GONAD MATURATION AND HORMONE-INDUCED SPAWNING OF THE GULF CROAKER, *Bairdiella icistia*[1]

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ABSTRACT

Successful methods of capture, transport, disease treatment, and laboratory maintenance of the gulf croaker, *Bairdiella icistia*, are described. Gonad maturation was achieved out of season by the use of appropriate controlled photoperiods, temperatures, and abundant feeding. Mature fish or fish brought to maturity in the laboratory were spawned with suitable hormone injections and the time of spawning could be accurately predicted. Eggs obtained from hormone-induced spawning were normal in all respects and the larvae were reared through metamorphosis using rotifers and brine shrimp nauplii as food; thus, the life history of this marine fish can, for the first time, be completed under controlled laboratory conditions. The techniques developed for croakers may have application to mariculture, biossay of marine pollution, and in more general research on marine fish reproduction.

Reproduction in fishes which spawn annually is controlled by the interaction of exogenous and endogenous stimuli, particularly the endocrine activity of the pituitary-gonadal axis which is indirectly influenced by environmental factors such as day length and temperature. Against this basic background, which determines the gonadal maturation cycle, the actual spawning act is controlled by neurohumeral and neuromuscular connections activated by rapidly changing environmental parameters and behavioral cues (Liley, 1969; Malven, 1970). This basic reproductive pattern and the lacunae in our understanding of it have recently been reviewed in detail (Breder and Rosen, 1966; Hoar, 1969).

Much of the work on reproductive physiology of fishes has been done with freshwater species of interest to aquarists (Wickler, 1966) or experimentalists (New, 1966; Hoar, 1969; Liley, 1969). There is, however, no reason to suspect that the basic features of reproduction, elucidated with freshwater species convenient to maintain, will differ significantly from those of marine forms, which require more elaborate facilities for study.

Because of increasing demand for fish as a source of protein, as well as for sport and for scientific studies on reproductive success of fishes in generally deteriorating natural environments, there are now intensive and extensive efforts to artificially culture commercially valuable freshwater species (Hickling, 1962; Hora and Pillay, 1962; Bardach, 1968). Recent interest has also focused on mariculture, the cultivation of marine species. Some success has been achieved in Great Britain (plaice and sole—Shelbourne, 1964 and 1970), Japan (bream and yellowtail—Harada, 1970), and in the United States (pompano—Finucane, 1970). However, all such cultures are started either with young fish caught at sea or with eggs spawned in the sea or stripped from mature fish captured during their normal spawning season. The field work necessitated by this method can be costly and unpredictable, drawbacks which are compounded by the difficulty usually experienced in simultaneously finding running-ripe fish of both sexes, especially females. These problems are avoided in the culture of salmonids because of the unique determination of the fish to return to the same place to spawn at a fairly predictable time (Leitritz, 1959). The grunion is a striking

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example of a marine fish showing similar determination (Walker, 1952).

The necessity of working with the reproductive products of one species only during its normal spawning season is one of the major obstacles faced by experimentalists and fish culturists. Most species of temperate and high latitudes show rather well-defined, short spawning seasons, probably related to the annual cycle of day length, temperature, and associated productivity. This seasonality generally means that experimental work proceeds at a hectic pace for a short time and then must be dropped or switched to another species available in breeding condition. This causes numerous difficulties, delays, and expenses for both research and researcher. The answer to this problem is to artificially induce maturation and spawning under controlled laboratory conditions.

To my knowledge, artificial gonad maturation under controlled conditions of light and temperature, hormone-induced spawning, and laboratory rearing of the fragile larvae through metamorphosis have not been accomplished for any single marine fish prior to this time. Pompano have been spawned with hormones, but the fertilized eggs did not hatch (Finucane, 1970). A euryhaline form, *Fundulus heteroclitus*, has been spawned with pituitary extract (Joseph and Saksena, 1966); however, only mature fish recently obtained from their natural environment were used.

Protocols for hormone-induced spawning of numerous species of freshwater fishes is well established (Dodd, 1955; Fontenele, 1955; Clemens and Sneed, 1962). A useful review of literature on the effects of hormones in fishes (Pickford and Atz, 1957) has been updated with a comprehensive, annotated bibliography (Atz and Pickford, 1964), and a timely review of various aspects of reproductive physiology of fishes is also available (Hoar and Randall, 1969, 1970).

Hormone-induced spawning is an accepted part of several commercial fish culture ventures and will be used in many more when techniques become reliably standardized for various species. Brazilians pioneered the use of hormones in spawning carp, while in Russia, where hydroelectric dams block the spawning migrations of sturgeons and salmonids, hormone-induced spawning has been practiced for many years (Atz and Pickford, 1959). In India, carps spawn naturally in flowing streams but must be injected with conspecific pituitaries before they will spawn in ponds (Chaudhuri, 1960; Das and Khan, 1962). Catfish respond to similar treatment (Sundararaj and Goswami, 1968). In the United States, several freshwater and anadromous species are spawned with hormones (Ball and Bacon, 1954; Clemens and Sneed, 1962; Stevens, 1966).

My investigation focused on the use of hormones in inducing maturation and spawning of the gulf croaker, *Bairdiella icistia* (Jordan and Gilbert), both for the specific purpose of obtaining eggs for physiological studies of salinity tolerance and for the more general purpose of studying factors which influence spawning in marine fishes. Once success had been achieved in spawning fish under controlled laboratory conditions, the influences of biological and physical factors on the spawning process were examined in detail.

THE SALTON SEA FISHERY

The Salton Sea is a large, saline, inland lake in the lower desert of southern California. The present body of water was formed when the flood waters of the Colorado and Gila Rivers broke through irrigation dikes in 1905 and poured into the then dry Salton Sink. The irrigation canals were repaired and the water again brought under control in 1907. Subsequently, the Salton Sea was declared an agricultural sump for the deposition of large quantities of irrigation waste water. This water leached large quantities of salt out of the surrounding agricultural land and carried it into the sea. Consequently, over the years the salinity gradually increased. Short-term fluctuations occurred—reflecting changes in inflow, annual rainfall, surface area, temperature, and evaporation. The salinity of the Salton Sea in 1970 is about 37 ‰.

During the period 1950-56, when the salinity of the Salton Sea approximated that of the ocean,

the California Department of Fish and Game transplanted a variety of fishes from the Gulf of California with the intent of establishing a productive sport fishery. The present fishery stems from the descendants of a total original introduction of 57 gulf croaker, 67 sargo, *Anisotremus davidsoni*, and 100 to 200 orangemouth corvina, *Cynoscion xanthulus*, transplanted to Salton Sea in 1951-52 (Walker, 1961; Whitney, 1967). The fishes thrived and developed large populations in the simple, man-made ecosystem, but increasing salinity now threatens the continuing existence of the famous fishery.

The Salton Sea Investigations was a joint Federal-State project whose overall goal was to predict a target salinity at which a water quality control project could be aimed that would not be detrimental to the present highly esteemed fishery. Engineering studies had suggested a method by which the salinity of the Salton Sea could be controlled, but the maximum permissible salinity levels still had to be established on the basis of biological information. Other sciaenid species live in the Gulf of Mexico in a wide range of salinities up to 75 ‰, but it is considered unlikely that spawning occurs in extremely high salinities or that the larvae could tolerate such osmotic stress (Gunter, 1967; Hedgpeth, 1959).

To attain our goal in the short time available, we have developed all of the necessary techniques for spawning Salton Sea fishes under controlled laboratory conditions. These techniques have proved successful for obtaining viable spawn for the necessary salinity tolerance studies of both croaker and sargo.[3] I had originally assumed that the close relationship of gulf croaker to corvina, both of the family Sciaenidae, would make it possible to apply croaker spawning techniques directly to the corvina. However, the carnivorous nature of corvina and the general difficulty of handling this powerful fish in the laboratory demanded special feeding and holding techniques and precluded successful laboratory spawning. Hormone-induced spawning has been achieved in our laboratory with other mature marine fishes, including *Eucinostomus* sp. (family Gerridae), *Genyonemus lineatus* (Sciaenidae), and sargo (Haemulidae). The techniques developed for inducing spawning with hormones in gulf croakers may thus have general applicability.

[3] Reuben Lasker and Richard R. Tenaza. 1968. Salton Sea fish larvae investigation progress report: techniques and preliminary experiments on osmotic stress (spring-summer 1968). Inland Fisheries Administrative Report No. 68-7, Oct. 1968, (Unpublished).

METHODS

CAPTURE, TRANSPORT, CARE, AND HANDLING OF FISH

Two year classes of gulf croakers were captured with a 60-m beach seine in May and October 1969. The first sample consisted of mature fish, 1 year old, and the second of young-of-the-year fish which were subsequently matured under controlled photoperiod and temperature conditions in the laboratory. The fish were captured on sandy beaches north of the U.S. Navy Base and north of the Salton Bay Marina, both on the west side of the Salton Sea. A beach seine is the most dependable method for capturing large numbers of these fish in good condition, except in midsummer and late winter, when fish are unavailable near shore.

The fish were transported to La Jolla, Calif., in a 500-liter tank equipped with aeration and filtration devices. In transport, and for several subsequent days in the laboratory, the fish were treated with Furacin antibiotic[4] at an initial concentration of 250 mg/3.8 liter. Several early failures showed that careful handling, high standards of water quality, and, especially, antibiotic treatment are all essential to high survival rates of Salton Sea fishes after capture.

In the laboratory, the fish were held in 2,000-liter rectangular tanks (2 m × 1 m × 1 m deep) supplied continuously from the seawater supply of the Fishery-Oceanography Center in La Jolla. A general description of this extraordinary facility is available (Lasker and Vlymen, 1969). A water temperature of 22 ± 1° C was main-

[4] Sharpe and Vejar, Los Angeles, Calif. Use of trade names is merely to facilitate descriptions; no endorsement is implied.

tained except for special studies or for short maintenance periods. An artificial photoperiod of 16 hr light:8 hr dark (16L:8D) was maintained by time clocks (Tork, Model 7100[6]) in a light-tight room with 400 w, white mercury lamps (Sylvania, H33-1-GL/C[6]), suspended 1.5 m above the water surface at the center of each tank. Deviations from this water temperature and light schedule are reported where appropriate.

Young fish, less than 13 cm in length, were fed 0.238 cm Oregon Moist Pellets[8] from Allen automatic trout feeders[7] activated with time clocks (Paragon Model 4001-0[6]). As fish grew larger their consumption of pellets diminished until they were fed exclusively on squid which was ground semifrozen into pieces ranging from 0.5 to 2.5 cm^2. All fish were fed squid ad libitum twice daily during the week and once a day on weekends. I consider adequate feeding to be an important factor in gonad maturation, but it was not a variable in this study. In the laboratory, young fish doubled their weight in 2 months and, later, added about 10 g each month, reaching 80 to 100 g by the end of the first year. Salton Sea fish weigh 40 to 50 g at the end of 1 year; the largest gulf croaker caught during this study weighed 420 g.

Initially, and for later studies of the effects of a series of hormone injections, fish were never disturbed without first draining the tanks to within 10 to 15 cm of the bottom and then adding anaesthetic until the fish could be touched without causing a sudden reaction. Fish to be injected were then removed and completely anaesthetized for 1 to 3 min in MS-222 (Tricaine Methanesulfonate)[9] at 0.6 g/3.8 liters. Treated fish were replaced in fresh seawater; if necessary, fish were held with gloved hands and moved rapidly back and forth at the surface to aerate the gills and assist their recovery. There is little danger of fish mortality when the anaesthetic is properly used, and no adverse effects on fish reproduction were found. Later, experience enabled us to net unanaesthetized fish rapidly and place them directly into buckets with MS-222 until they were unconscious. This was done routinely when spawning techniques were thoroughly known.

Fish were individually marked with subcutaneous injections of a 65 mg/ml stock solution of Bismark Brown or Fast Turquoise PT.[10] Hormone, antibiotic and other injections were carried out with 0.5- to 1.0-cc disposable syringes fitted with 25- to 26-gauge, 1.27- to 1.90-cm (½-¾ inch) needles. Needles 0.95 cm (⅜ inch) long proved useful for intraperitoneal (ip) injection where internal damage was possible, but these allowed too much fluid to escape when intramuscular (im) injections were used. In most cases the needle was carefully slipped into the skin between scales, and carrier fluid (oil and saline) was slowly injected into the deep muscles of the back adjacent to the dorsal fin. Slow withdrawal and pressure over the wound site helped to retain most of the fluid. Sesame oil was used in most cases as a carrier, but no essential differences were noted between oil and Holtfreter's saline injections.

LIST OF HORMONES AND THEIR PREPARATION

1. Oxytocin (Pitocin, Pituitary grade I).[11] Injectable solution, 20 IU/ml, used as obtained and stored at 4° C.
2. Deoxycorticosterone Acetate (DOCA, grade II).[11] Injected as a slurry in sesame oil.[12]
3. Human Chorionic Gonadotropin (HCG, stock No. CG-2).[11] Anhydrous powder was made to volume with Holtfreter's saline just prior to injection.
4. Gonadotropin from Pregnant Mare's Serum (PMS).[11] Powder dissolved in distilled water and stored at —10° C.
5. Carp Pituitary (freeze-dried powder).[13]

[6] Pacific Wholesale Electric Company, San Diego, Calif.
[8] R. V. Moore Company, La Conner, Wash.
[7] G-Z Company, Sacramento, Calif.
[8] J. F. Zwiener Company, San Diego, Calif.
[9] Crescent Research Chemicals, Scottsdale, Ariz.

[10] Allied Chemical Company, San Francisco, Calif.
[11] Sigma Chemical Co., St. Louis, Mo.
[12] A. Sahadi Co., Moonachie, N.J.
[13] Stoller Fisheries, Spirit Lake, Iowa.

Powder was homogenized wt/vol in sesame oil or Holtfreter's saline.

6. King Salmon, *Oncorhynchus tshawytscha*, Pituitary.[14] Glands removed and placed in acetone for extraction within 15 min of spawning. Whole glands were homogenized wt/vol in sesame oil or Holtfreter's saline.

Multiple intramuscular (im) or intraperitoneal (ip) injections were accompanied with 200 IU Potassium Penicillin G and 0.025 mg active Streptomycin Sulfate. Fish that were handled several times, with disposable plastic gloves, were routinely treated with Furacin water-mix antibiotic (Vet. grade), at an initial concentration of 250 mg/3.8 liters.

PREPARATION OF SALMON PITUITARIES

Early in the project my attention was directed to the possible usefulness of fish pituitaries for inducing spawning (Pickford and Atz, 1957; Clemens and Sneed, 1962; Atz and Pickford, 1964). Carp pituitaries are commercially available, but there is no assurance that these are removed from spawning fish, a time at which the glands are assumed to have high titers of spawning hormones. Glands from *Bairdiella*, taken during the spawning season, would be ideal, but the bony nature of the brain case makes their removal difficult and their small size makes the effort relatively unrewarding. Spawning grunion, *Leuresthes tenuis*, are seasonally abundant locally but, again, collecting a large number of glands would require great effort. Salmon provide a convenient source of fish pituitaries, since they are available in large numbers at fixed locations (fish hatcheries). Each gland is of considerable size (about 13 mg dry weight), and the bony brain case is reduced to a soft cartilage by decalcification near the time of spawning. Each fish is graded at the hatchery so that spawning females are only sacrificed when at the peak of running ripeness. Fish return to spawn at different times at various hatcheries which allows some flexibility in the timing of collecting operations. All these advantages, plus the fact that the glands proved useful in spawning croakers, justify a detailed discussion of the method developed by Nimbus Hatchery personnel for removing the pituitary from king salmon. I thank W. H. Jochimsen and D. R. Von Allmen for originally demonstrating this technique to me.

A technique for removing pituitary glands from salmon with a special tool exists (Tsuyuki, Schmidt, and Smith, 1964), and similar equipment was available at the Nimbus Hatchery. However, hatchery personnel have developed a simple and rapid technique which allows one person, with a little practice, to directly remove 100 to 200 pituitary glands during the course of a morning's spawning activities at the hatchery. The number of fish spawned limits the number of glands obtained; several hundred fish are spawned each week during November, the peak season at Nimbus Hatchery. The largest numbers of fish enter the hatchery ponds from the river on overcast days or during winter rains.

Female and male pituitary glands were removed from spawned fish within 15 min of death (unfortunately, following death, a severing of the head artery which bleeds the fish often destroys the pituitary in the process). The fish to be used for pituitary extraction are held upright with the gill cover slipped over a sharp stake clamped to a table. A large, sharp butcher knife is used to slice through the cartilaginous tissue of the brain case, parallel to the jaw and just above the level of the eye (a metal glove, as worn by fish-market personnel, would be useful for this step, which is carried out while the neuromuscular system of the large fish is still active). A rubber coat and rubber boots are also necessary accouterments at this gross stage of dissection. The cut exposes the brain, or, if at the appropriate depth, the pituitary stalk (sometimes the gland itself) is severed and the gland can be removed from its cavity with a pair of forceps or a narrow scoop. Some cuts, at odd angles, sever nerve cords as well as the pituitary stalk and expose three cavities. Once learned, the location, consistency, and color of the pituitary differentiate it from nervous or other fatty tissues with which it might be confused. At first, shallow cuts can be made to

[14] Nimbus Fish Hatchery, Rancho Cordova, Calif.

expose the brain; the gland's cavity and stalk are readily seen when the brain is lifted from its cavity and the entire gland can be removed with forceps. A direct approach is more appropriate to the speed with which spawning activities proceed and 1 day of practice is sufficient to enable one person to remove glands as fast as fish are available without undue loss of damaged or partial pituitaries.

The glands are placed directly in chilled acetone until spawning and gland-taking activities are finished. The acetone is decanted and replaced 2 or 3 times over a period of 3 days until all loose material is washed away. The final wash of acetone is decanted, and the glands are blotted gently on filter paper, placed in tightly stoppered vials, and kept at —10° C in a jar of desiccant. Glands stored 1 or 2 years by this method showed no detectable loss of activity.

It was found that pituitaries were easier to homogenize if they were not completely dried. Just prior to preparing a stock solution, the glands were removed from vials, air-dried for a few minutes, and weighed. They were then immediately placed in glass tissue grinders and homogenized in sesame oil or saline. The resulting brei was finely divided and was used unfiltered without clogging 25- and 26-gauge needles. Stock solutions (1-10 mg/ml) were stored in rubber-capped serum bottles (5 ml) at —10° C. All injections were 1.0 to 0.5 ml im and up to 1 ml ip. I used oil rather than saline in most cases on the assumption that oil may slow the rate of absorption and more evenly distribute the hormone.

EVALUATING THE EFFECTS OF HORMONE TREATMENT

Three basic criteria—gonad index, spawning, and fertilization of eggs—were chosen to test the effects of gonadotropin injections. Development of fertilized eggs to hatching is also a useful test of the absolute success of the hormone treatment used to obtain spawn, but hatching success is also very sensitive to other factors, e.g., salinity, temperature, dissolved oxygen, and bacterial contamination.

A commonly used measure of the effects of hormone treatment is the gonosomatic index (GSI) which expresses wet gonad weight as a percent of total wet body weight. The GSI is a reasonable measure of the state of reproductive maturity of a fish. Its measurement, of course, requires that the fish be sacrificed. Histological examination of gonads or measuring egg diameters to assess the stage of maturation are more elaborate approaches which were not used after it was found that GSI accurately predicted spawning readiness of the fish. McInerney and Evans (1970) have shown a direct relationship between GSI and the histological index in female threespine stickleback. Among more recent approaches to this problem is Stevens' (1966) development of a technique for removing eggs from striped bass by means of a catheter. He was able to determine the stage of the eggs and predict the time of optimal ripeness with a fair degree of accuracy.

Samples of 4 or 5 fish were used to assess the effects of hormone treatment. Controls of uninjected or sham-injected groups of fish were maintained where these were appropriate to understanding the effects of hormone treatments on GSI. The GSI of laboratory-held fish varied, understandably, through time, and the effects which this had on the results obtained are discussed where appropriate. The dates of most experiments are given to help explain this variation.

Two fundamental processes in spawning, hydration and ovulation, were evaluated separately with respect to various hormone treatments. Hydration was measured as an increase in total body weight over a period of 1 to 2 days after injection, a process distinct from gonad growth which occurs over longer time periods. This rapid weight gain is due mostly to water uptake by the fish and is reflected in much higher GSI values, as most of the water appears to go into the gonad. Externally, a hydrated fish is grossly bloated, and, in some cases, the fish are listless and remain motionless on the bottom of the tank. Ovulation was assessed by attempting to strip eggs from fish at various times after injection. Eggs may be forced from nearly ripe fish, but these eggs invariably are surrounded

by a single layer of vascularized ovarian tissue. Overripe eggs have no covering but are spotted with areas of coagulated yolk. Usually, eggs taken within 1 to 2 hr of ovulation are perfectly round, 0.7 to 0.8 mm in diameter, and have the single oil drop characteristic of this species. These eggs float in normal Salton Sea water (37 ‰) and are perfectly transparent and viable.

Fertilization of viable eggs and development to hatching was followed for random samples of eggs obtained from hormone treatments. Eggs which did not cleave were considered unfertilized. In most cases, test fertilizations were conducted in charcoal-filtered Salton Sea water, which has an ionic composition different from that of normal seawater (Carpelan, 1961). Fertilization and hatching served as final criteria of the usefulness of various preparations for spawning.

EXPERIMENTAL RESULTS

The reproductive process in fishes can be experimentally divided into four phases: (1) gonad maturation—measured as a slow increase in GSI over several weeks; (2) gonad hydration —the final preparation of reproductive products for spawning; (3) the actual spawning act—releasing eggs and sperm; and (4) fertilization and development of the eggs. These phases are interdependent and proceed in a timed sequence as part of a cycle keyed to external and internal cues which are integrated by the fish. Thus, spawning is the culmination of a whole series of events, each of which has particular physical and biological requirements for successful completion.

Although each process in the spawning cycle can be separately evaluated with appropriate experiments, it is important to keep in mind that the results must be judged against the general reproductive status of the organism. For example, the effects of hormone injections differ among croakers with different GSI's. For reasons explained in the following section, most of the experiments were focused on the reproductive processes of female croakers.

GONAD MATURATION (MALES)

Effects of Photoperiod and Temperature

Male croakers became running ripe in the laboratory under all combinations of experimental conditions. Fish of 20 to 30 g, captured in October, well beyond the normal breeding season of the species in Salton Sea, became running ripe within 3 weeks after being placed in tanks with various combinations of 14° C and 22° C water and 16L:8D or 8L:16D photoperiod schedules. Adult fish, captured at the height of the breeding season in May and June, were still ripe under all laboratory conditions in November and remained in this state until the end of the experiments in July of the following year. In the field, males become running ripe at least 1 month prior to the natural spawning season and can thus serve as indicators of the approaching breeding season in Salton Sea. They are not ripe prior to this time or after about 1 month following the spawning season, although water temperatures and day lengths are similar to those maintained in some laboratory situations in which the males did remain running ripe. It may be that either the absence of the normal Salton Sea cycle of light and temperature conditions (in which the fish experience very warm water—25°-30° C—followed by a period of winter dormancy), or the omission of the normal spawning act in the laboratory, helped to maintain the fish in running ripe condition. The threshold at which environmental factors induce spermiation may also be quite low, and in the laboratory, food, light intensity, photoperiod, and temperature acting in concert may have exceeded this level.

Effects of Hormones

Hormone injection of 1 mg salmon pituitary caused a seminal thinning response similar to that discussed by Clemens and Grant (1964). In comparison, the milt taken from uninjected fish was quite viscous and formed clots which had to be mechanically broken up to disperse the sperm. However, quantitative injections of smaller doses of salmon pituitary caused no apparent increase in the percent water of the testes (Table 1). In males, the reaction to hormones

TABLE 1.—Total weight, GSI (gonad weight/body weight × 100) and percent water in testis of male *Bairdiella icistia* injected with various dosages of salmon pituitary.

Treatment	Wet weight of fish (mean ± SD)	GSI (mean ± SD)	% water in gonad (mean ± SD)	N
Control: 0.01 ml/g sesame oil	67.96 ± 13.38	7.03 ± 1.13	85.2 ± 1.08	5
Salmon pituitary 0.005 mg/g	59.97 ± 11.67	5.25 ± 1.61	86.0 ± 1.20	5
Salmon pituitary 0.01 mg/g	59.34 ± 6.99	5.53 ± 1.74	85.8 ± 1.14	5

was not comparable to hydration in females and was not further pursued. Possibly stronger doses of pituitary would give a positive response. The time scale and extent of the seminal hydration and thinning response might be useful in assessing the effect of various hormones and dosage relationships where female fish are at a premium and males would otherwise be in excess. This bioassay technique has been used for salmon pituitary gonadotropin (Yamazaki and Donaldson, 1968a, 1968b).

GONAD MATURATION (FEMALES)

Seasonal Maturation Cycle in the Salton Sea

A series of croaker samples was taken from the Salton Sea at monthly or more frequent intervals during the year. GSI values were less than 2 % from January to mid-March 1969, prior to spawning, and less than 1 % from mid-June to December 1969, following spawning. From mid-March to mid-April, the mean GSI increased rapidly from 2 % to 10 % and reached a high of 12 % in mid-May (Fig. 1); at this time, individual females were caught with GSI's of more than 17 %. Peak spawning in the Salton Sea was observed in May and early June in 1969. Less frequent sampling and observations confirmed a similar pattern of events in 1970. In the years 1955, 1956, and 1957, Whitney (1961) found that the peak abundance of croaker eggs and larvae in the Salton Sea fell in middle and late May.

Laboratory Cycle and Effects of Photoperiod, Water Temperature, and Food

Immature fish.—Young-of-the-year croakers were captured in October 1969, placed in 14° C or 22° C water on a 16L or 8L photoperiod, and given an abundance of food (Oregon moist chow and chopped squid). These fish grew to maturity in a little less than 4 months at 16L:8D and 22° C (Table 2, group 1). Similar fish kept on 8L:16D at 14° C did not grow as rapidly as warm-water fish and did not mature under these conditions (compare groups 1 and 2, Table 2, 16-II-70). The lack of maturity was not due to the slower growth rate of the fish kept in cold water, as Salton Sea fish of lower average weight showed higher GSI values at their capture during the spawning season (Table 2, group 3). Short days, therefore, inhibited the maturation process. These same fish (Table 2, group 2) did mature rapidly, in 2 months, when the water temperature was increased to 22° C along with increased photoperiod. Young fish in their first year do not have as high a percentage of gonad as fish older than 1 year (Table 2, groups 3 and 4).

Mature fish.—A sample of fish more than 1 year old, captured in November 1968, 6 months

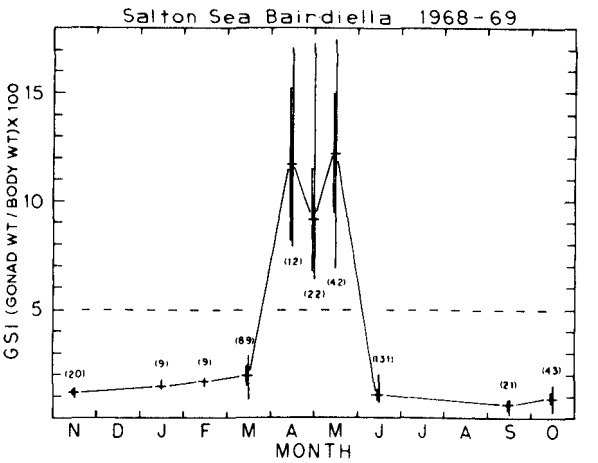

FIGURE 1.—Seasonal change in GSI of female *Bairdiella icistia* captured in the Salton Sea. Horizontal line indicates the mean GSI value; vertical line indicates range; on either side of the mean, open bar indicates the standard deviation, and closed bar two standard errors of the mean of each sample. The number of fish sampled is given in parentheses. Horizontal dashed line indicates 5 % GSI for comparison with laboratory fish (Fig. 2).

TABLE 2.—GSI values of female *Bairdiella icistia* captured in Salton Sea compared with captured fish matured in the laboratory.

Group	Date of sample	Weight (mean ± SD)	GSI (mean ± SD)	N
1	23- X -69[1]	10.47 ± 3.56	0.96 ± 0.28	9
	5-XII-69	34.30 ± 5.15	0.93 ± 0.15	11
	19-XII-69	35.40 ± 4.78	1.07 ± 0.46	10
	16- II -70	60.14 ± 11.84	5.46 ± 3.32	5
2	23- X -69[1]	10.47 ± 3.56	0.96 ± 0.28	9
	5-XII-69	31.90 ± 8.04	0.92 ± 0.14	10
	19-XII-69	33.10 ± 4.94	0.80 ± 0.09	9
	16- II -70	39.40 ± 7.16	1.28 ± 0.22	5
	4-IV-70	49.30 ± 5.01	9.69 ± 3.46	5
3	14- V-70[1]	32.38 ± 8.32	5.90 ± 3.84	25
4	14- V-70[1]	170.4 ± 84.1	7.93 ± 1.35	24
5	19-III-69	161.6 ± 22.8	10.2 ± 1.94	23

Treatments

Group 1: Laboratory stock fish sampled irregularly. All fish kept on 16L:8D photoperiod at 22° C.

Group 2: Laboratory stock separated from group 1 and maintained on 8L:16D photoperiod at 14° C until 16-II-70, when they were switched step-wise (15 min/day) to 16L:8D and 22° C.

Group 3: Young fish captured during their first breeding season in Salton Sea.

Group 4: Fish more than 1 year old, captured during the breeding season in Salton Sea.

Group 5: Fish more than 1 year old, captured following the breeding season in Salton Sea and matured early under laboratory conditions of 15L:9D and 14° to 16° C.

[1] At capture.

before their normal breeding season, reached maturity in the laboratory sometime prior to being sampled in mid-March 1969 (Table 2, group 5). These fish experienced 15L:9D and ambient La Jolla seawater temperature (14°-16° C) during 4 months in the laboratory. Cold water may slow down the maturation process, but it is evidently not as important as the stimulation of long days.

A second sample of adult fish was captured in mid-May (at the peak of the breeding season), subjected to various experimental laboratory conditions and sampled every 2 weeks to determine the status of their GSI (Fig. 2). Fish maintained on long days (16L:8D) at 14° C and 22° C showed a slow decline in GSI from a high of 12 % at capture to below 5 % by late-August and September. The GSI values at 22° C were more variable and, in general, showed a more rapid decline than those at 14° C. Similar fish given 10L:14D at high and low temperatures showed a similar but much more rapid decline in GSI, and their GSI also declined to a lower overall level (1-2 % by mid-August) than that of fish which never experienced short-day conditions.

Both groups of short-day fish subsequently showed a slow but steady increase in their GSI in response to having the photoperiod increased 15 min/day from 10L to 16L. Although this increase was not followed through a complete cycle, it was evident that exposure to long days for 2 to 3 months would have been required to bring the fish up to the GSI level necessary for spawning (about 5 %—see below).

It is likely that adult fish brought into the laboratory just prior to the normal increase in GSI observed in the Salton Sea would respond rather quickly, I estimate within 1 month, if they were given adequate light, temperature, and food. It may also be possible to mature fish rapidly after they have gone through their natural GSI decrease (Fig. 1), but this was not tested.

Effects of Hormones on Maturation of Fish Maintained in the Laboratory

Groups of fish maintained on various light and temperature regimes were subjected to hormonal treatments to enhance gonad maturation. Adult fish captured prior to the breeding season were not available for these experiments, which were conducted after the spawning season on fish undergoing a decline in GSI, as in Fig. 2. Nonetheless, the results obtained probably indicate the extent to which maturation can be influenced by hormone treatment. In croakers, the technique of hormone-induced maturation is of relatively little practical importance, since fish can be matured by appropriate manipulation of photoperiod, temperature, and feeding schedules. The results are, therefore, reported for their possible application to other species in which maturation proves more intractable.

Since the treatments were carried out on fish being used for photoperiod and temperature experiments, the results can only be evaluated in relation to the GSI value of the population under each set of conditions. In some cases, sham-injected controls were used while in others uninjected fish sampled for the light-temperature

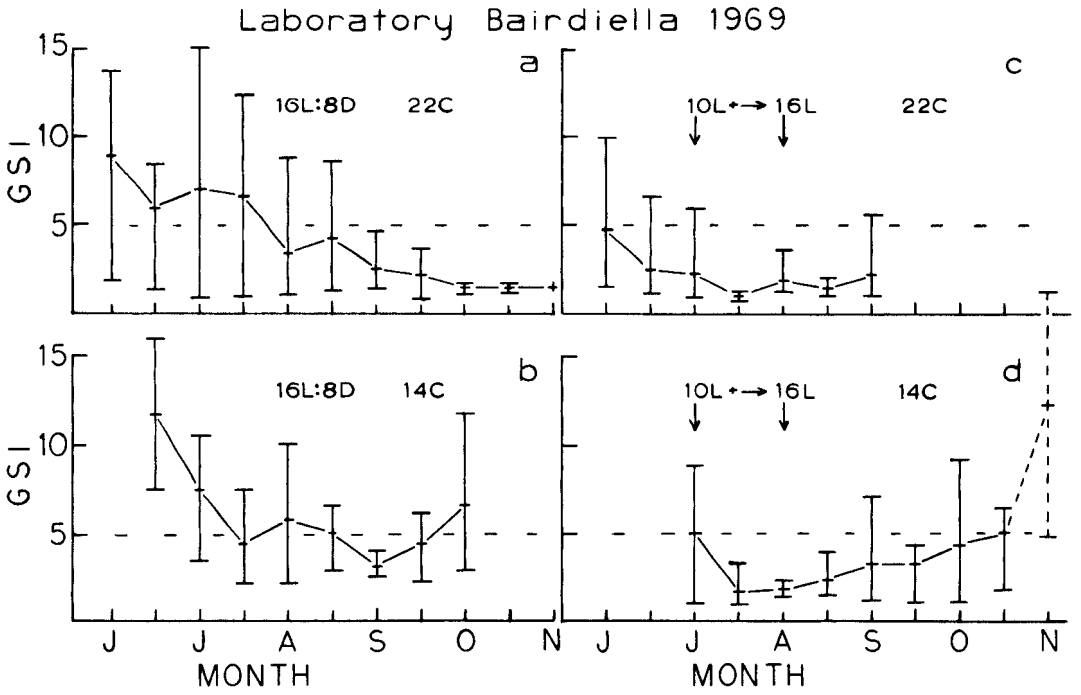

FIGURE 2.—GSI of female *Bairdiella icistia* captured during the spawning season and maintained under the following laboratory conditions: (a) 16L:8D, 22° C; (b) 16L:8D, 14° C; (c) 10L:14D, 22° C for 2 months; then, photoperiod was increased 15 min/day to 16L:8D; (d) 10L.14D, 14° C for 2 months; then, photoperiod was increased 15 min/day to 16L:8D. Mean GSI of all fish at capture (May 13, 1969) was 12.2 %. Dashed value in (d) indicates GSI of five fish injected with 1 mg of salmon pituitary 1 day prior to last sampling period. Horizontal line at 5 % GSI indicates approximate minimum level necessary for successful hormone-induced spawning.

studies served as controls when they were scheduled to be sampled at the same time as the injected fish. In all cases the gonad index responded positively to hormone treatment. Differences seen in the tabular data (Tables 3, 4, and 5) were due to the number of injections, strength and type of hormone injected, the water temperature and the GSI value of the fish at the beginning of the experiment.

In Tables 3, 4, and 5 the symbols (S, E, +, —) record the initial (2 days after the first injection) and the maximal (at some point during the series of injections) response noted for each group of fish. Two factors, water temperature and GSI, were found to have an important bearing on the results observed following hormone injection. Fish kept in 14° C water showed the most consistent and largest positive response to long-term hormone injections which were giv-

TABLE 3.—GSI of *Bairdiella icistia* given five injections, every other day for 9 days (17-26-IX-1969). Photoperiod was 16L:8D and temperature 14° C during this experiment. The response of the fish to injection was assessed every day and the maximal response is indicated by S (= spawned viable eggs), E (= obtained nonviable eggs), + (= swollen papillae observed, no eggs obtained), or — (= no observed response). The second column under each heading indicates the reproductive status of the fish 2 days after the first injection. The third column (in parentheses) indicates the maximum reaction noted during the experiment.

Treatment	1 mg salmon pituitary extract	100 IU HCG	5 mg DOCA	Uninjected control
GSI	12.4 + (E)	12.1 — (E)	11.4 — (E)	4.1 (—)
	11.6 + (E)	10.0 — (E)	6.1 — (E)	3.6 (—)
	11.1 + (E)	8.8 — (+)	5.2 — (E)	3.2 (—)
	10.9 — (—)	6.6 — (+)	4.6 — (+)	2.7 (—)
	6.0 — (+)	5.9 — (—)	male	2.7 (—)
$\bar{X} \pm SD$	10.4 ± 2.6	8.7 ± 2.5	6.8 ± 2.7	3.2 ± 0.6

TABLE 4.—GSI of *Bairdiella icistia* given six injections over a 15-day period (18-VIII-69 — 2-IX-69). Each injection consisted of 1 mg salmon pituitary extract. Fish in groups 1 and 2 experienced a 16L:8D photoperiod; groups 3 and 4 experienced 10L:14D for 2 months (to 28-VII-69) followed by day length increases to 16L:8D (by 22-VIII-69). Groups 1 and 3 were maintained at 22° C, groups 2 and 4 at 14° C throughout the experiments. Symbols (S, E, +, −) are the same as in Table 3.

	Group 1 (16L, 22° C)		Group 2 (16L, 14° C)		Group 3 (10L-16L, 22° C)		Group 4 (10L-16L, 14° C)	
	Injected	Control	Injected	Control	Injected	Control	Injected	Control
	6.5 E (E)	10.2 (−)	11.3 + (E)	10.2 (−)	5.6 − (E)	3.6 (−)	7.3 − (E)	2.4 (−)
	5.0 E (E)	3.2 (−)	9.4 + (E)	6.0 (−)	4.9 − (E)	1.6 (−)	3.3 − (+)	2.0 (−)
	4.2 + (E)	1.9 (−)	9.1 + (E)	5.5 (−)	1.8 − (+)	1.4 (−)	1.9 − (−)	1.9 (−)
	4.2 + (+)	1.3 (−)	9.1 + (E)	5.2 (−)	1.6 − (−)	1.4 (−)	1.7 − (−)	1.6 (−)
	3.6 − (−)	0.9 (−)	7.6 + (E)	2.3 (−)	1.2 − (−)	1.3 (−)	1.2 − (−)	1.4 (−)
$\bar{X} \pm SD$	4.7 ± 1.1	3.5 ± 3.8	9.4 ± 1.3	5.8 ± 2.8	3.2 ± 2.0	1.9 ± 1.0	3.1 ± 2.5	1.9 ± 0.4

TABLE 5.—GSI of *Bairdiella icistia* given three injections, one every other day for 7 days (16-IX—23-IX-1969). Photoperiod was 16L:8D and temperature 22° C during this experiment. Symbols (S, E, +, −) are the same as in Table 3.

1 mg salmon sesame oil	1 mg salmon Holtfreter's	1 mg carp sesame oil	0.1 mg salmon sesame oil	Control sesame oil
5.5 S (S)	5.0 S (S)	8.1 + (E)	6.0 − (E)	4.9 (−)
4.9 S (S)	5.0 S (S)	6.3 + (E)	3.3 − (+)	1.4 (−)
4.7 − (E)	4.6 − (E)	2.7 − (E)	3.0 − (+)	1.4 (−)
4.1 − (E)	3.6 − (E)	2.7 − (+)	2.5 − (+)	1.1 (−)
1.8 − (−)	1.4 − (−)	2.5 − (+)	1.5 − (−)	1.0 (−)
$\bar{X} \pm SD$ 4.2 ± 1.4	3.9 ± 1.5	4.5 ± 2.6	3.2 ± 1.7	2.0 ± 1.6

en every other day for 1 to 2 weeks (Table 3; Table 4, groups 2 and 4). Fish in 22° C water also showed a positive response (Table 4, groups 1 and 3; Table 5); however, this response is less clear because warm-water fish occasionally shed their eggs prior to sampling and this reduced the observed GSI.

In general, fish with GSI values below 5 % did not respond to the first injection (—), responded with a weak swelling in the genital area (+), or gave nonviable eggs (E) only after several injections. This indicated that threshold values of GSI and temperature exist below which "growth" and above which hydration and ovulation occur in response to hormone injections. These values will be further discussed in the section on ovulation. Here it will suffice to point out that hormone treatment does give rise to increases in gonad size which can perhaps be considered the equivalent of gonad growth.

The tabular data indicate that salmon pituitary caused the greatest increase in GSI of fish kept in cold water. Salmon was followed by HCG and then DOCA (Table 3). Salmon pituitary produced smaller increases in warm water than in cold (Tables 3, 4, and 5), and the response tended to vary in proportion to the dosage used (Table 5). The relatively small response at 22° C in Table 5 was probably due in part to the lesser number of injections (3) in this batch and in part to the fact that three of the fish spawned relatively large quantities of eggs (see qualitative responses in Table 5), thus reducing their GSI values, which were measured after testing for the presence of viable eggs. However, the results of the longest series of injections (Table 4) suggest that there is a general pattern of greater increase in GSI in cold water, except in the case of fish with a very low initial GSI.

In warm water (Table 4, group 1; Table 5), fish produced viable eggs (S) or nonviable eggs (E) which could be forced out the day after the first injection. Cold-water fish (Table 3; Table 4, groups 2 and 4) required several injections to produce a response and never spawned viable eggs on stripping.

In a further experiment, young-of-the-year fish collected in October were injected shortly thereafter with various concentrations of salmon pituitary to assess usefulness of immatures as a bioassay in testing dose-response relationships. Each fish received five injections over a 10-day period. At each dose level a large number of fish (20) was injected, but, because of the difficulty in identifying the sex of these immature fish, the number of females actually injected was somewhat smaller. Also, the gonads were quite small, and the overall GSI response was slight. This made any meaningful analysis difficult. How-

TABLE 6.—GSI of young female *Bairdiella icistia* after five injections of various concentrations of salmon pituitary extract over a 10-day experimental period (4-XI-14-XI-69).

Dose	Weight (g) mean ± SD	GSI mean ± SD	*N*
Control sesame oil	26.07 ± 5.78	0.79 ± 0.08	13
0.1 mg salmon	23.90 ± 6.23	0.82 ± 0.22	10
0.5 mg salmon	28.40 ± 4.46	0.88 ± 0.18	13
1.0 mg salmon	26.97 ± 6.88	0.99 ± 0.19	17

ever, the results (Table 6) do indicate a general increase in GSI corresponding to dose. That these fish were not too small to respond to treatment is indicated by the high GSI observed in similar sized fish captured in Salton Sea during the breeding season (Table 2, group 3). It is possible that the rather small response observed was due in part to the fact that the fish were handled frequently and did not feed readily during the course of the experiment. This test would probably be more successful if carried out with 30- to 50-g fish kept under short-day conditions at 14° C.

GONAD HYDRATION

Water Uptake Following Hormone Injections

Weight gain on various hormones.—Short term changes in body weight occurred 1 to 2 days following the injection of various hormones. This weight change was recorded as a percent of the initial total body weight (Table 7) and was found to vary from slightly negative values to positive values of over 13 % of the body weight. Single injections were given, and fish were weighed prior to, and 30 hr after, injection. With few exceptions, the fish showed little or

TABLE 7.—Effects of hormones of GSI of *Bairdiella icistia* at 30 hr post-injection. (1 = did, 0 = did not hydrate or ovulate.)

Preparation, dose, and date	Initial body weight (g)	Weight change (% initial weight)	GSI (% final weight)	Hydrate	Ovulate
Carp pituitary 10 mg					
10-VI-70	51.07	+10.99	9.34	1	0
	70.46	+5.42	19.80	1	0
	72.79	+11.72	25.50	1	0
	57.22	+12.82	28.60	1	0
Carp pituitary 5 mg					
16-VI-70	71.40	--	5.42	0	0
	54.22	--	8.14	0	0
Salmon pituitary					
0.1 mg, 5-VI-70	48.33	−0.93	2.88	0	0
	55.90	−2.45	3.60	0	0
	49.40	+2.39	10.97	(1)	0
	66.14	+0.06	12.41	0	0
Salmon pituitary					
0.5 mg, 2-VI-70	65.18	−2.59	3.83	0	0
	62.77	−1.96	5.02	0	0
	74.83	−0.64	5.18	0	0
	56.22	+8.75	21.76	1	1
Salmon pituitary 1 mg					
28-IV-70	96.12	+9.40	15.90	1	1
15-V-70	65.50	+13.40	--	1	1
18-V-70	79.90	+13.10	--	1	1
15-V-70	131.20	+6.00	--	1	1
Salmon pituitary 2 mg					
10-IV-70	93.30	--	24.47	1	1
23-III-70	68.92	--	25.67	1	1
10-IV-70	100.03	--	28.08	1	1
Salmon pituitary 5 mg					
28-V-70	66.1	+7.35	20.79	1	0 (few eggs)
	65.4	+6.11	21.23	1	0 (few eggs)
Salmon pituitary 5 mg—Con.					
	91.7	+10.09	25.94	1	1 (poor eggs)
	62.3	+8.06	26.64	1	0 (few eggs)
PMS 100 IU					
27-IV-70	86.8	--	21.00	0 (?)	1
	--	--	--	0 (?)	1
12-V-70	88.7	+4.18	--	1	1
7-V-70	173.6	+11.98	24.02	1	0
PMS 50 IU					
9-VI-70	80.38	−3.06	2.90	0	0
	54.89	+4.10	14.60	1	1
	69.90	+7.77	18.50	1	1
	62.18	+12.06	24.10	1	1
HCG 100 IU					
28-IV-70	68.5	--	28.76	1	0
14-IV-70	87.5	--	29.37	1	0
15-IV-70	84.5	--	30.29	1	0
HCG 50 IU					
16-VI-70	78.51	−2.09	2.69	0	0
	57.03	−1.95	3.65	0	0
	67.91	−1.29	3.68	0	0
	80.41	+7.15	19.80	1	1
Oxytocin 20 IU					
18-VI-70	59.12	−0.70	1.60	0	0
	65.66	−1.02	3.37	0	0
	72.11	−0.11	3.95	0	0
	60.08	+2.39	11.97	1	0
DOCA 5 mg					
15-VI-70	54.78	−0.51	4.07	0	0
	53.70	−3.07	4.28	0	0
	55.33	−0.55	7.98	0	0
	73.10	−1.20	9.78	0	0

no response to 0.1 and 0.5 mg salmon pituitary, 5 mg carp pituitary, 50 IU HCG, 20 IU oxytocin, and 5 mg DOCA. On the other hand, 1 to 5 mg salmon, 10 mg carp, and 100 IU HCG, gave uniformly positive results, all fish showing weight gains of 5 to 13 %. Variable results were observed with 50 and 100 IU PMS. Although most PMS fish which were weighed showed some gain in weight, this was in general less than that observed with carp, salmon, and HCG. In fact, it was noted that two fish injected with 100 IU PMS spawned freely without ever appearing grossly bloated, a characteristic of all fish which were spawned with other preparations.

The time scale of weight gain.—A comparison was made of the weight gained by fish given one injection of 5 mg salmon, 10 mg carp, and 50 IU PMS (Table 8). The time span of hydration was arbitrarily divided into the weight gained between 7 and 23 hr post-injection and the total weight gained, including that added between 23 and 30 hr. At 30 hr ovulated eggs, if present, were stripped from the fish. Generally, all fish lost weight in the first 7 hr, probably because of handling and lack of feeding during the experiment. The weight gains are due mostly to water uptake and movement of water into the gonad.

TABLE 8.—Effects of hormones on time-course of weight gain.

Preparation and dose	Initial fish weight	Percent weight gain	
		7-23 hr post-injection	7-30 hr post-injection
	G	%	%
Salmon pituitary 5 mg	66.1	4.58	7.35
	91.7	7.72	10.09
	65.4	4.59	6.11
	62.3	4.41	8.46
Carp pituitary 10 mg	57.22	7.38	13.13
	51.07	4.17	10.44
	72.79	6.76	13.11
PMS 50 IU	54.89	0.13	5.25
	62.18	4.86	13.11
	69.9	2.88	9.69

The results show that the weight increase in the final 7 hr prior to spawning is less than 50 % of the total increase with salmon pituitary, more than 50 % with PMS, and about equal when carp pituitary is used. This evidently reflects some fundamental difference in the way these preparations affect the physiological mechanism causing hydration. The time-course of hydration (Table 8) may be important in determining the condition of eggs at ovulation (Table 7). It should be noted that among the three groups tested for the time-course of hydration, viable eggs were obtained only from the PMS-injected fish (Table 7); unfortunately, there is no comparable data on the time-course of weight gain in fish given 1 mg salmon, which also produces viable eggs.

Factors Affecting Hydration

GSI.—It is apparent from Table 7 that the gonad must be close to 5 % of the body weight to respond to an otherwise adequate dose of hormone. Although GSI could not be measured prior to injection, almost all fish which failed to respond had final GSI's below 5 %. Table 9 presents further confirmation of this. These fish, injected with 1 mg salmon, came from a stock which had shown a general decline in GSI, because of being kept on a long photoperiod for an extended time. Of four injected fish, three hydrated and one of these subsequently spawned. The fish that neither hydrated nor spawned had a final GSI of just under 3 %. Only 1 of 11 uninjected fish from this same stock showed a GSI above 5 %, while 3 more were above 4 %.

TABLE 9.—GSI of *Bairdiella icistia* measured on 26-VI-70.

A. Fish injected with 1 mg salmon pituitary, after GSI had declined (1 = did, 0 = did not hydrate or ovulate).

Initial body weight	Weight change (% initial weight)	GSI (% final weight)	Hydrate	Ovulate
53.10	+14.73	24.42	1	1
65.38	+7.40	10.55	1	0
60.14	+4.21	6.94	1	0
79.89	−2.21	2.77	0	0

B. Uninjected fish from same tank.

Total weight	GSI
67.65	1.63
53.20	1.79
93.28	0.05
58.86	4.38
63.48	1.27
61.90	4.57
78.60	1.70
74.70	2.08
53.24	2.59
69.29	7.86
59.46	4.80

Hormone dosage.—In general, there appears to be a threshold response to dosage. Fish with high GSI values and between 50- and 100-g body weight hydrated after one injection of 10 mg carp but not 5 mg (Table 7). With salmon, 1 to 5 mg were adequate doses for 50- to 350-g fish while 0.1 and 0.5 mg were inadequate except in one case. In the case of HCG, 100 IU caused hydration while 50 IU did not except in a single case. Note, however, the low GSI value measured for three of the fish given 50 IU HCG.

It must be noted that the highest dosages used were adequate for hydration but inhibited spawning (see section following on ovulation). This was true for 10 mg carp, 5 mg salmon and, especially, 100 IU HCG where the fish continued to gain weight and eventually died in the tank without ovulating a single egg. These results would suggest that it is important to determine the lowest possible dosage which will consistently bring about hydration.

Temperature.—A temperature threshold underlies the entire spawning process. Between 14° and 17° C the fish did not hydrate in response to an otherwise adequate dosage of 1 mg salmon pituitary. Two days later these same fish spawned within 30 hr when given a second 1-mg-salmon injection 24 hr after being transferred to 22° C water.

OVULATION

Ovulation and Hydration as Separate Events

Some early results with 100-IU-PMS and 100-IU-HCG injections led to speculation that the two hormones were acting on different physiological processes. PMS brought about ovulation without gross hydration while HCG hydrated fish to the point of death without ovulation (Table 7). This result was not confirmed with 50-IU doses, but, in general, the impression gained was that PMS produced high quality eggs with less hydration than either HCG or salmon pituitary.

In sharp contrast to the PMS results, HCG caused uniform hydration but, with one exception, failed to bring about ovulation. In a preliminary test, it was found that oxytocin (20 IU) or salmon (1 mg) caused some eggs to be ovulated when the injection was given 24 hr after the fish were injected with HCG. Oxytocin and salmon pituitary had the same effect on carp-injected fish which otherwise did not ovulate.

Ovulation without apparent hydration was also achieved by using multiple, subthreshold doses of 0.1 and 0.5 mg salmon pituitary, but the time of ovulation could not be accurately predicted, and therefore the eggs obtained usually were not viable. (The importance of obtaining eggs just at ovulation is discussed in the section on fertilization.)

In summary, carp pituitary, HCG, DOCA, and oxytocin were uniformly inadequate for bringing on ovulation except in the case of one fish treated with HCG. Both carp- and HCG-injected fish hydrated, some becoming grossly distorted. On the other hand, salmon pituitary and PMS regularly brought about ovulation. Dosage appeared to be critical in the case of salmon pituitary, as nonviable eggs resulted from injections of 5 mg and no spawn could be obtained with a single dose of 0.1 and 0.5 mg. A 1-mg-salmon dosage seems to be optimal for fish of 50- to 100-g total weight. Both 50- and 100-IU dosages of PMS gave good results with remarkably clear eggs obtained from all fish. In one case, a 50-IU dose of HCG was adequate for spawning, but 100 IU appeared to be inhibitory to ovulation.

The Time Scale of Ovulation

The combined results from 28 fish which produced viable eggs following injection with a single dose of salmon (1-3 mg) showed that the average time elapsed from injection to spawning was 30.4 hr, with a standard deviation of 3.3 hr and a range of 24.5 to 35.5 hr (Fig. 3). This 30-hr latent period following injection generally held regardless of the type of hormone used, its dosage, or the time of day the injection was made. In the case of a few large fish (100 to 300 g) given 1 mg salmon, a second 1-mg injection was given 24 hr later, but this did not affect the time of spawning. Five fish given 5 mg salmon spawned 28 hr after injection, but their eggs were not viable. In one experiment,

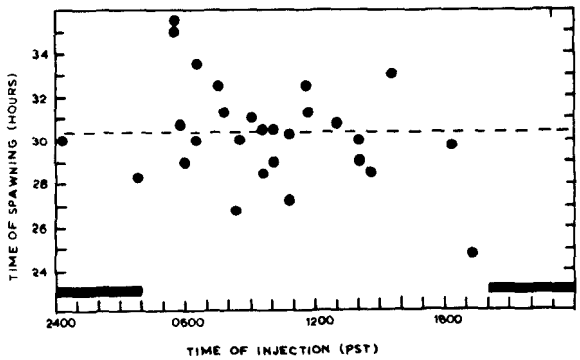

FIGURE 3.—Time of injection (Pacific Standard Time) and time of spawning (hours post-injection) in relation to photoperiod. All injections were 1-3 mg salmon pituitary extract and all fish produced viable eggs. Solid black bar indicates dark period; dashed line indicates mean time of spawning.

five fish were injected with 1 mg salmon at a time corresponding with the beginning of the laboratory dark cycle. All these fish spawned 24 hr later, indicating a possible enhancement by the normal diurnal cycle of glandular activity. Natural spawning in the Salton Sea is related to the normal diurnal light cycle, with most spawning occurring in the early evening.

Factors Affecting Ovulation

It has already been shown that GSI level, hormone dosage and type of hormone are critical interacting factors which must be considered in any spawning effort. Injection of high levels of salmon (5 mg) may possibly assure a more uniform hydration response (see GSI of Table 7), but the nonviable eggs which result speak against using more than the minimal dose found to give consistent results.

The effect of temperature on ovulation per se was not studied. Hydration is effectively blocked at temperatures lower than 17° C, but this effect was reversed after 24 hr acclimation at 22° C. In the cases in which this transfer was carried out, a second injection was given 24 hr after transfer, and spawning took place approximately 30 hr later.

As a matter of practical interest, it was found that fish could be injected and spawned twice (tried, successfully, with two fish) or three times (one fish) with a period between spawnings of 3 to 4 weeks. This is in contrast to the much longer time required for maturation after fish had slowly resorbed their gonads in photoperiod experiments (Fig. 2). Apparently the rapid emptying of the gonad consequent upon hormonal injection quickly leads to a renewed cycle of egg maturation.

The direct and indirect effects of various injections on the gonad were assessed by biopsy following spawning or the lack of spawning. These qualitative observations are listed in Table 10; no attempt is made to interpret these results, except to point out that fish injected with salmon pituitary extract had gonads most closely resembling those of naturally spawning fish.

TABLE 10.—Appearance of mature *Bairdiella icistia* ovaries during natural spawning and 30 hr after various hormone injections, and color reaction of fish to injections.

1. Salton Sea fish at spawning
   Gonad color white, light yellow or red-orange. Consistency of *mature gonad is granular with patches of transparent eggs which* are close to being ovulated or are lying free in the ovarian lumen.
2. Salmon pituitary extract
   Gonad color and consistency very close to naturally spawning fish. *Most eggs ovulated and free in lumen. Fish blanch on injection.*
3. Carp pituitary extract
   Gonad color red-orange; few eggs ovulated. In fish given 5 mg dose, blood clots appeared to be blocking oviducts near vent. *Fish blanch on injection.*
4. PMS
   Gonad whitish, translucent; strikingly different from other preparations. Most eggs ovulated and free in lumen. Possibly, greater degree of ovulation with less hydration makes gonad appear lighter in color. Fish do not blanch on injection.
5. HCG
   Eggs either not ovulated or partially ovulated; those not ovulated appear as white patches in the ovary. Many vacuoles and dispersed oil drops appear in eggs. Fish do not blanch on injection.
6. Oxytocin
   Ovary was very bloody. Eggs white (not hydrated); different sized eggs (mostly large) apparent in ovarian folds. Fish do not blanch on injection.
7. DOCA
   Fish showed no observable reaction.

FERTILIZATION

Relationship of Egg Viability to the Time of Ovulation

Shortly before ovulation, eggs could be squeezed from females by applying strong pressure to the abdomen, but eggs obtained in this way still had an investiture of blood vessels and ovarian tissue and could not be fertilized. An analysis of viability in relation to the time after

TABLE 11.—Fertilization and viability of *Bairdiella icistia* eggs tested over a 4-hr period following first ovulation. Fish was injected with 1 mg salmon pituitary extract at 0930 1-V-70; first eggs expressed with some difficulty at 1530 2-V-70.

Time of fertilization	Fertilization	Development to early tailbud	Hatching
	%	%	%
1530 hr (not quite running ripe)	100	91	84
1630 hr (running ripe)	100	91	83
1730 hr	100	78	72
1830 hr	90	62	44
1930 hr (eggs spotty, opaque)	5	52	34

ovulation was made in the case of one fish which remained ripe for 4 hr (Table 11). To check for viability, eggs were test-fertilized at hourly intervals following the first sign of ovulation, taken as the earliest time when normal eggs could easily be expressed from the fish by gentle pressure applied to the abdomen. Fertilization and early cleavage remained above 90 % up to 3 hr post-ovulation. By 4 hr the eggs looked crinkled, opaque, and spotty, and less than 5 % could be fertilized. A further measure of viability was made by culturing 100 early cleaving eggs from each batch until hatching. A decrease in hatching success was noted in the eggs obtained 2 hr after the initial ovulation, and hatching decreased still further in the 3- and 4-hr post-ovulatory samples. It appears that the maximum grace period for egg-taking is about 2 hr. In another experiment I studied eggs from a larger sample of 10 fish determining fertilization success as a function of time after ovulation; the optimum time for taking eggs was 1 hr after the fish first showed signs of running ripeness and gave viable eggs.

Although eggs rapidly deteriorated when kept in the ovary following hormone-induced ovulation, it was found that they retained their ability to be fertilized up to several hours after they were placed in a moist storage chamber. Eggs placed in seawater remained fertile for several minutes; in one case, a few cleaving eggs resulted from fertilization carried out after the eggs had been in seawater for 30 min.

Viability of Sperm

Although eggs kept in seawater remained viable for several minutes, sperm were no longer able to fertilize eggs 30 sec after the sperm mass had been introduced into seawater. It is thus readily apparent that croaker sperm and eggs should be mixed immediately after the sperm is obtained, in order to achieve maximum fertilization. Microscopic examination showed that sperm were immediately activated by addition of water and retained motility for a period of 1-5 min. In some tests it was apparent that water from the Salton Sea caused greater activity for a longer time than water taken from the ocean at La Jolla, Calif., but there was great variability between males, and a proper technique of quantifying this relationship awaits further studies.

Number of Eggs Obtained by Hormone Treatment

The number of eggs obtained by hormone injection varied between 700 and 1,000 per gram of fish wet weight (Table 12). This provided 50,000 to 100,000 eggs for experiments from each fish of 50 to 100 g used in this study.

TABLE 12.—Number of eggs obtained from hormone-induced spawning of *Bairdiella icistia*.

Wet weight		GSI	Ripe eggs		Actual count (1 g eggs)	Eggs/g fish weight	Approximate total eggs/ fish
Total fish	gonad		Body weight	Gonad weight			
G	*G*		%	%			
82.0	17.7	21.6	16.0	74.0	4,700	841	69,000
86.6	17.6	20.3	15.5	78.1	--	[1]793	[1]69,000
91.7	26.0	25.9	19.7	76.1	5,590	1,101	101,000
342.7	62.1	--	--	75.0	--	[1]699	[1]240,000
441.5	129.7	--	17.1	--	--	[1]878	[1]388,000

[1] Indicates values calculated from measured parameter and mean number of eggs per gram counted.

DISCUSSION

MATURATION

Many experiments have shown that gonad maturation can result from hormone therapy (for reviews see Pickford and Atz, 1957; Ahsan and Hoar, 1963; Atz and Pickford, 1964; and Hoar, 1969), but in most cases this is a long and tedious approach and has proved of practical use only on a short-term basis for elucidating mechanisms of hormone action. A recently described catheter implant technique (Frogner and Hendrickson, 1970), allowing frequent or continuous administration of hormones, has been used with partial success to mature mullet, *Mugil cephalus*, with a minimum of damage from excessive handling (Shehadeh, personal communication). A mass of tangled catheters is envisioned if this technique were applied to commercial fish production, but the ease of this method may have considerable merit for experimental situations. Implanted pituitary glands might also be used to enhance maturation, and this could easily be tested in croakers.

In the present study, a slight increase in GSI, possibly reflecting enhanced gonad "growth," followed 1 to 2 weeks of hormone injections given every other day to fish held in 22° C water. Even greater "growth" enhancement was observed in 14° C water, and these fish could have been spawned using techniques which were fully developed later in the study. However, for practical purposes, it proved simpler to mature croakers in large groups using appropriate schedules of long days, warm water, and abundant feeding.

The fact that fish kept in cold water respond to hormones by gonad enlargement without subsequent hydration or ovulation indicates that different temperature thresholds exist for these various processes. It is possible that the rate of absorption of hormone is considerably slowed in cold water, as some fish do develop a slight reaction following several days of injection at 14° C.

The general relationship of light and temperature to gonad maturation is well known (see Harrington, 1959; Henderson, 1963; Wiebe, 1968; and Hoar, 1969, for reviews) and requires no lengthy discussion here. It is sufficient to note that long-day photoperiod (16L:8D) and high temperature water (22° C) induce gonad maturation in croakers several months prior to the normal breeding season observed in the Salton Sea. Also, a combination of long days and low temperature (14° C) will retard the normal GSI decline when the fish have been captured at the peak of breeding. This technique may prove useful for maintaining fish in a mature state for prolonged periods; such fish may be subsequently spawned following transfer to warm water (22° C) for a period of 1 day.

Studies on the relationship between maturity and spawning indicate the existence of a GSI threshold value of about 5 %, below which hormone injections are ineffective. Also, fish brought to maturity with photoperiod and temperature control eventually resorb gonadal tissues if they are not subsequently spawned. This resorption process requires several weeks, and the gonad will not grow in response to photoperiod and temperature during this time. Fish which are spawned with hormones do not show a refractory state and can be respawned within a few weeks. The practical implication of these findings is that the GSI of maturing fish should be frequently checked so that spawning can begin soon after the 5 % GSI threshold is reached and the fish should be spawned before they reach the maximum GSI value and begin gonad resorption. A useful approach would be to hold stock supplies of fish on short days at low temperature and mature separate groups as needed for experiments.

Samples of croakers taken throughout the year from Salton Sea showed that maturation is quite rapid, the GSI increasing from 2 to 10 % in a little over 1 month. It is probable that the increased light, temperature, and food stimulation available in the laboratory could bring about even more rapid maturation, but the proper fish (early spring) to test this were not obtained during this study.

HYDRATION

Hormone-induced gonadal hydration is a relatively rapid phenomenon which is completed

in a little over 1 day following injection under laboratory conditions. In croakers, the total water uptake is reflected primarily in increased gonad weight and may amount to more than a 10 % increase in total body weight. A detailed study of the gonadal hydration of carp, *Cyprinus carpio*, and goldfish, *Carassius auratus*, following injections of carp pituitary extracts showed a similar pattern of water movement into the gonad with respect to time (Clemens and Grant, 1964). These authors measured the increased water content of the gonad following injection and found that, in the case of males, the peak of seminal fluidity was 24 hr after a single ip injection. A similar response was observed with im injection. Goldfish females injected with 10 mg/g carp pituitary extract showed similar responses, increasing gonad water by up to 7.2 % over carrier-injected controls. Unfortunately, the changes they describe are in the relative water content of gonads and various other tissues including blood, and no mention is made of any increase in total body weight resulting from water taken up from the external medium.

Hydration under laboratory conditions results in a grossly distorted appearance in females, the abdominal cavity becoming bloated several hours prior to spawning. In the Salton Sea, females appear plump but never grossly enlarged at spawning. It is possible that naturally spawning females hydrate and ovulate frequently but in small amounts over the course of the breeding season and that the laboratory fish show the maximum hydration and ovulatory response because of unnatural overstimulation with the injection of salmon pituitary. Use of 10 mg carp pituitary and 100 IU HCG caused an equally strong hydration response, but generally this did not culminate in ovulation when these preparations were used alone. A dose threshold for response was indicated by the inability of 1 to 5 mg of carp pituitary to cause hydration. With 100 IU HCG, continued hydration without ovulation evidently overstressed the fish and led to their eventual death. On the contrary, PMS gave somewhat variable results, but appeared to have less effect on gonad hydration while, at the same time, proving to be a potent ovulating agent. Subthreshold doses of salmon pituitary do not appear to cause hydration, but a sequence of injections given at daily intervals will eventually lead to ovulation of small quantities of eggs. This may reflect the response of exceptionally ripe eggs which are able to hydrate and ovulate.

Thresholds of GSI (above 5 %), water temperature (between 17° and 22° C), and hormone dose (e.g., 1 mg or more of salmon pituitary for 50- to 100-g fish) exist, and if any one of these factors is below its threshold, hydration does not occur.

OVULATION

Ovulation in croakers is a rapid process, taking 1 to 2 hr for completion when induced with hormones in the laboratory. The period between injection and spawning includes the hydration phase and culminates in ovulation at about 30 hr post-injection. Stevens (1966) found a similar 30-hr latent period for fully mature striped bass, *Roccus saxatilis*. Clemens and Sneed (1962) found a shorter latent period of 15 hr for goldfish. Fontenele (1955) gave injections to several Brazilian fish species at 6-hr intervals. He stated that spawning usually occurred just prior to the 5th injection (i.e., close to 30 hr after the first injection), although in most cases the fish were allowed to spawn naturally in ponds and were not tested by stripping. Indian carp are also allowed to spawn naturally in ponds after injection. Chaudhuri (1960) states that spawning may come 6 to 8 hr after the first injection of very mature fish; if a second injection is necessary, the total elapsed time may be 14 to 18 hr. It would appear that in most recorded cases (see above and Pickford and Atz, 1957, Table 46) hormone injection will bring about final maturation and spawning within 1 to 2 days if the gonads are fully mature. In only a few cases (e.g., Joseph and Saksena, 1966) have longer series of injections been successfully used to produce viable eggs.

A constant time period for spawning latency was found to hold for croakers used in this study. When GSI, water temperature, and hor-

mone dosage all exceed certain threshold levels, the fish spawned viable eggs an average of 30 hr after the first injection. Hormone dosages above threshold and the type of preparation had no apparent effect on this result, which exhibited only small variability. Subthreshold doses of salmon pituitary did delay ovulation, but this delay could not be accurately predicted, and therefore the spawn obtained was never viable. Subthreshold doses might theoretically be useful if females were to spawn naturally in captivity, but croakers never exhibited complete spawning behavior in tanks following hormonal injections. Injection of oxytocin in hydrated females and in males appeared to cause heightened prespawning behavior, with males following and touching the vent of females, but actual spawning was not observed. Hydrated females eventually expelled their eggs into the tanks if they were not hand-stripped shortly after ovulation. Actually, the relatively constant latency and the fact that fish must be hand-stripped are highly advantageous to scheduling laboratory operations.

At Salton Sea, eggs of gulf croakers sampled from the plankton and staged at various times during the day and night showed that there is a diurnal pattern of spawning, most early cleavage stages appearing in the early evening (Whitney, 1961). The same diurnal pattern was found in a closely related species from the East Coast, *B. chrysura* (Kuntz, 1914). When the effect of this diurnal pattern on laboratory spawning was tested by injecting fish at a time corresponding to the beginning of the laboratory dark cycle, all five injected fish spawned just at "dusk," 24 hr after injection rather than the usual 30 hr. However, such enhancement was not found in any subsequent spawning attempts carried out at many different times of day and night. Clemens and Sneed (1962) found no change in latency in goldfish, groups of which were injected at 2-hr intervals over a period of 12 hr. Evidently, the injection of hormones usually overrides any effect of diurnal spawning patterns.

Clemens and Sneed (1962) found that the latent period decreased with increasing temperature, doubling from 12 hr at 30° C to 25 hr at 10° C. Croakers spawn above 20° C in the Salton Sea, and eggs develop to hatching between 20° and 30° C in the laboratory (Robert C. May, Scripps Institution of Oceanography, personal communication). However, all laboratory spawning was accomplished at 20° to 22° C, and no tests were run to determine if higher temperatures would decrease the latent period. At temperatures below 17° C, the croaker does not hydrate or ovulate in response to hormone injection.

For 50- to 100-g croakers, with GSI levels above 4 to 5 %, a single injection of 50 IU PMS, 50 IU HCG, or 1 mg salmon pituitary proved adequate to induce spawning. A dosage of 100 IU PMS and 2-3 mg salmon also produced viable eggs, but 5 mg salmon produced nonviable eggs in the four fish tested. A single injection of 10 mg (not 5 mg) carp pituitary or 100 IU HCG was adequate for hydration but not for ovulation.

An injection of 20 IU oxytocin or 5 mg DOCA apparently had little or no effect on hydration, although DOCA may have caused some slight change in the GSI.

Oxytocin may affect spawning directly. Liley (1969) reviews evidence that the spawning reflex is controlled by behavioral stimulation of the CNS which releases oxytocin. Oxytocin is used up during the reproductive season of fishes, e.g., *Fundulus* and *Oncorhynchus* (Perks, 1969).

The possibility that a second hormone, acting (in concert or independently) directly on ovulation, was absent from HCG or in too low a concentration in carp pituitary was evaluated in a preliminary way by injecting 10 mg carp or 100 IU HCG fish with 20 IU oxytocin at 30 hr or an otherwise inadequate dose (0.1 mg) of salmon 24 hr after the initial injection. Evidence was obtained for ovulation shortly after injection (oxytocin) or at 30 hr (salmon), although the eggs usually were not viable. These experiences indicated that hydration alone was not sufficient to initiate ovulation and that the latter may be a separately controlled process.

The apparent contrast observed with respect to the different abilities of HCG and PMS to hydrate and ovulate fish may possibly be

interpreted as additional evidence that a two-hormone system exists for reproductive control in fishes similar to the FSH-LH system of birds and mammals (see Ahsan and Hoar, 1963; Hyder, 1970, for details). HCG is LH-like while PMS is FSH-like; fish pituitary extracts show strong LH and slight FSH activity in mammals,, but there is a great deal of conflicting evidence and interpretation (Sundararaj and Goswami, 1966; Hoar, 1969). Other evidence indicates that PMS acts like a combination of FSH and LH when tested in mammals (Ball, 1960), but this effect can be modified by the dosage used. Hoar (1969) presently considers it likely that teleost pituitaries contain only a single gonadotropin.

Sundararaj and Goswami (1966) demonstrate how wide the range of conflicting results can be, when they report that hypophysectomized catfish, *Heteropneustes fossilis*, spawned ripe eggs after injection with appropriate concentrations of LH, HCG, PMS, and DOCA, while FSH brought about ripening but no spawning (LH contamination was possible). PMS did cause ovulation in striped bass (Stevens, 1966). HCG has been used successfully in other fish spawning studies (e.g., Sneed and Clemens, 1959; Stevens, 1966). The fact that both PMS and HCG can lead to successful spawning and, yet, reflect basically antagonistic systems in mammals should make these hormones prime targets in future experiments.

It is quite evident that considerable work remains to be done to untangle the connections between hydration and ovulation, which are certainly related events, but may be controlled by different hormones acting at different threshold levels.

The puzzling fact that one out of four 50 IU HCG fish hydrated and spawned while three out of three 100 IU fish hydrated but never spawned, might be explained by postulating that a "critical dose" exists, with doses above or below this level being unable to induce the complete sequence of spawning events. The three 50 IU fish which did not hydrate would perhaps have spawned if their GSI had been above 5 %. A "critical dose" phenomenon might also be involved in the observed difference in hydration and the complete lack of spawning obtained with 5 mg and 10 mg carp pituitary, as both groups showed GSI values above 5 %; in this case the "critical dose" might lie between 5 mg and 10 mg.

Carp is considered a universal donor by Clemens and Sneed (1962) and was successfully used to spawn several species of freshwater fishes. Bioassay with goldfish showed 100 IU HCG to be equivalent to 0.5 mg acetone-dried carp pituitary (Sneed and Clemens, 1959), and ovulation was obtained with 100-1600 IU HCG and 0.5-3.0 mg carp. Most other workers also report no inhibition of spawning from very large doses, but they all point out the critical nature of exceeding some lower threshold dose to initiate ovulation. The strength of pituitary extracts for spawning is assumed to be related to the reproductive status of the donors, a datum not given by the company selling the carp pituitaries used in the present experiments. Salmon pituitaries, however, were removed only from fish graded at the hatchery for optimal ripeness and the glands were taken within 15 min of death. Nonetheless, from the results of this study 1 mg of salmon pituitary appears to be 5 to 10 times more potent than 1 mg carp and about equal to 50 IU HCG or PMS, although definite qualitative differences in response exist. A truly valid comparison of the strength of various fish pituitary preparations can of course be made only by standardized bioassay (for reviews of methods see Clemens and Sneed, 1962; Das and Kahn, 1962; and Yamazaki and Donaldson, 1968a and 1968b).

It is clear that the effects of hormones vary with the GSI level of the experimental fish. Most of these spawning experiments were carried out over a 2-month period beginning in mid-April and ending in mid-June, while the GSI was gradually decreasing in the stock of fish used for the experiments. Thus, it is difficult to directly compare the effects of 50 IU and 100 IU HCG, as they were tested almost 2 months apart and the average GSI values of the experimental fish may have been somewhat different. The effect of the population's declining GSI is clear in the

case of a standard 1 mg dose of salmon pituitary, which caused hydration, but produced eggs from only one fish in the last test (late June 1970) carried out with the same stock of fish which had been spawned regularly with the same dose over the prior 2 months.

FERTILIZATION

Several early attempts to fertilize gulf croaker eggs all ended in failure. These eggs were obtained from hormone-induced spawning, and they appeared normal in all respects; however, the sperm mass was dispersed in the water some time prior to adding the eggs. Later studies showed that no fertilization resulted when the sperm and eggs were mixed more than 30 sec after sperm had been placed in water, while eggs retained their ability to be fertilized for several minutes when kept in water and for several hours when stored in moist chambers. The early failures to fertilize eggs thus resulted from not utilizing diluted sperm quickly enough. It is well known that sperm may be stored for long periods of time if it is maintained in concentrated form or is not activated by the diluent. The rapid decrease in the viability of sperm in water is probably important for maintaining the genetic integrity of the spawners; its significance for practical laboratory work is that sperm should be added to the eggs and not vice versa.

Sperm tended to be more active and to remain motile longer in water from the Salton Sea than in ordinary seawater from La Jolla, Calif. Moreover, developing eggs always floated in Salton Sea water (salinity in 1970, about 37 ‰ on the basis of total dissolved solids), while they sank in La Jolla seawater (33.5 ‰). These observations may have important implications for the salinity tolerance and adaptability of Salton Sea fishes (transplanted originally from the Gulf of California), matters of crucial interest in the initiation of this study of fish reproductive physiology.

Several batches of eggs obtained from hormone-induced spawning were allowed to develop to hatching, and a few of the resulting larvae were reared to metamorphosis in the laboratory on a diet of rotifiers, *Branchionus plicatilis*, followed by brine shrimp, *Artemia salina*, nauplii. Thus the entire life history of the gulf croaker can probably be completed under controlled laboratory conditions. This opens up the possibility of using this species for many other biological studies where large numbers (50,000-100,000) of pelagic eggs are desired from a marine species of known genetic history. Some of these studies are now in progress (Robert C. May, Scripps Institution of Oceanography, personal communication). It is hoped that future studies will include comparative work on this species, especially with respect to the possible adaptations of Salton Sea croakers since their separation from the Gulf of California population.

SUMMARY

1. Adult and immature gulf croakers captured by beach seining in the Salton Sea were transported to the Fishery-Oceanography Center laboratory in La Jolla, Calif., and used in laboratory studies on gonad maturation and hormone-induced spawning.

2. A bacterial disease which invariably developed on recently captured or frequently handled fish was successfully treated with Furacin antibiotic.

3. Long photoperiods (16 hr of light per 24 hr) and warm water (22° C), along with optimal feeding, accelerated the gonadal maturation of females captured prior to their natural cycle of gonadal maturation. These fish were ready to spawn in the laboratory 1 to 3 months prior to the spawning season observed in the Salton Sea. Male fish became ripe under all combinations of laboratory conditions and remained ripe throughout the study.

4. Concomitant field studies confirmed earlier work showing that female croakers ripened in April, while day length was increasing, and spawned when the water temperature reached about 20° C; peak spawning occurred in May of 1969 and 1970.

5. Mature fish, captured during the spawning season at the Salton Sea, quickly resorbed their gonads when held under short photoperiods (10 hr of light) in the laboratory, but similar fish

maintained on long photoperiods (16 hr of light) remained in spawning condition for 2 months (at 22° C) or 3 months (at 14° C) beyond the normal season.

6. At 14° C, injection of mature fish with salmon pituitary, carp pituitary, chorionic gonadotropin from human pregnancy urine (HCG), and deoxycorticosterone acetate (DOCA) caused increases in gonad size over sham-injected or uninjected fish.

7. A single injection of 1 mg (acetone dried) salmon pituitary, 50 IU of gonadotropin from pregnant mare serum (PMS) or 50 IU of HCG induced spawning in mature croakers (50-100 g) with gonad index values about 5 %. Fish with gonad index values below about 5 % did not respond to otherwise adequate hormone doses. Hormone-spawned fish could be spawned a second or third time at 1- to 2-month intervals.

8. Eggs could be stripped from the fish an average of 30.4 hr following injection. This latent period consisted of a slow hydration phase of water uptake followed by a rapid ovulation phase which released eggs from the follicles into the ovarian lumen.

9. The eggs remained viable only for 1 to 2 hr following ovulation, unless they were stripped from the fish and stored in moist chambers. Each female produced 700 to 1000 eggs per gram of wet body weight.

10. Sperm are viable for less than 30 sec after dispersion in water.

11. Low dosages (0.1 mg) of salmon pituitary were insufficient to cause hydration, while very high dosages (5 mg) caused hydration but, evidently, inhibited ovulation. High dosages (100 IU) of HCG caused fish to overhydrate and eventually die without having ovulated.

12. Carp pituitary caused hydration but was inadequate for ovulation. Deoxycorticosterone acetate and oxytocin, given alone, had little or no effect on the fish.

13. Fish did not respond to single hormone injections if the water temperature was at or below 17° C. One day of acclimation to a higher temperature was sufficient to prepare fish from cold water for spawning.

14. A few larvae hatched from eggs obtained by hormone-induced spawning were reared through metamorphosis; thus, the entire life cycle of the gulf croaker can be completed under laboratory conditions.

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