HORMONAL-INDUCED OVULATION OF THE WINTER FLOUNDER, *PSEUDOPLEURONECTES AMERICANUS*

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ABSTRACT

The response of winter flounder, *Pseudopleuronectes americanus*, to human chorionic gonadotropin (HCG), oxytocin, pregnant mare serum gonadotropin (PMSG), deoxycorticosterone (DOCA), and freeze-dried carp pituitary is described. HCG and PMS were successful in some instances in producing viable eggs and larvae while carp pituitary was successful in all instances. These were the first known successful attempts to induce maturation and spawning of winter flounder artificially in the laboratory.

Larvae obtained from these hormonal-induced spawnings were normal in all respects and were reared in the laboratory through metamorphosis. Wild plankton obtained from Narragansett Bay, brine shrimp nauplii, and chopped clams were fed as food. The early life history of this flatfish can, for the first time, be completed under controlled laboratory conditions.

The winter flounder, *Pseudopleuronectes americanus* (Walbaum), an important species in local New England commercial and sport fishing industries, occurs from Chesapeake Bay to the northern shore of the Gulf of St. Lawrence (Bigelow and Schroeder 1953). Winter flounder spawn from January through April in Rhode Island estuaries with a peak spawning period in February. Demersal eggs are produced which range from 0.74 to 0.85 mm in diameter.

Investigations to evaluate methods of inducing spawning of winter flounder with the aid of hormones under controlled laboratory conditions were undertaken at the National Marine Fisheries Service, Northeast Fisheries Center, Narragansett Laboratory in 1970 and continued over a 3-yr period. The use of hormone injections for inducing spawning in other species of fish has been well documented by Pickford and Atz (1957). The induction of spawning in winter flounder in the laboratory provides a practical method of supplying viable eggs and larvae for physiological studies. By controlling water temperatures, photoperiods, and injecting hormones, the research time for this species can be extended. As far as is known, these were the first reported successful attempts to artificially mature and spawn the winter flounder in the laboratory.

MATERIALS AND METHODS

Adult winter flounder were captured by otter trawling in Narragansett Bay in the autumn of 1970, 1971, and 1972, and were brought to the Narragansett Laboratory in a 380-liter live car equipped with an aerator. In the laboratory, the fish were held in 1,890-liter circular aquaria (1.2 m in diameter; water depth, 0.8 m). A continual supply of filtered seawater was pumped to the aquaria from Narragansett Bay.

After acclimating in the laboratory, the fish were segregated by sex, measured and weighed, and tagged with numbered plastic pennants secured through the caudal peduncle with a double barbed stainless steel wire. Winter flounder adults may be sexed easily by placing the fish on its back and running a hand down the white underside. Females are quite smooth while the males are very rough to the touch. Winter flounder lend themselves quite readily to handling and have proven to be a durable fish. No anesthesia was required prior to injecting hormones as the fish rarely struggled.

An artificial photoperiod of 9 h light and 15 h darkness (9L:15D) simulating seasonal light conditions was maintained for all experiments by time clocks controlling four banks of 80-W cool white fluorescent lights suspended 1.2 m above the tanks.

Since winter flounder fast during the spawning season, as many other fish do, their food regimen was not difficult to maintain. A varied diet of clams, squid, chopped menhaden, and silversides

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was provided. Very few females fed actively. However the males, a majority of which were running ripe, fed throughout the experiments.

Hormone injections were carried out with 0-2 cc syringes fitted with 20 gauge, 3.85 cm (1.5 inch) needles. All injections were intramuscular. Intraperitoneal injections were ruled out for fear of injuring or killing the experimental fish. Injections were made into the back muscle below the dorsal fin. Inserting and withdrawing the needle slowly aided in retaining most of the fluid in the fish (Figure 1). After injection, the flesh of the fish was massaged to diffuse the fluid into the muscles. A saline solution of isotonic sodium chloride was used as a carrier for all hormone injections except with deoxycorticosterone, which was mixed with sesame oil and injected as a slurry. Hormones tested in the various studies were: human chorionic gonadotropin (HCG), oxytocin, deoxycorticosterone (DOCA), pregnant mare serum gonadotropin (PMSG) and carp pituitary (freeze-dried powder). The criteria chosen to test the effectiveness of the hormones were gonad index, spawning, fertilization of eggs, and hatching success. Hormones were prepared on the day of injection, and dosages were established by the weight of each individual fish.

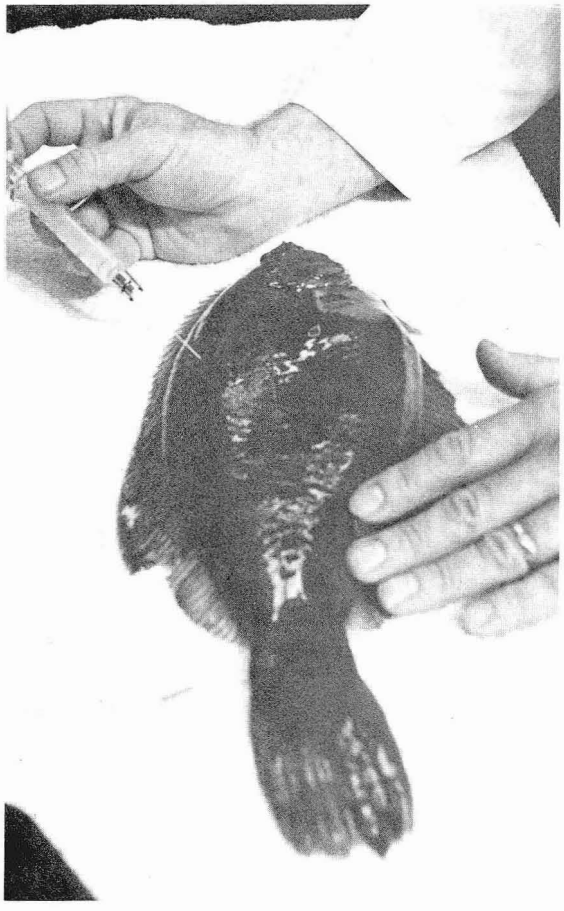

FIGURE 1.—Winter flounder receiving hormone injection.

Running ripe fish were stripped by hand. Winter flounder spawn adhesive demersal eggs which form clumps under experimental conditions. Spawned and fertilized eggs were handled and treated according to the separation techniques of Smigielski and Arnold (1972).

Human chorionic gonadotropin was the first hormone selected for evaluation. Stevens (1966) reported successful spawnings of striped bass with dosage levels of this hormone ranging from 31 through 403 IU/pound fish. Two hormone dosage levels and two time sequences of injecting were administered to three groups of test fish numbering five per group at dosage levels of 150 IU/454 g fish injected daily, 300 IU/454 g fish injected daily, and 300 IU/454 g fish injected every other day. A fourth group served as controls. Water temperatures during the first testing trial ranged between 5° and 7°C with a mean of 6.2°C.

A second series of experiments was initiated when the water temperature fell to 4°C. Water temperatures during these trials ranged between 3° and 5°C with a mean of 3.2°C. A test group numbering 15 fish was established and held in three 1,890-liter aquaria. Dosage levels of 150 IU/454 g fish were dispensed daily and administered over a time period of 12 days.

Further experiments were initiated to evaluate oxytocin, DOCA, and PMSG at the two water temperature ranges of 6°-7.5°C and 3°-5°C. Mean water temperatures during the trials were 7.2° and 3.2°C. Because of variable results obtained with the hormone HCG in prior experiments, HCG was included in these trials for further testing. Three dosage levels of each hormone were evaluated and a total of five injections administered over a period of 10 days. The dosage levels were: oxytocin at 10, 20, and 40 IU/454 g fish; DOCA at 5, 10, and 20 mg/454 g fish; PMSG at 55, 110, and 220 IU/454 g fish; and HCG at 100, 200, and 400 IU/454 g fish. Female test fish were measured, weighed, tagged, and placed into four 1,890-liter circular tanks supplied with a continual supply of seawater. Each tank contained a total of

16 fish. The three differing dosage levels of each test hormone were injected into groups consisting of four fish per group, and the remaining four fish in each tank served as uninjected controls. At the termination of the trial, test fish were sacrificed, ovaries examined, and gonosomatic index (GSI) levels recorded.

A final series of experiments was initiated to evaluate the effectiveness of carp pituitary (freeze-dried powder) at the dosage levels of 5.0 mg and 0.5 mg/454 g fish injected daily. Two groups of test fish with each group consisting of three trial fish and three uninjected controls were established and held in two 1,890-liter tanks. During the trials, water temperatures ranged between 1.5° and 3.5°C with a mean at 2.5°C.

RESULTS AND DISCUSSION

In the group of test fish that received HCG injections at 300 IU daily, one fish hydrated after a single injection (Table 1). Hydration, an increase in total body weight due to water uptake by the ovaries resulting in higher GSI levels, was rapid, and the following morning this fish was grossly bloated. Externally, hydrated fish may become slightly swollen or grossly bloated. The majority of the eggs stripped were opaque and misshapen, and approximately 5% of the total egg mass in the ovaries were viable. These eggs, numbering approximately 10,000, were fertilized but embryonic development ceased in the blastula stage and none survived to hatch. This rapid hydration may have been responsible for the poor quality of the eggs. A long development period for oocytes with a minimum of 2 or 3 yr from the time oocytes become histologically recognizable until they are spawned has been suggested for winter flounder by Dunn and Tyler (1969). Our observations in the past have noted that the natural hydration and ovulation process of winter flounder can occupy a lengthy period of time (unpubl. data). The remaining fish in this test group were refractory after receiving a total of 12 injections.

TABLE 1.—Effects of human chorionic gonadotropin (HCG) on *Pseudopleuronectes americanus*, temperature range 5°-7°C ($\bar{x}$ 6.2°C). Photoperiod 9L/15D.

Hormone and dosage	No. of fish	No. of injections	Number hydrated	Number ovulated	Fertilization (%)	Hatch (%)
300 IU HCG/454 g fish daily	1	1	1	1	[1]5	0
300 IU HCG/454 g fish daily	4	12	0	0	—	—
300 IU HCG/454 g fish every 48 h	5	7	1	[2]1	95	0
150 IU HCG/454 g fish daily	5	12	2	0	—	—
Controls	6	0	1	0	—	—

[1]Approximate.
[2]Ovulated and spawned 10 days after last injection.

The group of five test fish that received injections of 300 IU every other day (for a total of seven injections in 13 days) contained three fish which were refractory at the conclusion of the trial; one fish displayed signs of slight hydration, but did not ovulate. The remaining fish in the group ovulated and was spawned 10 days after the last injection. Approximately 95% of the eggs were fertilized but development ceased at the blastula stage and none survived to hatch.

The group of test fish which received daily injections of 150 IU for 12 days contained three fish which were refractory and two which hydrated slightly but did not ovulate. Of the six fish that served as uninjected controls, five displayed no signs of hydration, and the remaining fish hydrated slightly but did not ovulate.

At the termination of the trials, the fish were not sacrificed. The only criterion for evaluating the success of the hormone was obtaining viable eggs. It was reasoned that the relatively warm-water temperatures (5°-7°C) coupled with suspected low GSI levels inhibited the effectiveness of the hormones. Observations made in the inner parts of the Gulf of Maine, (Bigelow and Schroeder 1953) have shown that extensive spawning of winter flounder does not occur in water temperatures above 6°C.

At the conclusion of the second series of experiments, four fish hydrated and ovulated after receiving from two to nine injections (Table 2). Fertilization was high for all fish but embryonic mortalities were high in the blastula and gastrula stages, and hatches were poor, ranging from 2 to 20%. Larvae obtained from these spawnings appeared to be normal in all respects and several were reared through metamorphosis. Three additional test fish became grossly bloated after receiving a total of 10 injections in 10 days and hydrated to the point of dying. Membranous plugs formed in their oviducts and the eggs were water hardened. The formation of these plugs is not understood as prior to receiving their last injection they were hydrating at a normal rate. Shehadeh and Ellis (1970) reported plugs forming in striped

TABLE 2.—Effects of hormones on *Pseudopleuronectes americanus*. Temperature range 3°-5°C ($\bar{x}$ 3.2°C). Photoperiod 9L/15D. Symbols (+ = Did, 0 = Did not hydrate or ovulate).

Hormone and dosage	Number of injections	Total length (mm)	Initial body weight (g)	Weight change at termination (% initial wt.)	GSI[1] (% final wt.)	Hydrated	Ovulated	Fertilization (%)	Hatch (%)
[2]HCG 150 IU/454 g fish daily	2	271	271	−3.32	1.29	+	+	80	2
	4	316	792	+0.76	1.08	+	+	90	5
	8	349	640	+0.63	1.24	+	+	80	10
	9	370	692	+0.58	1.15	+	+	80	20
	10	360	545	+3.67	32.74	+	[3]0	—	—
	10	290	275	+5.45	30.34	+	[3]0	—	—
	10	421	982	+2.75	30.13	+	[3]0	—	—
	12	391	670	+0.60	16.02	+	0	—	—
	12	334	571	−0.35	8.08	0	0	—	—
	12	377	486	+0.62	7.57	0	0	—	—
	12	317	562	+1.60	16.46	+	0	—	—
	12	342	454	+0.44	10.13	0	0	—	—
	12	280	352	+1.42	15.13	+	0	—	—
	12	336	599	+0.33	9.48	0	0	—	—
	12	352	800	+0.50	20.15	+	0	—	—
Control	0	341	721	+1.05	1.14	+	+	85	75
	0	330	540	+1.11	19.60	+	0	—	—
	0	321	449	+1.56	19.08	+	0	—	—
	0	337	497	+1.01	18.73	+	0	—	—
	0	271	299	−0.67	10.24	0	0	—	—
	0	269	356	+2.25	12.09	0	0	—	—

[1]Gonadosomatic index.
[2]Human chorionic gonadotropin.
[3]Plug formed, fish became grossly bloated and died, eggs were water hardened in ovaries.

mullet, *Mugil cephalus*, while attempting induced spawning.

The findings at the conclusion of the second series of experiments indicated that the injection of HCG, although more effective at the lower water temperatures, resulted in poor egg quality and egg survival, the formation of membranous plugs, and gross hydration causing death.

Oxytocin at all three dosage levels tested had little effect when water temperatures were above 5°C. Three fish hydrated but none ovulated or contained matured eggs (Table 3).

DOCA administered at the lower dosage levels of 5 and 10 mg/454 g fish and at water temperatures above 5°C, resulted in several fish hydrating but none ovulating. At the higher dosage level of 20 mg/454 g fish, two test fish hydrated, and although their GSI levels were high, no ovulation occurred and no mature eggs were present in their ovaries.

PMSG at the three dosage levels of 55, 110, and 220 IU/454 g fish showed low activity when administered at water temperatures above 5°C. Although some fish had hydrated, none ovulated and GSI levels were low in all of the test groups.

HCG was ineffective at the dosage levels of 100 and 200 IU. Dosage levels of 400 IU resulted in three fish hydrating at low GSI levels. Another fish ovulated and was stripped 3 days after the last injection. Egg fertilization was high and approximately 80% of the eggs hatched. The larvae obtained from this induced spawning appeared to be normal in all respects and several were reared through metamorphosis.

The data gleaned from this trial at water temperatures in the 6°-7.5°C range substantiated results derived from earlier experiments, suggesting that water temperatures above 6°C inhibit maturation of winter flounder, and for the most part hormones are ineffective. The manner in which hormones exert their effects on fish is poorly understood, and dosage levels are probably meaningless as the largest fish may not necessarily be the most sexually mature and may differ in receptibility to hormone injections.

Haydock (1971) has observed a temperature threshold of 17°C below which the gulf croaker, *Bairdiella icistia*, would not hydrate or ovulate. It is very probable that a similar temperature threshold exists for winter flounder above 6°C. Observations in the past at our laboratory have noted that gravid female flounder perished in water temperatures of 10°C, and ova from these fish were stunted and misshapen. Male flounder held under the same conditions suffered no apparent ill effects.

In the lower temperature range tested (range 3°-5°C, mean 3.2°C), oxytocin produced slightly better results (Table 4). Three fish hydrated but none ovulated nor contained mature eggs. GSI levels were higher at the lower temperatures at all dosage levels tested. No abnormal hydration was

TABLE 3.–Effects of hormones on *Pseudopleuronectes americanus* given five injections over a 10-day period. All fish experienced a 9L:15D photoperiod, water temperature range 6°-7.5°C (x̄ 7.2°C). Symbols (+ = Did, 0 = Did not hydrate or ovulate).

Hormone and dosage	Total length (mm)	Initial body weight (g)	Weight change (% initial wt.)	GSI[1] (% final wt.)	Hydrated	Ovulated	Fertilization (%)	Hatch (%)
Oxytocin	386	716	+3.91	13.87	+	0		
10 IU/454	336	495	0	15.25	0	0		
g fish daily	308	344	+0.87	17.87	0	0		
	333	504	−0.79	14.00	0	0		
Oxytocin	323	453	+2.21	17.17	+	0		
20 IU/454	305	350	−0.29	14.90	0	0		
g fish	345	477	−1.68	9.81	0	0		
	370	662	−0.30	12.00	0	0		
Oxytocin	306	409	+0.98	14.53	+	0		
40 IU/454	338	377	−1.06	13.94	0	0		
g fish	308	404	−0.50	8.83	0	0		
	349	471	0	16.99	0	0		
Control	346	443	+0.45	13.82	0	0		
	296	327	+1.22	13.14	0	0		
	302	389	+3.08	13.19	+	0		
	370	634	+1.58	16.61	0	0		
[2]DOCA 5 mg/454	340	456	+2.63	1.39	0	0		
g fish	345	549	+3.10	15.37	+	0		
	372	540	+5.74	14.97	+	0		
	299	310	+9.68	13.09	+	0		
[2]DOCA 10 mg/454	287	270	+13.70	11.24	+	0		
g fish	283	272	+7.35	8.39	+	0		
	300	354	−0.28	12.75	+	0		
	345	525	+5.71	15.05	+	0		
[2]DOCA 20 mg/454	392	726	+1.38	4.08	0	0		
g fish	306	338	+4.73	7.20	0	0		
	376	726	+5.79	19.79	+	0		
	375	516	+15.70	28.14	+	0		
Control	295	327	+1.22	12.24	0	0		
	323	408	+3.67	14.07	+	0		
	356	526	+1.71	24.58	+	[3]0		
	342	480	+2.92	17.21	+	0		
[4]PMSG 55 IU/454	315	448	+1.79	11.84	+	0		
g fish	321	420	−0.24	9.07	0	0		
	381	652	+0.46	10.23	0	0		
	358	582	+6.19	16.34	+	0		
[4]PMSG 110 IU/454	294	318	+1.57	0.93	[5]0	0		
g fish	349	546	−0.92	12.29	0	0		
	349	219	+1.83	9.42	0	0		
	303	341	+4.69	13.45	+	0		
[4]PMSG 220 IU/454	335	488	+2.46	18.00	+	0		
g fish	321	389	−1.03	3.64	0	0		
	315	390	+5.64	13.23	+	0		
	342	480	−0.21	6.26	0	0		
Control	357	474	+0.63	1.23	0	0		
	424	989	+1.01	16.52	0	0		
	310	351	+1.14	0.70	0	0		
	265	263	+1.90	1.12	0	0		
[6]HCG 100 IU/454	321	326	−1.23	6.83	0	0		
g fish	323	455	−0.22	13.00	0	0		
	358	517	0	10.35	0	0		
	350	530	+1.89	12.50	+	0		
[6]HCG 200 IU/454	315	300	−0.33	13.55	0	0		
g fish	316	345	+2.03	9.09	0	0		
	282	247	+4.05	11.67	0	0		
	292	275	+4.36	11.32	0	0		
[6]HCG 400 IU/454	337	549	−1.82	0.74	+	+	98	80
g fish	330	323	+3.10	2.25	+	0		
	388	706	+4.39	8.07	+	0		
	330	391	+2.30	9.75	+	0		
Control	301	309	−0.65	10.75	0	0		
	296	339	−0.88	14.14	0	0		
	282	346	−0.29	8.41	0	0		
	281	229	+6.99	18.57	+	[3]0		

[1]Gonadosomatic index.
[2]Deoxycorticosterone.
[3]Matured eggs in ovaries.
[4]Pregnant mare serum gonadotropin.
[5]Sexually immature.
[6]Human chorionic gonadotropin.

TABLE 4.—Effects of hormones on *Pseudopleuronectes americanus* given five injections over a 10-day period. All fish experienced a 9L:15D photoperiod, water temperature range 3°-5°C (x̄ 3.2°C). Symbols (+ = Did, 0 = Did not hydrate or ovulate).

Hormone and dosage	Total length (mm)	Initial body weight (g)	Weight change (% initial wt.)	GSI[1] (% final wt.)	Hydrated	Ovulated	Fertilization (%)	Hatch (%)
Oxytocin	378	578	−0.35	19.36	0	0		
10 IU/454	306	321	−1.25	13.72	0	0		
g fish	412	752	−3.32	20.50	0	0		
	340	488	+0.41	13.76	0	0		
Oxytocin	335	481	−1.66	12.90	0	0		
20 IU/454	373	654	+0.92	24.62	+	0		
g fish	308	356	−1.69	16.29	0	0		
	391	774	−0.65	18.66	0	0		
Oxytocin	305	352	+4.83	17.21	+	0		
40 IU/454	284	290	−4.92	14.88	0	0		
g fish	338	440	+3.30	20.34	+	0		
	352	478	−0.42	11.40	0	0		
Control	292	327	−1.21	12.08	0	0		
	235	184	+0.54	22.55	+	[2]0		
	316	360	0	17.92	+	0		
	300	399	−0.50	16.67	+	0		
[3]DOCA 5 mg/454	320	430	+3.49	11.24	0	0		
g fish	332	419	+0.48	16.79	+	0		
	360	619	+2.75	23.58	+	0		
	335	517	+0.77	23.03	+	0		
[3]DOCA 10 mg/454	314	270	−3.70	10.96	0	0		
g fish	374	649	+4.31	16.84	+	0		
	323	460	+4.35	24.79	+	[2]0		
	271	225	+8.44	11.07	0	0		
[3]DOCA 20 mg/454	333	528	+7.39	19.22	+	0		
g fish	402	1,003	+9.07	32.45	+	[2]0		
	337	468	+2.78	12.68	0	0		
	340	546	+2.01	23.07	+	0		
Control	284	314	+5.37	11.78	0	0		
	295	279	+2.20	11.83	0	0		
	396	800	+2.30	13.25	0	0		
	347	579	+0.35	24.27	+	0		
[4]PMSG 55 IU/454	334	693	+5.34	18.36	+	0		
g fish	336	435	+2.30	18.71	+	0		
	425	1,105	−0.09	18.98	+	0		
	321	539	+7.98	25.17	+	+[5]	[6]0	0
[4]PMSG 110 IU/454	327	467	+5.14	14.97	+	0		
g fish	340	498	+1.20	14.58	+	0		
	324	411	−2.92	14.44	0	0		
	303	372	−4.30	13.34	0	0		
[4]PMSG 220 IU/454	360	593	0	13.07	0	0		
g fish	261	207	−8.70	20.11	0	+[5]	0	0
	350	541	+0.74	17.61	+	0		
	437	1,173	+6.05	41.52	+	+[5]	20	18
Control	295	334	−6.59	14.74	0	0		
	329	500	+1.00	13.74	+	0		
	287	346	+1.45	15.10	+	0		
	310	350	−1.71	1.16	+	+	98	70
[7]HCG 100 IU/454	400	722	+2.08	11.13	0	0		
g fish	343	462	−5.19	18.04	0	0		
	335	446	−6.95	12.29	0	0		
	402	812	+2.83	20.12	+	0		
[7]HCG 200 IU/454	340	519	+0.39	19.67	+	0		
g fish	352	500	−4.20	18.58	0	0		
	331	462	+6.93	23.99	+	0		
	290	320	+9.69	1.21	+	+	98	80
[7]HCG 400 IU/454	285	298	−1.34	17.01	0	0		
g fish	348	516	+5.04	18.91	+	0		
	330	513	+3.12	27.03	+	+[5]	15	0
	296	336	−5.65	14.67	0	0		
Control	362	249	+1.20	14.48	0	0		
	324	548	+2.24	21.81	+	0		
	397	943	+1.18	24.13	+	0		
	318	500	+4.82	13.10	0	0		

[1]Gonadosomatic index.
[2]Matured ova in ovaries.
[3]Deoxycorticosterone.
[4]Pregnant mare serum gonadotropin.
[5]Plug formed in oviduct.
[6]Eggs water hardened.
[7]Human chorionic gonadotropin.

noted nor did any plugs form in the test fish. A weight loss occurred in almost all of the test fish at the lower temperatures.

Administering DOCA at lower temperatures resulted in GSI levels being higher in test fish at all three dosage levels. Several fish hydrated and although no ovulation occurred, some matured eggs were present in the ovaries of two test fish that received injections at the higher levels of 10 and 20 mg. No plugs were present in any of the test fish and no abnormal hydrations were observed.

Tests with PMSG in the lower temperature range resulted in some fish at all three dosage levels hydrating but not spawning. Plugs formed in the oviducts in two of the fish that received dosage levels of 55 and 220 IU respectively. Eggs were water hardened in one and were nonfertilizable. Approximately 20% of the total eggs obtained from the second fish were fertilized. These eggs were normal in size and the majority of them developed and hatched. The larvae obtained from this induced spawning appeared to be normal in all respects and several were reared through metamorphosis.

HCG administered at the level of 200 IU at lower temperatures produced ovulation in one fish, and 4 days after the last planned injection it was spawned. The eggs obtained were normal in size and appearance. Approximately 95% were fertilized and approximately 80% developed and hatched. The larvae obtained from this hormone-induced spawning appeared normal in all respects and many were reared through metamorphosis. One other fish that received the higher dosage level of 400 IU had a plug form in the oviduct and approximately 15% of the eggs present in the ovaries were mature. These eggs were spawned and fertilization was successful. All development halted at the blastula stage and none survived to hatch.

The limited success that was obtained with the hormones oxytocin, DOCA, PMSG, and HCG administered alone is apparent. It is hoped future studies will evaluate the synergistic actions of various hormone combinations administered to winter flounder.

Two test fish that received injections of carp pituitary at the level of 5.0 mg ovulated and were stripped (Table 5). The eggs obtained from these induced spawnings were in the normal size range of winter flounder eggs and approximately 90-95% were fertilized. The majority of them developed normally and approximately 85% hatched. The larvae appeared normal in all respects and many were reared through metamorphosis. At the dosage level of 0.5 mg, three fish ovulated and were spawned after receiving three to six injections. During the time of injecting these fish hydrated normally. The eggs obtained from these induced spawnings were normal in size and approximately 95% were fertilized. Their development was normal and approximately 70-85% survived to hatch. Larvae obtained appeared normal in all respects and several were reared through metamorphosis.

All of the uninjected controls at the conclusion of the trials displayed signs of hydrating. Four out of the six fish were sacrificed and GSI levels recorded. The remaining two fish that displayed the best signs of hydration were spared and

TABLE 5.—Effects of carp pituitary on *Pseudopleuronectes americanus*. All fish experienced a 9L:15D photoperiod, water temperature range 1.5°-3.5°C ($\bar{x}$ 2.5°C). Symbols (+ = Did, 0 = Did not hydrate or ovulate).

Hormone and dosage	No. of injections	Total length (mm)	Initial body weight (g)	Weight change (% final wt.)	GSI[1] (% final wt.)	Hydrate	Ovulate	Fertilization (%)	Hatch (%)
Carp pituitary 5.0 mg/454 g fish	3	410	830	+1.20	1.11	+	+	90	85
	3	415	1,069	+2.99	1.06	+	+	95	85
	3	316	453	−0.04	1.60	[2]0	0		
Carp pituitary 0.5 mg/454 g fish	3	325	509	+2.55	1.11	+	+	95	75
	6	357	556	+2.16	1.10	+	+	95	80
	6	345	509	+3.54	1.32	+	+	95	70
Control	0	360	494	+3.64	20.04	+	0		
	0	347	462	+4.11	21.21	+	0		
	0	339	510	+4.31	21.37	+	0		
	0	310	381	+6.04	20.47	+	0		
	0	298	321	+2.18	2.18	+	+[3]	95	30
	0	359	492	+2.64	1.11	+	+[3]	95	90

[1]Gonadosomatic index.
[2]Sexually immature.
[3]Ovulated and spawned 46 days after termination of testing trials.

allowed to continue to develop without interruption. After 46 days they ovulated and were stripped.

The ability of carp pituitary as an aid in inducing spawning in winter flounder was dramatically shown in these trials. At least six additional weeks of research time was able to be realized when carp pituitary was administered in conjunction with low water temperatures (1.5°-2.5°C). It is hoped that future tests will be initiated to evaluate the effectiveness of various dosage levels and at warmer water temperatures. By controlling photoperiods and water temperatures and injecting carp pituitary it may be possible to extend the research time on the eggs and larvae of this species of flatfish in the laboratory for several additional months through induced spawnings.

In conclusion, it would appear that water temperature may be the most critical factor in producing ovulation; and while the administering of hormones was effective in producing hydration, hydration alone is not sufficient to initiate ovulation. Low GSI levels below 12% in conjunction with water temperatures above 6°C resulted in the majority of test fish not hydrating regardless of the hormone administered.

Hormone treatments had a range of effects depending on the degree of ovarian maturation; test fish in the later stages of development responded while less mature fish developed higher GSI levels but did not ovulate and spawn. No evidence was discovered indicating that spawning induced by hormones produced abnormal larvae.

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LITERATURE CITED

BIGELOW, H. B., AND W. C. SCHROEDER.
1953. Winter flounder *Pseudopleuronectes americanus* (Walbaum) 1792. *In* Fishes of the Gulf of Maine, p. 276-283. U.S. Fish Wildl. Serv., Fish. Bull. 53.

DUNN, R. S., AND A. V. TYLER.
1969. Aspects of the anatomy of the winter flounder ovary with hypotheses on oocyte maturation time. J. Fish. Res. Board Can. 26:1943-1947.

HAYDOCK, I.
1971. Gonad maturation and hormone-induced spawning of the Gulf croaker, *Bairdiella icistia*. Fish. Bull., U.S. 69:157-180.

PICKFORD, G. E., AND J. W. ATZ.
1957. The physiology of the pituitary gland of fishes. New York Zoological Society, N.Y., 613 p.

SHEHADEH, Z. H., AND J. N. ELLIS.
1970. Induced spawning of the striped mullet *Mugil cephalus* L. J. Fish. Biol. 2:355-360.

SMIGIELSKI, A. S., AND C. R. ARNOLD.
1972. Separating and incubating winter flounder eggs. Prog.-Fish Cult. 34:113.

STEVENS, R. E.
1966. Hormone-induced spawning of striped bass for reservoir stocking. Prog.-Fish Cult. 28:19-28.