AN EVALUATION OF TRIPLOID FLATHEAD CATFISH

(*Pylodictis olivaris*) PRODUCTION

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AN EVALUATION OF TRIPLOID FLATHEAD CATFISH (*Pylodictis olivaris*) PRODUCTION

Andrew E. Gima

A Thesis

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AN EVALUATION OF TRIPLOID FLATHEAD CATFISH

(*Pylodictis olivaris*) PRODUCTION

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VITA

Andrew Edward Gima was born on April 17th, 1982 in Merrillville, Indiana. Andrew is the oldest child of Gregory and Marcia Gima. He grew up in Merrillville, Indiana with his younger brother Alan Gima. He and his brother are both graduates of Merrillville Senior High School. Andrew is the eldest of two grandchildren of his mother's parents Edward and Beverly Maywald. Andrew is one of six grandchildren of his father's parents George and Bernice Gima. Andrew graduated from Purdue University of West Lafayette, Indiana in May of 2006 and entered the graduate school of Auburn University in January of 2006. He married Megan Rosinski in 2008.

THESIS ABSTRACT

AN EVALUATION OF TRIPLOID FLATHEAD CATFISH

(*Pylodictis olivaris*) PRODUCTION

Andrew E. Gima

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The overall objective of this research was to develop an effective protocol to produce triploid flathead catfish, *Pylodictis olivaris*. To accomplish this goal, data was gathered depicting characteristics of flathead catfish provided by Georgia Department of Natural Resources and the fry produced from these fish. The gamete production of male and female flathead catfish, ovulation techniques, egg incubation methods, and characteristics of fry produced was examined.

Eleven males and twenty eight females were used in this study. The average weight of the male catfish was 3.27 kg with the females averaging 4.55 kg. The mean grams of usable testes produced by male catfish were 1.24 g. Larger males produced a greater amount of usable testes demonstrating a strong positive correlation that exists between the traits. Females in this experiment produced an average of 350.0 g of eggs.

The total number of eggs produced by each female was 10,826 eggs from strip spawning, and a linear relationship exists between egg number and body weight, r^2=0.70. Relative fecundity of the females decreased with size with a linear regression equation with fecundity=3,511.1-209.89(female BW).

Females that received a combination of implant and injection had a percent ovulation of 37.5 and 50% over ripe eggs. The implant only treatment had 33.3% ovulation. The percent of viable eggs that were not over ripe for this treatment was 83.3%. The mean latency of the implant only treatment, 81.3 hours was longer than that for the combination treatment, 63.3h. Two incubation techniques were used to assess any differences that may exist between the two treatments. The first technique utilizes a standard paddle wheel in a flow thru trough. The second technique utilized hatching jars to incubate the eggs. The mean percent hatch for the troughs was 12.4 % was lower than the mean percent hatch in the jars, 22.1. Five different pressure treatments, 5,500, 6,000, 6,500, 7,000, and 7,500 psi were administered. The pressure treatment of 6,500 psi was the most effective treatment with a % hatch of 16.7 and 100% triploidy

The diploid fry grew over this entire period from a minimum of 4.6 to a maximum of 28.5 grams. This period produced triploid fry from a minimum growth of 3.9 and a maximum of 33.7 grams. These values were not proven to be significantly different from each other (P=0.72). Triploid fry appeared less aggressive during confinement in aquaria and trough with fewer deaths in their tanks being attributed to cannibalism.

Key words: flathead catfish, invasive species, triploidy, polyploidy, hydrostatic pressure, cannibalism, predatory, fry growth, super males, screw fly, spawning.

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INTRODUCTION

Many effects, often detrimental to existing fauna, can be seen when a non native species is introduced into a new habitat. When the exotic species is the apex predator in the system, effects can be amplified significantly. Impacts such as disturbance of native food webs can have ramifications that can negatively impact an existing population and thus possibly alter the ecosystem. One of the North American fish species that is documented for negatively impacting a native ecosystem when established outside its natural range is the flathead catfish *Pylodictis olivaris*. This primarily piscivorous species has been documented in non-selectively feeding upon prey items that exist in its habitat (Pine III *et al.* 2005). Controlling such non-endemic nuisance species is often a challenge once they have an established population outside their natural range.

During the past century populations of flathead catfish have been introduced into numerous Southeastern ecosystems. Illegal introductions of flathead catfish in the Satilla River during the 1990's and in the Altamaha River system, in Georgia during the 1970's has adversely impacted an existing fishery causing great concern to natural resources or fisheries managers. The native redbreast sunfish *Lepomis auritus*, a highly sought sport species, has seen a drastic population decline since the introduction of the flathead catfish. The introduction of this species has also negatively impacted other native species such as bullhead catfish including the brown bullhead *Ameirus nebulosus*, yellow bullhead *Ameirus natalis*, and snail bullhead *Ameirus brunneus.*

Additionally, species diversity has been negatively impacted as abundance of native fauna has decreased as biomass of flathead catfish and other exotic species such as the blue catfish *Ictalurus furcatus* has increased. These changes threaten important native fisheries, and as a result threaten certain economies in South Georgia. Examination of effective means of controlling flathead catfish has begun by Georgia Department of Natural Resources.

No one single approach exists to address this problem but a combination of approaches may effectively address the issue. Currently, physical removal of this nuisance species via electro-fishing is one option that has had some success, but total extirpation of flathead catfish by this method remains costly and ineffective. However, some new innovative approaches are available for evaluation. Combinations of such approaches have potential to diminish nuisance populations with a more cost effective result. Introduction of sterile triploid males into the affected ecosystem could result in reduced numbers of annual diploid recuirts.

Theoretically, triploid males would be capable of courtship and mating behaviors, stimulating the females to mate and release their eggs, but would be unable to successfully fertilize the females' gametes. Many approaches can be used to produce triploid individuals. Hydrostatic pressure is the preferred method to produce triploid channel catfish (Rezk 1988). With the combination of this approach and other techniques, such as manual removal, the effectiveness reducing the negative impacts of the flathead catfish in a non-native habitat might be greatly enhanced.

In triploid animals, the somatic cells contain three sets of chrosomes arising from the manipulation of the newly fertilized developing egg. Artificial production of triploid fish was first documented in the 1940's (Makino and Ozima 1943). However it was not until years later that triploid fish were raised to adulthood and their life history characteristics assessed (Swarup 1959).

Induction of triploidy is not always 100% effective, but it is viewed to be the most practical measure to sterilize a population of fish (Devlin and Nagahama 2002; Pandian and Koteeswaran 1998). At the onset of sexual maturity, reduced or inhibited gonadal development may allow energy normally used in reproductive tissue and behavior, to be used for growth of somatic tissue (Thorgaard and Gall, 1979; Lincoln, 1981; Wolters *et al*., 1982). The use of sterile triploids can prevent the permanent establishment of exotic species in otherwise restricted geographical locations (Shelton and Jensen, 1979). Other potential uses include: 1) supplemental stocking of natural populations without compromising the genetic integrity of the resident population, 2) disruption of reproduction in nuisance species and, 3) sterilization of transgenic fish. All are mechanisms for reducing environmental risk and applying genetic conservation (Dunham 2004). Theoretically, triploid males are capable of courtship and normal mating behaviors, stimulating females to mate and release eggs, but are unable to successfully fertilize the female's gametes. This "screwfly" approach (Klassen *et al.* 2003) may be a mechanism to control reproduction of nuisance species.

Triploidy is achieved by allowing normal fertilization to occur followed by disruption of the mechanisms of cell division thus forcing retention of the second polar body (Flajšhans et al. 1993).

The second polar body is retained by applying temperature (hot or cold), hydrostatic pressure, anesthetics or chemical shocks shortly after fertilization (Thorgaard *et al*., 1999; Wolters *et al*., 1982; Chourrout and Itskovich, 1983; Benfey and Sutterlin, 1984; Chourrout, 1984; Cassani and Caton, 1986; Curtis *et al*., 1987; Johnstone *et al*., 1989).

Due to the increased chromosome number in cells of triploid individuals their red blood cell size is consequently larger than that of a diploid (Gheyas *et al.* 2001). Nitrous oxide application induced 80% triploidy in Atlantic salmon, *Salmo sala* (Lilyestrom et al. 1999). Freon was moderately effective, halothane and ethane induced less than 10% triploidy, and cyclopropane was ineffective for inducing triploidy in Atlantic salmon (Johnstone *et al*., 1989). Regarding physical consequences from use of hydrostatic pressure treatments is perceived to be less intrusive upon the developing fish embryo (Peruzzi and Chatain 2000). Hydrostatic pressure produces more consistent results, survival of treated eggs and percent triploidy than does temperature shocks and other treatments that have been evaluated (Cassani and Caton, 1986; Bury, 1989; Dunham 2004).

The cellular mechanism of triploidy induction with hydrostatic pressure has been examined in the loach, *Misgurnus fossilis*. When the pressure shock is applied, the polar body has already started to extrude, but then merges again with the egg cytoplasm and the meiotic spindle is destroyed, resulting in two female pronuclei (Betina *et al*., 1985). The male pronucleus then fuses with the female pronuclei, resulting in formation of the triploid zygote.

At the beginning of first cleavage (anaphase, furrow formation), the blastodisc in untreated control eggs is thicker than in the triploid zygotes and the nuclear transformations are accelerated in the triploid eggs.

The success one has in inducing polyploidy depends upon several key factors. The timing of the shock, the magnitude of the shock and the duration of the shock all play roles in the effectiveness of the treatments (Ihssen *et al*. 1990).

The ideal time for one to initiate the shock varies widely among different species of fish and is related to the rate of development of each species, specifically, the time of the second meiotic division normal for the species. This development and time frame is temperature dependent, thus, the timing of the meiotic divisions can vary depending upon temperature the embryo is developing in and environmental conditions at the time of fertilization. However, by standardizing for temperature related developmental shifts, consistent results can be obtained. One method is to apply the shocks at a certain accumulated number of temperature degree minutes (Palti *et al*., 1997). Often the hatch of embryos that have been tripilodized is lower than that for normal 2n individuals. Atlantic salmon triploids were found to have a 10 to 30% lower survival than diploid controls (Benfey and Sutterlin 1984). This has also been demonstrated in other species such as turbot, *Scophthalmus maximus* and sea bass, *Dicentrarchus labrax* (Piferrer *et al*., 2000).

When viability of bighead carp eggs, *Hypophthalmichthys nobilis*, was above 59%, high rates of triploidy were produced, but, when the viability was less than 40%, no triploids were produced (Aldridge *et al*., 1990).

Delaying fertilization of ovulated grass carp eggs did not affect the rate of triploid induction (Cassani and Caton, 1986), thus eggs may be held for short periods of time so as not to rush the procedure and to allow repeated shocking and maximum utilization of the available eggs.

Strain or family effects may have implications upon the ability to effectively produce triploid individuals. Various strains of rainbow trout may react differently towards different temperature shocks to induce triploidy (Anders, 1990). This is not surprising because the genetic differences among strains can affect the rates of embryonic development, thus changing the optimal parameters for manipulation of the embryo. The effectiveness of ploidy induction for triploid and tetraploid rainbow trout was found to be affected by sire components (Blanc *et al*. 1987).

Several studies confirm the sterility of triploid fish. One-year-old triploid male and female common carp had gonads that were undeveloped and were sterile (Cherfas *et al*., 1994). Long-term sterility was demonstrated in white bass and hybrid striped bass hybrids, which had reduced and dysfunctional gonads at 5 years of age (Kerby *et al*., 2002). Triploid female silver barbs did not undergo vitellogenesis (Koedprang and Na-Nakorn, 2000), and female triploid European sea bass had lower hepatosomatic indices, possibly indicating a lack of oestradiol-mediated hepatic synthesis of vitellogenin (Felip *et al*., 2001). Experimentally, there have been extremely rare occasions where triploid males produced small numbers of viable progeny (Dunham 2004). Female and male triploid walleye and yellow perch were found to have retarded gonadal development, thus leaving individuals' reproductively inactive (Malison *et al*. 1996).

Functional sperm from triploid males results in aneuploid individuals that are unviable (Arai, 2001). Triploid and diploid European sea bass males had similar testicular development; however, triploid males never spermiated (Felip *et al*., 2000). Pre-adult triploid channel X blue hybrid catfish had GSIs equivalent to those of diploids.

However, diploids had normal gonadal development and histology, whereas triploid hybrids had abnormal gonadal development (Lilyestrom *et al*., 1999). The abnormal gonadal histology of the triploid individuals leads to no sperm present the seminiferous tubules.

In triploid yellowtail flounder *Limanda ferruginea* triploid males were found to have translucent under developed testes and sperm ducts containing miniscule amounts of viable milt (Manning *et al.* 2004). Females were found to have only one ovary 36% of the time. Experimentally, there have been extremely rare occasions where triploid males have produced small numbers of viable progeny.

True sterility of triploid fish has occasionally been an issue. The probability is extremely low but it is possible for triploid males to produce progeny. However, even if progeny were produced, they would not likely result in a reproducing population, as resulting progeny would probably be sterile. Triploid rosy bitterling, *Rhodeus ocellatus ocellatus*, develop nuptial coloration regardless of their spermiation ability (Kawamura *et al*., 1999). Mud loach *Misgurnus mizolepis* was documented to have suppressed gonadal development when triploidized (Nam *et al.* 2004). Sperm density of the triploids was less than 2% of that of diploids, and normal spermatids and spermatozoa were rare inside the testes of triploid rosy bitterling (Kawamura *et al*., 1999).

One embryo developed from the mixing of sperm from triploid males with 1500 eggs from diploid females resulting in embryos that had a ploidy level of 2.5N (Kawamura *et al*., 1999). If this embryo was to survive to adulthood, it is likely that the line would die. However, exceptions to the fertility rule may exist. It has been documented that triploid male grass carp can produce small numbers of viable diploid progeny when artificially mated with diploid females (Eenennaam *et al*., 1990). When diploid females were artificially inseminated with milt from triploid males fertilization rates were significantly lower. The fertilization rate was found to be one-half to one-third of that for diploid mating.

Embryos of this mating were also found to hatch with smaller bodies and smaller yolk-sacs. They also utilized their yolk at a slower rate when compared to the diploid embryos. Most hatched diploid by triploid embryos had notochord deformities and died before first feeding. However survival did occur with 0.1–0.2% of the embryos living to 5 months (Eenennaam *et al*., 1990).

Chouinard and Boulanger (1988) were also able to artificially backcross a triploid rainbow trout–brook trout hybrid with brook trout and produce viable progeny. If only triploids are in the system the reduced fertility of the individuals should ensure that no breeding population will become established. This would be brought on by the decreased gonadal development of adult individuals in the population. If diploid fish are already present in the system, an effective alternative would be to use monosex female triploid populations since the triploid females have not been known to produce any progeny. Also triploid females have decreased reproductive hormonal levels.

One complication regarding these experiments that demonstrate fertility of triploid males is the fact that artificial insemination was used to achieve fertilization. Possibly, these male triploid grass carp or triploids of other species might not have the capability to naturally produce progeny. Videos taken by Dr Cassani in Florida of triploid grass carp show that the triploid males exhibit normal courtship behavior in spawning tanks but have no visible ejaculate upon the ovulation by the female. This may be evidence that the carp may not be able to produce progeny if they have been triploized without the assistance of manual hand-stripping.

Triploidy not only affects sterility and gonad development, but sex hormone production and profiles as well. Generally, triploid females have minimal production of sex hormones. However, triploid males have sex-hormone profiles that mimic those of diploid males despite their sterility.

Testosterone levels were found to be similar for diploid and triploid European sea bass males (Felip *et al*., 2000a). Gonadal steroid and gonadotrophin hormone profiles were similar for diploid and triploid Atlantic salmon (Cotter *et al*., 2000). This similarity in sex hormone production profile apparently leads to normal sexual behavior. Triploid male ayu, *Plecoglossus altivelis,*(Inada and Taniguchi 1991), grass carp (J. Casani, personal communication) do not produce sperm, however they were found to exhibit normal courtship and sexual behavior and effectively induce females to ovulate without ejaculation and fertilization of eggs in laboratory settings. Diploid males were not in competition with triploid males in these mating experiments. Theoretically, these sexually active triploid males could be used in a similar manner as sterile screwflies (Klassen 2003).

The release of massive quantities of males, sterilized via irradiation, was used to eradicate the screwflies by inducing females to mate with the sterile males thus producing an unfertilized embryo. However, due to limited data and research on the flathead catfish's reproductive traits and early life history, the feasibility of a triploid approach of controlling flathead catfish production is not known. Several steps are involved to produce triploids in flathead catfish, and ultimately reach a stage to evaluate the reproductive behavior of and impact of triploid males on diploid reproduction to control nuisance populations.

To increase our understanding on these issues induced ovulation coupled with ploidy manipulation of fertilized gametes were analyzed. Triploid embryo production requires induced spawning, little is known concerning the propagation of flathead catfish using this approach. Examination of incubation techniques and the survival of fry can give insight into cost effective techniques needed to maximize number of triploid fry. Growth comparisons were also assessed among juvenile diploid and triploid flathead catfish produced in this project. The objectives of this study were to 1) determine the effectiveness of hormonally induced gamete production of male and female flathead catfish, and 2) compare Luteinizing hormone releasing hormone analogue (LHRHa) implants with LHRHa implants combined with Carp Pituitary Extract (CPE) to induce ovulation and produce flathead catfish fry, 3) determine the relative effectiveness of jar and paddlewheel trough incubation, 4) determine the relative growth and survival of diploid and triploid flathead catfish fingerlings and 5) determine the most effective hydrostatic pressure treatment for production of triploid flathead catfish. The overall objective of was to develop effective protocol to produce triploid flathead catfish.

GAMETE PRODUCTION AND INDUCED SPAWNING OF MALE AND FEMALE FLATHEAD CATFISH *Pylodictis olivaris*

INTRODUCTION

Flathead catfish have been found to rapidly populate environments in which they are introduced (Fuller et al. 1999). Males mature in 3-4 years while females take slightly longer with average maturity at the age of 4-5 years (Etnier and Starnes 1993; Jackson 1999). In a study of introduced flathead catfish in North Carolina it was found that after 15 years of inhabiting an area of the Cape Fear River system this species comprised 64% of the total biomass of the system greatly reducing the native species population (Guier *et al.*1981).

Production of triploids by artificial hormone induced ovulation, and their use in a "sterile screw fly approach" might be a mechanism to ultimately reduce their numbers in a natural environment. However limited data on the reproduction of captive flathead catfish in a controlled environment is available to assay appropriate techniques to spawn this species to enable maximum triploid juvenile production. Carp pituitary extract (CPE) and reproboost implants have been used in similar species such as the channel catfish to successfully induce ovulation (Dunham et al. 2000). However, hormone induction to ovulate flathead female has not previously been previously examined.

The objectives of this study were to 1) determine the gamete production of male and female flathead catfish, and 2) compare LHRHa implants with LHRHa implants combined with CPE to induce ovulation and produce flathead catfish fry.

MATERIALS AND METHODS

Experimental Fish

Male and female flathead catfish were captured on May 10th and 22nd, 2007 from sampling locations on the Satilla River by the Georgia Department of Natural Resources and were delivered to E. W. Shell Fisheries Center one day prior to inducing of ovulation. Upon arrival the sex of the individual catfish was determined and the males and females were segregated and placed into separate raceways. Twenty eight females were used to assess the hormone treatments and their effects upon this species of catfish. Females delivered on the first date were weighed and placed into individual mesh holding bags. The second delivery of fish was segregated by sex and were identified by a series of barbel clips and kept in raceways of two to three individuals. Hormone treatments were randomly assigned to individual females on each delivery event.

Males were held in a raceway until 1 to 2 hours prior to the expected female ovulation and stripping of eggs from the female. The individual body weights and weights of freshly extracted testes of each of the eleven males were documented.

Preparation of Sperm

Males were euthanized and an incision was made on the abdomen to access the abdominal cavity. The testes were removed from the abdominal cavity being careful to minimize the amount of mesenteric tissue attached to the testes. Once the testes were removed, they were placed in a Petri dish, containing a saline solution comprised of 8.5g pickling salt per 1 liter of distilled water, and then rinsed to remove any remaining blood. The sample was then gently blotted dry and weighed. Care was taken during this time not to release any of the milt within the removed testes.

Only testicular tissue of good quality was kept. Based on prior experiences, only testes containing milt and possessing a pale white color were considered usable and weights were recorded.

The testes were then processed to release sperm by macerating them in a fine mesh bag allows milt (sperm) to pass through, but retaining any testicular tissue. The milt was strained from the testes into a clean Petri dish. The processing bag was then rinsed with a minute amount of the saline solution, saving any milt that may still be in the bag. The Petri dish was then rinsed into a test tube containing the saline solution of 10ml per gram of testes. Sperm of multiple males was combined to make a stock solution. The extended milt solution was then refrigerated until it was used.

Hormone Administration

Females were implanted a reproboost luetinizing hormone releasing hormone analogue (LHRHa) implant at a dosage of 100µg per kilogram of body weight. Half of the implanted females also received an injection with a solution of CPE (carp pituitary extract) at a dosage of 2mg per kilogram of body weight. Marked females and those that were placed into laundry bags were monitored in attempt to identify the individual fish that had begun ovulating. Females in bags were selected for striping when eggs were present upon the holding bag material. Those that were not placed into the bags were left in the raceway, a PVC pipe which functioned to attract and adhere to any ovulated eggs enabling monitoring of the onset of ovulation. Tanks were checked 6 hours prior to expected ovulation and in 3 hour intervals thereafter.

Female Ovulation

Temperatures and flow rates of the water in each raceway were documented to maintain a consistent value. Flow rates averaged 23.3 L/min and the temperature ranged from 23.9-28.1 ° C.

Upon onset of ovulation individual females were anesthetized using a solution of 200 ppm of MS-222 (tricaine methane sulfate) buffered with 200 ppm of sodium bicarbonate. Females were then rinsed and dried to remove all excess water from her body. If this is not done properly fresh water coming in contact with the egg causes premature hardening of the chorion. Care was taken not to lose any eggs prior to stripping by covering the genital opening with a finger. After the female was dried, the eggs were stripped into an aluminum pie pan greased with Crisco vegetable shorting. Strippable eggs were removed from each female. Stripping of the eggs stopped once the eggs no longer appeared visually viable. After stripping if blood was present in the pan, eggs were rinsed carefully with a saline solution until no blood was present. The eggs were then weighed and divided into the desired amount for ploidy manipulation by pressure treatments. Fifty eggs from each female were counted and weighed to assess the average size of an egg from a given female. Characteristics of the eggs were noted in to assess quality. Any female that died in this procedure also had her eggs examined. These female's egg characteristics were incorporated into the analysis only if they had recently died. An incision was made into all females and all the eggs were removed to attain a total weight. The eggs were then classified as being under ripe, ripe, or over-ripe.

Fertilization

Fertilization was accomplisher by adding 5.0 ml of the previously collected sperm solution to every 200 g of eggs. Eggs were fertilized utilizing sperm from multiple males. The sperm and egg mixture was gradually mixed for the next 1 to 2 minutes and then set aside. Five minutes after fertilization occurred eggs were given the prescribed pressure treatment. Once treatment was administered, eggs were returned into the pan. The pans were then placed in a water hardening bath for at least 15 minutes.

DATA ANALYSIS

Male Data

The statistical analysis program SAS version 9.1 was used to analyze the data. A means procedure was used to determine the average, maximum, minimum, range, and standard deviation for traits noted. Regression analysis model was developed to assess any relationship that may exist between the grams of testes and kilo gram body weight.

Female Data

A means procedure was run on the data from the females to determine the average, minimum, maximum, range, and standard deviation. Regression analysis was utilized to assess any relationship that may exist between grams of eggs given, fecundity, average egg weight, and egg count with female body weight. A series of t-tests on the means for grams of eggs produced and latency to determine differences between treatments, impact of using an implant alone and the combination of injection and implant.

RESULTS

Male Traits

The average weight of the male flathead catfish was 3.27 +/- 0.83 (SD) kilo grams. (Table 1) The maximum body weight was 5.04 kilo grams and minimum was 2.11 kg. Mean grams of testes was 4.11 +/-1.2 (SD) with a range of 2.69 to 6.87g. Mean grams of testes per kg was 1.24 +/- 0.08 (SD) with a range of 1.12 to 1.36. Grams of testes increased with body weight in a linear fashion ($P<0.0001$), g of testes= -0.5385 + 1.4203 kg/BW, $r^2 = 0.95$ (Fig. 1). The coefficient of variation, 8.3, for g testes/kg male body weight was relatively small, indicating uniformity in testicular size.

Female Traits

The weight of the females ranged from 2.10 to 8.56 kg with an average of 4.55 kg. (Table 2) Egg characteristics were highly variable (Table 2), however, this was a reflection of the variable size of the females that were utilized. The range of the grams of eggs for individual females ranged from 149.8 to 833.3 grams with a mean of 350.0 g.

Table 1. Sample number, mean, range, standard deviation (Std Dev), and coefficient of variation (CV) for grams of testes, body weight (BW) of males kilograms, and grams of testes per kilo gram male body weight (g testes/kg BW) for flathead catfish, *Pylodictis olivaris,* in the Satilla River, Georgia

Variable	N	Mean	Range	Std Dev	C V
g Testes	11	4.1	2.7- 6.9	1.2	29.4
BW (kg)	11	3.3	2.1- 5.0	0.8	25.4
g Testes/kg BW	11	1.2	1.1- 1.4	0.1	8.3

Figure 1. Relationship of grams (g) of testes and male body weight (Kg BW) for flathead catfish, *Pylodictis olivaris,* in the Satilla River, Georgia

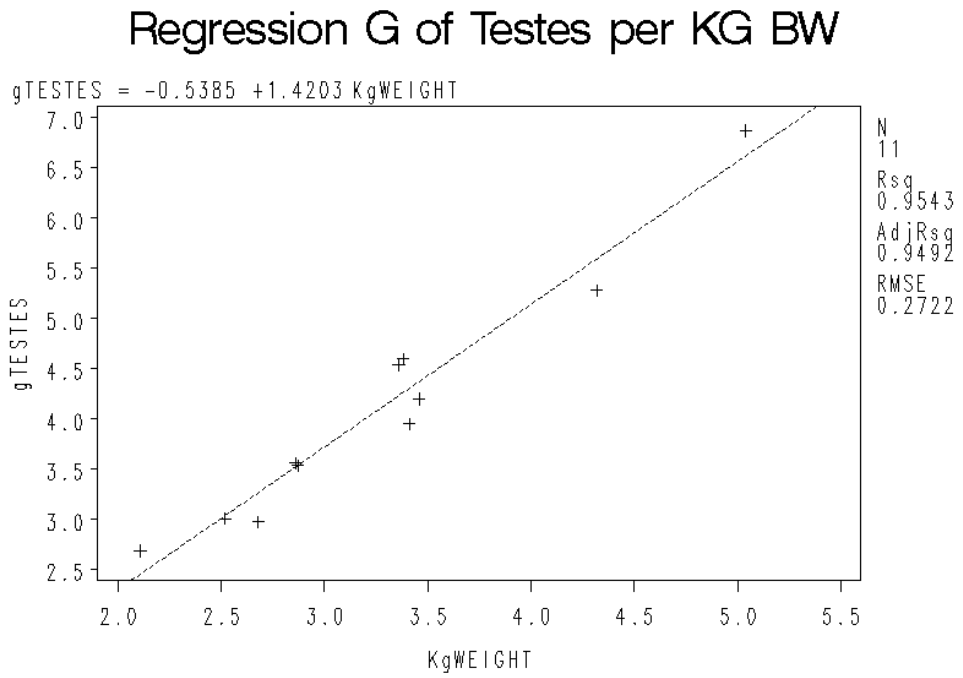


Table 2. Sample number, mean, range, standard deviation (SD), and coefficient of variation (CV) for body weight (BW), latency (hr), total grams of eggs, egg weight (g), eggs count for females, and fecundity (eggs/kg BW) for flathead catfish, *Pylodictis olivaris,* in the Satilla River, Georgia

Variable	N	Mean	Range	Std Dev	C V
BW	28	4.6	2.1-8.6	2.0	43.8
Latency	28	70.5	39.3-113.0	24.9	35.3
g of Eggs	28	350	150-833	200	57.2
Egg Weight	28	0.03	0.02-0.05	0.008	26.5
Egg Count	28	10,828	6,809-17,628	3,659	33.8
Fecundity	28	2,555	1,548-3,989	630	24.7

The egg count per female yields a maximum of 17,628 and a minimum of 6,809 eggs with the mean being 10,826 eggs (Table 2). As expected, larger females produced greater total weight of eggs (g of eggs = -77.69 + 93.913 kg BW, $r^2 = 0.88$, Fig. 2). Larger females also produced larger sized eggs as the range of egg size was 2.5- fold from 0.02-0.05g, and female body weight ranged 4-fold, 2.1-8.6 kg (Table 2, Fig. 3).

A strong linear relationship existed between egg weight and female body weight, egg weight = 0.0131 + 0.038 kgBW, $r^2 = 0.89$. ($P < 0.0001$) (Fig. 3). The observed linear relationship between egg number and body weight was, number of eggs = 3,815.8 +1540.1 kgBW, $r^2 = 0.71$ (Fig. 4). Relative fecundity decreased with size, fecundity was moderately variable with a coefficient of variation of 24.7 fecundity=3,511.1-209.89 female BW, $r^2 = 0.44$ (Fig 5).

Of the 28 females that were induced to ovulate, ten produced eggs that were over ripe at stripping time. Of these ten females, eight had received a combination of injection and implant. With sixteen females receiving this combination, only half produced viable eggs that could be used for the pressurization. Of the twelve females that received only an implant only two of them were overripe. This treatment resulted in 83.4% of the females having viable eggs that were not overripe.

Figure 2. Relationship between grams (g) of eggs and body weight (KG BW) for flathead catfish, *Pylodictis olivaris,* in the Satilla River, Georgia

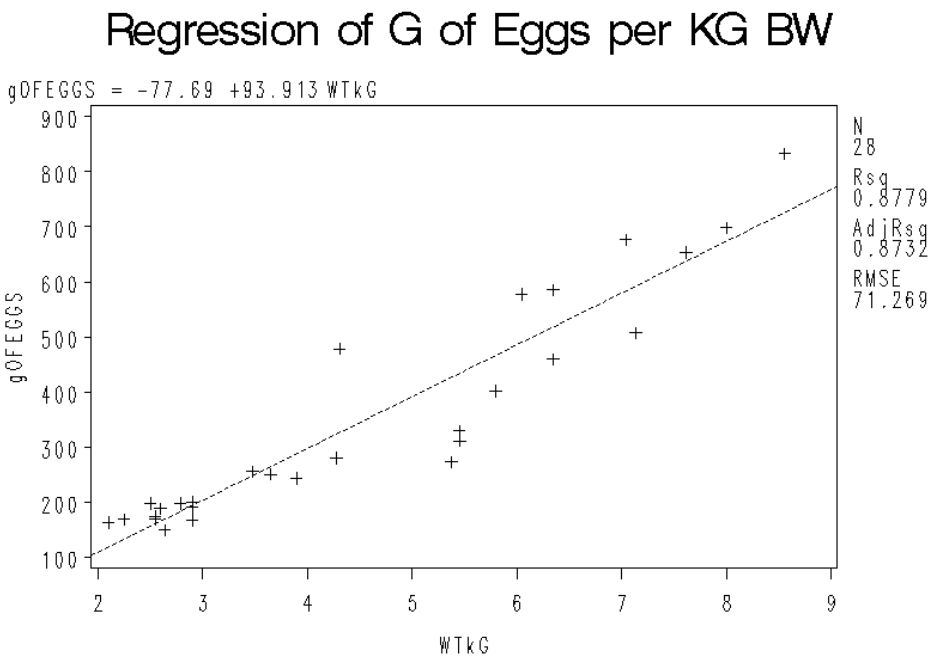


Figure 3. Relationship between egg weight in grams (g) and body weight (KG BW) for flathead catfish, *Pylodictis olivaris,* in the Satilla River, Georgia

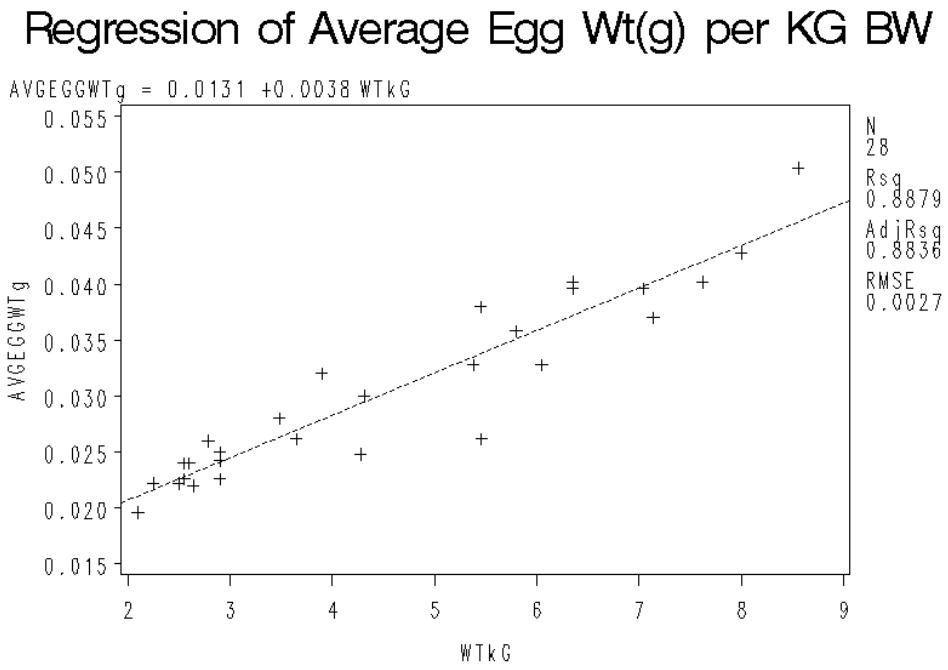


Figure 4. Relationship between egg count and body weight (KG BW) for flathead catfish, *Pylodictis olivaris,* in the Satilla River, Georgia

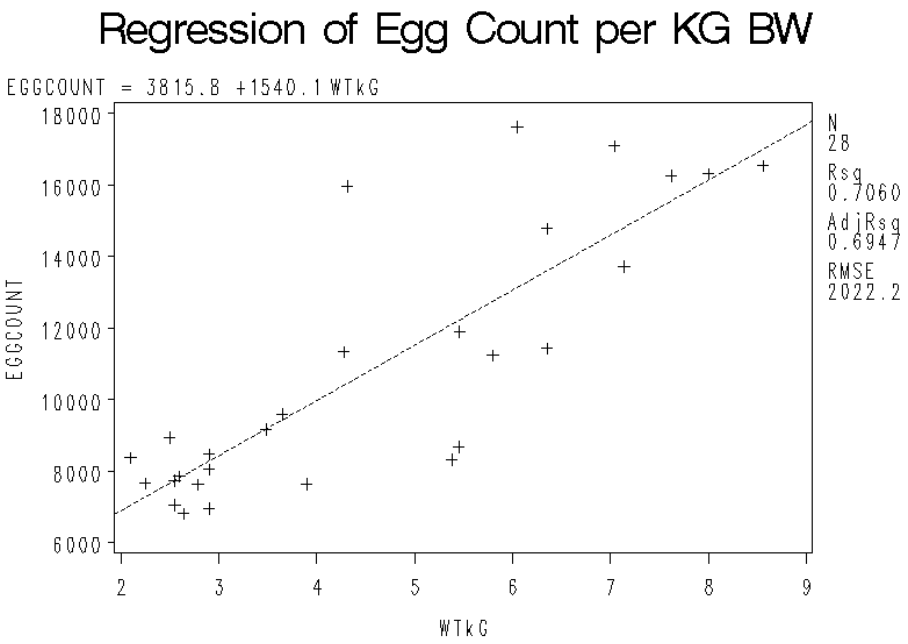
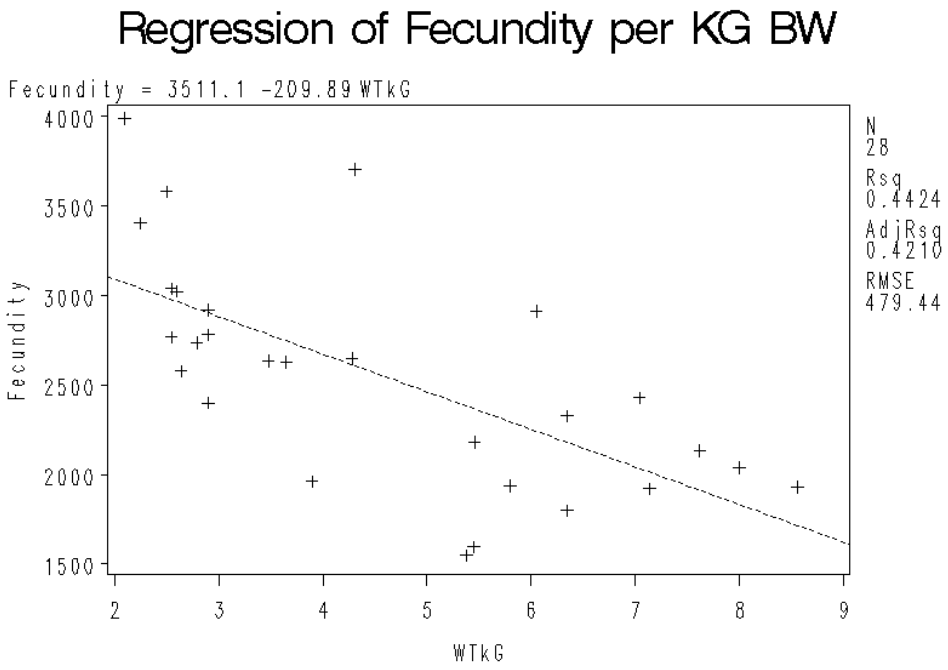


Figure 5. Relationship between fecundity and body weight (KG BW) for flathead catfish, *Pylodictis olivaris,* in the Satilla River, Georgia

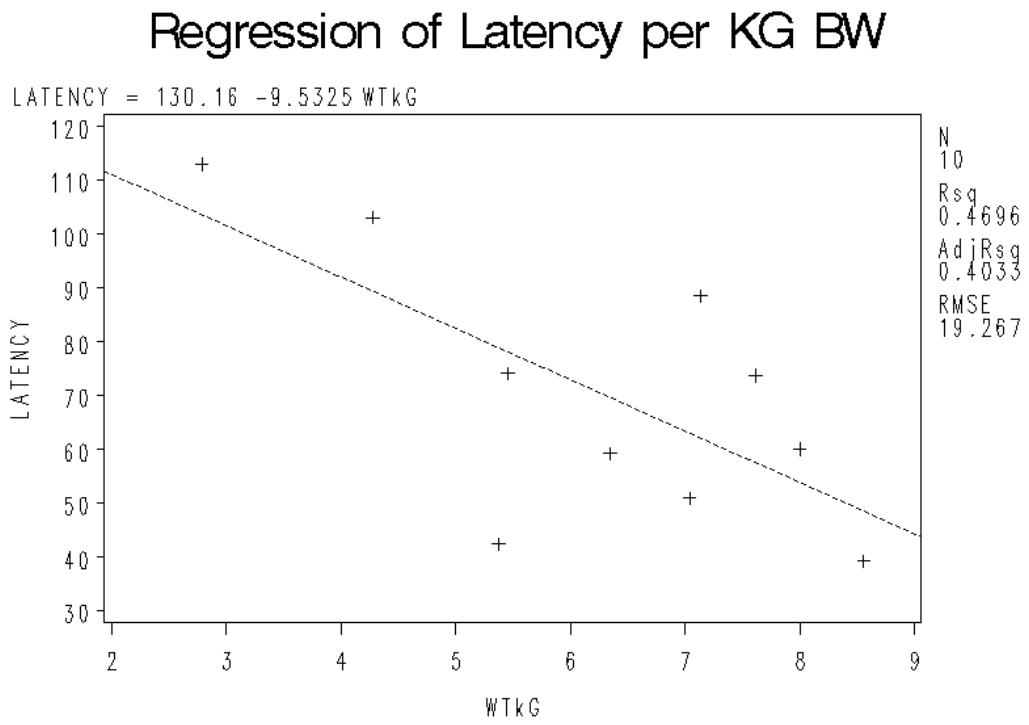


Mean latency for the implant plus injection was 63.3 hours, which was different (P=0.03) from the injection alone 81.3 hours (Table 3). The observed linear relationship between latency and body weight was, latency = 130.1 – 9.5 kgBW, r^2 =0.47 (Fig 6). The grams of eggs produced by the treatment implant plus injection 396.7 was not different P=0.15 than from implant alone, 287.6 g. The mean fry per kg for the implant alone was 1068.2. The value for fry per kg for the treatment implant and injection was 462.5.

Table 3. Sample number, percent ovulation, percent over ripe eggs, latency, grams of eggs, and fecundity (g of eggs/kg female BW) for flathead catfish, *Pylodictis olivaris,* in the Satilla River, Georgia either implanted with 100ug/kg BW with luteinizing hormone releasing hormone (LHRHa) implants(implant) or 100ug/kg BW LHRHa implants and combined with injection of 2 mg/kg common carp pituitary extract (implant&injection)

Variable	N	% Ovulation	% Over Ripe Eggs	Latency	g of Eggs	Fecundity
Implant	12	33.3	16.7	81.3 ± 18.9 (59.3-103.1)	287.6	2,537.3 ± 509.3 (1,922.2-3,80.2)
Injection & Implant	16	37.5	50.0	63.3 ± 27.4 (39.3-113.0)	396.7	2,568.9 ± 723.9 (1,47.6-3,989.2)

Figure 6. Relationship among latency and female body weight (KG BW) for flathead catfish, *Pylodictis olivaris,* in the Satilla River, Georgia



DISCUSSION

Males had uniform testicular size and reproductive characteristics across a range of fish sizes. Egg characteristics were highly variable; however, this was a reflection of the variable size of females that were utilized. Females had a large difference in egg size as compared to channel catfish, *Ictalurus punctatus.* A determination of the extent of maternal effects on various early life traits would be interesting. Number of eggs produced increased with the size of female. Relative fecundity decreased with female body weight, so if culture or hatchery space is limited the use of smaller females may increase fry output. Latency was significantly different between the two treatments with injection alone having a mean value of 18 more hours. Eight of the females that received a combination of injection and implant produced eggs that were overripe. Weight of eggs produced did not differ between the two treatments. Though many of the parameters assessed did not differ between treatments, the impact of using the wrong procedure for ovulation was that four times the number of females produced overripe eggs that were unusable for production. The most effective treatment for artificially producing flathead catfish fry was the use of the 100ug/kg female BW implant. Future research should examine a variety of doses of the implant to further increase fry production.

COMPARISON OF PADDLE WHEEL TROUGH AND McDONALD JAR INCUBATION FOR ARTIFICALLY PRODUCED DIPLOID AND TRIPLOID FLATHEAD CATFISH EMBRYOS

INTRODUCTION

Research on the growth and development of triploid and diploid flathead catfish *Pylodictis olivaris,* fry has been stimulated by the interest in the possible eradication of nuisance exotic populations. One approach is using triploidy to sterilize individuals. The reproductive fitness of treated males is theoretically not altered and mating behaviors seem to proceed normally under controlled conditions. However triploidized individuals do not produce viable gametes, therefore, preventing development of offspring from sexually active triploid male and diploid female pairs. No research has examined the effects that a pressure shock treatment has upon the development of the flathead catfish fry. The treatments to induce triploidy can result in poor hatch rates.

Fertilized channel catfish eggs as well as those of other ictalurids are traditionally incubated in paddle troughs. However, under some research and commercial conditions, channel catfish eggs have been successfully incubated in McDonald jars. Fresh ictalurid eggs are extremely adhesive and form a congealed mass. However application of a pressure treatment to induce polyploidy, the eggs looses some of the binding ability.

This results in a mass that is not easily maintained within the trough like the untreated eggs. Eggs break apart from the mass and settle at the bottom of the hatching trough thus decreasing their chance of survival. Incubation within a typical hatching jar suspends the eggs within the water column, which may circumvent the problem of free eggs which have been pressure treated.

This methodology consistently and gently rolls the eggs preventing them from settling to the bottom of the jar. This may also be beneficial in the control of fungus.

No data is available to determine the preferred method to incubate the triploidized flathead catfish eggs. The primary objective was to compare diploid (untreated) and pressure treated egg masses incubated in hatching jars and paddle wheel troughs for hatching rates. The ultimate goal is to make the production of triploid flathead catfish fry more efficient.

MATERIALS AND METHODS

Experimental Design

Eggs and sperm were obtained from the female and male adult flathead catfish to produce triploid fry. Eggs were stripped and fertilized with the sperm five minutes prior to the pressure treatment. Sperm was a mixture from several males to fertilize each batch of eggs to eliminate confounding factors such as poor sperm production or quality from an individual male. Fresh water was immediately added following the sperm being mixed with the eggs to activate sperm and accomplish fertilization.

Five minutes after the sperm and water was added, the egg mass was placed into a cylindrical tube that could be manually pressurized to a desired PSI. Hydrostatic pressure was exerted upon the eggs to prevent the extrusion of the polar body from the egg. This pressure chamber holds approximately 100 grams of fertilized flathead catfish eggs.

Several different treatments were administered to each batch of eggs produced from a single female. Treatments were in pressure intervals of 500 PSI and included zero, 5500, 6000, 6500, 7000, and 7500 psi. The sample egg masses were pressurized to the desired treatment and continually maintained at that pressure for duration of five minutes.

Incubation and Hatching

Upon completion of the pressure treatment the egg masses were emptied from the pressure chamber into pie pans and placed into a water hardening trough for twenty minutes. Once the masses were hardened they were removed from the pie pans and placed into either a hatching trough or a hatching jar to incubate.

The hatching trough consisted of a standard trough with a set of hanging mesh baskets placed directly below the surface of the water.

Each trough was equipped with a paddle wheel system to gently agitate the egg mass. This mimics the male behavior of fanning the eggs during incubation. The trough had a constant flow of water, averaging 12 L/min, from a reservoir pond ensuring that metabolites would not become an issue during the incubation. Compressed air was diffused into each of the hatching troughs.

Formalin was administered at 100 ppm to the eggs in the hatching troughs to prevent fungal infestations. Treatments began 24 hours after fertilization. It was then administered three times a day until just before hatching began. Dead eggs and fungal matter were removed from the egg masses on a daily basis. Eggs were allowed to incubate and upon hatching sac fry were able to fall through the basket mesh to the bottom of the trough.

Notes were taken as to when eyes became apparent and when the majority of the fry hatched. At this time, the paddle wheels were removed from the trough. Total number of fry that hatched was obtained for each hatching trough.

Hatching jar methodology was compared to the paddle wheel trough incubation. Each jar was set up with water that continuously flushed thru from a reservoir which averaged 4 L/min.

Water flow rates were maintained to minimal levels allowing gentle movement of the eggs inside each jar. Supplemented air was not used in this design. No anti-fungal treatment, such as formalin, was administered to the hatching jars. Dead eggs and unwanted debris were removed from the hatching jar daily. Viable eggs remained in the jar until hatching.

Upon this time the sac fry were removed and placed into a trough that did not have paddle wheels. Again the days to eyes appearing and days to hatch were recorded. The total number of fry hatched was then documented for each hatching jar.

Individual eggs were monitored during this period to estimate the time to development of eyes and emergence from the egg. Development time of the eye was defined as the time for over 75% of the individuals to form a fully developed, black eye spot. Hatching time was defined as the time for over 75% of the individuals in a treatment to free themselves from the egg chorion.

DATA ANALYSIS

A means procedure was performed upon the data on the incubation techniques providing mean, maximum, minimum, standard deviation, and range for the traits measured. A t-test was also preformed to determine differences between means for the jar and trough treatments for the traits fry hatched, hours to hatch, and hours to eye.

RESULTS

The embryos in the hatching jar had an average hour to eye of 136.0 hours. (Table 4) The average hours to hatch was 165.2 hours. The average hours to eye for eggs in the hatching trough was 139.3 hours, and was not different (P=0.42) from the mean for the jars. The average hours to hatch was 161.9 hours for the egg masses hatched with this technique, and was not different (P=0.10) from the mean for the jars.

The average number of fry hatched in the jars was 22 with the trough being slightly lower with an observed value of 13 individuals, which was not different (P=0.14) from the mean for the jars. This 76.9% difference was not significantly different (P>0.05), because of the high amount of variation. The average percent hatch for eggs in paddle wheel troughs was 12.4 and for individuals in the hatching jar was 22.1 was significantly different (P<0.05).

Table 4. Sample number, hours to eye, hours to hatch, percent hatch, and number hatched for flathead catfish fry triploid and diploid, *Pylodictis olivaris* when incubated in a hatching jar or a paddle wheel hatching trough

Variable	N	Hr to Eye	Hr to Hatch	% Hatch	N Hatched
Paddle Wheel Trough	**33**	139.3 ± 9.5 (120.0-168.0)	161.9 ± 8.7 (144.0-172.0)	12.4	13.5 ± 13.0 (0.0-50.0)
Hatching Jar	**6**	136.0 ± 6.2 (132.0-144.0)	165.2 ± 3.4 (160.0-168.0)	22.1	22.3 ± 10.0 (6.0-45.0)

DISCUSSION

Freshly fertilized eggs were extremely adhesive. However, upon the application of the pressure treatment the eggs lost some of their binding ability. In troughs, this resulted in eggs breaking free from the mass and settling to the bottom of the trough decreasing their chance of survival. The hatching jar method suspended the eggs within the water column. The observed mean for numbers of individuals hatching in MacDonald jars was higher, 23, than for eggs hatched in standard hatching troughs, 13. This 76.9% difference was not significantly different ($P>0.05$), because of the high amount of variation. Hatching percentage was also almost double for the eggs in the MacDonald jars compared to the eggs in the paddle wheel troughs. The significant difference between the percent hatch for these techniques could extrapolate into larger a larger output of triploid flathead catfish fry.

This comparison should be repeated. If the 76.9% increase is repeatable, hatchery, output of triploid flathead catfish would be significantly increased. Not only did the jars prevent the eggs from lying motionless upon the base of the jar, it also inhibited the outbreak of fungal activity. The eggs in jars were not treated with formalin like the eggs in troughs. Although, no fungal growth was detected in the jars, a combination of jar hatching and formalin treatment could be beneficial as potential adverse effects of bacteria may also decrease hatch rate and should be evaluated in the future.

THE EFFECTS OF PRESSURE TREATMENTS ON TRIPLOID INDUCTION, GROWTH AND SURVIVAL OF FLATHEAD CATFISH FRY

INTRODUCTION

During the past century many populations of flathead catfish, *Pylodictis olivaris,* have been introduced into many Southeastern ecosystems. Illegal introductions of flathead catfish in the Satilla River during the 1990s and in the Altamaha River system, Georgia during the late 1970's has greatly impacted an existing fishery causing great concern. The native red breast sunfish has seen a drastic population decline since the introduction of this predatory species as well as other species. Additionally, species diversity has been impacted as abundance of native fauna has decreased as biomass of flathead catfish and other exotic species has increased.

Introduction of sterile triploid flathead catfish males into the effected ecosystem could result in reduced numbers of the nuisance, exotic, diploid conspecifics. Theoretically, triploid males would be capable of courtship and mating behaviors, stimulating diploid females to mate and release eggs, but would be unable to successfully fertilize the females' gametes, resulting in disrupted spawning and reduced population numbers.

Hydrostatic pressure is the preferred method for producing triploid channel catfish, (Rezk 1988), and would likely be a successful technique to induce triploidy in flathead catfish. However, no information is available for triploid induction in flathead catfish or on the development and early life history of triploids of this species.

The first objective was to evaluate various pressure treatments to maximize effectiveness of triploid induction and hatch of triploid flathead catfish embryos. The second objective was to determine differences in development and growth of diploid and triploid fry.

MATERIALS AND METHODS

Triploid induction

Eggs and sperm were obtained from the female and male adult flathead catfish to produce triploid fry. Eggs were fertilized with the sperm five minutes prior to the pressure treatment. Sperm was utilized from several males to fertilize each batch of eggs. Fresh water was immediately added following the sperm being mixed with the eggs to activate sperm and accomplish fertilization. Five minutes after the sperm and water was added the egg mass was placed into a cylindrical tube that could be manually pressurized to a desired pressure. This pressure chamber held approximately 100 grams of fertilized flathead catfish eggs. Pressure treatments compared were 0; 5,500; 6,000; 6,500; 7,000 and 7,500 psi. Eggs from a single female were given multiple treatments. Treatment duration was five minutes.

Incubation and Hatching

Upon completion of the pressure treatment the egg masses were emptied from the pressure chamber into pie pans and placed into a water hardening trough for twenty minutes. Once the masses were allowed to harden, they were removed and placed into a hatching trough or a hatching jar to incubate. Time to development of the eyed stage and time of majority of the fry hatching was recorded. Total number of fry hatched was determined.

Culture of fry and fingerlings

The swim up fry were first fed a mixture of dry fry feed and freeze dried krill. When the fry responded to the supplemental feed they were divided into smaller groups.

Each group was placed into an aquaria determined by the treatment given and the ploidy level of the fry. Once in the aquaria, the fish were fed to saturation three times daily. Live mosquito fish were placed into each aquarium to supplement the dry feed and krill. Structures in the form of PVC pipe segments were placed in the bottom of each aquarium to deter aggression and cannibalism within the aquaria. These aquaria had an initial stocking density of 30 fry per aquaria. Once the fry began to show signs of aggression towards each other they were split into smaller groupings of 5 to 10 fish.

Ploidy determination

Once the fry reached an appropriate size to measure ploidy level, putative triploids and a sample of diploids were taken to assess their ploidy level. This size was estimated to be approximately 2 to 3 inches of total length. A small sample of blood was drawn from each fish at the base of their caudal fin. Fish of this size were appropriate for this collection of three to four droplets of blood.

The blood sample was then analyzed using a Coulter counter to determine blood cell size. The steps of usage for this technique include usage of a lytic reagent to lyse red blood cells from the sample taken. The cells were then measured by DC impedance. This is a measurement constructed in a non-focused flow aperture. Nucleated red blood cells are then differentiated from additional cell types that may be present in the sample. Measurements of the size of the each fish's blood cells allowed determination of the ploidy level. The increased chromosome number present in the cells of triploid individuals results in a size difference among diploid controls.

Growth and survival

Fry were sampled and weighed monthly from July 5th to September 3rd. As the fry grew, they were divided into several other aquaria to allow more space to alleviate cannibalism.

After ploidy determination, the fry were grouped into two batches: triploid or diploid. Triploids were all combined and placed into three large troughs with structure on the basin. The diploid fry were also grouped and divided into three troughs again with accompanying PVC structure. The fingerlings were fed dry feed three times daily and supplemented continually with live fish. Weights were taken for duration of 28 weeks. The juvenile catfish were in the larger troughs, approximately 450 L, from October 1, 2007 until April 16, 2008. Once fingerlings were large enough for determination of their sex they were divided into tanks that were same sex and same ploidy level.
Male diploids, female diploids, male triploids, and female diploids were segregated into four large troughs with flow thru water (450 L). At the end of the period, fingerlings were weighed a final time and were stocked into a grow-out pond to allow for maturation.

DATA ANALYSIS

A means procedure was performed upon the data for the treatments applied to the egg masses to determine means, maximums, minimums, and standard deviations. A regression for the traits assessed was also performed to determine the relationship between the various traits and the pressure treatments.

This also produced an r^2 value that allowed for determination of any correlation that may exist. Differences among means were evaluated with a t-test.

RESULTS

The average hours to eye for the diploid eggs was 136.4 with an average of 157.5 hours to hatch (Table 5). The average percent hatch for diploid individuals was 21.5 with the individuals that were pressure treated having an average of 10.9. The average hours to eye on the pressure treatment of 5,500 PSI was 144.0. These individuals took 168.0 hours to hatch. Less than ten percent of the 5,500 PSI eggs hatched and none survived to the first weight assessment. The 6,000 PSI treatment had an average of 140.0 hours to eye and 165.3 hours to hatch. Just over twelve percent of the fry hatched and 81.8% of the fry tested positive for triploidy (Table 5). The pressure treatment of 6,500 psi had value of 138.0 hours until eyes were present and 164.0 hours to hatch. The hatch rate was 16.7% and 100% of the individuals were positive for triploidy. (Table 5) The pressure treatment of 7,000 psi resulted in embryos that took 137.1 hours to eye and 162.9 hours to hatch. Less than six percent of the eggs treated at this pressure hatched, and only two survived to the final sampling. The 7,500 psi treatment resulted in embryos that took 144.0 hours to eye and 168.0 hours to hatch. Approximately, 3% of the eggs hatched with only one individual surviving to 3 months of age.

Table 5. Sample number, hours to eyed stage, hours to hatch, hatching rate, embryo's testes for triploidy, positive number of triploid embryos, and percentage triploid for the pressure treatment for flathead catfish, *Pylodictis olivaris,* and embryos treated with 0-7,500 psi of hydrostatic pressure 5 minutes after fertilization

Pressure	N	Hr to Eye	Hr to Hatch	% Hatch	Embryo Tested	3N Embryo	% 3N
0	11	136.4	157.5	21.5	-	-	-
5,500	2	144.0	168.0	3.5	0	-	-
6,000	9	140.0	165.4	12.2	11	9	81.8
6,500	6	138.0	164.0	16.7	36	36	100.0
7,000	7	137.1	162.9	5.3	2	2	100.0
7,500	4	144.0	168.0	3.3	1	1	100.0

The regression of hours to eye on the pressure treatments resulted in the following equation, hours to eye=136.46+0.0005 pressure treatment ($r^2 = 0.027$, Fig 7). The variation of hours to eye within the pressure treatments can be seen in figure 8. The regression of hours to hatch by pressure treatment was hours to hatch=157.65+0.0011pressure treatment ($r^2 = 0.166$, Fig 9). Figure 10 shows the variation in hours to hatch by the different treatments administered. The relationship of fry hatch by pressure treatment=28.606-0.0029 pressure treatment ($r^2 = 0.444$ Fig 11).

Figure 7. Relationship among average hours to eye by pressure treatment for flathead catfish fry, *Pylodictis olivaris*

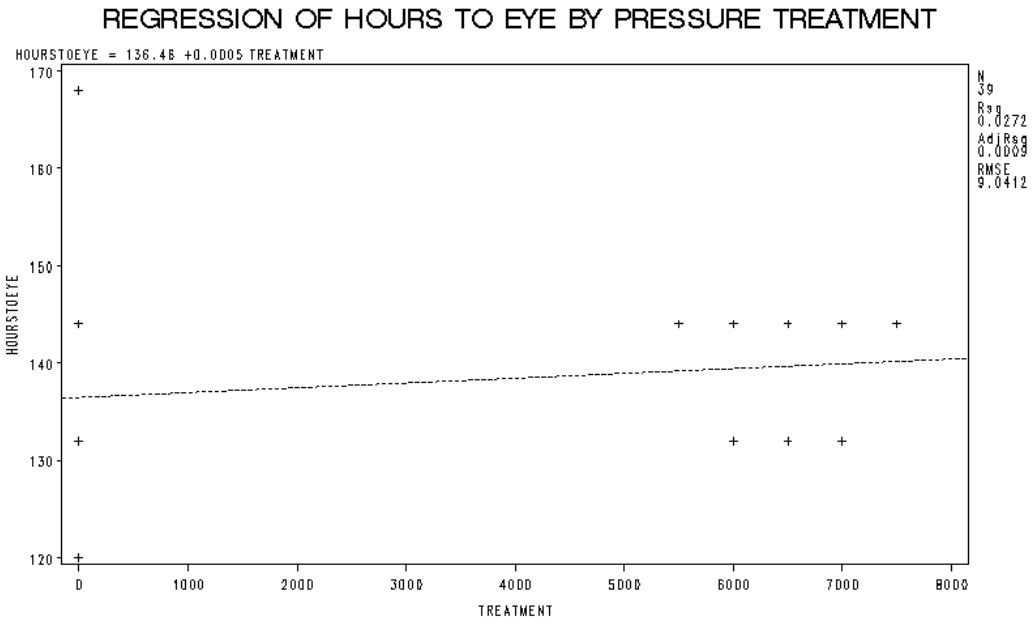
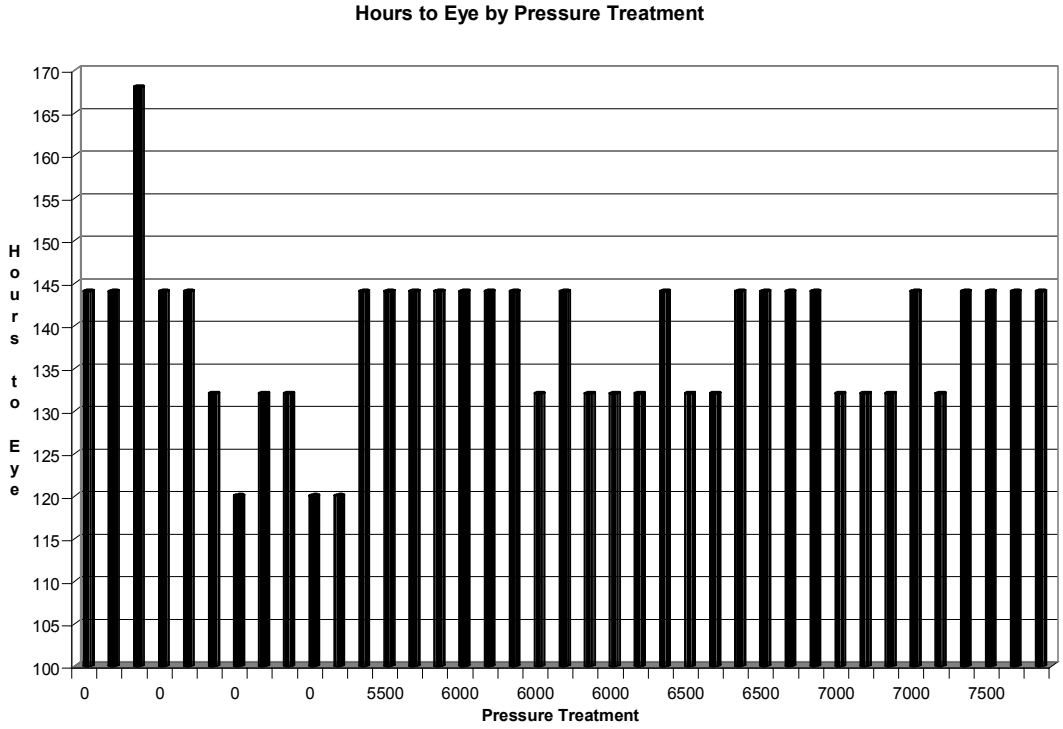


Figure 8. Hours to eye by pressure treatment for flathead catfish fry, *Pylodictis olivaris*


Hours to Eye by Pressure Treatment
Hours to Eye
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100
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0
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0
5500
6000
6000
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7000
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Pressure Treatment

Figure 9. Relationship among average hours to hatch by pressure treatment for flathead catfish fry, *Pylodictis olivaris*

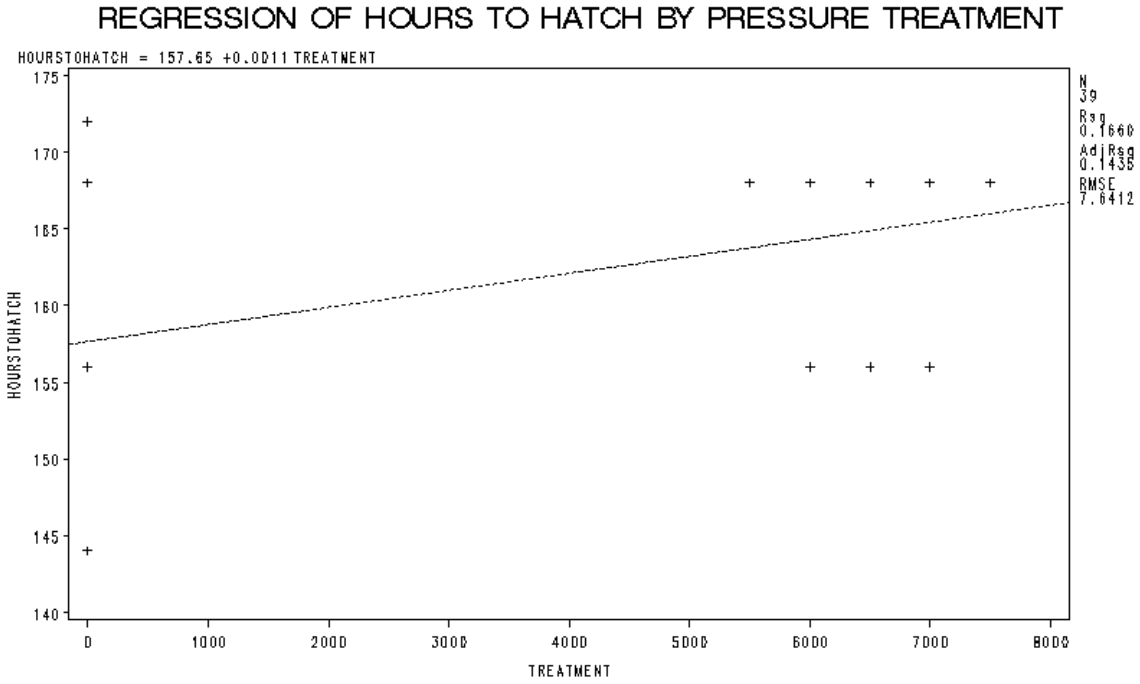


Figure 10. Hours to hatch by pressure treatment for flathead catfish fry, *Pylodictis olivaris*

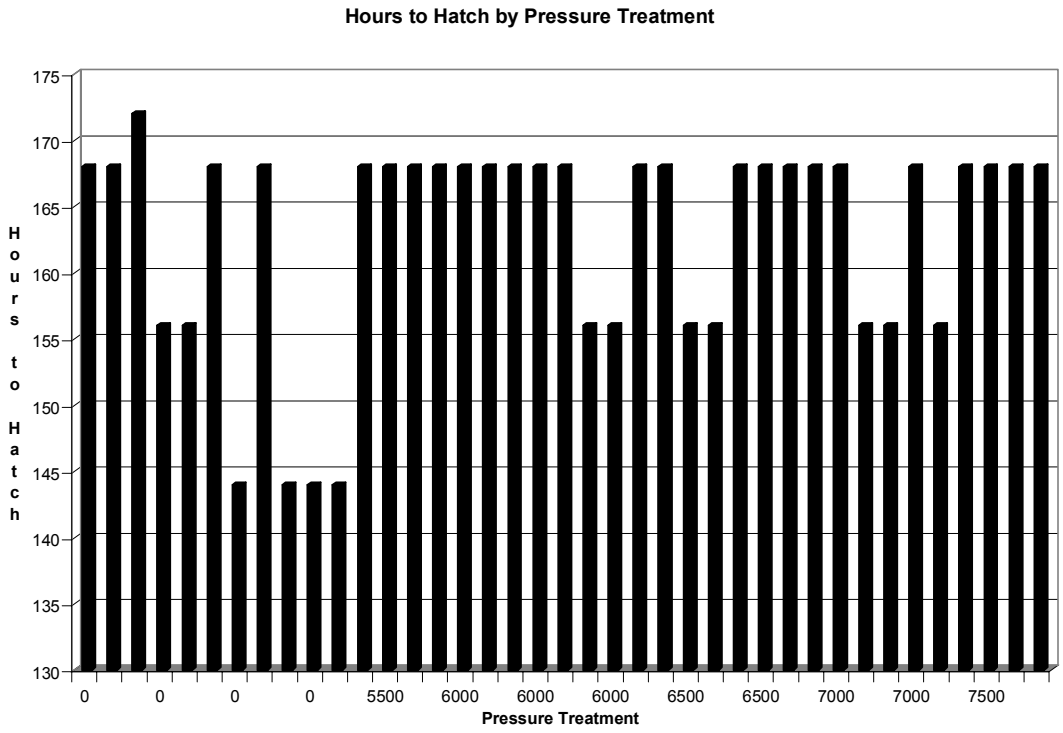


Figure 11. Relationship among fry hatched by pressure treatment for flathead catfish fry, *Pylodictis olivaris*

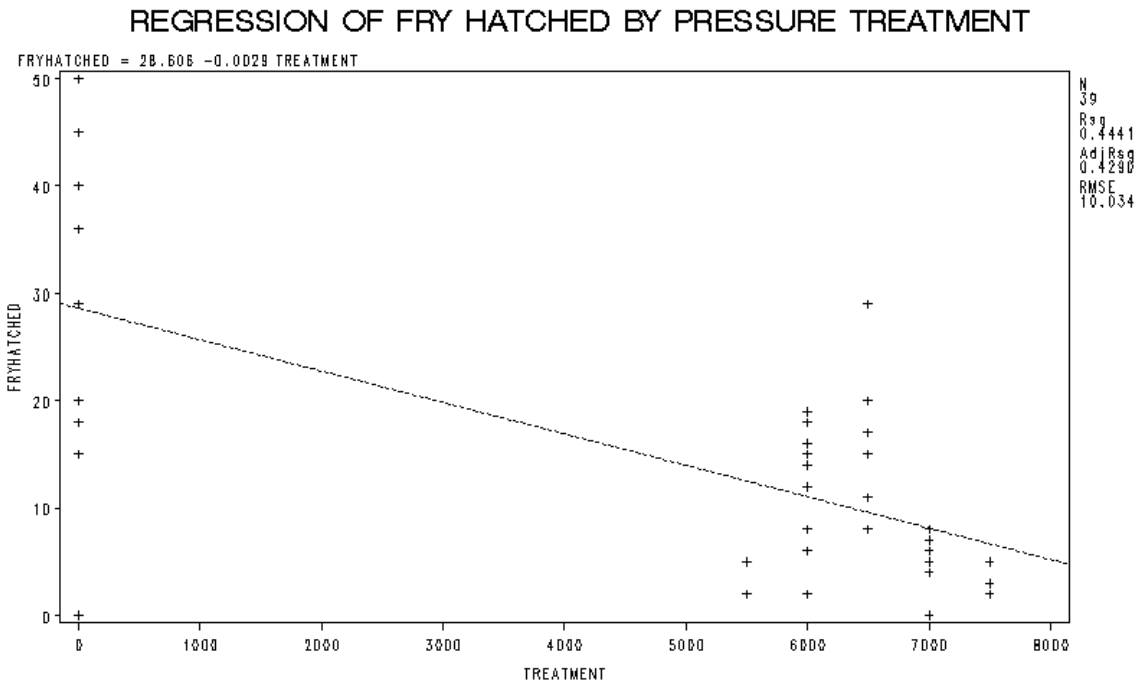


Figure 12 shows the relationship of percent hatch by pressure treatment. The equation derived from this model is as followed; percent hatch=0.2202 - 174 E^{-7} treatment. Initially diploids were stocked into aquaria with an average weight of 0.27 g. The initial stocking weight of the triploids into the aquaria was 0.30 g. At the end of the eight week period the diploids had increased in size to an average of 13.5 g. The triploid individuals growth was not as drastic with their average when moved from the aquaria was 8.5 grams. However the differences observed in the growth rates was not different (P= 0.75) at this time. The maximum body weight for untreated diploid individuals was 18.2 grams while n the aquaria. The triploid treatment had a maximum total body weight of 16.4 grams. Total growth for triploid individuals was 16.4g and not different (P=0.30) from diploid individuals weighing 18.2g.

Individuals pressurized with 6,000 psi had an average total weight gain 9.8 g while in the aquaria and a maximum of 15.5 g. The pressure treatment of 6,500 psi had an average total weight gain of 8.05 g and a maximum over the two month period of 16.4 g. The pressure treatment of 7,000 psi had an average total growth of 10.1 g and a maximum of 10.1g. The pressure treatment of 7,500 only had one fish that survived having a weight of 12.1g.

Figure 12. Relationship among percent hatch by pressure treatment for flathead catfish fry, *Pylodictis olivaris*

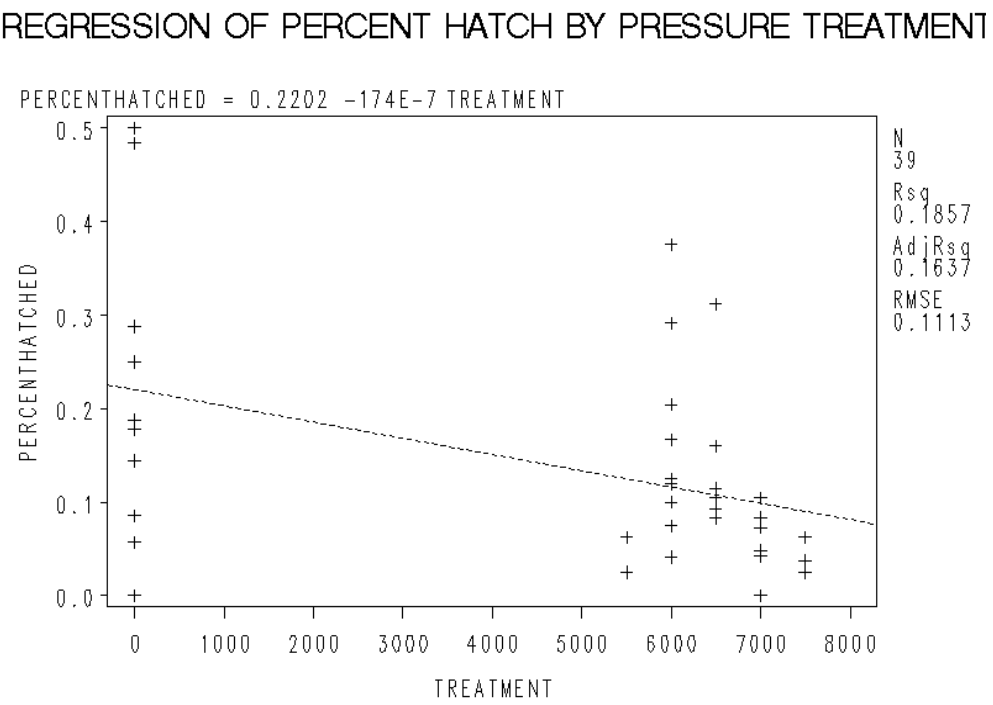


Figure 13. Average (Avg) Growth (wt in g) triploid flathead catfish, Pylodictis olivaris, fingerlings in tanks fed a combination of forage and supplemental feed

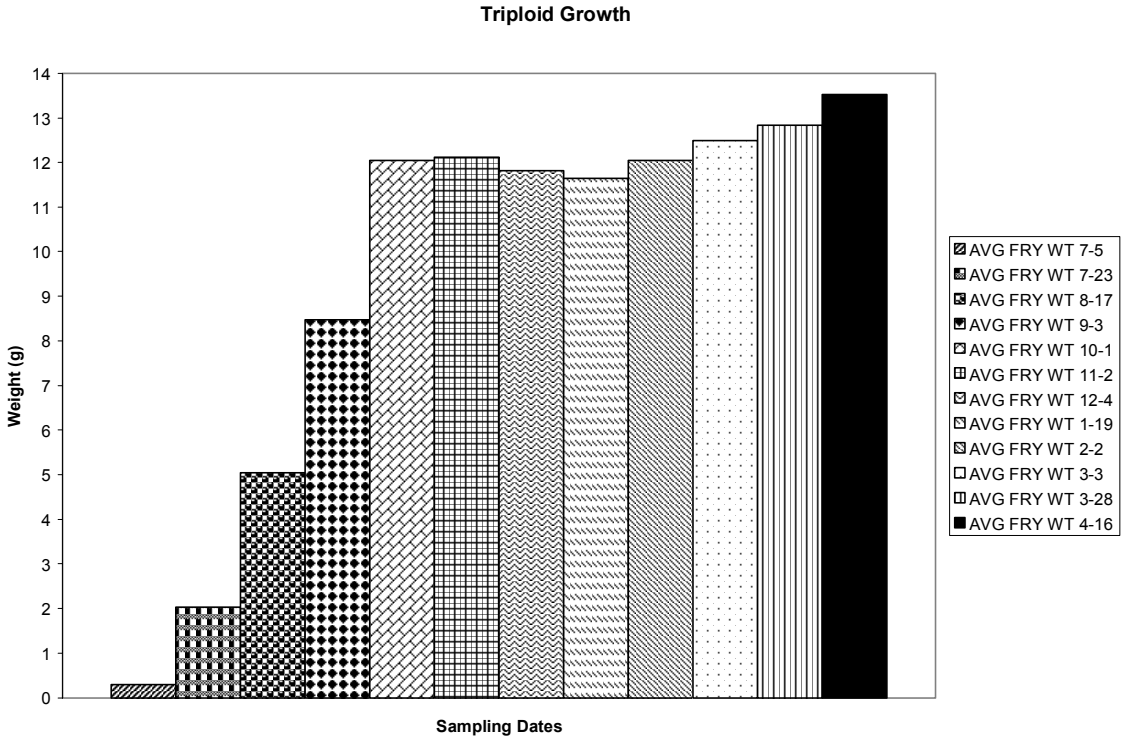
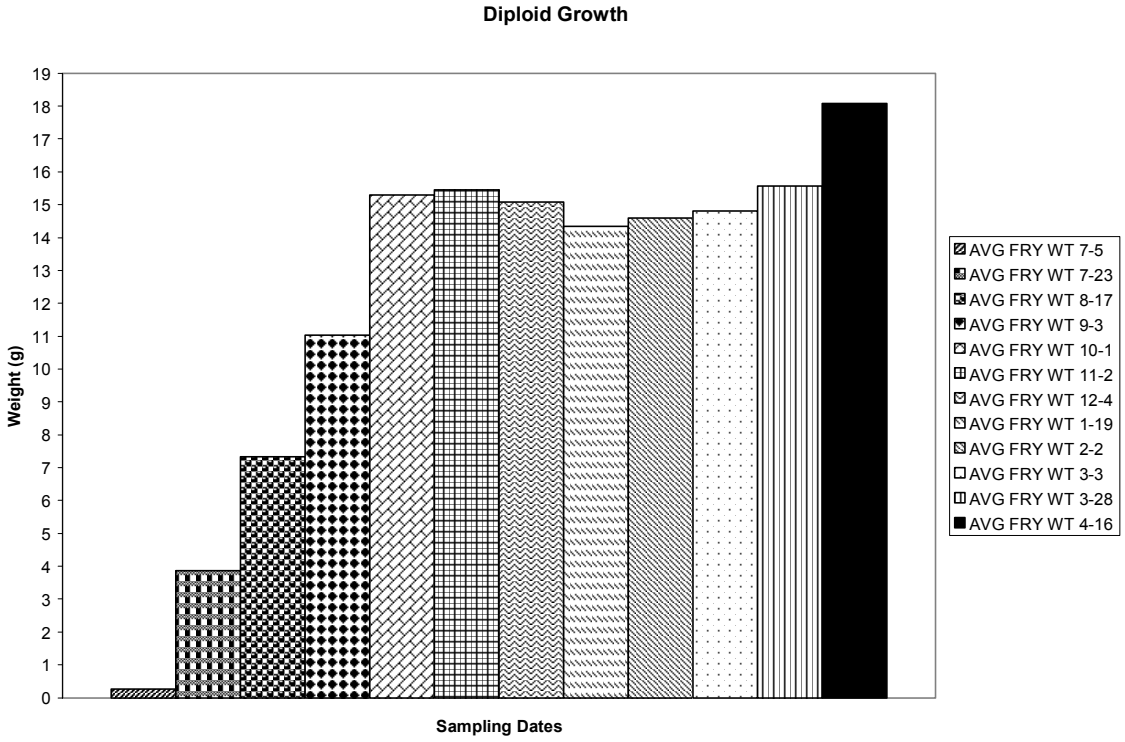


Figure 14. Average (Avg) Growth (wt in g) of diploid flathead catfish, Pylodictis olivaris, fingerlings in tanks fed a combination of forage and supplemental feed



From early October 2007 to mid April 2008 the juvenile catfish were maintained in 450 L troughs. The initial average weight at the time of the first sampling in these troughs for the diploids was 15.3 g. The triploid individuals were slightly smaller having a mean of 12.0 g. At the end of this period the average weight of a diploid fingerling was 18.1 g with a maximum total weight of 28.5 g. The average total weight triploid individual was 13.5 g and the largest having a weight of 33.7 g.

The body weights of 2N and 3N were not different (P= 0.48). Cumulative fry survival in aquaria demonstrated by figure 16 shows survival was increased in triploid individuals with a value of 30.3% compared to the diploid value of 11.7%. The cumulative fry survival for the period while in troughs showed similar results (Fig 17) with diploid individuals having a value of 23.3% and diploid being 6.9% at the final sampling date of April 16th 2008.

Figure 15. Cumulative survival of diploid and triploid flathead catfish, Pylodictis olivaris, fingerlings in aquaria 7-5-7 to 9-3-7tanks fed a combination of forage and supplemental feed.

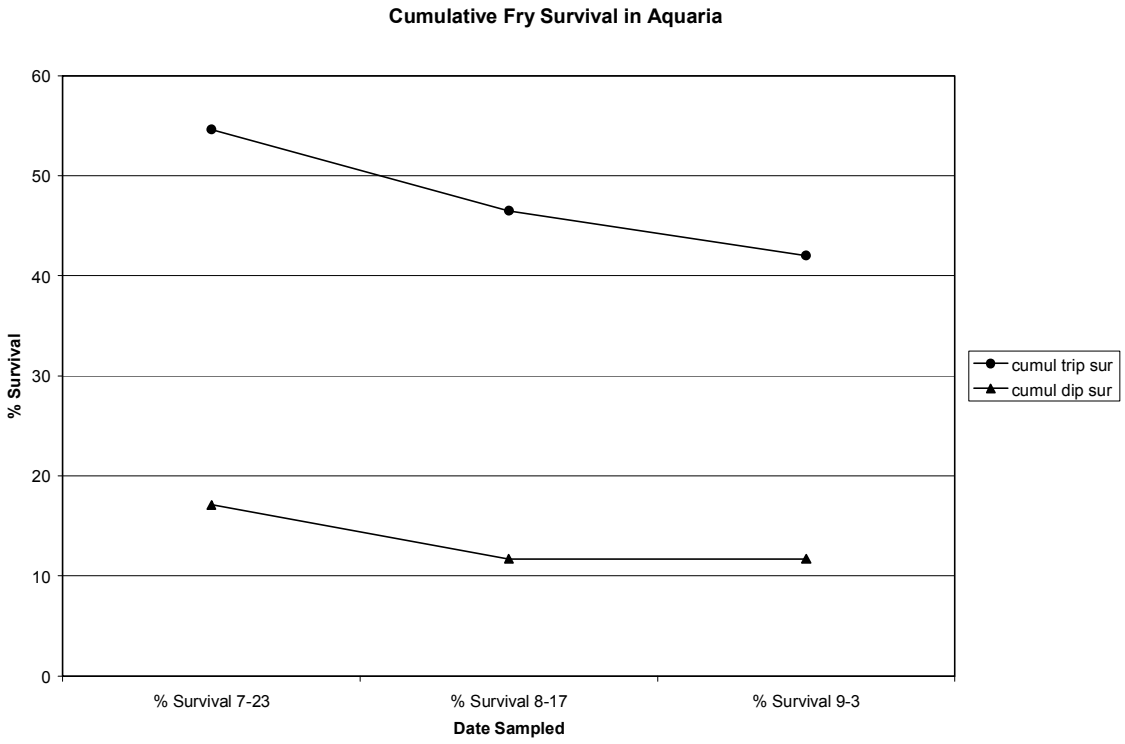
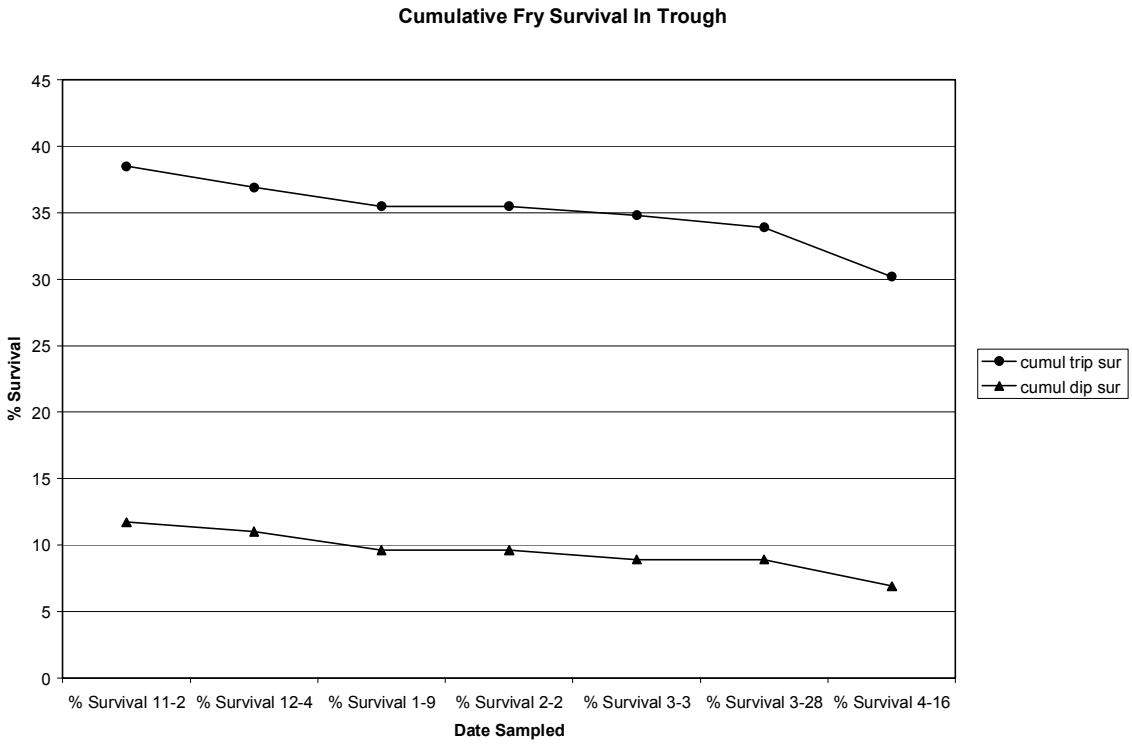


Figure16. Cumulative survival of diploid and triploid flathead catfish, Pylodictis olivaris, fingerlings in troughs 10-1-7 to 4-16-8 fed a combination of forage and supplemental feed.



Over the 28 week period mean total weight gain for diploid individuals was 18.7 g. The maximum weight gain seen in the diploid controls was 28.5 g. The average weight gain for a triploid individual was 13.5 g. The maximum weight a triploid individual gained over this period was 33.7 grams. No differences in body weight was observed (P= 0.72). The observed change in body weight during this early fall to spring period was higher 17.6% for diploids than for triploids, 11.5%.

DISCUSSION

Assuming no temperature effects, developmental rates for pressure shocked putative triploid embryos was slightly slower. This is consistent with the fact that larger cells have altered metabolic rates because of the lower surface area to volume ratio (Rezk 1988). Control eggs had the highest observed hatch rate, 28.5%. Hatch rate for treated eggs increased with pressure, peaking at 6,500 psi, which was fortunately the optimum pressure for inducing triploidy, and then declining with increased pressure. All treatments with surviving fingerlings, 6,000; 6,500; 7,000 and 7,500 psi produced a high percentage of triploids with only 6,000 psi (81.8% triploids) producing less than 100% triploid fingerlings. Hydrostatic pressure is a very efficient mechanism for producing triploid flathead catfish.

Early growth rate of diploid and triploid flathead catfish was similar up to 3.5 g when the fry were approximately three to four months of age. Weight measurements taken from that point forward, diploids grew faster than triploids with the diploids being 24.2% larger by the time the fish reached 10-12 g. From October 1st 2007 to mid April 2008, fish were in tanks with cover and habitat to minimize cannibalism.

The flathead catfish juveniles were offered minnows continuously and three times daily they were fed supplemental feed in the form of krill and pellets. The observed change in body weight during this early fall to spring period was higher (18.3%) for diploids than for triploids, (12.5%). Diploids were growing faster, but this may have been confounded by lower survival, although densities in the tanks were very low. Triploids were more variable in size. Some fish did not grow over the winter.

However, three triploid individuals increased total weight 50% over the winter accounting for almost all of the growth and contributing to the variability in size of the triploids. Without these 3 individuals, growth of diploids would have been even more dramatic compared to triploids.

It appears that the triploids are less aggressive and less cannibalistic. This was determined by the lower mortality observed in triploid tanks and the absence of injury from fighting. Triploid flathead catfish survival, 86.7+/- 9.4%, was higher than that of diploids, 59.0 +/- 7.0 during this early fall to spring period. When no carcasses were found the mortality was attributed solely to cannibalism. Diploids were eliminating themselves over time. At the conclusion of the experiment, there were 2.5 times more triploids left than diploids.

Survival of treated eggs increased with pressure, peaking at 6,500 psi, which was fortunately the optimum pressure for inducing triploidy, then declining with increased pressure .Triploid flathead catfish were effectively produced, 100% triploidy, utilizing 6,500 psi of hydrostatic pressure 5 minutes after fertilization.

These fish should be grown to sexual maturity and their reproductive behavior studied. If triploid flathead catfish exhibit normal courtship and are able to induce diploid females to deposit eggs, a screwfly approach to controlling flathead catfish reproduction can be tested.

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