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PRELIMINARY EVALUATION OF
TRIPLOID CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA)
IN THE GREAT LAKES

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PRELIMINARY EVALUATION OF TRIPLOID CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA) IN THE GREAT LAKES

By

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ABSTRACT

PRELIMINARY EVALUATION OF TRIPLOID CHINOOK SALMON STOCKED IN THE GREAT LAKES

By

William P. Young

Approximately 880,000 triploid chinook salmon (Onchorynchus tshawytscha) were stocked into the Great Lakes from 1985 through 1990 in an attempt to produce a trophy salmon fishery. Triploidy was induced with a heat shock of 28.5 C. for 10 minutes at 10 minutes after fertilization. Female chinook salmon have not returned to the monitored release sites at Little Manistee River or Swan Creek. Six mature male triploids have returned to these sites. Triploid males were the same size and similar in appearance diploid fish, but no milt produced. GSI values of triploid males were significantly lower than that of diploids male chinook. Returns of triploid males was significantly lower than returns of diploid male chinook salmon from the same year class. A small population of triploid chinook salmon were held in a pond at the Wolf Lake Hatchery until maturity. Triploid female chinook were not significantly different in size, showed no secondary sexual characteristics, produced no mature oocytes and their GSI was significantly lower than diploid females. Unfortunately, no male triploid chinook remained in the population due to a hatchery stocking error.

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INTRODUCTION

In 1985, Michigan State University, in cooperation with the Michigan Department of Natural Resources, initiated a program to create a trophy salmon fishery in the Great Lakes. A successful trophy salmon fishery would produce a few salmon each year that are larger than the current largest salmon in the Great Lakes. This program involved producing triploid chinook salmon (Oncorhynchus tshawytscha) for the Great Lakes stocking program. Triploid fish contain three sets of chromosomes, instead of the normal two. It was hoped that these fish would not return to the river, spawn and die, but would remain in the lake and grow to trophy size. Since 1986, approximately 800,000 triploid chinook salmon have been stocked into the Great Lakes (Jim Copland, MDNR per. comm.). The purpose of this thesis was to evaluate the success of the triploid chinook salmon project the Great Lakes.

The overall goal of this project was to produce a trophy chinook salmon fishery in the great Lakes. Although much work has been done concerning growth and development of triploid anadromous salmonids, little information is available concerning the migration tendencies of triploid

fish. This project was based on the hypothesis that the reduced gonadal development observed in triploid salmonids (Lincoln and Scott, 1984) will prevent migration and postspawning mortality of chinook salmon. The longer life span of the triploid fish should increase their ultimate size. The Touly River strain of chinook salmon stocked in the Great Lakes matures at three or four years of age. It is unknown how long triploid salmon may live, but since they have indeterminant growth they should continue growing their entire lives (Beverton and Holt, 1957).

Triploid chinook salmon have three sets of chromosomes compared to the normal two sets found in diploids. All triploid fishes appear to be sterile (Thorgaard and Allen, 1986). Sterile fish are useful for fisheries management and fish culture. They can be used to prevent overpopulation and stunting or to protect endemic stocks from genetic introgression in a put and take fishery. Triploids may be useful in preventing maturation of salmonids and extend the life span of Pacific salmon (Ihssen et al. 1990). Growth of triploids compared to diploids varies widely between species (Ihssen et al., 1990) Development of gonads in triploids varies between male and female of a species. Females gonadal development is much more reduced than males. Males often show secondary sexual characteristics associated with maturity and even produce watery milt (Thorgaard and Gall, 1979; Benfy et al.,1986).

Triploidy is induced by shocking the egg which disrupts meiosis and prevents extrusion of the second polar body. The diploid ovum is then fertilized by the haploid sperm to form a triploid organism. A temperature, pressure or chemical shock given at the correct time of development will induce triploidy. For the triploid salmon project, the method chosen was heat shock. A heated water bath was easy to control and 90-100% effective at inducing triploidy in large batches of eggs (Westerof, 1988). Chemical induction often produces mosaics and pressure shock is limited by the number of eggs which can be treated in the pressure chamber (Thorgaard, 1986).

This thesis is divided into two sections, evaluation of growth and maturation of triploid chinook salmon and a review of the Michigan DNR production runs of 1989 and 1990. First, I will detail the research done to determine the success of induced triploidy in the prevention of migration, spawning and death of chinook salmon. This involves fish from two sources. One group of 500 triploid and 500 diploid chinook were held in captivity at Wolf Lake Fish Hatchery until maturation. These fish were sampled to compare growth and development of triploid chinook salmon relative to diploids. The second group of fish was the triploid chinook salmon stocked into the Great Lakes. Fish from the Great Lakes were sampled from angler creel returns from both Lake Michigan and Lake Huron. Triploids that returned at

maturation were also sampled at the Little Manistee River weir and Swan Creek for growth and development. Percent returns were calculated to determine migration tendencies of triploid chinook salmon. Second, I will report on the continuation of the MDNR production runs which annually produces between 100,000 and 200,000 triploid chinook salmon for stocking into the Great Lakes.

LITERATURE REVIEW

Identification of Triploid Fishes

Identification of sterile triploid fish is very important for most management applications to prevent fertile diploids from entering the population. In some instances, such as stocking of exotics, 100% accuracy is needed. For aquaculture purposes pure triploid populations may not be necessary, although a very high percentage of triploids is more valuable at harvest time. There are many methods available to determine ploidy, each with advantages and disadvantages and varying degrees of accuracy.

Triploid fish usually cannot be distinguished by appearance alone. Triploid progeny from a mating of female scattered scaled pattern carp (Cyprinus carpio) and male normal carp could be distinguished from the hybrid by their intermediate scale pattern (Gervai et al., 1980). Bonar, et al (1988) measured morphometric characteristics of triploid and diploid grass carp (Ctenopharyngodon idella). This procedure was not recommended for ploidy determination because it was only 60-85% effective in distinguishing triploid from diploid fish.

Silver staining cells in order to count nucleoli was somewhat effective in identifying triploid coho salmon (Oncorhynchus kisutch), chinook salmon and rainbow trout (Oncorhynchus mykiss) (Phillips et al., 1986). Triploid

fish cells contained one, two or three nucleoli while diploid fish never contained more than two nuceoli. Many cells from each individual must be scored. Draw backs to this method were that it was ineffective for fish species which contain more than one nucleolar organizer region (i.e. brook trout Savelinus fontinalus, lake trout Salvelinus namaycush) and it was time consuming and labor intensive.

A very reliable, but time consuming method to determine ploidy, was karyotyping. Karyotypes can be easily obtained from solid tissues (Kligermen and Bloom, 1977; Blaxall, 1983), embryos (Myers, 1986) and cultured lymphocytes (Wolters et al., 1981) and are a very accurate measurement of ploidy. The major drawback to this method was that it was labor intensive (Utter, et al, 1983).

The cells of triploids are larger than cells of diploids. Two methods have been used that identify triploids by their larger cell size: Direct measurement under a microscope and a coulter counter and channelizer.

A microscope has been used to directly measure the diameter of the erythrocyte nucleus in order to identify triploid fish (Swarp, 1959; Cimino, 1973; Wolters et al., 1982; Johnstone and Lincoln, 1986). This technique may lead to misclassification of some fish since the range of measurements often overlap. This method may misclassify triploid and diploid mosaics. Mosaics reported in some early studies (Refstie, et al 1977; Allen and Stanley 1979;

Lemoine and Smith, 1980) may actually have been an artifact of this identification method (Utter, et al, 1983; Benfey et al., 1984). Wolters et al. (1984) compared the accuracy of a flow cytometer to erythrocyte nucleus measurement and determined erythrocyte measurement would misclassify approximately 8% of the fish tested.

A more accurate method of ploidy determination is measurement of erythrocyte volume with a coulter counter and channelizer. The increased accuracy results from measurement of thousands of cells from each fish compared to only a few cells per fish using direct measurement. Some fish are still misclassified which is probably due to the fragility of the cellular shape and volume (Johnson et al., 1984). Measurement of erythrocyte nuclear volume with a coulter counter and channelizer has remedied this problem and has proven to be 100% effective (Wattendorf, 1986).

The most accurate and rapid method to identify triploids uses a flow cytometer (Thorgaard, et al, 1982; Allen, 1983). A flow cytometer measures the amount of DNA in a cell relative to a known control. Triploid cells contain 50% more DNA than diploid cells. Sample preparation is quick and easy. Erythrocyte samples are usually used, due to the ease at blood collection, but sperm (Benfey et al., 1986) and crushed fry (A. Westmaas, per. comm.) can also be used. The major drawback to flow cytometry is the

cost of equipment, however flow cytometers are becoming more common in research laboratories.

Gynogenesis

All maternal inheritance is termed gynogenesis. It is induced by fertilizing the egg with inactivated sperm followed by the doubling of the maternal genome. Sperm is inactivated with gamma or ultraviolet radiation and contributes no genetic material to the zygote (Purdom, 1969). Doubling of the maternal genome is accomplished by shocking the egg in the same method used in polyploidy induction. This prevents extrusion of the second polar body or suppression of the first cleavage, depending on the time of development when the shock is applied. Retention of the second polar body results in some heterozygosity at distant loci due to crossing over. Nearly total homozygosity results from a suppression of the first cleavage (Purdom, 1983; Streisinger et al., 1981).

There are two major applications for gynogenesis. Production of inbred lines which can be used for scientific studies or fish culture. Second, since the female is the homogametic sex in most fish species, all female progeny can be obtained and used for fish culture and fisheries management (Purdom, 1983; Chourrout, 1982).

Androgenesis

All male inheritance is called androgenesis. This is induced by irradiating the eggs with gamma or ultraviolet radiation, fertilizing these eggs with normal sperm, then suppressing first cleavage to return diploidy (Purdom, 1969; Parsons and Thorgaard, 1984). Two applications for androgenic fish are scientific study where inbred line are needed and recovery of fish lines from cryopreserved sperm (Thorgaard, 1986).

Natural Polyploids

Polyploidy is a fairly common occurrence among plants (Strickberger, 1985). Polyploidy is not very common in vertebrates. In fish, spontaneous triploidy has been observed in rainbow trout (Thorgaard and Gall, 1979; Cellular, 1972), brook trout (Allen and Stanley, 1978) and roach (Hesperoleucus symmetricus) (Gold and Avise, 1976). Some species of the genus Poecilia are viable and fertile triploids (Schultz, 1967). Fish of these species reproduce by parthenogenesis, producing all female progeny. Sperm from a closely related diploid species stimulates the egg to develop. It appears the chromosome compliment doubles to hexaploid, then is reduced to triploid by meiosis. Only the maternal genome is passed on to the gametes (Schultz, 1968).

Triploid Hybrids

Hybrids of closely related species are often used in fisheries management. Hybrids often have better growth rates than either parent and can be sterile. Triploid hybrids had lower natality and grow better than diploid hybrids of the some fishes. (Scheerer and Thorgaard, 1983; Chevassus et al., 1983). The presence of two maternal chromosome sets in triploid hybrids enhances developmental stability compared to diploid hybrids (Scheerer and Thorgaard, 1987). Triploid hybrids have three advantages for fish culture and fisheries management: First, hybrids often show increased growth and induced triploidy increases the viability of hybrid progeny; Second, triploidy and hybridization together offer two forms of sterilization for added security against reproduction. Sterile hybrids are often stocked in a system to provide sport fishing while ensuring reproductive isolation and prevent introgression of genetic material into the native fish population; Finally it may be possible to mix the positive traits of two species into a hybrid (Utter et al, 1986).

Triploidy Induction

Triploidy induction is accomplished with a shock given to the egg during meiosis II that causes retention of the second polar body (Purdom, 1983). When the haploid sperm fertilizes the diploid egg a triploid organism is produced.

The shock can be temperature, pressure or chemical. Timing of the shock is very critical and varies between species (Ihssen et al, 1990).

Faulkhauser and Griffiths (1939) induced triploidy in the amphibian Triturus viridescens using cold shocks and laid the basis for later triploidy induction in fish. Swarp(1959) induced triploidy in the sticklback with a cold shock and completed the first comprehensive study of growth and development of triploid fish. Triploid induction was not widely used for fish culture and management until the 1980's.

Although successful in plants, chemical shock has generally produced a mixture of diploid and triploid mosaics (Allen and Stanley, 1979; Smith and Lemoine, 1979; Refstie et al., 1977; Refstie, 1981), although this may have been an artifact of the ploidy determination method. The chemicals used were colchicine and cytochalasin B. Recently the anesthetic nitrous oxide (NO3) has been successfully used to induce triploidy in Atlantic salmon (Johnstone et al., 1989) and rainbow trout (Sheldon et al., 1986). Eggs exposed to nitrous oxide under pressure (optimal of 11 ATM) produced up to 100% triploidy in each species. Neither nitrous oxide or pressure used alone produced triploid fish.

Temperature shocks are the most widely used technique to induce triploidy. It is easy to do and it doesn't require special equipment. Generally, heat shock was more

effective for coldwater fish and cold shock more effective for warm water fish (Thorgaard and Allen, 1986). Triploid induction rates were positively correlated to mortality. For this reason, high triploid yields which don't result in 100% triploid induction but have high treatment survival may be more efficient at obtaining maximum numbers of triploids(Johnson et al., 1985).

Cold shock treatments resulted in production of diploid and triploid mosaics in Atlantic salmon (Lincoln, 1974) and brook trout (Lemoine, 1980). Cold shock has proven to be very effective at inducing triploidy in blue tilapia (Tilapia aurea)(Don and Avtalion, 1988), common carp (Gervai et al., 1980), catfish (Wolters et al, 1981) and bluegill sunfish (Lepomis micromis) (A. Westmaas, MSU per. comm.).

Heat shock has produced up to 100% triploidy in many fishes (for review see Table 1). Physiologically, heat depolymerizes the microtubules necessary for meiosis (Rieder and Bajer, 1979).

Hydrostatic pressure shock successfully produces triploids in many species of fish (Lou and Purdom, 1984; Chourrout, 1984; Benfey and Sutterlin, 1984a). Pressure induced triploidy results in better survival and induction rates. Pressures of 8,000-10,000 psi for short periods of time given at the proper developmental stage are used. Originally, a drawback of pressure shock was

Table 1. Examples of Induction of Triploidy by Heat Shock in Fishes

Species	Heat Shock Fert. (min)	Time After Fert. (min)	Duration (min)	Ploidy %	Surv. %	Primary Author and Year (1900)
sturgeon	34	1-60	3	52.3	---	Vasetski 67
tilapia	32-38	14	60	10	50	Valenti 75
trout	27-30	60	10	50	---	Chourrout 80
trout	37-38	10	1	50	10	Thorgaard 81
trout	26	25	20	90-100	63	Chourrout 82
trout	27-28	40	10-15	100	---	Lincoln 83
coho	24-30	10	10	85	47	Utter 83
pink	29	10	10	72	84	Utter 83
chinook	29	10	10	60	88	Utter 83
Atlantic	32	5	5-15	100	80	Benfey 84a
trout	28	35-40	10	90	50	Lou 84
trout	26-28	40	10	90-100	50-57	Solar 84
catfish	40	80-90	1	13	---	Bidwell 85
chinook	28.5	10	10	100	70	Hill 85
Atlantic	30	20	10-12	100	67	Johstone 85
grass carp	40	5	1	8	81	Cassani 85
trout	38	40	10	90	40	Bye 86
trout	29	10	10	91-96	48-79	Thorgaard 86
tilapia	39.5	3	3.5-4	60	60	Don 88
chinook	28.5	10	10	100	45	Westerhof 88
chinook	28.5	10	10	95	17	Spruell 89
crappie	36	5	5	10	30-60	Curtis 89

modified from Spruell (1989)

that only small volumes of eggs could be treated at a time in the pressure chamber(Thorgaard and Allen, 1987). A larger pressure shock unit which can treat up to one liter of eggs has been produced, but this is still too small for large batches of large eggs, like salmonid eggs. No reduction in survival or induction rate was seen compared to the smaller unit (Benfy et al, 1988).

Growth and Development

Polyploidy results in an increase in cell size and is the physiological reason behind many "giant" varieties of plants (Strickberger, 1985). In fish, the increased cell size is offset by a reduction in cell number (Purdom,1972; Fankhauser, 1945). Growth of triploid fish varies from species to species. Triploid channel catfish were heavier and had a better feed conversion than diploid catfish at eight months of age (Wolters et al., 1982). Similarly, seven month old triploid fancy carp (Taniguchi et al., 1986), fourteen week old triploid european catfish (Krasznai and Marian, 1986) and fourteen week old triploid tilapia (Tilapia aurea) (Valenti, 1975) all exhibited increased growth compared to diploid fish.

No difference in growth was observed in diploid compared to triploids of carp (Gervai et al., 1980), chinook salmon (Spruell, 1989) or Atlantic salmon (Salmo salar) (Benfey and Sutterlin, 1984b). Johnson et al. (1986) found

no difference in growth or conversion factor in coho salmon from 18 to 30 months of age. In the same species, Utter et al. (1986) found a significant increase in growth of diploid coho salmon compared to triploid salmon through 17 months of age. Diploid grass carp grew faster than triploid when they were reared together, but there was no difference in growth for diploid and triploids reared separately (Cassani and Caton, 1986). Growth of 3.5 year old female triploid rainbow trout was significantly higher than that of diploid rainbow trout. Rainbow trout grow very little after sexual maturity and spawning occurs, but triploids did not exhibit this reduction in growth (Thorgaard, 1986).

Reduced gonadal development was observed in triploids of all species of fish tested (Ihssen et al. 1990). Ovarian development was much more reduced than testicular development. Gonadal development was measured by gonadal somatic index (GSI). Triploid male GSI was usually reduced 50% compared to diploid males. Triploid female ovaries are less than 10% the size of diploid ovaries. Gonadal development appears to be normal until gametogenesis begins (Benfey and Sutterlin, 1984b). The proliferation of primary spermatocytes in the testes before gametogenesis begins was probably the reason triploid testes obtain a larger size at maturity compared to triploid ovaries.

Significant reduction in GSI was observed in triploids compared to diploids in channel catfish Ictalurus punctatus

(Wolters et al., 1982), fancy carp Cyprinus carpio (Taniguchi et al., 1986), rainbow trout (Thorgaard and Gall, 1979; Solar et al., 1984), common carp (Gervai et al., 1980), Atlantic salmon (Benfey and Sutterlin, 1984b) and coho salmon (Johnson et al., 1986). Secondary sex characteristics were often observed in triploid males, making them less valuable for aquaculture (Thorgaard and Gall, 1979; Lincoln and Scott, 1984; Lincoln, 1981a). Secondary sex characteristics were not observed in triploid coho salmon (Johnson et al., 1986) or channel catfish (Wolters et al., 1982) and the sex of the triploids could not be determined from external appearance, which was possible in diploids.

Triploid males have occasionally produced thin watery milt (Lincoln and Scott, 1984; Benfey et al 1986). Flow cytometrey determined the sperm of triploid Atlantic salmon were aneuploid (Benfey et al., 1986). Levels of testosterone were similar in diploid and triploid rainbow trout. Male triploids may exhibit spawning behavior even though they are functionally sterile (Lincoln and Scott, 1984). Allen et al. (1986) induced triploid grass carp to spermiation with hormones to determine the chance a euploid sperm could develop from a triploid fish. Flow cytometry revealed the sperm to be 1.5n, 3n, and 6n. The hexaploid cells were considered to be premeiotic unreduced spermatagonia. The third chromosome set should segregate

randomly during meiosis and this was indicated by the high variance in DNA content found in the 1.5N cells. It was determined that the probability of a euploid sperm being produced was 4×10^{-11} for every meiotic reduction.

Females appear to be physiologically sterile. GSI value of triploid females has usually been less than 10 % that of diploids. Production of hormones associated with maturation and spawning were extremely reduced in rainbow trout (Lincoln and Scott, 1984). Some maturing oocytes have been seen in ovaries, but these are not in advanced stages of development. Most of the ovary consisted of undifferentiated connective tissue (Lincoln and Scott, 1984; Benfey and Sutterlin, 1984b).

CHAPTER I. EVALUATION OF GROWTH AND MATURATION OF TRIPLOID CHINOOK SALMON

MATERIALS AND METHODS

Captive Triploid Chinook Salmon Population

A sample of 500 triploid and 500 diploid chinook salmon from the first production run (1985) were held in a spring fed pond at Wolf Lake Fish Hatchery, Mattawan, MI. These fish were initially sampled annually to determine growth and maturation relative to diploid chinook salmon from the same captive population. The first two years the fish were reared under intensive hatchery conditions in linear tanks. In 1986, the fish were moved to outdoor rearing ponds due to space limitations at the hatchery. Fish primarily relied on endogenous feed items during the pond rearing cycle since little feed was given to the fish. In 1988 the pond was inadvertently harvested and the fish were stocked as part of a put-and-take fishery. The remaining fish were not sampled until 1989 when they were four years old.

The pond was harvested on 1 Nov. 1989. This is near the end of the time when diploid chinook salmon from the Great Lakes stocks normally spawn. All fish were squeezed to induce expulsion of eggs or milt. Blood was taken to determine ploidy by flow cytometry (see Appendix A). The fish were sampled for length and weight, and any secondary sexual characteristics were recorded. The gonads were

removed, weighed and preserved in formalin for later histological analysis. Condition factor (Piper et al., 1982) and gonadal-somatic index (GSI) (Benfey and Sutterlin, 1984) were calculated.

K=(fish weight/length3) x 1000

GSI=(gonad weight/fish weight) x 100

Diploids were compared to triploids for length, weight and condition factor. Triploids were compared to immature diploid females for length, weight, condition factor and GSI. Triploids were compared to mature diploids for GSI. Means of diploid and triploid chinook were compared to identify significant differences for length, weight and condition factor using a students t test (Gill, 1978). GSI means were compared using a Kruskal-Wallis k-Sample Test (Steel and Torrie, 1980). This test is a nonparametric rank test and was used because of imprecise measurements of the gonad weights of the triploids. The triploid gonads all weighed less than one gram, but the scale used could only measure to the gram. This resulted in an upward bias of the GSI of the triploid fish.

Summer Creel Census

Before triploid chinook salmon were stocked they were microtagged and adipose fin clipped by the Michigan Department of Natural Resources (MDNR). The microtag is a very small wire tag, marked with a binary code indicating

ploidy, place and date of stocking and was inserted into the cartilage in the snout of the fish. All chinook salmon which are adipose fin clipped and have no other fin clips contain a microtag. Tag loss occurs in approximately 10% of the tagged fish, although it can vary greatly depending on the species of fish and person doing the tagging (Paul Gelderbloom, MDNR per. comm.). Because of possible tag loss, all adipose fin clipped chinook found were examined. Any abnormal looking gonads were checked histologically to determine ploidy.

During the summers of 1989 and 1990, anglers' catches were checked for adipose clipped fish by the MDNR creel census workers and by me. On weekends, catches at the Michigan port cities of Ludington and Manistee were surveyed. Charterboat captains were interviewed at fish cleaning stations and reminded to check chinook for adipose fin clips. In addition, five fishing tournaments were surveyed during the 1989 and 1990 fishing seasons:

Blue water tourn.	Port Huron, MI	1989
Blue Water Tourn.	Port Huron, MI	1990
Pro-King Tourn.	St. Joseph, MI	1990
Brown Trout Festival	Alpena, MI	1990
Rogers City Tourn.	Rogers City, MI	1990

All fish were checked for adipose fin clips at the tournament weigh-in. When an adipose fin clipped chinook

salmon was found, the length, weight and area caught were recorded. The gonads were removed, weighed and fixed in formalin for subsequent histological analysis. GSI was calculated using the above formula. The heads were removed and stored frozen for subsequent microtag recovery. A sample of diploid chinook (not adipose fin clipped) were sampled for the same parameters at each tournament for comparison.

Little Manistee River Weir

Chinook salmon were checked for adipose fin clips during the fall chinook salmon egg collection at the Little Manistee River weir. The weir is the main source of chinook salmon eggs for the MDNR and all fish are handled and sorted before spawning and/or harvest. When an adipose fin clipped chinook salmon was found, the length, weight, and external appearance were recorded. The fish was squeezed to induce the expulsion of eggs or milt. The GSI was calculated and the gonads were fixed in formalin for later histological analysis. The head was removed and stored frozen for subsequent microtag recovery.

Ten male diploid chinook were sampled to calculate GSI for comparison to the triploid fish. Lengths and weights of diploid chinook salmon were obtained from the MDNR Little Manistee River biosample. The MDNR biosample is calculated by sampling 100 chinook per week for length and weight at

the Little Manistee River weir during the spawning run (Sept.-Nov.). Sex was recorded, but scales were not taken to determine age at the time of sampling because they become embedded in the skin when chinook salmon mature. Age classes were calculated from a length-age frequency distribution. The length-age frequency distribution was determined from creel samples of fish caught in the late summer. At this time scales could be collected and ages directly determined. The lengths of fish caught in late summer correspond closely to the lengths of the fish collected at the weir (Kelly Smith, MDNR, per. comm.).

Return percentages were calculated by dividing the number of fish which returned by the number released for each year class of fish. Suspected triploids were confirmed by both microtag recovery and gonadal morphology. Diploid returns were calculated by the MDNR. Male and female chinook return percentages were calculated separately.

Swan Creek Returns

MDNR also harvests chinook salmon at Swan Creek, a the Lake Huron tributary near Rogers City, MI. In the spring of 1987, 24,198 triploid and 127,502 diploid microtagged and adipose fin clipped chinook salmon were stocked at this site. This created a situation were the return of microtagged triploid chinook could be compared to the return of microtagged diploid chinook. In collecting these

chinook, tag loss and percentage of adipose fin clipped fish missed by weir technicians were assumed to be equal for diploids and triploids.

Fish where harvested by MDNR workers using seines and trap nets at the mouth of the tributary (Tom Rozich, MDNR, per. comm.). This is done to obtain eggs and prevent pollution of the creek by the decaying salmon carcasses. Percent returns were calculated by dividing the number of tagged fish returning by the number of tagged fish stocked. Some microtag loss occurs. Since I was not present to confirm ploidy and the gonads from the adipose fin clipped fish were not collected, all fish with a tag from a triploid lot were considered triploid from this collection site. Length, weight and sex were recorded by MDNR personal and the heads were removed for microtag recovery. Triploid data was compared to diploid data for length, weight and GSI using only the data from fish possessing a microtag identifying them as triploid or diploid chinook salmon released at Swan Creek. Lengths and weights were compared using a students t test. The return percentages for male and female chinook were analyzed separately. The return of triploids was compared to the diploid (control) return by a contingency Chi Square test with 1 degree of freedom.

Histological Analysis

Gonads from adipose fin clipped fish were fixed in formalin. Pieces of gonad were dehydrated, embedded in wax, sectioned at a thickness of 6 um and stained with hematoxylin-eosin by Joao Machado, (MSU, Dept. of Pathology, per. comm.). I examined the slides using a light microscope. Research has shown histological examination of the gonads could determine ploidy, stage of gametogenesis and sex in fish (Thorgaard, 1979; Lincoln et al, 1984;). Male and female diploid chinook salmon gonads were also analyzed for comparison.

Microtag Recovery

All microtags from adipose fin clipped fish were read at MDNR Charlevoix Fisheries Station, Charlevoix, MI. The heads were passed over a sensitive metal detector to confirm tag retention. A large butcher knife was used to cut the head in half. Each half was tested again. The half which retained the tag was then halved again and each half was again checked with the metal detector. This process was repeated until only a small piece of tissue remained. This was held up to a fiber optic lamp to locate the tag, which was then removed using a scalpel. Tags were read using a light microscope by MDNR technician, Paul Gelderbloom.

RESULTS

Captive Chinook Salmon

Thirteen chinook salmon remained in the pond at Wolf Lake Fish Hatchery after most were inadvertently stocked as a put and take fishery. Flow cytometry and histological analysis revealed two mature diploid males, one mature diploid female, five immature diploid females and five triploid females. No triploid male chinook salmon were found in the pond. The five immature diploid and five triploid females were bright silver with loose scales (Figure 1). One of the triploids had a spinal deformity and was the smallest fish in the sample. The gonads of the triploids were thin and string-like and the sex could not be determined by external morphology. Sex of triploid chinook was determined by histological analysis. The gonads of the immature females were larger with oocyte development apparent by external morphology. The two diploid males showed secondary sexual characteristics (darkening, kype formation and embedded scales) and milt production. The ripe female was dark with embedded scales and expelled eggs when squeezed. External morphology of diploid compared to triploid chinook can be seen in Figure 2.

Histology revealed the diploid ovaries from the immature chinook salmon contained large numbers of postmeiotic oocytes (Figure 3). The five triploid ovaries

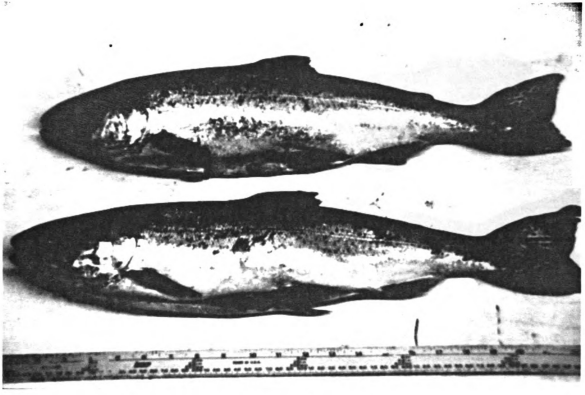

Figure 1. Four year-old immature female diploid (top) and four year old female triploid chinook salmon (bottom).

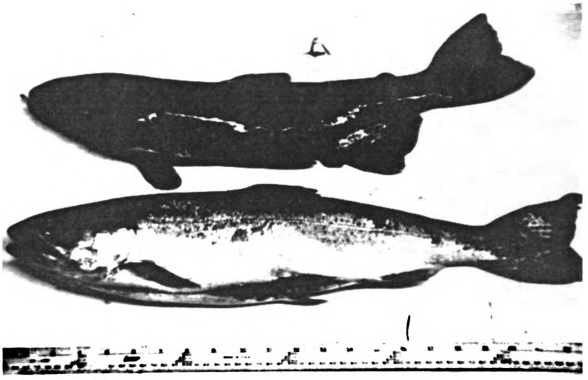

Figure 2. Mature diploid female (top) and four year-old female triploid chinook salmon (bottom).

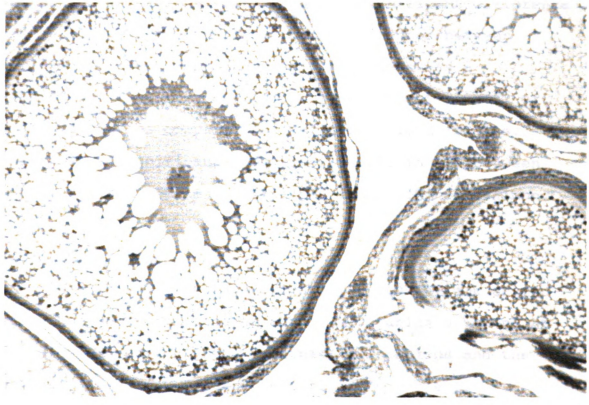

Figure 3. Section of an ovary from an immature diploid chinook salmon.

mainly consisted of connective tissue and unidentifiable cells (Figure 4). One triploid ovary contained one postmeiotic oocyte that was similar to the oocytes found in the diploid ovaries (Figure 5). The testes from the ripe males were packed with spermatatids and spermatazoa.

Means of weight, length, GSI and condition factor are summarized in Table 2. There was no significant difference ($P<.05$) in weight, length or condition factor between diploid and triploid chinook salmon. There was a significant reduction ($P<.05$) in gonad weight and GSI of triploid females compared to immature diploid females. There was a significant reduction ($P<.05$) in GSI between mature diploid female and triploid female chinook salmon.

Summer Creel Census

In 1989 and 1990, approximately 3,500 chinook salmon were checked at tournaments and fish cleaning stations at two Michigan port cities. Tournament locations and the number of fish checked are summarized in Table 3. Approximately 30 adipose fin clipped chinook salmon where found and sampled. Analysis of the microtags revealed that none of the chinook salmon were from triploid treatment lots. Some of the adipose fin clipped fish did not retain a tag. Since tag loss might have prevented a triploid fish from being identified, a gonadal examination was done on all adipose fin clipped chinook. None of the fish examined

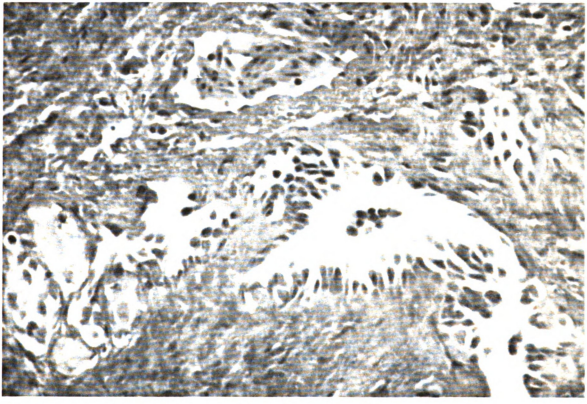

Figure 4. Section of an ovary from a four year-old triploid chinook salmon.

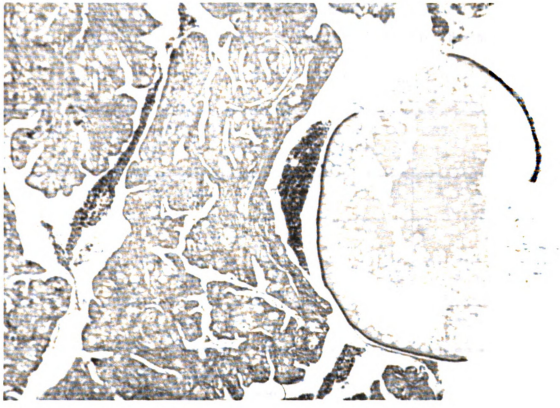

Figure 5. Section of an ovary from a four year-old triploid chinook salmon which contained a developing ooctye.

Table 2. Average lenght (mm), weight (g), gonad weight (g), gonadal-somatic index[1] (GSI) and condition factor[2] of four year old diploid (2N) and triploid (3N) chinook salmon held at Wolf Lake Fish Hatchery. Standard deviation of the means are presented in parenthesis. Different superscripts in columns indicate significant differences in the means ($P < 0.05$).

type	length (mm)	weight (g)	gonad weight (g)	GSI	condition factor
3N females	489 (103)	1121 (530)	≤1	0.12[a] (0.08)	0.88 (0.09)
all 2N	524 (34)	1383 (191)			
immature female 2N	547 (10)	1506 (102)	10 (2.3)	0.69[b] (0.14)	0.93 (0.19)
mature 2N	487 (27)	1177 (73)	118 (43)	9.94[c] (3.0)	1.02 (0.11)

[1] GSI = (gonad weight/fish weight) X 100
[2] Condition Factor = (weight/length^3) X 1000

Table 3. Tournaments and estimated number of chinook salmon checked for adipose fin clips during the summers of 1989 and 1990.

Tournament	Lake	Year	Number of Chinook Checked
Blue Water	Huron	1989	350
Blue Water	Huron	1990	400
Pro-King	Michigan	1990	28
Brown Trout	Huron	1990	900
Roger's City	Huron	1990	1800
TOTALS 5	2		3478

appeared to be triploid from gonadal examination. The female chinook found had developing oocytes or eggs and all but one of the male testes appeared normal. This fish was identified as a diploid by microtag analysis.

Three young of the year (YOY) triploid chinook salmon were caught by an MSU researcher (Rob Elliot, per. comm.) in Lake Michigan. One was caught in June off Ludington MI, another was caught off South Haven, MI in June, and the third was caught off Ludington in Aug. These fish were from a group of 77,444 smolts with triploid tags stocked into Little Manistee River at 9 Mile Bridge on 27 April, 1990. At that same date and place, 108,000 microtagged diploid chinook salmon were also stocked. During the summer of 1990, eight of the tagged diploid fish were captured in Elliot's samples. The chinook tagged as triploid made up 42% of the tagged fish stocked on 27 April at 9 Mile Bridge. Three triploids were caught out of eleven total 27 April, 1990 microtagged chinook caught that summer. Triploids therefore made up 28% of the microtagged fish from Little Manistee River caught in the summer of 1990. There was no significant difference between the percentage of microtagged fish stocked on 27 April, 1990 and percentage of fish caught in the lake during the summer. No triploid fish were returned and identified by either MDNR creel census workers or Michigan anglers.

A list of triploid chinook salmon caught by anglers in Wisconsin is summarized in Table 4. Lengths, weights, place stocked and date stocked are included in the table. The sex of these fish was unknown and no information was available concerning gonadal development. No statistical analysis comparing diploid and triploids for length and weight has been done due to lack of diploid control data.

Little Manistee River Weir Returns

Prior to 1989 no verified triploid chinook salmon returned to either the Little Manistee River weir or Swan Creek during the fall salmon spawning migration. In 1989, three adipose fin clipped female chinook salmon with microtags identiying them as triploid returned to Little Manistee River weir. All three were originally stocked at the Little Manistee River. One was stocked in 1986 (four years old) and the other two were stocked in 1987 (three years old). All three were ripe with eggs and exhibited all the characteristics of diploid fish. Normal development began when eggs from these fish were fertilized. Tests were not done to confirm the ploidy of these fish, but all observations indicated that the fish were diploid. No male adipose fin clipped chinook salmon returned in 1989.

In 1990 no adipose fin clipped female chinook salmon returned to the Little Manistee River weir. However, five adipose fin clipped chinook salmon males returned to the

Table 4. Length, weight, capture site and date and stocking site and date of triploid tagged chinook salmon caught in Lake Michigan from 1988-1990.

Fish	Length (cm)	Weight (kg)	Capture site (WI) (date)	Plant site (date)
1	54.6	1.6	Washington I. (7/1988)	L. Manistee R. (1987)
2	66.0	4.1	Manitowoc (8/1988)	L. Manistee R. (4/1986)
3	68.6	3.2	Cedar River (7/1989)	L. Manistee R. (1987)
4	76.2	4.5	Sturgeon Bay (8/1989)	Swan Creek (1987)
5	64.8	2.8	Sheboygan (8/1989)	L. Manistee R. (1987)
6	68.6	3.2	Manitowoc (8/1989)	L. Manistee R. (1987)
7	88.9	8.2	Sheboygan (7/1990)	Van etten Cr. (4/1987)
8	61.0	2.7	Sheboygan (7/1990)	L. Manistee R. (4/1988)

weir on the Little Manistee River in 1990 (Table 5). All five fish exhibited secondary sexual characteristics common to mature diploid chinook salmon males (darkening, kype and embeddedscales). Three contained a microtag identifying them as triploid fish. One was a ripe male which produced normal thick white milt when squeezed. Microtag analysis revealed this fish was stocked in 1987 (three year old). The other two tagged fish produced no milt when their abdomen was squeezed. Fish number two from Table 5 had and spinal deformity and was very thin. One of the fish that was not tagged did not produce milt when squeezed, while the other untagged fish was ripe and produced thick white milt. The testes of the three fish which did not produce milt were similar in shape to the testes of normal diploid fish but felt firmer and were grey in color. The testes of diploid chinook salmon were white and much softer. Histological examination revealed the three fish that did not produce milt were triploid since the testes had extensive connective tissue and gametogenesis was only progressed to the spermatid stage (Figure 6). The testes from the diploid chinook were packed with spermatatids and spermatazoa (Figure 7). GSI of the triploids was significantly lower ($P<.05$) than the diploid GSI (Table 6). No significant difference ($P<.05$) was found in length or weight between diploid and triploid chinook salmon.

TABLE 5. Length, weight, gonadal somatic index (GSI), ploidy and sex of triploid microtagged chinook salmon which returned to the Little Manistee River weir in 1990. (3N= triploid, 2N= diploid; M=male, F=female)

Fish	Length (mm)	Weight (kg)	GSI[1]	Ploidy	Sex	Date Stocked
1	904	6.35	1.89	3N	M	May 1987
2	655	2.04	2.63	3N	M	May 1987
3	813	5.31	6.12	2N	M	May 1987
4	851	5.40	-	3N	M	May 1987
5	823	5.08	-	2N	M	May 1988

[1] GSI = (gonad weight/fish weight) x 100

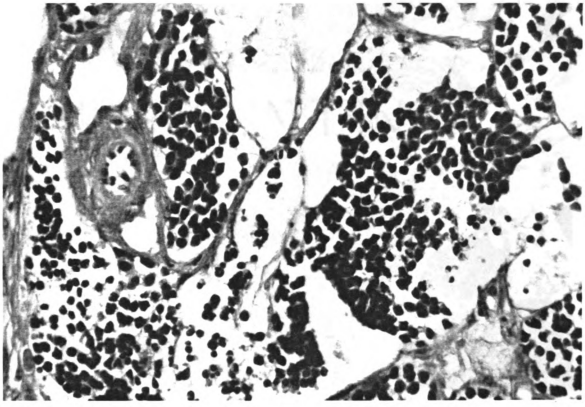

Figure 6. Section of a testis from a four year-old triploid chinook salmon.

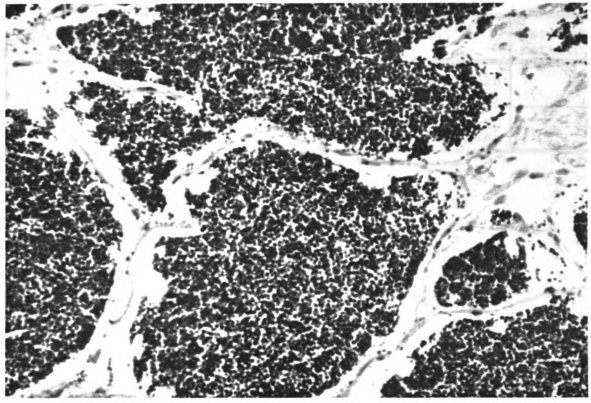

Figure 7. Section of a testis from a four year-old mature diploid chinook salmon.

Table 6. Average length, weight, gonadal somatic index (GSI) and return of triploid (3N) and diploid (2n) male chinook salmon at the Little Manistee River weir in 1990.

Ploidy	Number	Length (mm) (S.D.)	Weight (Kg) (S.D.)	GSI[1,2]	percent return[1]
3N	3	803 (131)	4.72 (2.4)	2.26 (n=2)	.01
2N	63	929 (68)	7.91 (1.2)	6.10 (n=8)	.35

[1] significant difference (P<.05)
[2] (gonad weight/fish weight) x 100

The return of four year old triploid chinook salmon males which returned to the Little Manistee River weir in 1990 was .0095%. The return of four year old diploid chinook salmon males returning to the weir in 1990 was .35%. The return of triploid chinook was significantly ($P<.05$) lower than the return of diploid chinook (Table 6).

Swan Creek Returns

In 1987 approximately 127,502 microtagged diploid and 24,198 microtagged triploid chinook salmon were stocked in Swan Creek. This provided a diploid control to compare to the triploid chinook salmon return. In 1989, no chinook salmon returned possessing a microtag from a triploid lot. In 1990, three 4 year old male chinook salmon returned to Swan creek which were identified as from a triploid lot by microtag analysis. Thirty two male chinook salmon returned from the diploid microtagged group stocked the same year. Length and weight data are summarized in Table 7. No significant differences ($P<.05$) were found in length or weight between male diploid and triploid chinook salmon.

Out of a total of 24,198 triploid chinook salmon stocked at Swan creek in 1987, 3 were identified by microtag analysis for a return of .00012 percent. Out of 127,502 microtagged diploid chinook salmon stocked at Swan creek in 1987, 32 were positively identified by microtag analysis for a return of .00025 percent.

Table 7. Length, weight and percent return of microtagged diploid (2N) and triploid (3N) chinook salmon at Swan Creek in 1990. Standard deviations of the means are in parenthesis.

Ploidy	Number	Length (cm)	Weight (kg)	Percent Return[1]
2N	32	92.0 (4.8)	7.7 (1.4)	0.050%
3N	3	82.0 (9.8)	6.5 (1.6)	0.025%

[1] Significant difference (P<.05)

Assuming the microtagged diploid return to be the control, the return of triploid chinook salmon to Swan Creek was significantly ($P<.05$) lower than the diploid return.

I was not present at Swan Creek during harvest, so no examination of fish returning was done. All tagged fish from a triploid lot were assumed to be triploid and no correction was made for tag loss from adipose fin clipped fish.

DISCUSSION

Captive Chinook Salmon

The results of this study revealed no difference between diploid and triploid chinook salmon in growth and condition factor at four years of age. This indicates triploids grow at the same rate as diploids up to sexual maturation at four years of age. This was expected based on other growth studies of triploid chinook salmon. Westerhof (1988) found no difference in growth and condition factor between diploid and triploid chinook salmon up to two years of age from this same population of fish. No significant difference in growth was found between diploid and triploid chinook salmon fry in an eight week growth study (Spruell, 1989). The results of other triploid salmonids show growth rates compared to diploid to be variable (for review see Ihssen et al., 1990).

Five out of six of the diploid females did not mature at four years of age. This was a very low rate of age four maturation. Based on data from MDNR, the strain of chinook (upper Touly) in the Great Lakes does mature at age five at a very low rate (<.01%). Possible reasons for the high incidence of postponed maturation include the abnormal habitat of the rearing pond or the fish being much smaller

than normal diploid four year old chinook salmon in the Great Lakes.

Based on GSI and the histological examination it appears triploidy successfully sterilized female chinook salmon. A significant reduction in gonad weight and GSI was observed between triploid female chinook and both mature and immature diploid females. This was in agreement with other studies. The GSI of triploid female coho salmon was significantly smaller than the GSI value of diploid coho at 30 months of age (Johnson et al., 1986). Lincoln and Scott (1984) found a significant difference in GSI between diploid and triploid rainbow trout at maturity.

Histological analysis indicated the ovarian development of triploid chinook was similar to the results reported for Atlantic salmon (Benfey and Sutterlin, 1984) and coho salmon (Johnson et al., 1986). The ovaries of the triploid fish observed in these studies were similar to the triploid chinook salmon ovaries in appearance, but differed slightly histologically. The ovaries observed in the previous studies all contained at least one postmeiotic oocyte, and often contained many more, although far fewer than ovaries from a diploid fish. In this study, only one chinook salmon ovary contained one postmeiotic oocyte. This may have been due to difference in rearing methods used or species differences. The fish in the previous study were reared by intensive culture with daily feed rations. After two years

of age, the chinook in my study were transferred to a spring fed pond and exogenous feeding was stopped. Ovaries from triploid rainbow trout raised to the age of maturity contained no postmeiotic oocytes (Lincoln and Scott, 1984). All thirteen fish had to be sacrificed for analysis, and it was unkown how long they would live or the size they would reach.

The absence of triploid male chinook salmon in the pond was puzzling. A predominance of females in the population was not noted by Westerhof (1988) during the first two years. Most likely the male chinook were removed when the pond was harvested in 1988. Precocial maturation as three year old fish was possible, though not likely. Westerhof (1988) did not see any male triploid chinook mature as two year olds in the same population, and no sub-four year old triploid males have returned to stocking sites, even though it was evident that at least some triploid males will return to the river at four years of age. Another possibility is that male triploids had a higher mortality than female triploids. A differential mortality was noted by Lincoln (1981b) when only one female was found in a sample of 21 triploid plaice.

Triploid Chinook Salmon in the Great Lakes

All the data from the lake survey and weir returns was collected to determine if triploidy would extend the life of

chinook salmon and create a "trophy" chinook salmon fishery. First I will detail some of the problems encountered in data collection, then discuss the status of the triploid chinook salmon in the Great Lakes.

One of the most important pieces of data which would provide proof that triploidy extends the life of chinook salmon in the Great Lakes would have been locating a five year old triploid chinook in 1990. There were several reasons why locating a five year old triploid chinook was extremely difficult. The first reason was the low survival of the 1985 chinook salmon year class and the low number of triploid chinook stocked that year. Estimating survival of chinook salmon is difficult. The best estimate of survival of a year class is based on the percent of fish returning to the weir when sexually mature. The 1985 year class of normal chinook had a total return to the Little Manistee River weir of 3.3%. The total return is calculated by summing the returns of each age class (age 2 returns + age 3 returns + age 4 returns) of a year class. This represented nearly a 50% decrease from the average of 7.2% per year age class from 1981-1983. Recent low angler harvests also indicated that the survival of the 1985 year class was low. The reason for the low survival of the chinook salmon in Lake Michigan is not known. Suspected causes include bacterial kidney disease (BKD), reduction in the forage base or increased predation of out-migrating smolts. Assuming

weir return is a close approximation of the survival of a year class, then survival of triploid chinook from the 1985 year class to age four was at best 3.3%. The true survival of triploids would be lower because 2.3% of the total year class return is made up of two and three year old fish. Angler harvest and natural mortality would have reduced the number of age two and three year old triploids that reach four years of age. Out of 24,000 triploid tagged chinook salmon planted in Lake Michigan, only 45% were triploid due to low triploid induction rate the initial year. That means approximately 10,800 triploid fish were actually stocked that year. With 3.3% survival, and assuming triploid and diploid viability was equal, at best only 350 triploid fish reached four years of age, and survived to the fall of 1989. Most likly survival was between one and two percent. Even though no triploid chinook salmon males were found at the weir in 1989, it appears some males may exhibit characteristics of mature diploid males and return to the river, which would reduce the number of surviving triploids even more. Based on this, the chances of a five year old triploid being caught and identified in 1990 was very small.

Adding to the problem of recovering triploid chinook from Lake Michigan was a significant decline in angler effort and harvest in 1990. Since the peak in the early 1980's, angling for and harvest of salmon has dropped approximately 60% (Myrl Kellor, MDNR per. comm.). The

reasons for the decline include a smaller population of salmon, as a result of decreased survival and decreased stocking, and consumption advisories warning of health risks from eating the fish. The number of tournaments on the Great Lakes, especially Lake Michigan, has decreased significantly in 1989 and 1990. Tournaments concentrated anglers at one port, and large numbers of chinook could be checked in short times. The surveys at the port cities of Ludington and Manistee, MI netted very poor results, often only 25-30 chinook salmon checked each weekend.

The third problem was lack of public awareness of the microtag recovery program in Michigan. Public eduction and participation in the tag recovery program was very important to properly monitor the triploid salmon project. The majority of the anglers interviewed were not aware an adipose fin clipped fish was microtagged or that the head should be returned to the MDNR. The charterboat captains knew about tag recovery and many tackle shops had signs to notify anglers to look for adipose fin clipped fish, but no triploid chinook were returned. The Wisconsin Department of Natural Resources (WDNR) also had very few returns of adipose fin clipped fish from the general fishing public. Most of the microtags were recovered by WDNR creel census workers (Tom Racosey, WDNR per. comm.) Both MDNR and WDNR officials indicated creel census workers occasionally miss identify adipose fin clipped fish.

Adipose fin clipped chinook returning to the weir were also missed due to the rapid pace of harvest and spawning. Mis-identification of females was probably minimal because all are handled during spawning, but males were quickly thrown into a crate. Unless the weir technicians were reminded to look out for adipose fin clipped fish, they worked quickly without paying much attention. The low number of triploid fish returned was at least partially attributed to these problems in data collection. In the future if a very large "trophy" triploid chinook salmon is caught the media attention it draws should aid identification.

Survival of Triploid Chinook Salmon in the Great Lakes

Although few triploid chinook salmon were found in the lake surveys or weir returns, most information indicates that survival rate of triploids were similar to that of diploids. In the Great Lakes, triploid and diploid chinook salmon were stocked together. After the triploid fish were stocked it is unknown what happens to the them. It appears they do go to the lakes and survive the first summer at the same rate as diploid chinook. The percentages of triploid and diploid microtagged YOY chinook caught in Lake Michigan during the summer of 1990 (28% triploid :72% diploid) was not significantly lower than the percentages when they were stocked in the Little Manistee River (42% triploid : 58%

triploid). Fron this it appears triploid chinook salmon smolts survive and out migrate with diploid fish.

From the data collected it was not possible to compare the survival of triploids to diploids in the lake. The only indication we had was from prior physilogical and culture studies concerning survival of triploid salmonids, which were variable. Also, a comparison of triploid and diploid survival in the captive chinook salmon population could not be done because it was unknown how many fish remained after the pond was harvested in 1988.

Physilogically, one factor which has been extensively studied in triploid fish is oxygen utilization. Triploid and diploid fish have equivalent haematocrits (fewer but larger erythrocytes) but triploid had lower total haemoglobin in their blood (Swarp, 1960; Benfey and Sutterlin, 1984c). It was originally thought that this might cause triploid fish a competitive disadvantage by lowering their ability to utilize oxygen, but results indicated no differences. However, no difference in oxygen consumption was found between diploid and triploid Atlantic salmon (Benfey and Sutterlin, 1984d). Graham et al. (1985) suggested triploids compensate for lower haemoglobin level by increasing cardiac output. If this were true, you would expect triploid fish to have less stamina than diploid fish. Small and Randall (1984), however, measured maximum sustained swimming ability (stamina) of triploid and diploid

coho salmon and found no significant difference. This would idicate that triploids were at no competative disadvantage in the lakes.

Stress from intensive culture has lead to increased triploid mortalities in one case. Utter et al. (1983) found a significant reduction in triploid numbers from one of two replicate groups of diploid and triploid coho salmon raised intensively in a net pen, but Johnson et al. (1986) found no increase in the mortality of triploid compared to diploid coho salmon. He suggested that the difference in survival in the two studies may have been due to the rearing method used. In the 1986 study, the coho were raised with reduced rearing densities and vigorous feeding which put little stress on the fish, whereas Utters study was more intensive and stressful to the fish. The only other reported case of increased mortality was when Johnson et al, (1986) gave a seawater challenge to diploid and triploid coho salmon at a time that didn't correspond with normal smoltification and caused osmoregulatory stress to the fish. Of 102 mortalities from the challenge, 100 of them were triploid smolts. At the normal time of out migration, triploid smolts given the seawater challenge survived at the same rate as diploids. Spruell (1989) fed half rations of feed to triploid and diploid chinook salmon both while isolated in separate tanks and competing in the same tank and found no significant difference in growth. It is possible that

no significant difference in growth. It is possible that stress factors on the Great Lakes chinook salmon population, which have decreased survival in recent years, may have resulted in a higher mortality rate in triploids than diploids, although the poor data recovery would give the same results.

Based on data from the captive chinook salmon population and returns to the Little Manistee River weir, female triploid chinook salmon did not show any characteristics of sexual maturation. No female triploid chinook salmon has ever returned to the weir at age four or five. Female triploid chinook from the captive population survived to four years of age and showed no signs of sexual maturation. Robertson (1961) extended the life span of kokanee (landlocked sockeye salmon,(Oncorhynchus nerka) by castrating them before maturation. The kokanee normally matured at four years of age. Most of the non-castrated fish matured and died at age four. Some of the castrated fish also died at ages four and five. Examination revealed the gonads of these fish had regenerated and promoted sexually maturation. One male and one female reached eight years of age. Growth of the castrated fish was fairly constant until it ceased 2-3 months before death. McBride and Van Overbeeke (1963) removed the gonads from sexually mature sockeye salmon. Some of them regained their silvery appearance and resumed feeding. It appears mature gonads

characteristics and postspawning mortality, and since triploid chinook females show no gonadal development, they should continue to live beyond four years of age. No secondary sexual characteristics were exhibited by adult triploid female salmonids (Thorgaard and Gall, 1979; Thorgaard, 1983; Lincoln and Scott, 1984; Benfey and Sutterlin, 1984b).

The ovaries of diploid fish produced estrogen which matured oocytes and led to development of secondary sexual characteristics (Fish Physiology, 1974). A significantly reduced estrogen level in triploids compared to diploids has been observed in other salmonids (Lincoln and Scott, 1984; Benfey et al., 1989). Benfey et al. (1989) suggested the occasional postmeiotic oocytes observed in triploid coho salmon did not grow to full maturity due in part to the diminished estrogen from the ovary. Based on the histology and appearance of the captive female triploid chinook salmon, they would not have sexually matured or exhibited secondary sexual characteristics. It is unknown what factor promotes migration in Pacific salmon.

Six triploid male chinook salmon migrated and exhibited characteristics of sexually mature diploid fish. The percentage of triploid male fish which returned to the river could not be directly estimated because it was unknown if any male triploid chinook remained in the lake. It was not known if the survival of male triploid chinook in the lake

was equivalent to diploid male chinook, but all observations indicated it to have been similar (see previous discussion on survival). Triploidy did appear to prevent precocial maturation of chinook salmon based on the fact than no age two or three chinook have been found at the weir. Four year old triploid males returned to the Little Manistee River weir and Swan Creek at significantly lower percentage than four year old diploid chinook. The fish which returned were similar in appearance to normal diploids. The males that did return were sacrificed, so it is unknown if the triploid chinook males would have exhibited normal spawning behavior (attempted to spawn) or demonstrated "postspawning" mortality. Benfey et al. (1986) observed that triploid male rainbow trout exhibited secondary sexual characteristics and some "postspawning" mortality. It is not known if triploid males did not migrate back to the rivers, but still showed signs of maturation while remaining in the lake. Triploid male rainbow trout were functionally sterile due to the production of aneuploid sperm (Benfey and Solar, 1986) but their testosterone level was not significantly different from diploids (Lincoln and Scott, 1984). Histologically, the testes of mature triploid male chinook salmon were similar to the testes of other diploid male salmonids and the GSI comparisons were similar (Thorgaard and Gall, 1979; Thorgaard, 1983; Lincoln and Scott, 1984; Benfey and Sutterlin, 1984b). The germ cells of triploid salmonids

usually only developed to the spermatid stage. Two out of twelve triploid male rainbow trout produced sperm (Lincoln and Scott, 1984). No triploid chinook salmon in this study produced sperm.

The growth and survival of triploid chinook salmon after four years of age in the Great Lakes could not be determined. Chinook salmon have indeterminant growth meaning they will continue growing their entire lives (Beverton and Holt, 1957). Triploid rainbow trout up to age five showed a significant increase in growth compared to diploids. Triploid rainbow trout grew an average of 300g/fish while diploid rainbow trout grew an average of 60g/fish from 2-3.5 years of age (Thorgaard, 1986). Castrated sockeye salmon survived past normal maturation time and continued growing at a constant rate (Robertson, 1963).

CHAPTER II. MICHIGAN DEPARTMENT OF NATURAL RESOURCES PRODUCTION RUNS

MATERIAL AND METHODS

Triploidy was induced in 1989 by applying a heat shock to the eggs as described by Westerhof (1988) and modified by Spruell (1989). Sperm was obtained from 8-10 males and held on ice in a plastic safety wash bottle. Females were artificially spawned by injecting air into the peritoneal cavity (Piper et al., 1982). Eggs from four to six females were combined in a five gallon bucket and fertilized with a sample of the pooled sperm. After two minutes, water was added to the eggs to increase fertilization (Piper et al., 1982). Before the eggs were placed into the heat shock unit they were gently rinsed. Rinsing the eggs removed excess milt and broken eggs and maintained high water quality in the heat shock unit. An increase in survival of treated eggs in 1988 was partially attributed to rinsing the eggs (Spruell, 1989). At ten minutes after fertilization, the eggs were placed in the heat shock unit containing 28.5 $\pm$1 C water. The heat shock unit consists of a 172 quart ice chest filled with water that was heated with a 1500 watt thermostatically controlled immersion heater and circulated up through the eggs with a submersible pump. Water temperature was constantly monitored during the entire procedure. After ten minutes in the water bath, the eggs were removed and poured into a bucket containing

intermediate temperature water which slow cooled them back to ambient stream temperature over a period of 15 minutes. The eggs were then water hardened for at least one hour before being transported to MDNR Wolf Lake Fish Hatchery for incubation and grow out.

For the 1990 production runs, a modification was made in the heat shock procedure in a attempt to increase survival. Normally when the eggs were moved from one step to the next, they were poured from one bucket to another. It was thought that pouring might have stressed the eggs and increased mortality. The modification involved collecting the eggs from the females in a screen bottom bucket similar to the one normally used in the heat shock unit. Instead of pouring the eggs from container to container between each step, the screen bottom bucket was transferred from step to step without pouring. After the eggs were fertilized in the screen bottom bucket it was placed in a bucket of water to activate and rinse. Ten minutes after fertilization, the bucket containing the eggs was removed from the rinse bucket, making sure all the water drained out, and placed into the heat shock unit. When it was removed from the heat shock unit, it was placed in another bucket to slow cool as described above. The final step was to pour the eggs into a bucket to water hardening and transport. The final step was the only time the eggs were poured in the entire heat shock procedure.

the only time the eggs were poured in the entire heat shock procedure.

Egg survival from the modified method was compared to egg survival from original method using three replicate heat shocks of each treatment. The eggs from ten female chinook were combined in a screen bottom bucket, fertilized and rinsed, making sure the eggs were well mixed. Half the eggs were poured into one heat shock unit. The bucket containing the remaining eggs was placed into the other heat shock unit. After ten minutes, the poured eggs were removed from the heat shock unit and poured into a bucket for slow cooling, then poured into a bucket for water hardening and transport. The screen bottom bucket containing the eggs which were not poured was removed from the heat shock unit, placed into a bucket for slow cooling and poured into a bucket for water hardening and transport.

Three samples of eggs shocked using each method were taken to the MSU Fisheries Laboratory for evaluation and comparison. The number of eggs in each sample was estimated by calculating the average volume of 25 eggs measured three times, then measuring the volume of the remaining eggs. The number of eggs was estimated by dividing the total volume of eggs by the average volume of twenty five eggs, and multiplying that number by twenty five. Dead eggs were periodically removed and counted to prevent the spread of fungus and calculate survival of the eggs until hatching was

complete. Ploidy induction rate was determined by flow cytometry.

The MDNR annually requested 200,000 triploid smolts which required heat shocking approximately 2,000,000 eggs. Triploid smolts were stocked in Lakes Michigan, Huron and Superior. The percentage of triploids in the population of fish to be stocked was determined annually by flow cytometry of the blood from 20 randomly sampled fish. This estimated the number of true triploids being stocked.

RESULTS

1989 MDNR Production Run

All shocked eggs were collected at Little Manistee River weir. A total of 1.5 million eggs were shocked in two days. On 7 Oct. 1990, 950,000 eggs were heat shocked, and on 10 Oct. 1990, another 500,000 eggs were heat shocked. Normally the MDNR requests 200,000 triploid chinook salmon smolts for stocking. Survival of smolts from treatment to time of stocking was 12.8 percent. This resulted in 178,137 triploid chinook salmon smolts, which were stocked in the spring of 1990. Of twenty smolts randomly chosen for ploidy determination by flow cytometry, eighteen were triploid, for a 90%+5% triploid induction rate. Rivers receiving and number of triploid chinook salmon stocked in each are summarized on Table 8.

1990 MDNR Production Run

All shocked eggs were collected at the Little Manistee River Weir in 1990. On 10 Oct. 1990, 985,000 eggs were heat shocked using the modified shocking technique. Survival of smolts from heat shock to stocking was 8.3%. This resulted in 80,000 triploid chinook salmon for stocking in the spring of 1991. Triploid induction was 65.5%, based on a sample of 20 fish tested by flow cytometry. All triploid chinook

TABLE 8. Number and location of triploid chinook salmon stocked by the Michigan Department of Natural Resources in Spring 1989. Data provided by the Michigan Department of Natural Resources Fish Division, Wolf Lake State Fish Hatchery.

Location stocked	Number stocked
Lake Michigan	
Little Manistee River	77,444
Lake Huron	
Van Ettan Creek	25,000
Swan Creek	25,000
Harbor Beach	25,000
Lake Superior	
Ontonagongan River	12,000
Carp River	11,000
TOTAL	175,444

salmon will be stocked at Little Manistee River to aid in future monitoring of the project.

In 1991, eggs were shocked using both the previous method (Spruell, 1989) and the modified method. The previous method involved pouring the eggs from step to step. The modification enabled a batch of eggs to go through the entire heat shock procedure and only get poured once. The triploid induction rate using the no-pour method was 54% and the survival compared to the control was 21%. The triploid induction rate using the pour method was 43% and the survival compared to control was 26.5%. No significant difference was found between the no-pour method or the pour method for triploid induction rate or survival, so it appears the modification offered no advantages over the previous method.

Three replicates were done and in each one the method using heat shock unit number two had higher survival. It appeared the buckets in the two heat shock units were slightly different and this may have influenced both survival and ploidy induction rate. Triploid induction rate between replicates was significantly different, ranging from zero to 100% for the sample groups, however, no significant difference was found within each replicate.

DISCUSSION

MDNR Production Runs

The survival in 1989 of triploid chinook salmon from heat shock to stocking was 12.3%. This was good survival relative to past production run results. Heat shocking has produced near normal survival of treated eggs of Atlantic salmon (Benfey and Sutterlin, 1984) and rainbow trout (Chourrout and Quillet, 1982). The reason for the low survival may have been due to the number of eggs shocked per batch, long transport distance and reduced egg care by the MDNR. Triploidy induction rate was 90%, which was similar to previous years.

The survival in 1990 of triploid chinook salmon from heat shock to stocking was 8.3%. The survival was lower than previous years and may have been due to the modified heat shock method. One problem encountered with the modification was the screen used for the construction of the screen bottom buckets had a mesh size that was to large. The large mesh enabled salmon eggs to form into the holes and block the water from draining while moving to each step. The weight the water may have compressed the eggs on the bottom of the bucket and increased mortality. Other causes of low survival are low egg quality, handling during heat shock and incubation and long transport time. Spruell (1989) suggested, after egg quality, care of the eggs was

the most important factor effecting survival of heat shock eggs.

The modified no-pour method and the pour method gave similar results and no advantage was gained using the new method. As long as care is taken when pouring the eggs from step to step, then survival will be equevilent Many problems were encountered which made quantification of the differences between methods difficult. First induction rate was extremely variable, ranging from 0-100%. A reason for the low triploid induction rate in some replicates may have been a result of the eggs sitting in the bucket for a half hour of more before fertilization. The eggs from ten females were needed for each replicate. The lack of ripe females caused the delay. Westerhof (1988) reported chinook salmon eggs which were delayed in fertilization for 6-8 hours had a triploid induction rate of zero. High induction rates were reported using delayed fertilization of other salmonid eggs (Utter et al., 1983) The strain of chinook salmon found in the Great Lakes may require prompt heat shocking to insure high induction rates.

The second problem encountered was, in each of the three replicates, one heat shock unit (number two) produced eggs with a higher survival and lower triploid induction, no matter which method was used. The bucket in the heat shock unit number two was slightly different then the bucket in unit number one. This may have enabled water to leak up

around the top of the bucket and not provide even circulation through the eggs. In future years the bucket should be changed to ensure optimum performance of the heat shock units.

CONCLUSIONS

1. Growth and condition factor of triploid and diploid chinook salmon were not significantly different.
2. Triploid chinook salmon females had significantly reduced GSI compared to mature diploid chinook salmon females.
3. Triploid female chinook salmon did not migrate to the river to spawn at four or five years of age.
4. Triploid male chinook salmon migrated and exhibited secondary sexual characteristics.
5. The percent of the surviving population that migrates is not known.

The overall goal of the triploid salmon project was to create a trophy chinook salmon fishery in the Great Lakes. The project will be successful if a few large chinook salmon (larger than forty pounds) were caught by anglers each year. Female triploid chinook salmon did not show signs of maturity and migrate to the rivers and, therefore, should continue to grow to trophy sizes in the Great Lakes. Based on the definition of a trophy salmon fishery, the number which survive may not be important as long as the ones which do survive reach "trophy" size. The ultimate size triploids may attain is unknown, but growth should continue as long as they live.

Few fish were recovered from angler creel surveys. If a better tag recovery program had been in place when the project was started in 1985, additional fish may have been recovered. Ways of improving data collection from the general angling public include: making it easy to return tags by putting head drop-off points at major ports, listing information about tag recovery in the fishing regulations of all the Great Lakes states and publicizing DNR research projects to increase knowledge and support of the DNR and it's projects.

The poor survival of the general Great Lakes chinook population has affected the results of this study. Low survival of chinooks reduced triploid numbers in the lakes and made monitoring difficult because of reduced angler effort and harvest of salmon. Many factors may be affecting the current size of the Great Lakes salmon population. Until more is known about the factors affecting chinook salmon survival in the Great Lakes, the triploid project should be a low priority of the MDNR.

In the future, if the triploid chinook salmon project is continued, methods of producing all female triploid chinook salmon should be considered. Based on the triploid chinook data from this study, and information from the reviewed literature, triploid Pacific salmonid females do not exhibit the degenerative characteristics of mature diploids, and would be better suited for a trophy salmon

project. The best approach to producing an all female stock of chinook salmon may be induction of triploidy followed by hormonal sex reversal. Use of a brood stock of gynogenic, sex reversed males for the production of all female progeny is not feasible for chinook salmon due to their long life span and postspawning mortality.

APPENDIX

APPENDIX A

Ploidy of chinook salmon were determined with a flow cytometer and was similar to the procedure used by Spruell, (1989). One modification to the procedure was bluegill blood was used as a internal standard instead of chicken blood. Bluegill blood histograms peak at the nearly the same position and was easier to obtain than chicken blood. The internal standard was used to monitor the drift of the machine which results due to sample differences. The protocol for sample preparation can be seen on table A1.

Chinook salmon ploidy was determined using an Orth Diagnostics Systems, Inc. Model 50-H dual laser Cytofluorograph located in Giltner Hall on the MSU campus. An Orth 2150 computer system was coupled to the cytofluorograph and the Orth Cytofluorograph Analysis for Cellular DNA content of Fixed Cells with DNA Doublet Discrimination program was used to analyze the data. Cell samples were run at an argon-ion laser setting of 488 nm with a 0.5 W output.

Pulse-height histograms were generated by an Orth 2150 computer system, coupled to the cytofluorograph, based on DNA measurements from 10,000 cells per sample. Bluegill blood was used as an internal standard. The flow cytometer

was adjusted so that peaks resulting from bluegill blood ran at 25-28 units of DNA fluorescence, diploid chinook salmon blood peaks ran at 85-90 units and triploid chinook salmon blood ran at 125-130 units of DNA fluorescence. The positions of peaks vary due to sample preparation or machine settings. Bluegill blood and diploid chinook salmon blood samples were run to establish the peak positions.

Table A1. Steps in the cell staining technique used to determine ploidy in chinook salmon.

Step	Description
1.	Blood was drawn from each chinook salmon by a cardiac puncture using a 25 gauge needle and a 1 ml syringe rinsed with sodium citrate buffer solution (CBS) (Appendix Table 3).
1a.	Bluegill blood was obtained by drawing blood by cardiac puncture using a 25 gauge needle and a 1 ml syringe rinsed with CBS. The bluegill blood was added directly to the chinook salmon sample.
2.	Blood was added to a 12 x 75 mm plastic test tube containing 0.5 ml of CBS by submersing the needle in the buffer and applying slight pressure to the hypodermic. Blood was added until samples were a faint pink color.
3.	Samples were stored on ice until further processing was completed.
4.	Samples were centrifuged at 2500 rpm at 10 C for 5 minutes and the supernant was discarded leaving a cell pellet.
5.	Cells were resuspended in 0.5 ml of CBS and vortexed until all clumps disappeared.
6.	Cells were fixed for approximately 15 minutes using 70% ethanol. Ethanol was stored on ice prior to use.
7.	Samples were centrifuged as described in Step 4, and the supernant was discarded.
8.	Cells were resuspended in 1.5 ml of propidium iodide solution (Appendix Table 3).
9.	0.5 ml of RNAse-A solution was added to each tube.
10.	Samples were run on the Orth Cytofluorogragh.

Table A2. Composition of reagents used in flow cytometric determination of ploidy level in chinook salmon.

Reagent	Composition	
Citrate Buffer Solution: (CBS)		
	8.55 g	sucrose
	1.17 g	trisodium citrate
	100.00 ml	distilled water
RNAse-A Solution:		
	1.0 mg	RNAse-A
	5.0 ml	Phosphate buffer solution (1X)
Propidium Iodide Solution:		
	2.5 mg	Propidium Iodide
	0.5 ml	Triton-X
	1.85mg	EDTA
	50.0 ml	Phosphate buffer solution (1X)

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