. Park, J.P. McVey, K. Takayanagi, and Y. Uekita (eds.), *Nutrition and Technical Development of Aquaculture*. UJNR Tech. Rep. No. 26, Univ. New Hampshire Sea Grant College Prog., Durham, NH.
- Smith, T.I.J., W.E. Jenkins and Louis D. Heyward. 1995. Hatchery performance and reuse of domesticated white bass broodstock. *Proceedings of the Annual Conference of the Southeast Association of Fish and Wildlife Agencies*. 49: 97-105.
- Smith, T.I.J., M. R. Denson, L.D. Heyward, Sr., W.E. Jenkins and L.M. Carter. 1999a. Salinity effects on early life stages of southern flounder, *Paralichthys lethostigma*. *Journal of the World Aquaculture Society*. 30(2): 236-244.
- Smith, T.I.J., D.C. McVey, W.E. Jenkins, M.R. Denson, L.D. Heyward, C.V. Sullivan and D.L. Berlinsky. 1999b. Broodstock management and spawning of southern flounder, *Paralichthys lethostigma*. *Aquaculture*. 176: 87-99.
- Wenner, C.A., W.A. Roumillat, J.E. Moran, Jr., M.B. Maddox, L.B. Daniel, III and J.W. Smith. 1990. Southern Flounder: *Paralichthys lethostigma*. Pages 2: 1-35. In: *Investigations on the life history and population dynamics of marine recreational fishes in South Carolina: Part I, Report to USFWS for Project F-37, Atlanta, GA.*