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INDUCED SPAWNING AND LARVAL REARING OF THE YELLOWTAIL FLOUNDER, *LIMANDA FERRUGINEA*

The yellowtail flounder, *Limanda ferruginea* (Storer), a commercially important flatfish, occurs in North American continental waters from the north shore of the Gulf of St. Lawrence southward to the lower part of Chesapeake Bay (Bigelow and Schroeder 1953). The yellowtail flounder spawns from March through August where water temperatures over its range vary from about 5° to 12°C (Colton 1972). The eggs are pelagic and lack an oil globule; diameter of the live eggs (range 0.79-1.01 mm) averages 0.88 mm (Colton and Marak 1969).

A program to obtain viable yellowtail eggs through hormone induction, to rear larvae through metamorphosis, and to determine the mechanisms of survival of early life stages under controlled laboratory conditions was undertaken. The successful induction of yellowtail flounder and subsequent rearing of the larvae through metamorphosis marks the first time the early life history of this flatfish has been completed in the laboratory.

Materials and Methods

Adult yellowtail flounder were captured by otter trawling in Block Island Sound in the winters of 1974, 1975, and 1976 and transported to the Narragansett Laboratory in a 380-l live car equipped with an aerator. In the laboratory the fish were held in a 28,000-l aquarium. A continual supply of filtered seawater was pumped to the aquarium from Narragansett Bay.

Individuals presumed to be sexually mature were selected by length. Available length-weight data (Lux 1969) indicated that yellowtail flounder in southern New England waters mature when they attain a length near 35 cm or an age of 3 yr (Lux and Nichy 1969). 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. Yellowtail flounder were sexed by holding the white underside to the light and looking through the flesh. The outline of the ovary extending posteriorly from the mass of viscera can readily be seen even in immature females (Royce et al. 1959). Yellowtail flounder are delicate and excitable. To minimize injury, the fish were anesthetized in a solution of tricane methanesulfonate (MS-222[1]) at a concentration of 1:20,000 (Leitritz and Lewis 1976) during each examination.

While the fish were held in captivity, a photoperiod of 11 h of light and 13 h of dark simulated spawning light conditions. Four banks of fluorescent lights (each bank composed of 16 40-W bulbs) were suspended 4 m from the ceiling and mechanically timed. The light banks were sequentially turned on and off in the morning and evening at 15-min intervals to simulate dawn and twilight. Prior to receiving hormones the fish were fed a daily diet of chopped frozen hake, whiting, or squid. During the trials the fish were not fed.

[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

The effectiveness of the pituitary preparations was evaluated by monitoring the gonosomatic index (GSI), ovulation, success of egg fertilization, and hatching success. Hormones were prepared on the day of injection, and dosages were established by the weight of each individual fish. A saline solution of isotonic sodium chloride was used as a carrier. All injections were administered (2-cm^3 syringe, 20 gage 3.85-cm needle) intramuscularly into the back below the dorsal fin. Inserting and withdrawing the needle slowly aided in retaining most of the fluid in the flesh. After injection, the flesh of the fish was massaged to diffuse the fluid into the muscles.

Sexually mature fish were hand stripped and the eggs fertilized in a polyethylene pan. Several thousand eggs were collected at each spawning, and the sperm of two males was used to fertilize the eggs from each female. Yellowtail flounder are nonsynchronous spawners (Bigelow and Schroeder 1953), and multiple spawnings occurred among most induced fish. The fecundity of yellowtail flounder increases with age and body length, and an individual female may yield from 350,000 to more than 4,000,000 eggs during the spawning season (Pitt 1971).

The state of ova maturation of the experimental fish was observed at the start and termination of each experiment. Before injecting, a polyethylene cannula was inserted into the oviduct and oocyte samples were orally withdrawn. The oogenesis of oocytes was divided by microscopic observation into three general histological stages:

Stage I - the primary oocyte stage, oocytes contained cytoplasmic vacuoles and measured between 0.1 and 0.25 mm.

Stage II - the yolk globule stage, cytoplasm of oocytes was filled with dense yolk granules and measured up to 0.6 mm.

Stage III - ripe stage, hyaline oocytes present and measured 0.75-1.00 mm in size.

Fertilized eggs were incubated in static, aerated, black-sided aquaria that had been inoculated with the green algae *Dunaliella* sp. A single application of penicillin (25 international units [IU]/ml) and streptomycin (0.02 mg/ml) at the concentration of 50 mg/l was effective in controlling bacterial contamination of the aquaria in almost all cases.

Three series of experiments were undertaken to determine the effectiveness of the hormone injections (Table 1). The first trial was conducted in winter 1975 to determine if induced spawning would occur at low winter water temperatures. The second and third were conducted in the springs of 1976-77 and coincided with the yellowtail flounder's natural spawning season.

Hormone dosage levels of 2, 5, and 10 mg/kg fish and frequency of injecting were dictated by previous successful results obtained with the summer flounder, *Paralichthys dentatus*, (Smigielski 1975a) and winter flounder, *Pseudopleuronectes americanus*, (Smigielski 1975b). After each trial the female fish were killed and reweighed, the ovaries were examined, and gonosomatic indices were recorded. Prior to receiving hormone injections, all the female test fish in the first trial were in Stage I of oocyte oogenesis, and most females prior to the second and third trials were in Stage II. Males were not injected in the second and third trials because they were sexually ripe.

Results and Discussion

First Trial

In the first trial (Table 1), most females in the group receiving 10 daily injections of 2 mg pituitary were refractory with low GSI values (7-13%). One fish hydrated but did not ovulate, and a small number of Stage II ova were found in the ovaries. Hydration is an increase in total body weight. The weight gain is due mostly to water intake and is reflected by higher GSI values as most of the water appears to go into the gonads. Excessive hydration is manifested by grossly bloated fish which in some instances can hydrate to the point of death without ovulating.

TABLE 1.—Hormone dosages, water temperatures, and number of yellowtail flounder in each trial.

	Trial 1 - January 1975 Water temperatures, 3°-6°C (Mean 5.1°C)			Trial 2 - April 1976 Water temperatures, 7°-10°C (Mean 9.2°C)			Trial 3 - April 1977 Water temperatures, 8.5°-12.5°C (Mean 10.1°C)		
Daily carp pituitary dosages	Number of females	Number of males	Uninjected controls	Number of females	Number of males	Sham injected controls	Number of females	Number of males	Sham injected controls
2.0 mg/kg fish	6	4	3	8	0	4	9	0	4
5.0 mg/kg fish	6	4	3	8	0	4			
10.0 mg/kg fish	6	4	3	7	0	4			

In the group of females receiving 10 daily injections of 5 mg, three fish were refractory with low GSI values (10-15%), and three hydrated but did not ovulate. Two of the latter fish contained a small number of Stage II ova; the other developed a cloacal plug of membranous tissue and Stage I ova.

In the group receiving 10 daily injections of 10 mg, all were refractory with low GSI values (9-11%), except for one fish that hydrated but did not ovulate. A membranous plug developed in the cloaca of this fish, and it was bloated. A very small number of Stage II ova were found in the ovaries. There was no indication of sexual ripening in the uninjected control fish, and their GSI values were low (10-13%). Copious semen was obtained from the males injected at all three dosages; however, fertilization was not attempted. It was reasoned from the first trial that low GSI values (7-15%) of females coupled with low water temperatures (3°-6°C, mean 5.1°C) that were less than optimum inhibited the effectiveness of the hormones, for although some fish hydrated, they did not ovulate.

Second Trial

The results obtained from the second trial were variable (Table 2). All but one fish receiving injections of 2 mg hydrated and ovulated. Two fish died during the trial; one, after yielding spawn on two occasions, developed a membranous plug and became grossly bloated.

In the group receiving injections of 5 mg, two fish ovulated but the eggs obtained were not fertile. Three other fish developed plugs and hydrated to the point of death. Injections were discontinued at the first sign of abnormal hydration, but the fish continued to imbibe water.

In the group that received 10 mg, five fish experienced excessive hydration manifestated by bloating, plug formation, and, in two instances, death. The membranous plugs were identical to those that developed in the test group that received hormone dosages of 5 mg. The controls had four fish with signs of hydration but no Stage III ova were found in their ovaries.

Third Trial

The results of the third trial paralleled those of the second trial at a dosage of 2 mg. Seven of the experimental females hydrated normally and ovulated (Table 3). Fertilization and hatching of these eggs were satisfactory and the larvae were normal. The remaining two fish died during the trial, and their ovaries had a small number of Stage III ova. The control fish neither hydrated nor ovulated; GSI values were fairly high, but Stage III ova were absent.

The anomalous hydration with bloating and formation of membranous plugs during hormonal induction is not unique. Clemens and Grant (1964) injected female goldfish, *Carassius auratus*, with carp pituitary and observed that the gonadal water content increased, apparently in association with ovulation. The hormone regulating the hydration process appeared to be a gonadotropin.

Shehadeh and Ellis (1970) reported the formation of plugs in the cloaca in striped mullet, *Mugil cephalus*, treated with a combination of salmon pituitary and Synahorin. Sinha (1971) studied the gonadal hydration response of *Puntis gonionotus* using the second fraction of molecular seived carp pituitary extract and suggested that the second fraction is involved in osmoregulation, since an injection of an additional amount enhances the rate of water transport resulting in maturation.

Hirose and Ishida (1974) studied the effects of Cortisol and human chorionic gonadotropin (HCG) in ayu, *Plecoglossus altivelis*, and reported that the water content of the ovary from hormone-treated fish increased by 6%. Smigielski (1975b) reported a similar response in winter flounder injected with pregnant mare serum (PMS) and HCG. Hirose (1976) demonstrated that gonadotropin-treated ayu imbided a greater quantity of water than control ayu. He suggested that gonadotropin may act on the sodium and potassium system or permeability of the egg membrane.

Hydration is a normal and necessary prelude to maturation and ovulation. The cases of abnormal hydration experienced with yellowtail flounder may be attributed to an adverse reaction to hormone dosage. Most of the test fish that hydrated abnormally and became bloated had an increase in body weight of more than 10%. The increase in body weight appeared to be a result of the fish imbibing an excess amount of water. An excessive amount of introduced hormone may upset the water transport or sodium potassium systems, resulting in more water bing imbibed.

In conclusion, it appears that water temperatures higher than 6°C and GSI values approaching 20% coupled with carp pituitary injections approximating 2 mg/kg of fish is an effective combi-

TABLE 2.—Effects of carp pituitary on yellowtail flounder receiving daily injections. All fish were exposed to 11L:13D photoperiod and water temperatures of 8.5°-12.5°C (Mean 10.1°C). Symbols: + = did, 0 = did not hydrate or ovulate.

Dosage	No. of injections	Total length (mm)	Initial body weight (g)	Weight change (% initial wt)	GSI (% final wt)	Effect		Date of spawning 1976	Fertilization (%)	Hatch (%)
						Hydrated	Ovulated			
2.0 mg/kg fish	8	348	396	+6.94	19.7	+[1]	0			
	7	340	392	−1.84	5.6	+	+	Apr. 6	80	70
								Apr. 7	70	75
	4	353	421	−3.30	5.1	+	+	Apr. 6	75	55
								Apr. 7	70	60
								Apr. 8	80	70
								Apr. 9	90	75
								Apr. 11	70	60
	1	435	817	−0.96	4.3	+	+	Apr. 4	75	80
								Apr. 5	80	75
								Apr. 6	70	60
								Apr. 7	70	50
								Apr. 9	75	75
	6	280	263	−0.49	7.0	+	+	Apr. 5	80	75
								Apr. 7	75	70
								Apr. 8	80	70
	4	350	381	+1.12	8.4	+	+	Apr. 8	80	60
								Apr. 9	80	50
								Apr. 11	70	80
	6	450	882	+9.54	24.0	+	+[2]	Apr. 1	80	75
								Apr. 3	75	70
	6	387	721	+0.98	11.2	+	+	Apr. 5	80	80
								Apr. 6	75	65
Controls		420	667	+2.12	18.6	0	0			
		392	611	+2.63	19.6	+	0			
		361	409	+1.09	10.5	0	0			
		381	562	+0.44	9.8	0	0			
5.0 mg/kg fish	5	401	683	+2.11	18.6	+	+[3]			
	6	501	1,489	+9.62	29.5	+[2]	0			
	4	307	566	+11.69	26.1	+[2]	0			
	5	435	1,131	+13.17	24.9	+[2]	0			
	10	348	762	−0.19	4.6	0	0			
	5	351	491	+8.47	30.8	+[4]	+[3]			
	10	336	418	+0.96	11.0	0	0			
Controls		361	521	+2.92	19.3	+	0			
		392	587	+2.87	19.5	+	0			
		406	702	+2.19	15.1	0	0			
		339	367	+1.87	12.9	0	0			
10 mg/kg fish	5	467	1,259	+11.15	28.6	+[4]	0			
	4	346	593	+13.57	27.6	+[2]	0			
	5	352	463	+15.11	29.6	+[4]	+[3]			
	5	433	1,245	+10.76	25.3	+[4]	0			
	7	341	485	+14.06	29.1	+[2]	0			
	10	360	501	+1.16	12.6	0	0			
	10	348	437	+1.84	14.2	0	0			
Controls		343	428	+1.23	15.1	0	0			
		389	672	+2.63	17.9	+	0			
		430	891	+4.78	19.3	+	0			
		369	551	+1.63	14.3	0	0			

[1]Died, ~ 10% Stage III ova in ovaries, not fertilized.
[2]Plug formed, fish became bloated and hydrated to point of death.
[3]Stage III ova in ovaries, not fertilized.
[4]Plug formed, fish became bloated.

nation for inducing spawning of yellowtail flounder.

Larval Rearing

Fertilized yellowtail flounder embryos were incubated in 64-l, rectangular, black-sided, static, well-aerated aquaria at a density of approximately 80 embryos/l. The incubating and rearing temperature was 10°C and the salinity 32‰. Banks of 40-W timed fluorescent lights suspended 1 m over the aquaria simulated a day and night regimen of 15 h light and 9 h dark (15L:9D). The aquaria were inoculated with green algae (*Dunaliella* sp.) which may have aided in the removal of metabolic waste products produced by the larvae. The algae also served to sustain the zooplankton introduced as food. A single application of penicillin (25 IU/ml) and streptomycin (0.02 mg/ml) was effective in controlling bacterial contamination in almost all cases.

At 10°C, hatching occurred 6-7 days after fertilization. Yolk absorption occurred 4-5 days after hatching, which coincided with first feeding. The larvae averaged 2.75 mm long upon hatching and possessed a completely formed gut. The eyes were

TABLE 3.—Effects of carp pituitary on yellowtail flounder receiving daily injections. All fish were exposed to 11L:13D photoperiod and water temperatures of 8.5°-12.5°C (Mean 10.1°C). Symbols: + = did, 0 = did not hydrate or ovulate.

Dosage	No. of injections	Total length (mm)	Initial body weight (g)	Weight change (% initial wt)	GSI (% final wt)	Effect		Date of spawning 1977	Fertilization (%)	Hatch (%)
						Hydrated	Ovulated			
2.0 mg/kg fish	3	391	552	+0.56	4.6	+	+	Apr. 12	75	80
	3	453	898	+9.13	21.2	+[1]	0			
	2	474	1,298	+4.70	6.9	+	+	Apr. 12	80	70
								Apr. 14	80	75
	4	424	878	+7.16	20.8	+[1]	0			
	5	412	733	+2.04	5.3	+	+	Apr. 16	50	60
								Apr. 17	60	40
								Apr. 18	60	45
	5	364	486	+0.97	10.7	+	+	Apr. 17	60	55
								Apr. 18	70	65
	6	396	598	+1.62	8.8	+	+	Apr. 17	70	60
								Apr. 18	70	55
	5	405	694	+2.79	7.7	+	+	Apr. 16	85	60
	3	437	945	+3.17	9.3	+	+	Apr. 14	70	80
Controls		392	601	+2.16	18.0	0	0			
		446	891	+2.79	18.9	0	0			
		409	667	+1.92	16.7	0	0			
		359	472	+2.19	18.4	0	0			

[1]Died; Stage III ova in ovaries.

pigmented at 1 day and the mouth was functional at 1-3 days after hatching. No abnormalities were observed in hormone-induced larvae.

Twenty larvae were sampled weekly (Table 4). The specific growth rates for the 63-day period from first feeding averaged 9.97%/day for dry weight (micrograms) and 2.75% standard length (millimeters). The first fish metamorphosed 54 days after hatching at 17.00 mm standard length. By the 63d day after hatching, all the larvae had completed metamorphosis, and average length was 17.40 mm.

Wild copepod nauplii were collected daily from a nearby estuary and fed to the larvae after being sieved to obtain the proper particle size. Larvae of the yellowtail flounder required small food organisms (<100μm in largest dimension) to initiate feeding. The most difficult aspect of rearing the larvae was the problem of obtaining enough food organisms in the size range required. Larval mortality was high for the first 2 wk of feeding, possibly caused by starvation. However, yellowtail flounder larvae are able to survive for considerable periods of days without exogenous food. Some larvae were maintained at 8°C and fed successfully and survived after being deprived of food for 10 days after hatching (Smigielski unpubl. data). As the larvae increased in size through metamorphosis, larger food organisms such as adult copepods, the rotifer *Branchionus plicatilis*, and the brine shrimp, *Artemia* sp., were offered.

TABLE 4.—Size of yellowtail flounder larvae reared artificially at 10°C from hac ing to metamorphosis. Average sizes of 20 larvae are followed by standard deviation.

Days after first feeding	Mean length (mm)	Mean dry weight (μg)
1	3.08±0.20	16.2±4.3
7	3.39±0.25	19.8±4.3
14	5.16±0.47	56.0±20.1
21	5.92±0.79	89.9±57.4
27	6.82±0.51	126.5±34.0
34	6.68±0.89	161.5±66.4
41	8.95±1.03	608.6±340.2
48	10.53±4.38	1,133.3±1,267.8
55	14.73±3.98	5,576.6±2,694.1
63	17.40±2.33	8,635.9±3,058.4

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TRACE METAL CONTAMINATION OF THE ROCK SCALLOP, *HINNITES GIGANTEUS*, NEAR A LARGE SOUTHERN CALIFORNIA MUNICIPAL OUTFALL[1]

Los Angeles County's submarine discharge of municipal wastewater from the Joint Water Pollution Control Plant (JWPCP) off Palos Verdes Peninsula is the single largest anthropogenic source of trace metals to the marine ecosystem off southern California. The 1974 annual mass emission rates of chromium, copper, and zinc via this discharge (4.8×10^{11} l/yr, which underwent primary treatment only) were about 400, 300, and 850 t, respectively; these were approximately 10 times the corresponding inputs measured in 1971-72 surface runoff from southern California (Young et al. 1973). As a result, bottom sediments around this submarine outfall system are highly contaminated by a number of trace metals (Galloway 1972; Young et al. 1975). Here we report abnormal levels of seven metals in three tissues of the filter-feeding rock scallop, *Hinnites giganteus*,[2] that was collected in the discharge zone and thus had been exposed to suspended wastewater particulates. (The adductor muscle of this bivalve mollusc is considered to be a delicacy, and scallops near the discharge are sought by sport divers.)

Procedures

During 1974, divers collected eight scallops within the size range generally consumed (10 to 25 cm in diameter) from depths of about 20 m at three stations in the discharge zone between Whites Point and Point Vicente: these stations were <1 km off Palos Verdes Peninsula. Six scallops in the same size range also were taken from control stations at similar depths off Santa Catalina and Santa Barbara Islands (Figure 1). To check our 1974 results, during 1976 eight specimens within this size range were again collected from this region in the discharge zone. However, we were not able to obtain additional island samples; therefore, five specimens were collected from each of two coastal stations located approximately 50 km to the north and south of Palos Verdes Peninsula. The samples were frozen in plastic bags after collection. Later, digestive gland, gonad, and adductor muscle tissues were excised from each specimen before it was fully thawed, using a new carbon steel scalpel and a cleaned Teflon[3] sheet; the tissues were placed in cleaned polyethylene vials. Care was taken to avoid contaminating the gonadal or muscle tissue samples with sediments or juices from the digestive glands.

Following dissection, each sample (1 to 2 g wet weight) was digested in 10 ml of a 1:1 nitric acid

[1]Contribution No. 85 of the Southern California Coastal Water Research Project.

[2]Formerly *Hinnites multirugosus* (Roth and Coan 1978).

[3]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.