FISHERIES SESSION

THE FAILURE OF LARGEMOUTH BASS, *Micropterus Salmoides Floridanus* (LeSUEUR), TO SPAWN IN EUTROPHIC, OVER-CROWDED ENVIRONMENTS[1]

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ABSTRACT

Nonreproductive populations of adult largemouth bass have been found in a number of lakes. In all cases, these environments are characterized as highly eutrophic and over-crowded with bream and forage species. Reproductive failure was demonstrated to be due to a refusal of the adult population to spawn. Ripened ovaries were retained long after the spawning season and the deteriorated ova eventually reabsorbed. In some cases, only a portion of the adult largemouth bass population spawned, yet produced substantial yearclasses. Reproductive inhibition was attributed to the excretion and build up of a hormone like repressive factor by over-crowded bream and forage species. Largemouth bass reproduction was induced by Human Chorionic Gonadotrophin injections in at least 1 and perhaps 2 nonreproductive lakes.

Eutrophic, over-crowded conditions, identical to those found in non-productive lakes, were created in four hatchery ponds. Stocked pairs of largemouth bass failed to spawn in a manner identical to that in non-reproductive lakes.

INTRODUCTION

The largemouth bass, *Micropterus salmoides,* is the most important freshwater gamefish in the State of Florida. There is little doubt that in many eutrophic areas this once predominant piscivore is declining both in its role in the aquatic community and its availability to the sport fishery. The most dramatic indication that this species is having problems in eutrophic environments is its failure to reproduce. As early as 1964, block net surveys regularly conducted in several lakes in Polk County, Florida, reported the failure of substantial adult largemouth bass populations to produce young-of-the-year (Horel, 1964). Initially, it was attributed to lack of a suitable spawning substrate. Reproductive failure was subsequently reported in following years and it became apparent that this problem should be fully investigated.

The initial objective of F-24-R was to document and delineate the failure of largemouth bass to reproduce in Lake Hollingsworth (Polk County, Florida) and to derive solutions. An investigation determined that there was no largemouth bass reproduction in 1968 and that reproductive failure was not due to predation, parasitism, mortality, toxic materials, lack of a suitable habitat or substrate or due to water turbidity. It demonstrated that failure to reproduce in 1968 (and probably previous years) was related to the fact that the largemouth bass in Lake Hollingsworth failed to spawn and eventually reabsorbed their ripened gonadal products. This type of reproductive failure has been reported in the literature for largemouth bass and other species but, predominantly in hat-

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chery ponds and aquaria (Breder, 1936; Swingle, 1954, 1956a and b; Green, 1966; Whiteside and Richan, 1969). In all cases, these environments were characterized by overcrowded, stunted fish populations and a closed water system with little or no flushing action. When crowding was reduced or the water flushed, reproduction would occur. Swingle (1956a) attributed reproductive failure to a buildup of metabolic waste materials and hypothesized the apparent excretion of a hormone-like repressive factor (X) for goldfish, carp, buffalo and bluegill-largemouth bass populations. Both Bennett (1954) and Swingle (1956b) demonstrated an inverse relationship between the number of bluegills/acre and the number of young bass produced. Thus, not only is the failure of reproduction of concern but also the possibility of reduced reproduction in other areas.

This report summarizes the results of our investigations and studies into the failure of largemouth bass to reproduce in eutrophic, over-crowded conditions and the attempts made to induce spawning. Some of the experiments failed while others succeeded. All are important in piecing together this complex ecological problem.

METHODS

Lake Surveys and Experiments

Specimens were collected using a 220 volt electroshocker, rotenone and a series of nets(gill, minnow seine and sweep nets). Generally, the entire shoreline would be shocked either day or night in lakes less than 400 acres. Rotenone was applied to either 50, 100 or 150 feet of the shoreline and outward approximately 30 feet. Minnow seine samples were made using 30′ x 6′ net with ¼ inch stretched bar mesh for a distance of either 50 or 100 feet. Mensuration included standard, fork and total length in mm and weight in hundredths of a gram. Adult largemouth bass were sexed by external examination of the urogenital opening similar to the procedures and descriptions of Parker (1971).

Hormone injections were given intramuscularly using a 25 gauge needle, one inch long. The hormone used was Human Chorionic Gonadotrophin (H.C.G.) obtained from several pharmaceutical companies. The standard dose injected was 1,000 International Units per pound with a minimum of 2,000 I.U.

Specimens were tagged using Floy FD-67 anchor tags and a Floy FD-67 modified tagging gun. Tags were inserted immediately below the dorsal fin with the anchor locked in the pterygiophores. Those specimens marked had the 4th dorsal spine clipped.

An attempt was made to locate possible bass nesting sites using a method of tagging proved successful by Hasler and Wisby (1958). Largemouth bass of both sexes were captured by electroshocker during what was believed to be the peak spawning period. A small pencil-shaped cork bobber, painted flourescent orange, was attached to each bass by a five foot long monofilament line of six pound test. The line was secured beneath the rays of the dorsal fin by passing a needle through the body. Each bass was released at the location of capture and followed. Each place a bouy was located that day and for the following week was marked by stake and the bottom searched for nests and eggs using a suction tube and SCUBA gear.

Substrate samples were collected in areas of possible bass nesting using a suction tube developed for that purpose. The suction tube consisted of a 15-foot polythelene tube (½ inch inside diameter) coiled near the bottom and taped to an eight foot cane pole. When in position, air was allowed to escape from the surface while the water pressure below forced sand, eggs, larvae, detritus and water into the coils.

To determine if there was sufficient forage for larval largemouth bass in Lake Hollingsworth, pelagic invertebrates were sampled at monthly intervals during 1968 to document abundance and availability. A plankton net 12 cm across the

mouth and having No. 10 mesh bolting cloth (109 threads to the inch) was towed just below the surface for 10 minute intervals. Each sample was estimated to have strained 6.623 m^3. Samples were preserved immediately in 10% buffered formalin and their location marked on a map of the lake. Organisms were identified and counted at the Eustis Laboratory under a binocular dissecting scope. The total sample was placed in a 100 cm^2 counting chamber divided into cms^2. After the sample was scanned for larger items, a count was made in each of four different cm squares. To assure random distribution, the material was stirred before each count. Identification was limited to major groups such as: copepods, cladocerans or rotifers.

To determine if largemouth bass eggs or larvae were being preyed upon, specimens of all the major fish species inhabiting Lake Hollingsworth were collected during what was judged to be the peak bass spawning period and their stomach contents analyzed. Stomachs were removed by a cut through the esophagus just anterior to the stomach and a cut through the intestine just postrior to the pylorus. Stomach contents were washed into a watch glass and percent fullness of each stomach estimated. Identification of food items was limited to major groups. Using a dissecting scope, counts were made of each food item, and the percent contribution of each group to the total contents estimated. Estimates of three parameters were used to describe the food of each fish species: frequency of occurrence, percent of total number, and percent of total volume. Since the only objective of this food habit survey was to document predation on bass eggs and/or larvae, any such material was identified as being bass or non-bass.

Hatchery Pond Eutrophication Experiment

Four 1.2 acre hatchery ponds were secured at the Richloam Hatchery for the eutrophication study. Two ponds were deepened to seven feet over ¾ of the bottom. Each pond was filled and stocked with bream species nine months prior to the introduction of largemouth bass and by the end of the initial study, the following materials had been added to each pond.

Juvenile bream	2,000
Adult bream	45
Fertilizer (Hydro-pac, 20-20-5)	1,400 pounds
Bales of hay	14
Domestic sewage	400 pounds
Eutrophic lake water	100 gallons

Minor inflow was required only twice to replace water loss due to evaporation and seepage. No flushing occurred. Pairs of adult largemouth bass were obtained from seven donor lakes just prior to the spawning season and stocked at varying rates in each of the ponds. In one shallow and one deep pond, largemouth bass were repeatedly injected with H.C.G. while the two remaining ponds (one shallow and one deep) served as controls.

RESULTS

Lake Hollingsworth

Lake Hollingsworth is a 365 acre lake located in the city of Lakeland, Polk County, Florida. There is little flushing of the water as the lake is situated in a nearly landlocked bowl having little inflow or outflow. Runoff and storm drainage from the surrounding residential area, city streets and a college constitute the only inflow. Excess water flows out through a culvert, down a small stream and into another small lake, Bentley.

The substrate of Lake Hollingsworth consists largely of deep muck and silt. Shallow water areas near the shoreline are sandy with a perceivable thin layer of flocculent plankton fallout that is stirred by wind and wave action. Deeper areas, once measured to 20 feet, have filled in with organic detritus such that the muck layer begins uniformly at about four feet.

Maidencane and cattail border the shore area and are about the only vegetation within the lake. For years, a heavy phytoplankton bloom has restricted visability to less than 20 inches. The objectionable water color and substrate have restricted recreational activity to only limited sailing, fishing and waterskiing.

The fish population has, for years, been characterized by an over abundance of forage fishes (Horel, 1962-1967). Gizzard shad, threadfin shad and stunted bluegill predominate. Largemouth bass, when stocked, grow fast and do well in the lake but, have not reproduced successful year-classes. In the years between 1951 and 1960, 202,000 largemouth bass fry were stocked in Lake Hollingsworth. The only recent stocking of record was 6,000 in 1965.

Lake Hollingsworth, 1968

Beginning in January of 1968, every conceivable aspect of the aquatic community in Lake Hollingsworth was investigated. Monthly zooplankton samples indicated an abundant invertebrate food supply available to larval largemouth bass. Lake Hollingsworth supported almost 10 times ($\bar{x}$- = 4858.16/m^3) the number of copepods as did another lake (Weir) in which comparable zooplankton samples were taken ($\bar{x}$- = 519.49/m^3). The cladoceran population of Lake Hollingsworth ($\bar{x}$- = 90.04/m^3) was quite similar to that in Lake Weir during the spawning months (Chew, 1970).

Stomachs from a total of 196 fishes from Lake Hollingsworth, obtained during what was thought to be the peak period of largemouth bass reproduction, were examined. None of the food items ingested by any species were largemouth bass eggs, larvae or juveniles. Over 40% of the 60 bluegill examined had consumed shad, *Dorosoma sp.*, eggs.

Repeated minnow seine, rotenone and sweep net samples during the spring and summer of 1968 failed to capture any individual of a 1968 yearclass. Numerous larval bluegill were captured in the summer months indicating that some centrarchids could successfully reproduce.

Gonads from ten female and six male adult largemouth bass were collected on May 8, 1968 and sent to Auburn University for examination. No parasites or diseases which could interfere with spawning were found. A few encrusted acanthocephala were found on the peritoneum of some of the ovaries but could not have prevented spawning.

No largemouth bass nests could be located by SCUBA gear, walking the shoreline or using the suction tube. Seventeen of the 22 corks applied to adult largemouth bass were located entangled in the dense maidencane. None were still attached. The suction tube was used to scour the bottom within a radius of three feet around the location of each cork. No nests, eggs or larvae were found. Rotenone and sweep net samples at these locations failed to locate any evidence of largemouth bass reproduction. Four of the bouyed bass were eventually recovered by shocker and none appeared to be suffering any ill effects from the monofilament still implanted in their back. Repeated electroshocker, minnow seine and rotenone samples in the fall of 1968 and early part of 1969, proved that there had been no production of a 1968 year-class of largemouth bass in Lake Hollingsworth.

The first indication of what was happening in Lake Hollingsworth came from the examination of ovaries from females sacrificed every two weeks from February through July, 1968. None of the 74 female largemouth bass examined from February through July had spawned. Large, ripe, mature, turgid ovaries

were carried long past the peak spawning period indicated by other nearby lakes. By May, the ova began to deteriorate, lose their spherical shape and became quite opaque. Genital arteries and veins became quite prominent with dark blood clots. The color of the ovary deepened from a yellow or light orange to a darker orange-red. From June to August, 1968, the ovaries were obviously going through a stage of reabsorption. The average weight of ovaries collected 6-20-68 from seven female Lake Hollingsworth largemouth bass was $\bar{x}$- = 33.89 grams. The average weight of ovaries from three comparable sized bass collected from a reproductive lake nearby on 6-24-68 was 2.35 grams.

The fact that no largemouth bass nests, eggs, larvae or juveniles were found by the many methods employed lends support to the observation that female largemouth bass never deposited their eggs in Lake Hollingsworth in 1968.

Lake Hollingsworth, 1969

Ovaries from Lake Hollingsworth bass were periodically examined during 1969 and developed as follows. In January, the ovaries were enlarging and judged to be developing. In early February, some of the females examined were ripe while others were nearly ripe. By the middle of February, most of the females examined contained large, turgid ovaries and were judged ripe and ready to spawn.

To determine if Lake Hollingsworth largemouth bass could spawn in a more conducive environment and how long it would take them to adjust, 29 adults were transferred on February 24, 1969 to pond #11 at the Richloam Hatchery. Three days after transfer, the fish had begun spawning behavior and the major portion (10 out of 16 males) of the transferred fish had spawned within five days. All nests were examined by SCUBA gear and viable eggs obtained. Water temperature recorded through this period averaged 59° F. Additional nests and spawning sites were observed in pond #11 on March 7, 19, and 24. A total of 15 nests were found containing viable eggs and larvae. Two of the nesting sites were reused after the initial spawn had left.

On March 28, 1969, an additional 37 adult largemouth bass were transferred from Lake Hollingsworth to Richloam Hatchery pond #14. A similar pattern developed with eggs and larvae being found in five nests a week later. Turbidity of the water and weed growth in pond #14 made observation difficult. It is felt that most of the fish in pond #14 also spawned within five days of transfer.

It should be noted that the adults from Lake Hollingsworth were ready to spawn both in February and a month later in March. The delay of a month did not apparently affect viability of the eggs. Examination of the adults transferred and 15 other adults sacrificed on March 28 indicated that none of those bass had spawned prior to that date. These initial hatchery pond experiments indicated that Lake Hollingsworth largemouth bass had difficulty spawning in Hollingsworth. But, when transferred to less crowded and cleaner water environments they spawn profusely and rapidly, concurrent with the findings in the literature.

Repeated examination of ovaries from largemouth bass in Lake Hollingsworth during January, February, and early March, 1969, indicated that the non-reproductive pattern of 1968 would be repeated. Since all of the fundamental physical processes governing development, growth, metabolism and reproduction are known to be controlled by hormonal means, it was felt that hormones could perhaps induce spawning even in inhibited populations.

From the literature reviewed (Chew, 1970), it appeared that a single, massive dose of Human Chorionic Gonadotrophin may be sufficient to induce spawning, provided the gonads were in an advanced state of maturation, certainly the case in Lake Hollingsworth. From March 20, 1969, to April 28, 1969, 147 male and female Lake Hollingsworth largemouth bass were injected with H.C.G., tagged for identification, and released. The 147 included 59

females and 88 males. Twentyfour were recaptured during the injecting period and were given an additional dose. Two bass tag numbers 16 and 28, were recaptured and injected twice making a total of three injections for each.

Beginning on April 29, 1969, Lake Hollingsworth was intensively sampled for injected bass for examination. Only 25 of the tagged bass were recovered and examined. Table 1 lists the length, weight, sex, date of injection and recovery, gonad weight, and gonad percent body weight for those bass recovered. Examination of the gonads of recaptured injected bass revealed little (Table 1). It was expected that the hormone injections might be successful for only a few of those injected. Indeed, this appeared to be the case. Females, numbers 100, 104, 157, and with the yellow tag, had not spawned. Females, numbers 20, 38, 106, and 132 were probably partially spent. Females 94 and 150 appeared totally spent. The female with the yellow tag was quite out of the ordinary. It was a small fish which had quite large ovaries with most of the eggs ovulated. The hormone injection may have helped it attain this condition.

Ovaries from injected females compared quite closely with those from non-injected females concurrently captured and sacrificed. Some of both appeared to have spawned while others did not. Not enough injected fish were recaptured to derive any positive indication of the hormone effect.

It was difficult to tell if the males had spawned. Testes from both injected and non-injected males appeared similar. The difference in weight of a teste before and after spawning is slight. Such a weight difference occurs commonly with individual variation between males of the same length or weight.

As indicated by the appearance of some spent and partially spent ovaries, largemouth bass reproduced in Lake Hollingsworth in 1969. Twelve juvenile bass, 25 to 50 mm F. L., were captured in four, one-acre block net samples taken 4-29.69 through 5-6-69. A rotenone sample on 6-11-69 captured 26 juveniles averaging $\bar{x}$- = 52.3 mm with a range of 33 to 79 mm F.L. Using an estimated growth rate and back calculating, the approximate spawning date was established as late March or during the injecting period. But, it is also certain from fully ripe ovaries obtained in April and May that not all Lake Hollingsworth females participated in spawning activity. Since both injected and non-injected females were found to have spawned, it was difficult to attribute spawning activity to the hormone injections. Of course, non-injected females could have spawned with injected males, but it is equally possible that non-injected pairs spawned.

Regardless of the portion of the adult population which spawned, it was sufficient to produce a strong 1969 year-class of largemouth bass in Lake Hollingsworth. Growth and condition of the juveniles was excellent and fully substantiates previous conclusions that there was sufficient forage, suitable spawning substrate, little predation, and no material toxic to largemouth bass in Lake Hollingsworth. By the time the 1969 year-class had reached one year of age, they were contributing significantly to the creel survey on Lake Hollingsworth (Ware, 1970).

Lake Hollingsworth, 1970

Largemouth bass again reproduced in Lake Hollingsworth in 1970 although H.C.G. was not injected. Reproduction in 1970 was similar to that in 1969 in that only a portion of the adult population participated. Large, turgid, unspent ovaries were found in some adult females as late as June, when reabsorption of the ova became evident.

Reproduction in 1970 can possibly be attributed to an improvement in water quality, either by rainfall and flushing action or due to the substantial reduction in the over-crowded fish population through large scale haul seining operations by F-12 personnel (Ware, 1970).

Table 1. Hormone injected Lake Hollingsworth largemouth bass recaptured as of August, 1969.

Females:

Tag Number	Fork length in mm	Weight in grams	Sex	Injection date	Recapture date	Gonad weight in grams	Gonad as percent of body weight
3	455	—*	F	3-20-69	--	--	--
20	427	1290	F	3-20-69	4-30-69	26.36	2.04
38	396	1093	F	3-21-69	5-13-69	20.94	1.92
94	420	1579	F	3-28-69	8-12-69	8.68	0.55
100	395	1180	F	3-28-69	6-17-69	40.00	3.39
104	348	617	F	3-28-69	5-14-69	21.91	3.55
yellow	265	308	F	3-28-69	4-29-69	13.61	4.42
106	435	1275	F	4-02-69	4-29-69	25.10	1.97
132	430	1342	F	4-02-69	6-17-69	18.23	1.36
150	451	1268	F	4-22-69	5-28-69	6.89	0.54
157	392	1021	F	4-28-69	5-13-69	31.70	3.11

Males:

22	420	--*	M	3-20-69	5-27-69	--	--
28	396	997	M	3-20-69	5-08-69	2.60	0.26
29	498	1082	M	3-20-69	5-13-69	1.92	0.18
42	348	640	M	3-21-69	5-13-69	1.13	0.18
123	364	744	M	4-09-69	5-13-69	1.95	0.26
130	365	670	M	4-09-69	5-13-69	1.03	0.15
137	370	785	M	4-22-69	4-30-69	1.37	0.18
141	355	828	M	4-22-69	4-30-69	3.04	0.37
147	400	1123	M	4-22-69	4-30-69	3.64	0.32
148	382	978	M	4-22-69	5-13-69	2.98	0.30
152	368	834	M	4-22-69	5-14-69	2.15	0.26
159	345	667	M	4-28-69	4-30-69	2.03	0.30
161	369	847	M	4-28-69	5-28-69	0.64	0.08
165	370	855	M	4-28-69	4-30-69	1.67	0.29

*Data is incomplete for specimens captured by other than project personnel.

While the problem was considerably narrowed down in Lake Hollingsworth, a number of factors complicated the results such that no definite conclusions as to the success or failure of hormone injections could be derived. Slightly higher than normal rainfall occurred in February and March of 1969 and 1970 and could have flushed the lake sufficiently to stimulate limited largemouth bass reproduction. The removal of a substantial portion (163 pounds/acre) of the over-crowded fish population by haul seine in the spring (April and May) of 1969 could have been responsible for the production of the 1970 year-class (Ware, 1970). Regardless of why reproduction occurred in 1969 and 1970, the results in Lake Hollingsworth were quite significant in that it was conclusively demonstrated that, first, no reproduction occurred in 1968; second, that reproductive failure in 1968 and probably previous years was due to the lack of spawning activity and not any of many other possible factors; third, that when production of a strong year class does occur in a lake, it does not necessarily mean that all or even most of the adults participated.

Lakes Lorna Doone and Lawsona

To further test the effectiveness of hormone injections to induce spawning of largemouth bass, additional non-reproductive lakes were sought. Injecting was to be performed in some while others were to be monitored as controls. Rigid requirements were established to eliminate as many variables as possible.

Forty-six lakes within three central Florida counties were surveyed by electroshocker, rotenone and minnow seine. Of these, 16 had little or no recent largemouth bass reproduction. However, 11 of the 16 were extremely degraded and contained too few adult largemouth bass for adequate reproductive potential. Three lakes each contained few juveniles and an exceptionally large adult population. But, the clear water and well balanced panfish populations suggested factors other than spawning inhibition (i.e., predation) may be responsible for low recruitment. The remaining two lakes, Lawsona and Lorna Doone, were selected as study areas.

Lake Lawsona is a nine acre lake located in the southeast section of Orlando. Drainage into the lake is limited to rainfall and storm sewer. The panfish population is crowded and stunted. Eight adult largemouth bass were captured in the initial shocker samples and 5, 4, 6 and 3 in subsequent samples. No juveniles were found in any of the shocker samples, in 20 minnow seine hauls or in three spot rotenone samples. Although there were too few adults for injecting purposes, the population was thought to be sufficient for natural reproduction to be possible, and Lawsona was selected as the control site.

Lake Lorna Doone (18 acres) is a bowl shaped, highly eutrophic lake located in the southwest section of Orlando. Rainfall and storm sewers are the only inflow into the lake. Lorna Doone became a fish management area controlled by the Game and Fresh Water Fish Commission in 1964 and was used to raise channel catfish for sport fishery purposes. The lake was selectively treated with rotenone and stocked with largemouth bass and channel catfish in 1964. The fish population soon became out of balance and is characterized by stunted panfish.

Shocking efforts captured 18 adult largemouth bass initially and 13 and 15 in subsequent samples. No juveniles were obtained by shocking, 17 minnow seine hauls or three spot rotenone samples. From length data collected from captured largemouth bass, it is virtually certain that no reproduction occurred in Lorna Doone in 1969 or 1970. Although adults in Lorna Doone were fewer than desirable, it was felt that injecting had a reasonable chance of succeeding.

Lorna Doone and Lawsona, 1971

From March 24 to May 6, 1971, the adult largemouth bass population of Lake Lorna Doone was sampled each week by electroshocker, tagged and injected with H.C.G. following the procedures used in Lake Hollingsworth. A total of 46

injections were administered during 8 sampling efforts. Of those, 12 were repeated injections made on recaptured fish. During the first day of injecting (March 24), 14 fish were captured, tagged and injected. On the following day, only five fish were captured, two of which had been injected the previous day. For the remaining samples, few fish were captured although night, early morning and evening time periods were shocked. Either the fish learned to avoid the shocker or heavy mortality occurred. Lorna Doone's entire shoreline was inspected for mortality at least twice a week during the injecting period. Only one tagged (injected) fish was found (tag #359). The only other known mortality was the largest known female in the lake (tag #360), injected on March 24, and caught by a fisherman on May 13, 1971. The recovery of four tagged specimens by fishermen and electroshocker more than a year after the injecting suggests that initial mortality was not significant.

Of the 34 specimens injected only seven were females. This is an extremely unsatisfactory sex ratio and the low number of females injected limited the chances for success. Only one female (tag #375) was recaptured for a repetitive dose. The chance that even one of the seven injected females was stimulated to spawn with a single dose in March is small. Some of the injected males recaptured were strip ripe and no captured non-injected males were strip ripe. This would suggest that the injections affected some of the fish as intended. Too few females were captured and too few fish given additional doses for much of a chance of success.

No largemouth bass reproduction occurred in either Lake Lorna Doone or Lawsona in 1971. Repeated sampling by rotenone, minnow seine and electroshocker during the summer, fall and winter of 1971 failed to capture any individual of a 1971 year-class. Additional samples in 1972 verified the lack of reproduction.

Lorna Doone and Lawsona, 1972

Since it was concluded that the endemic adult largemouth bass populations of both Lorna Doone and Lawsona may have been too few for a successful injection or control program in 1971, pairs of adults were obtained from Lakes Dora and Eustis in March and April of 1972 and stocked in Lorna Doone and Lawsona. Those placed in Lorna Doone were injected with H.C.G. while those placed in control Lake Lawsona were not injected. All stocked largemouth bass had the fourth dorsal spine clipped for identification.

Females from Lakes Dora and Eustis had been sacrificed just prior to the stocking efforts and the examined ovaries indicated that those populations had not spawned to any significant degree. All ovaries examined were large, turgid and most were classified as "ripe" or "nearly ripe" and contained many ovulated eggs. At least six of the stocked females (some into each lake) were known to be "ripe" since light pressure to the abdomen resulted in the ready extrusion of ovaluated eggs.

A total of 55 pairs of adult largemouth bass were captured in Lake Dora, injected and stocked in Lake Lorna Doone between March 30 and April 12, 1972. The entire shoreline of Lorna Doone was shocked twice on April 19, 1972 in an effort to recapture and reinject some of the stocked adults. The six specimens (three females, three males) recaptured were comprised of five stocked and one endemic largemouth bass. A total of 24 pairs of adult largemouth bass were stocked into Lake Lawsona by April 20, 1972.

No reproduction occurred in control Lake Lawsona in 1972. Extensive rotenone and minnow seine samples on June 22 and 23 failed to capture any juveniles of a 1972 year-class.

Substantial largemouth bass reproduction occurred in Lake Lorna Doone in 1972. A small (50-foot) rotenone sample on June 22, 1972 produced nine juveniles of a 1972 year-class. Five short minnow seine hauls (50 foot each) captured a total of 35 young-of-the-year. Hauls were made at separate locations

around the lake shore and juveniles occurred in every haul (13, 3, 6, 5, and 8, respectively). An electroshocker sample on July 8, 1972 captured eight. Every indication points to a large 1972 year-class of largemouth bass in Lake Lorna Doone, the first in three years. It is thought that reproduction could only have been as a result of reproductive activity of the stocked-injected adults but perhaps in conjunction with the few endemic adults. Using an estimated growth rate derived for juvenile bass in similar lakes and back calculating from the average length of the specimens collected on June 22-23, 1972 ($\bar{x}$- = 39.22 mm), the approximate spawning date was estimated to be on April 13, 1972. April 13, 1972 was the day following the final stocking-injecting effort in Lorna Doone. Even though this is only an estimate based upon probable growth rates, it is at least accurate enough (within several weeks) to demonstrate that reproduction occurred in close proximity to the stocking of injected adults. Since similar but non-injected adults stocked in Lawsona did not reproduce, it would seem that the injections were a factor.

Eutrophic Hatchery Ponds, 1972

From the results of Bennett, 1954 and Swingle, 1954, 1956b and our findings in Lake Hollingsworth, it appeared that over-crowded, eutrophic lake conditions could be created in hatchery ponds. Such ponds would be ideal, easily controlled environments in which to inhibit largemouth bass reproduction and attempt to induce spawning by hormone injections. The procedures described under methods to establish eutrophic, crowded conditions were extremely successful. A heavy phytoplankton bloom continued throughout the study in all ponds and experienced only minor fluctuations in density. All four ponds maintained an extremely dense, stunted bream population. The composition of the bream population varied only slightly from pond to pond. In each case, the population was entirely dominated by stunted bluegill followed by stunted redear. An average number of 44,996 forage fishes and weight of 519 pounds was recovered from each of the four ponds during draining on May 17-19, 1972. This would be approximately 37, 497, or 433 pounds per acre. It would appear that the stocked bass did not seriously crop the bream population.

Pairs of adult largemouth bass were obtained by electroshocker from seven different donor lakes and stocked into all hatchery ponds between February 10 to 16, 1972. One of the deep ponds (A) and one of the shallow ponds (B) were used as injecting areas, while deep pond C and shallow pond D were controls. Stocking rates in ponds A and D were 11 pairs and 7 pairs in ponds B and C. Cool water temperature, inclement weather, surveys of lakes in the central Florida region, and sacrificing of numerous specimens made it virtually certain that largemouth bass had not spawned to any significant degree prior to their stocking in the hatchery ponds. Those bass stocked in injecting ponds (A and B) were sexed, tagged, and injected with H.C.G. as they were released. Specimens placed in control ponds (C and D) were sexed but never injected. Those placed in pond C were not tagged.

A fish kill observed on March 3, 1972 was caused by low D.O. and resulted in a known loss of 5 fish in pond A and 7 in pond C. Lake Dora was used as a source for replacement pairs. Inspection of ovaries from 15 females sacrificed on March 8, 1972 by three biologists indicated that Lake Dora largemouth bass had not yet spawned. Ovaries examined were large, turgid, classified in ripe or near ripe condition, and contained many ovulated eggs. Four replacement pairs were tagged and released in pond C on March 8. Four pairs were tagged, injected and released in pond A on March 10.

Weekly efforts were made to recover and reinject stocked fish from the experimental injecting ponds beginning February 24 and ending March 21, 1972. During the first two weeks, February 24 and March 1, the use of only one shocker boat resulted in poor recovery. Employment of two boats on March 9

and three boats on March 21 resulted in substantial recovery. Since most of the brood bass held in Richloam rearing ponds had spawned by March 21, it was felt that the injecting should be terminated.

On May 17 through 19, 1972 all four ponds were drained and the total fish population recovered. The first objective of the study was successful in that no largemouth bass reproduction occurred in any of the four ponds.

All of the largemouth bass recovered from all of the ponds were in exceptionally fine condition. No sores or parasites, often seen in eutrophic conditions, were present. All of the specimens were extremely heavy bodied and contained a large amount of fat in the peritoneal cavity. When opened, fat within every specimen covered the intestines, stomach, pyloric caeca, and air bladder. It is known, from observations of sacrificed specimens at the time of stocking, that these were not as robust when obtained. They could have only attained this condition by foraging on the abundant bream population available in each pond.

Table 2 lists the summarized information regarding stocking, injecting, and recovery of largemouth bass in the four ponds. A total of 53 injections, including the initial injections at stocking, were given to 30 specimens stocked in pond A. Nine females and seven males were recovered. In pond B, 21 additional doses were administered to the 14 stocked adults. Recovered were seven females and five males. Only one male survived the low D.O. in pond C and was recovered. Twentyone out of 22 stocked largemouth bass were recovered in pond D.

Ovaries from all of the 23 females immediately recovered were examined and none appeared to have released their eggs. Ovaries were medium sized to large, yellow to light orange in color and were filled with ova in various stages of maturation. The ovaries appeared quite similar to developing or pre-ripe ovaries which would commonly be found several weeks prior to spawning except that the ova was not as healthy in appearance. Toward the lumen in many of the ovaries there were large ova which had probably ovulated but were now deteriorating. It appeared that in most cases the ova were being reabsorbed. None of the characteristics associated with spent ovaries were observed.

Six brood females from Richloam rearing ponds were captured and sacrificed at the same time. These specimens had been obtained from donor Lake Dora and stocked at approximately the same time as the experimental fish. Ovaries from these brood females were spent, flaccid, pink or light purple in color, contained few eggs, and were entirely different from the ovaries contained in females recovered from the experimental eutrophic ponds. The average weight of the spent ovaries examined was 3.81 grams and they ranged between 2.94 and 4.97 grams. In contrast, the unspent ovaries ranged between 10.18 and 54.18 grams and averaged 26.82 grams.

An analysis of variance test comparing the weights of collected ovaries between the three experimental ponds in which females were recovered indicated no significant mean differences ($F = 2.029$). This supports the previous observations that there were no major differences in the size, color, texture, or contents of the ovaries from any females examined either within or between ponds. It also demonstrated no differences between the ovaries from the two injecting ponds and the one control pond. The fact that ovaries from the injected females had higher mean values ($\bar{x} = 32.09$ and $\bar{x} = 25.91$ grams) than the non-injected ovaries collected in pond D (20.94 grams) could be related to the influence of the hormone. The hormone could have either developed slightly larger ovaries or, more likely, it would have maintained the ovaries longer and retarded reabsorption.

The weights of ovaries from females from the three experimental ponds were pooled and compared to the six brood females by a "t" test. Highly significant mean differences are indicated ($t = 9.230$) as had been apparent in the raw data.

All four ponds were consistent in that they maintained eutrophic conditions, a crowded-stunted bream population and no largemouth bass reproduction.

Table 2. Stocking, injecting and recovery of male and female largemouth bass in four eutrophic ponds, 1972.

Pond	Date	Injection and Stocking		Recapture and Reinjecting		Recovery 5/17-19/72	
		Female	Male	Female	Male	Female	Male
A	2/10/72	11	11				
	3/10/72	4	4	12	11	9	7
B	2/14-16/72	8	6	13	8	7	5
C	2/11-16/72	7	7		Not	0	1
	3/8/72	4	4		attempted		
D	2/11/72	11	11		Not attempted	10	11

From the appearance of the ovaries it can only be concluded that the bass failed to spawn or spawned very little in any of the ponds. By comparisons with the spent brood fish, obtained at the same time from the same donor lake, we know that spawning was inhibited by a factor contained only in the eutrophic ponds. It is also known that the bass used were healthy in every respect and able to improve their condition in the experimental ponds. The fact that the injections failed to induce spawning does not detract from the demonstrated fact that reproduction is inhibited in eutrophic, over-crowded conditions.

SUMMARY

Results of surveys and experiments in Lakes Hollingsworth, Lorna Doone, Lawsona and hatchery ponds suggest the presence of a repressive factor which inhibits largemouth bass reproduction. The problem is associated with eutrophic environments, over-crowded with bream and forage species. It is easily discernable by the retention of enlarged, ripened ovaries long past the spawning period. It is by no means a rarity and is being observed in an increasing number of lakes in central Florida. It is accelerated by nutrient pollution and aggrevated by recent water management practices in many areas to stabilize water levels. The stabilization of water levels by water control structures prevents natural flushing produced by alternate periods of drought and heavy rainfall. Stabilized bodies of water then serve as nutrient traps for the massive out-pouring of wastes from sewage treatment plants, agriculture, and processing plants.

Preliminary surveys into some of the larger bodies of water and drainage systems indicate a serious reduction in largemouth bass reproduction. In Lake Parker (2,291 acres, Polk County), a lack of reproduction has been documented for years by the same personnel that reported it in Lake Hollingsworth. A small amount of largemouth bass recruitment is thought to enter Lake Parker by canals from nearby phosphate pits. In Lake Apopka (30,630 acres, Orange County), female largemouth bass with large turgid ovaries were examined more than a month after the spawning season had passed. In Lakes Dora and Eustis (3,660 and 7,200 acres, respectively, Lake County), female largemouth bass also retained their ova for an abnormal length of time. Extensive sampling with minnow seine and rotenone that should have produced hundreds of young-of-the-

year captured only nine in Lake Dora in 1972. The substantial adult largemouth bass populations which remain within these lakes are not thought to spawn. Recruitment probably enters from marsh, swamp, or canal areas bordering these lakes. However, the problem is not as simple as just a eutrophic, overcrowded lake. Some highly eutrophic lakes surveyed contained large numbers of crowded bluegill and yet were found to have substantial yearly largemouth bass reproduction. Perhaps flushing in these areas is sufficient to prevent a buildup of the inhibiting factor.

Injections with Human Chorionic Gonadotrophin appear to have induced reproduction in at least 1 and perhaps 2 inhibited populations. It is, however, not a fool proof method and is in need of refinement. If there are levels of the repressive factor (X) which will permit spawning and levels which will permit only a portion of the adult population to spawn, then there are possibly high levels at which injecting will be ineffective.

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