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TRIPLOID CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA)
FOR THE GREAT LAKES

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DEVELOPMENT OF TECHNIQUES TO PRODUCE TRIPLOID CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*) FOR THE GREAT LAKES.

By

Rick Eugene Westerhof

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ABSTRACT

DEVELOPMENT OF TECHNIQUES TO PRODUCE TRIPLOID CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA) FOR THE GREAT LAKES.

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Sterile triploid salmonids may grow larger than normal diploids due to an increased life expectancy. There are many potential applications in aquaculture and sport fishery management for triploid salmonids if an inexpensive, simple, and effective technique to produce large numbers (200,000) of triploid fish can be developed.

A technique has been developed that produces 90-100% triploid chinook salmon with 53.1% survival to hatch, using readily available, inexpensive equipment. The heat shock system consists of a 172 quart waterbath (ice chest), a 1500 watt immersion heater with a thermostat (accurate to $\pm$ 1° C), a corrosion free submersible pump (3 gpm), two modified 5 gallon plastic pails, and tygon tubing. Up to 30,000 eggs can be treated at one time facilitating production of a large number of triploids.

Triploidy was induced using a 28.5° $\pm$ 1° C heat shock, applied 10 minutes after fertilization for 10 minutes, followed by a 15 minute slow cooling to ambient temperature.

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INTRODUCTION

The Great Lakes salmon fishery has provided important recreational and economic opportunities for the Great Lakes states. The success and interest in this fishery depends on continued production of quality fishing and management practices. Developing new methods to enhance the quality of fishing may improve the appeal of the fishery program and economy of the Great Lakes region. One possible way of improving the quality of fish available to anglers is production of larger sized "trophy" salmon. Trophy sized salmon can be produced by sterilizing salmon currently stocked in the Great Lakes.

All Pacific salmon during the processes of maturation and spawning are affected by reduced growth rates, flesh degradation, and total mortality (McBride and van Overbeeke, 1971; Gjedrem, 1976; Refstie et al., 1977; Lemoine and Smith, 1980; Utter, 1983). These changes have been associated with the production of the gonadal steroids 11-ketotestosterone, 17α-methyltestosterone and estradiol (van Overbeeke and McBride, 1971). Therefore, sterilizing salmon may prevent tissue deterioration and mortalities that coincide with natural reproduction.

There are several advantages to sterilizing salmon. First, production of sterile fish should result in better

flesh quality, increased growth and reduced mortalities (Thorgaard et al., 1981; Purdom, 1983; Utter et al., 1983). Second, sterile fish may exhibit increased growth due to a longer life expectancy. Since fish have indeterminant growth (Beverton and Holt, 1957), sterile salmon should may continue to grow beyond their normal life span and they will not put energy into the production of gonads and gametes. Both factors should contribute to producing bigger ("trophy") and older fish. A third advantage of stocking sterile fish would be the prevention of establishing an unwanted breeding population or disruptions of genetic adaptions of indigenous gene pools through interbreeding (Utter et al., 1983; Benfey and Sutterlin, 1984; Purdom et al., 1985).

A cost effective way of sterilizing large numbers (200,000) of salmonids was needed to create a "trophy" fishery for the Great Lakes. Sterilization of salmonids can be accomplished by surgical castration (Robertson, 1961; van Overbeeke and McBride, 1971), steroid hormone treatments (Goetz, et al., 1979) and inter-specific hybrid crosses. Unfortunately, surgical castration and steroid hormone treatments aren't feasible in large hatchery operations because of the time and labor involved to produce large numbers of sterile fish. Steroid hormone treatments require special feeding throughout the hatchery rearing cycle and may cause concern over the use of chemicals in altering the fish. Variations in viability, survivability, behavior, degree of sterility and musculoskeletal deformities of hybrid crosses negate this choice for mass production of sterile fish (Blanc

and Chevassus, 1979; Blanc and Chevassus, 1982; Sweet, 1986).

Another, relatively simple way of producing sterile salmon is to induce triploidy. Triploidy results from a physical stress applied shortly after fertilization that prevents the second polar body from being extruded during the second meiotic division (Thorgaard et al., 1981; Purdom, 1983; Chourrout, 1984). A triploid (3N) fish has three sets (two maternal and one paternal) of chromosomes unlike, a diploid or normal (2N) fish that has two sets (one maternal and one paternal) of chromosomes. Triploid fish are expected to be infertile because homologous chromosomes cannot pair properly during meiotic development of gametes, which usually results in defective gamete formation (Benfey and Sutterlin, 1984; Cassoni et al., 1984).

The major goal of this research was to develop techniques to produce triploid chinook salmon (Oncorhynchus tshawytscha) for stocking in the Great Lakes. Chinook salmon was chosen because preliminary work at Michigan State University on coho (Oncorhynchus kisutch), chinook, and their reciprocal hybrids resulted in unacceptable triploid induction of coho salmon (Sweet, 1986). Plus, it is the most popular sport fish and largest introduced Pacific salmonid available from the Great Lakes system.

The first year objectives were:

1. To determine the effect of heat shock applied to eggs 10 minutes post-fertilization for varying times followed by rapid or slow cooling on induction of triploidy and survival of

chinook salmon.

2. To develop flow cytometry techniques for identifying triploid chinook salmon.
3. To develop a practical technique to produce production lots (200,000 eggs) of triploid chinook salmon.

In the second year, two additional objectives were investigated.

1. To determine the effect of time of egg take during the spawning season (early, mid or late season) on triploid induction and mortality of chinook salmon.
2. To determine the effect of delayed egg fertilization techniques on induction of triploidy and survival in chinook salmon.

Continuing objectives were to observe differences in the growth and survival of triploid and diploid chinook salmon held at Wolf Lake State Fish Hatchery, Michigan Department of Natural Resources, Fish Division and Michigan State University's Aquaculture Laboratory, and to follow the development and distribution of micro-tagged triploid chinook salmon released in the Great Lakes as a result of this study.

LITERATURE REVIEW

Natural Polyploids

In general, polyploidy is found more often in plants (bananas, winesap apples, and European pears) than animals (Strickberger, 1985). Thirty to 50% of all plants are polyploid. A few examples of animal species that are polyploid are the snout beetles (Curculionidae), psychid moths (Solenobia), lizards (Cnemidophorus), brine shrimp (Artemia), and sow bugs (Trichoniscus). They produce offspring through a special form of asexual reproduction called parthenogenesis (Maslin, 1962; Strickberger, 1985).

There are a couple of sexually reproducing polyploid animals (amphibians and fish) found in nature that are considered to be autopolyploid. Each of these sexually reproducing populations maintains a specialized mechanism for producing offspring. An example is the mating between the Jefferson salamanders (Ambystoma jeffersonianum) and the blue-spotted salamanders (Ambystoma laterale), which produces all-female gynogenetic triploid hybrids called the silvery salamander (Ambystoma platineum) and the Tremblays salamander (Ambystoma tremblayi) (MacGregor and Uzzell, 1964). These salamanders lack a meiotic reductional division and sperm from one of the hybrid paternal species only stimulates the egg to develop (Behler and King, 1979). Triploid axolotl

(Ambystoma mexicanum) females also produce viable offspring. They have no special meiotic mechanism to conserve normal chromosome numbers because the surviving offspring contain variable chromosome numbers (Uzzell, 1964). This demonstrates that axolotls have some degree of tolerance to aneuploidy (Fankhauser and Humphrey, 1950). The viviparous fish species, Poeciliopsis (Poecilidae) produces all-female triploid offspring devoid of paternal characteristics (Schultz, 1967; Cimino, 1972). These fish reproduce gynogenetically, in which no fusion occurs between the ova and the sperm. Triploidy is maintained by an endomitotic division that raises the chromosome number to hexaploid prior to meiosis (Schultz, 1967). Recombination does not occur and results in the progeny being all-female triploids.

Spontaneous Polyploids Found in Nature

Several polyploids have been found to occur spontaneously in nature. Spontaneous triploids have been reported in many bisexual vertebrates. For example, in salamanders such as Triturus viridescens, Eurycea bislineata, Triturus pyrrhogaster (Fankhauser 1938; 1940; et al., 1942), and Triton taeniatus (Book, 1940), the frogs Rana esculenta (Hertwig, 1920; Dalcq, 1930), and Rana fusca (Dalcq, 1930), and in a single chicken Gallus domesticus (Ohno et al., 1963; Sarvella, 1970; Abdel-Hameed and Shoffner, 1971). Gold and Avise (1976) identified one triploid California roach (Hesperoleucus symmetricus) from nine in the Russian River. One spontaneous triploid rainbow trout (Salmo gairdneri) was

found among 18 other fish by Cuelluar and Uyeno (1972). Another (rainbow trout) was discovered by Thorgaard and Gall (1979) from the McCloud River strain. Utter et al. (1983) also discovered three spontaneous pink salmon triploids (Oncorhynchus gorbuscha). Finally, one polyploid brook trout (Salvelinus fontinalis) was found in a hatchery stock of fish at the Maine Phillips Hatchery.

Triploid Induction Methods

There are a variety of techniques that have been used to induce triploidy in fish. For example, chemicals, cold shocks, hydrostatic pressure and heat shocks all have been successful to some degree in producing triploid individuals.

Chemicals that inhibit mitosis generally have shown little success except for producing mosaics. Mosaics are fish with diploid and polyploid cells. Two researchers reported mosaics in Atlantic salmon (Allen and Stanley, 1979) and rainbow trout (Refstie et al., 1977) treated with cytochalasin-B shortly after fertilization. Smith and Lemoine (1979) produced similar mosaic embryos and fry in brook trout after an early colchicine treatment. Neither one of these chemicals produced consistent results. The fish were either diploid, triploid, tetraploid or mosaics.

Cold shock techniques have ranged from completely effective to totally ineffective in producing polyploid fish. In flat fish, considerable variability was observed between egg batches; but, when triploids were produced, the incidence was 100% (Purdom, 1973). Swarup (1959a) reported high

frequencies of haploid, diploid and mosaic embryos in sticklebacks that were cold shocked. Tilipia aurea triploids had a survival rate of 90% from hatch with a 75% induction rate (Valenti, 1975). A cold shock applied to carp one to nine minutes after fertilization produced triploid and haploid offspring with low survival rates (Gervai et al., 1980). However, channel catfish (Ictalurus punctatus) subjected to a cold shock at five minutes post-fertilization for 1 hour resulted in 100% triploids and 79% hatching success (Wolters, 1981). Cold shocks have not been successful in producing polyploid salmonids. Repeated attempts at induced gynogenesis and polyploidy in rainbow trout and Atlantic salmon (Salmo salar) by cold shock were unsuccessful (Lincoln et al., 1974). Lemoine and Smith (1980) were unable to induce triploidy in brook trout using cold shocks, but did produce abnormal mosaics. Reftsie et al. (1982) produced no triploid coho salmon when subjected to a cold shock for four hours.

Hydrostatic pressure has been used to induce triploidy in animals. It has been used successfully to produce gynogenetic diploid zebra fish (Brachydanio rerio) (Streisinger, 1981) and triploid amphibians (Ferrier and Jaylet, 1978; Muller et al., 1978; Tompkins, 1978; Gillespie and Armstrong, 1979). Atlantic salmon that were exposed to a hydrostatic pressure shock of 7.0×10^4 kPa for three to six minutes produced 100% triploids with 80% survival relative to controls (Benfey and Sutterlin, 1984). In other experiments,

80 to 90% induction rates for triploid rainbow trout were achieved when pressure was applied at 40 minutes post-fertilization (Chourrout, 1984; Lou and Purdom, 1984).

Heat shocking has been the most effective and widely employed method of inducing triploidy (Table 1). Both triploids and tetraploids have been produced depending upon the time after fertilization the heat shock was applied to the eggs. Eggs shocked within the first hour after fertilization normally produced triploids, while eggs shocked anywhere from one to five hours post-fertilization or during the first mitotic division usually resulted in tetraploid individuals (Thorgaard et al, 1981).

Triploidy was induced in various urodeles and anurans with a heat shock of 35.0° C, applied to newly fertilized eggs for four to seven and a half minutes (Briggs, 1947). The optimal time observed by Briggs for the heat shock was 20 minutes post-fertilization. This parallels metaphase of the second meiotic division of the egg nucleus, which implies that the heat shock might partially denature the spindle fibers (Briggs, 1947; Fankhauser and Godwin, 1948), allowing the extra genetic material to be retained. Heat shocks also caused depolymerization of tubulin polymers that form microtubules, which are essential for the formation of the spindle apparatus (Rieder and Bajer, 1979). Ferrier and Jaylet (1978) used diploid female newts (Pleurodeles waltlii) that had a pericentric inversion of chromosome six as a chromosomal marker to show that the origin of triploidy was

caused by the suppression of the second meiotic division of the egg. The resulting offspring possessed two sets of maternal chromosomes and one set of paternal chromosomes. The optimal time to apply the heat shock in fish has been between 10 and 25 minutes post-fertilization for a time period of 10 to 20 minutes. The optimal heat shock temperature ranges from 26° C to 30° C with 28° C being most effective in terms of triploid induction and survival rates (Chourrout, 1980; Lincoln and Scott, 1983; Utter et al., 1983; Benfey and Sutterlin, 1984b; Solar et al., 1984; Johnstone, 1985). Hill (personal communication, 1985) reported 100% triploidy in chinook salmon with very acceptable survival rates of 70% with a 10 minute heat shock at 28.5° C applied 10 minutes after fertilization followed by slow cooling of the eggs back to ambient temperatures.

Higher temperatures and longer heat shock periods have increased the induction of triploids; but have also decreased the percentage of survivors. Lower temperatures and shorter heat shock periods have increased fish survival but resulted in lower proportion of triploids. Johnstone (1985) stated that the pursuit of optimal triploid induction rates disregarding high mortality, may not be the best scheme for producing large production runs of triploid fish. He found that triploid rates and mortality were positively correlated. He suggested using triploid yield (triploid induction rate x survival at hatch) as a better method of producing and reporting triploid success, instead of induction rates.

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

Species	Heat Shock Temp. (C)	Time After Fert.	Duration (min.)	Ploidy %	Surv. %	Author and Year (1900)
sturgeon	34	1-60	3	52.3	----	Vasetskii 67
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	74	Utter 83
chinook	29	10	10	60	88	Utter 83
Atlantic	32	5	5-15	100	80	Benfey 84
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	Johnstone 85
trout	28	40	10	90	40	Bye 86
trout	29	10	10	91-96	48-79	Thorgaard 86

Sterility in Triploids

Sterility is usually observed in adult triploids because of the abnormal development of the gametes. Since only two homologues can be present in any region at the time of meiotic pairing, the third homologue (especially if small) becomes a univalent after it fails to pair with either of the other two homologues (Lincoln, 1981; Solar et al., 1984; Strickberger, 1985). Sometimes the third chromosome might pair over a very short area to form a trivalent, which still may be randomly distributed to the gametes. This would result in some gametes having two homologues and others only one. The proximity of the third homologue may interrupt pairing, forming three univalents, that would be randomly separated to the gametes (Beatty, 1957; Strickberger, 1985; Myers, 1986).

Darlington and Mather (1949) found a triploid strain of hyacinths containing the make-up 3N = 8 + 8 + 8 = 24 that produces a variety of gametic chromosomes numbers ranging from 8 to 16. Many of these gametes unquestionably included unbalanced amounts of homologous chromosomes (three of one and zero of another), so fusion between them usually leads to the presence of some chromosomes in multiple dosage and the absence of others (Strickberger, 1985). These unevenly balanced chromosomes then impair the normal development of gametes, which require the appropriate genetic material. Practically, all sexually reproducing organisms that contain an odd-numbered set of chromosomes (triploids, pentaploids and septaploids) are sterile (Cassoni, et al., 1984; Strickberger, 1985).

Most triploid male fish are functionally sterile even though the reduction in gonadal development is less noticeable than in females. Other organisms, such as amphibians, have shown this type of male development (Fankhauser, 1940, 1941; Kawamura, 1951a, 1951b). This could be attributed to the larger size of the testes than the ovaries when their cells enter meiosis, at which point triploid gametogenesis is presumably disrupted (Benfey and Sutterlin, 1984a). Meiosis begins in the teleost ovary when oogonia are converted into primary oocytes and in the testes when primary spermatocytes become secondary spermatocytes (Nagahama, 1983). The meiotic process occurs later in testicular tissue of salmonids and after substantial mitotic proliferation (Robertson, 1953; Nakamura, 1982; Newport and Kirschner, 1982; Johnstone, 1985).

Growth of Triploids

It has been suggested that larger cell size and more DNA of triploid individuals should result in faster growth and larger sizes than diploids (Purdom, 1973). Reports on growth of numerous triploid species tends to be highly inconsistent. This is probably a result of differences between species, induction methods, experimental design and tolerances to changes in gene dosage. For example, triploid rainbow trout had slower growth rates than diploid trout (Solar et al., 1984). On the other hand, Atlantic salmon triploids showed no significant differences in growth from the diploids (Benfey and Sutterlin, 1984). Other papers report that triploid fish seem to grow at a slower rate than diploid fish (Refstie, 1981;

Thorgaard and Gall, 1979; Utter, 1983). While, Valenti (1975) observed triploid Tilapia aurea were larger than diploid fish at 14 weeks of age. This result could be misleading because of the small sample size that lived to the end of the 14 weeks.

Triploids shouldn't grow faster than diploids, but they may outgrow them during and after sexual maturation because the triploids won't be using energy on the production of gonads. Lincoln (1981) observed that sterile triploid plaice hybrids continued to grow while the diploid fish stopped growing four months prior to spawning. However, the diploids caught up with the triploids by the end of the 14 month experiment and there was no significant difference in growth or weight. Triploid channel catfish were significantly heavier compared to diploids at eight months or older, which corresponds with sexual maturity in catfish (Wolters, 1982). After the age of maturity (3.5 years), triploid rainbow trout had superior growth and higher mean weight compared to diploid fish of the same age (Thorgaard, 1986). In another experiment, triploid coho salmon at 30 months (onset of sexual maturation) showed no differences in length, body weight or condition factor contrasted to diploids (Johnson et al., 1986).

Gonadal Development

Triploid individuals usually develop non-viable gametocytes and often exhibit severely retarded gonadal development because of their sterility. For example, at seven months of age, triploid carp (Cyprinus carpio) showed retarded sex differentiation and the gonads were 0.7 $\pm$ 0.4% of the total

body weight compared to normal diploid gonads that were 10-25% of the total body weight, indicating the fish were sterile (Gervai, et al., 1980). In another experiment using fancy carp, triploids had less gonadal development at the age of 20 months than diploids (Taniguchi, 1986). Testes of triploid catfish were also smaller than diploid catfish and diploid ovaries were three to four times larger than triploid ovaries after eight months (Wolters et al., 1982). Krasznai et al., (1982) reported abnormal gonadal development in four and five year old triploid grass carp (Ctenopharyngodon idella Val. x Aristichthys nobilis Rich.), but some of the four year old fish exhibited secondary sexual characteristics. In triploid plaice (Pleuronectes platessa) x flounder (Platichthys flesus) hybrids and triploid hybrids between turbot (Scophthalmus maximus) x brill (Scophthalmus rhombus), female ovary growth was reduced and egg production was entirely suppressed (Lincoln, 1981b). However, there was normal gonad development in the male hybrid fish (Purdom, 1972; Lincoln, 1976; Lincoln, 1981, 1981a). Tabarini (1984) reported that triploid bay scallops (Argopecten irradians) were sexually underdeveloped compared to diploid scallops.

In triploid Atlantic salmon the GSI (gonadosomatic index) for females was only 7.7% of that of female diploids, and in males it was 52% of that of diploid males resulting in reduced gonad size (Benfey and Sutterlin, 1984b). In both sexes of triploid coho salmon gonadal development was severely impaired at 30 months. The average GSI of triploid coho females and

triploid coho males was 11.8% and 35.7% of diploids, respectively (Johnson et al., 1986). Gonad development of triploid female rainbow trout contained no oocytes, but the triploid male rainbow trout displayed no difference in structure and size from the diploid males (Lincoln and Scott, 1983; Lincoln and Scott, 1984; Solar et al., 1984). Thorgaard and Gall (1979) also noted in "spontaneous" occurring triploid rainbow trout that females showed no evidence of gonad formation; While, male triploids were identical to diploid males.

Survival of Triploid Hybrids

A possible advantage of triploid induction is enhanced survival of triploid hybrids. Triploidy provides one complete set of maternal chromosomes that could possibly increase survival of inter-generic and intra-generic hybrids. Sometimes essential hereditary material is missing in diploid hybrids because each parent supplies only a haploid set of chromosomes. This doesn't happen in triploid hybrids because the fish has one complete set of maternal chromosomes for development. Therefore, deficiencies seen in diploid hybrids are offset in triploid hybrids.

Diploid hybrids of bullfrog (Rana catesbiana) x green frog (Rana clamitans) all died during gastrulation, whereas triploid hybrids thrived (Elinson and Briedis, 1981). Scheerer and Thorgaard (1983) induced triploidy in brown trout (Salmo trutta) x brook trout, and rainbow trout hybrids. The triploid hybrids had higher survival rates than the diploid hybrids. In

some cases the triploid hybrids had a higher mortality to the eyed stage of development, but had better survival to the beginning of feeding. The increased initial mortality was probably a result of the heat shock rather than the triploidy. Parsons et al. (1986) reported that triploid rainbow trout females x coho salmon male hybrids showed increased survival to the fry stage relative to diploid hybrids. They also displayed a notable increase in IHN (infectious haematopoietic necrosis) immunity when compared to pure-species rainbow trout. Triploid hybrids were also reported to have increased viability in other species (Allen and Stanley, 1983; Chevassus et al., 1983).

Identifying Triploids From Diploids

Triploids usually don't differ morphologically from diploids. Diploid and triploid sticklebacks differed in certain body features but were identical in every other aspect (Swarup, 1959b). Rainbow trout triploids were exactly the same as diploids in physical structure but were smaller in average size (Solar et al., 1984). Triploid carp were phenotypically indistinguishable from normal carp excluding a minor disturbance in scale pattern (Gervai, et al., 1980). Triploid frog embryos and larvae had larger cell size and fewer number of cells than diploids, but still developed normally and were identical to normal larvae (Briggs, 1947).

On the cellular level diploid and triploid individuals can be distinguished. Karyological analysis reveals that triploids possess an extra haploid set of chromosomes over the normal diploid set. Generally, the nuclear size increases in

proportion with an increase in chromosome number (1/3 more DNA), while the nucleo-cytoplasmic ratio is maintained. In animals, larger cell size doesn't affect the ultimate size of the animal (Fankhauser, 1945; Swarup, 1959b). This regulation of the size of the individual is the result of a decrease in the total number of cells (Purdom, 1973).

Ploidy Analysis Methods

There are several different techniques available for determining the ploidy level of an organism. Chromosome preparations (karyotyping), erythrocyte parameters (Coulter Counter), microfluorimetry or microdensitometry and flow cytometry.

Solid Tissue Karyotyping

Solid tissue karyotyping (STK) is inexpensive and requires very little specialized equipment (Kligerman and Bloom, 1977). However, it can be very difficult or unsuccessful (Thorgaard et al., 1982; Sweet, personal communication, 1986). Preparations made from embryos (Myers, 1986) or very young fish produce the best results, unless tissue culture techniques are used. STK is more difficult in teleost fish than other vertebrates due to the small size and large numbers of chromosomes (Blaxhall, 1983). More importantly, STK is very time consuming, and labor-intensive and the number of fish that can be processed are limited.

Lymphocyte and Tissue Culture Karyotyping

Lymphocyte and tissue culture karyotyping may give better results than solid tissue techniques because a mitotic stimulant (phytohemagglutinin - PHA) is used to synchronize cell division (Wolters et al 1981a), which can then be followed by a colchicine treatment to stop cells at the same time in metaphase. STK only uses colchicine to stop normally dividing cell in metaphase. Some tissue cultures that use trypsin to emulsify tissue into single cells, also produce good metaphase spreads when treated with PHA and colchicine (Blaxhall, 1975). The major problem with these two methods is that they require more sophisticated equipment (culture media, centrifuges, and incubators) than STK and only a limited number of samples can be processed.

Erythrocytes for Triploid Identification

Erythrocytes can be used in a number of ways to identify triploid individuals. Triploids have an additional haploid set of chromosomes (more DNA) and therefore have larger nuclei. Nuclear measurements in fishes are often done on erythrocytes since they are nucleated and can be easily obtained by drawing blood. This method has been used to identify triploidy in Atlantic salmon (Allen and Stanley, 1979; Benfey et al., 1984), Poeciliopsis (Cimino, 1973), rainbow trout (Refstie, 1981; Lincoln and Scott, 1983), and channel catfish (Wolters et al., 1982c). There are several others measurements that are reliable in determining ploidy: cell major axis, cell surface area, nuclear major and minor axis and nucleus volume (Beck and

Bigger, 1983).

The only disadvantage to erythrocyte measurement methods is the less than 100% accuracy of determining the ploidy level (Thorgaard and Gall, 1979; Wolters et al., 1982). The Coulter Counter and Channelyzer allow rapid (5,000 cells/minute) separation of diploid and triploid fish based on median erythrocyte volume (Benfey and Sutterlin, 1984a) but is less accurate than flow cytometry in identifying mosaicism.

Microfluorimetry and Microdensitometry

Microfluorimetry or microdensitometry can also be used to determine ploidy. This technique measures the DNA content of single cells in a microscope from a blood smear (Gervai et al., 1980). The major disadvantages are that it is labor-intensive and time consuming, but it is a reliable way to identify triploids (Johnstone, 1985).

Flow cytometry

Flow cytometry can be used to analyze the DNA content of large numbers of cells in interphase with accuracy and speed surpassing that of other quantitative methods (Callis and Hoehn, 1976). Flow cytometry measures laser excited fluorescence of stained nuclear DNA (Allen, 1983). A typical flow cytometer has four major component systems: a light source, (usually a laser), a simple chamber and optical assembly, a set of associated electronics (to convert light impulses to digital signals) and a computer system to control instrument operations, collect data, and perform analytical

routines (Downing et al., 1984; Lovett et al., 1984).

The flow cytometer (Downing et al., 1984) suspends monodispersed cells in an aqueous solution (sheath solution) that are transported to the detection area by air pressure or vacuum rates at hundreds to thousands of cells per second. Laminar flow forces the cells into a single file in the center of the sheath solution and electrical and optical signals are recorded. Electrical signals are based on the Coulter Counter idea that cells will produce a pulse proportional to the cell volume when suspended in an electrically conducting medium. The laser or light source produces the optical signals as the cells intercept the focused light. The cells may absorb or scatter the incident light or if labelled with a fluorescent dye (eg. propidium iodide) emit fluorescent light at a lower energy and a longer wavelength that results in a different color being produced. Propidium iodide, that was used in this study, is a fluorescent dye that intercalates into double-stranded DNA and RNA. The flow cytometer can measure both light scatter (90^{o} or forward) and absorption, light scatter and fluorescence or the electronic cell volume and fluorescence (Downing et al., 1984). All three were measured in the study. In measuring the amount of DNA the number of cells in the various phases of the cell cycle are recorded and the presence of cells that contain abnormal (aneuploid) amounts of DNA as compared to normal diploid cells (Downing et al., 1984).

Flow cytometry has been widely used to determine ploidy of fish (Thorgaard et al., 1982; Allen, 1983; Allen and Stanley,

1983; Utter et al., 1983; Hill, personal communication, 1985; Johnson et al., 1986; Myers and Hershberger, 1986; Seeb et al., 1986). In a recent study comparing the reliability of a Coulter Counter with a flow cytometer for ploidy analysis of Pacific salmon, the flow cytometer accurately determined the ploidy of 200 diploid and triploid fish, while 11% of the Coulter Counter histograms were unreadable (Johnson et al., 1984).

MATERIALS AND METHODS

The Materials and Methods has been divided into two separate sections: experimental runs and Michigan Department of Natural Resources (MDNR) Production Runs. The experimental runs were small scale (10,000 eggs) research projects designed to determine the best technique to induce triploidy in chinook salmon. The MDNR Production Runs were designed to produce large numbers (50,000-200,000) of triploid chinook salmon for stocking in the Great Lakes.

All eggs and sperm were collected from spawning chinook salmon at the Little Manistee Weir (Stronach, MI) by the Fish Division of the MDNR. The Little Manistee Weir is an egg collection station (Figure 1). The weir and a fish ladder direct returning coho and chinook salmon to outdoor holding ponds where they are kept until ripe. Ripe fish are stripped and eggs are water hardened in a specially designed egg-take building. There are no provisions for on site egg incubation, so all eggs are transported to hatcheries for incubation and rearing.

Eggs from the experimental runs were incubated and reared at the Michigan State University Aquaculture Laboratory, in East Lansing, MI. The distance to MSU's Aquaculture laboratory is approximately 170 miles requiring three to four hours of transport. All MDNR Production Run

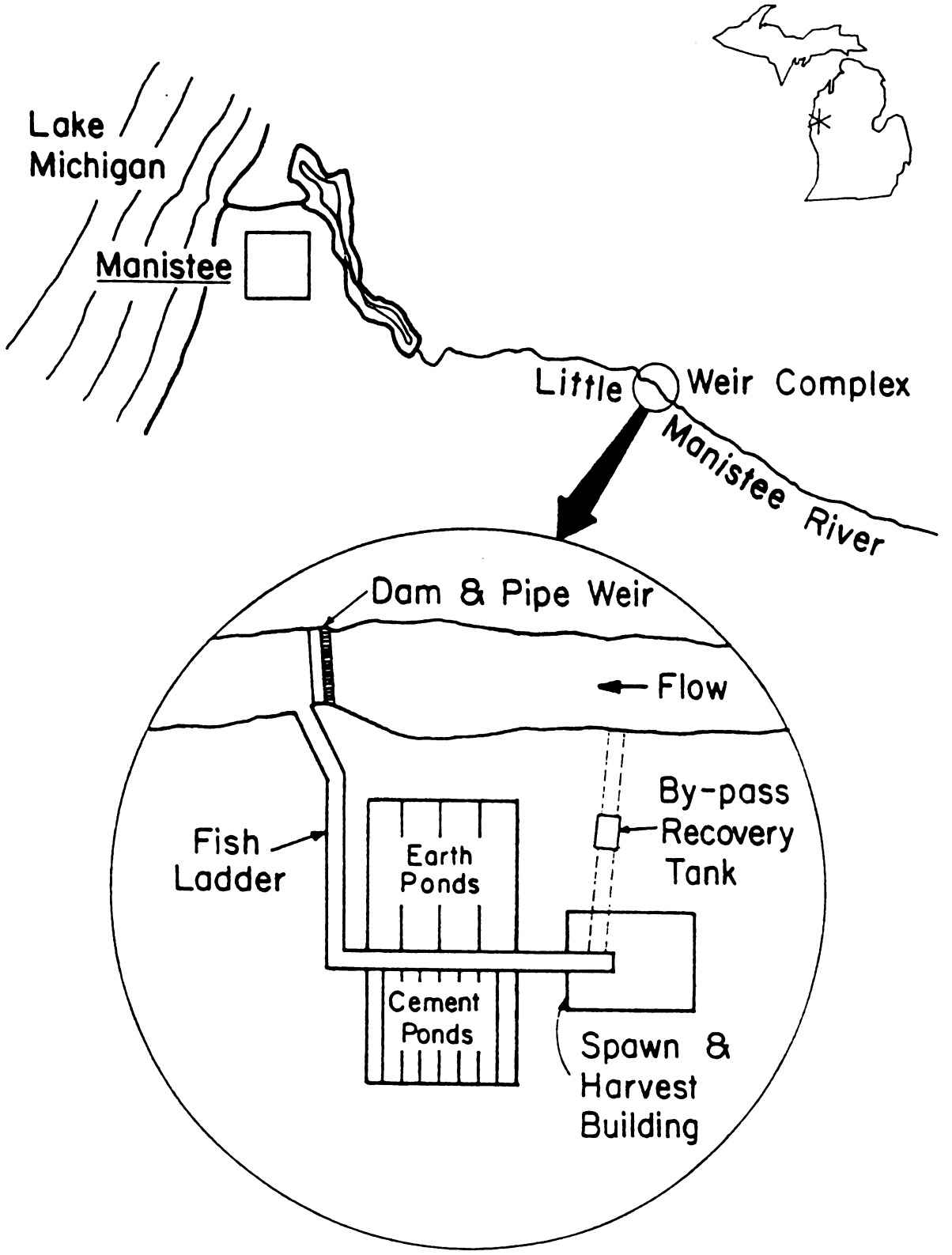


Figure 1. Location and schematic diagram of the Little Manistee River weir complex (Hay, R.L. 1986).

lots were incubated, reared, and micro-tagged at Wolf Lake State Fish Hatchery, Mattawan, MI. The distance to Wolf Lake State Fish Hatchery is approximately 180 miles and also requires three to four hours of transport. Stocking decisions for the MDNR Production Runs were made and carried out by the MDNR, Fish Division according to overall Great Lakes Management Objectives.

Experimental Runs

In 1985, experimental runs were designed to determine the optimal duration of heat shock and type of cooling method for producing "trophy" triploid chinook salmon for stocking the Great Lakes. A portable and inexpensive recirculating heat shocking system was developed to produce triploids at the Little Manistee Weir. Experimental runs in 1986 were designed to determine the differences in triploid yield and survival rates of eggs fertilized at the weir and eggs delayed fertilized at the laboratory during early, mid, and late runs.

1985 Experimental Runs

Chinook salmon eggs and sperm were kept in separate dry containers on ice until fertilization. Eggs and sperm were mixed using the dry fertilization method (Piper et al., 1983). They were water activated and washed in 10° C river water at two minutes post-fertilization. Five minutes later the eggs were divided into five groups containing approximately 2400 eggs each, and placed into five floating

screen baskets (5" x 5" x 5" with styrofoam collars) in one gallon square pails filled with 10° C river water.

At 10 minutes post-fertilization four groups were simultaneously placed in a 28.5 ± 1° C recirculating heat shock system for 0, 5, 10, 15, or 20 minutes (Table 2). The heat shock system consisted of a 172 quart ice chest (Gott Inc. 45" x 19" x 21") filled with river water. The water had been heated with a 1500 watt thermostatically controlled (sensitivity ± 1° C) immersible heater (Heet Grid, George Ulanet Company, Newark, NJ) to 28.5° C (approximately two hours) and maintained. Two corrosion free submersible pumps (Little Giant Pumps, Oklahoma City, OK) kept the water recirculating continuously into four one gallon square pails at 1.5 gallons per minute. The floating screen baskets were placed in the recirculating gallon pails, which were three-quarters submerged in the water bath. The water was pumped up from the bottom and through the eggs. The water flowed over the top edge of the pail and back into the bath. The flow was sufficient to suspend eggs in the water column, but not strong enough to wash eggs out of the floating screen baskets. All four pails with screen baskets and eggs were set on top of weighted gallon pails for support.

Each treatment group was rapidly cooled by being placed in a 120 quart ice chest filled with river water at ambient (10° C) temperature for five minutes. After five minutes the baskets were removed and the eggs were poured carefully into gallon pails containing river water for water hardening at

Table 2. October 3, 1985 research design for heat shocking chinook salmon eggs at 10 minutes post-fertilization in 28.5 ± 1° C water with rapid or slow cooling.

Heat Shock Treatment and Cooling Method		
Group	Heat shock Duration (min)	Cooling Method
Control R[1]	0	Rapid
1	5	Rapid
2	10	Rapid
3	15	Rapid
4	20	Rapid
Control S[1]	0	Slow
1	5	Slow
2	10	Slow
3	15	Slow
4	20	Slow
Control[2]	0	None

1 Sham heat shocked identical to heat shocked groups.

2 Normal hatchery techniques applied.

ambient river water temperature.

Another five groups of 2000 eggs each were fertilized, separated and heat shocked as described above. However, these groups were cooled slowly for approximately 30 minutes (Hill, personal communication, 1985). After heat shocking the eggs, all screen baskets were placed into a five gallon bucket that contained two gallons of heated water (28.5° C). Then one gallon of river water (10° C) was added by hand to the five gallon bucket at three minute intervals up to 18 minutes (starting at time zero). At 21 minutes, two gallons of ambient river water were added up to 27 minutes, at which time water was no longer added. Temperature readings were recorded at one minute intervals following addition of water. All control lots were sham-heat shocked by moving and immersing the eggs for a similar period of time in ambient water.

The eggs were removed from the bucket and carefully poured into gallon pails containing river water at ambient temperature to water harden. All eggs were water hardened for more than one hour before transport to the Michigan State University Fish Culture Laboratory.

The eggs were transported in plastic one gallon pails half filled half with water. The egg transport pail was placed inside an empty one gallon plastic pail that was placed on ice inside of a 172 quart cooler.

At the lab, each group was split into four sub-samples and randomly placed in one of 24 egg incubation trays

contained in three Heath incubation systems (Heath Tenca Inc.). Each tray was divided into four equal sections. Each stack of eight trays was supplied with 11° C aerated well water at a rate of one gallon per minute. Dead eggs, which appeared white were removed frequently to prevent potential mortalities caused by the spread of fungus. After hatching, sac-fry were held in the incubator trays until they reached the swim-up stage. Swim-up fry were then transferred to 30 gallon aquariums and reared on a soft pelleted feed (Bio Diet #'s 3 & 4). At the fry stage, the fish were fed Purina Trout Chow (#'s 3, 4, and 6) until 150 days post-fertilization.

On December 3, 1985 approximately 75% of all fish remaining were taken to Wolf Lake State Fish Hatchery because lack of space at MSU's Aquaculture Laboratory. The fish were held under the supervision of John Hnath. After ploidy analysis, by flow cytometry, the fish were stocked in outdoor ponds.

Survival of eggs and fry were recorded regularly from fertilization to 150 days post-fertilization. Mortality was divided into four development stages (day one, eyed egg, hatching, and fry) and recorded as cumulative percent mortality.

1986 Experimental Runs

The 1986 research objectives were designed to test differences in triploid yield and survival rates during early, mid, and late runs. Eggs were either fertilized and heat shocked at the weir or transported to the Michigan State

University Fish Culture Laboratory for delayed fertilization (four to five hours after collection) and heat shock. Standard methods for dry and delayed fertilization were used (Piper, et al., 1983). All treatments are outlined in Table 3.

The early season heat shock treatments were applied on September 19, 1986. The river water temperature was 11° C at the weir and at MSU's Laboratory the well water was 13° C. Cooling temperatures were nearly identical (± 2° C) for each treatment at their respective heat shocking sites. All groups were cooled to ambient temperatures within 15 minutes.

The mid season run was taken on September 30, 1986. The procedure used for egg collection, fertilization, heat shocking, cooling and rearing fry were identical to the methods used in the September 19 experiment. The water temperature was 18° C at the weir and 13° C at the laboratory.

The late season heat shock with fish that were held for 7-10 days after the last fish entered the Little Manistee Weir, was taken on October 24, 1986. It was very similar to the other experiments (heat shocking and cooling), except the delayed effect was separated from the transportation effect by heat shocking the eggs at exactly the same time, both at the weir and at the laboratory. The water temperature at weir and at the laboratory was 11° C and 13° C respectively.

Eggs and sperm were collected from six females and six males for each experimental run. The eggs and sperm were

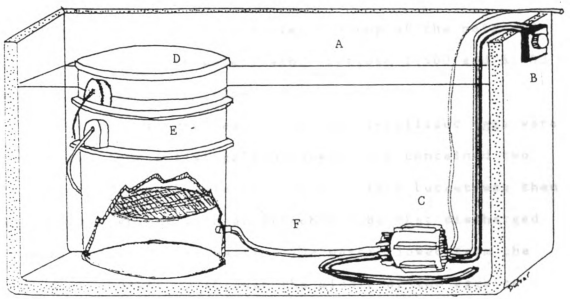
Table 3. Research design of 1986 experiments to determine the optimal time of egg take during the spawning season and the effects of delayed fertilization on triploidy induction and survival of chinook salmon eggs.

	Number of Fertilization Treatments				
	Heat Shocked		Control		Delayed
	Weir	Delayed at Lab	Weir	Delayed at Lab	Weir
Time of egg take during spawning					
early season	2	2	2	2	0
mid season	2	2	2	2	0
late season fish held 7-10 days	2	2	2	2	2

split into two groups: One group was fertilized and heat shocked immediately at the weir and the second group was used for delayed fertilization and heat shocking at the lab. Unfertilized eggs and sperm were stored in dry containers and transported on ice to MSU's Fish Culture Laboratory. Eggs were divided into four groups (two heat shock and two control) at the weir and at the laboratory. The entire process for fertilization and heat shocking (10 minutes post-fertilization for 10 minutes in 28.5 $\pm$ 1^{o} C) at the weir and at the laboratory was identical to techniques used for the 1985 experimental runs.

The heat shock system that was developed consisted of a 172 quart water bath (Gott Inc., ice chest), a 1500 watt immersion heater ((Heet Grid, George Ulanet Company, Newark, NJ) with a thermostat accurate to $\pm$ 1^{o} C, a corrosion free submersible pump (Little Giant Pumps, Oklahoma City, OK), two modified two and one half gallon plastic buckets, and half inch tygon tubing (Figure 2). The heat shock chamber was constructed by attaching a male tubing fixture to a hole made in the lower side of a two and one half gallon bucket. The chamber was partially submersed in the water bath and received a constant upwelling of heated water by connecting it to the submersible pump with tygon tubing. The pump was placed in the heater loop and rested on the bottom of the 172 quart water bath.

Fertilized eggs were placed in another two and one half gallon plastic pail that had the solid plastic bottom removed



A. 172 QUART ICE CHEST (45" x 19" x 21")
B. 1500 WATT THEMOSTATICALLY CONTROLLED IMMERSION HEATER
C. CORROSION FREE SUBMERSIBLE PUMP
D. SCREEN BOTTOMED FIVE GALLON BUCKET
E. MODIFIED FIVE GALLON BUCKET
F. TYGON TUBING (1/2 INCH)

Figure 2. Portable heat shocking system (recirculating).

and replaced by fiberglass screening cemented to the bottom rim with silicone. The pail was placed in the heat shock chamber (two and one half gallon pail with pump attached) and then the eggs were carefully poured in the double bucket arrangement. The heated water was forced through the eggs at a constant rate (1.5 gpm), which continuously bathed the eggs. The heated water exited over the top of the pail and back into the 172 quart water bath. Between 1,500 and 3,000 eggs were treated at one time.

After the 10 minute heat shock, the fertilized eggs were gently poured into a five gallon bucket that contained two gallons of heated bath water (28.5° C). Each bucket was then placed under a spigot with an attached tube that discharged one gpm of ambient river water. The water flowed from the bottom of the bucket up through the middle of the eggs to slowly cool them to ambient temperature. The water temperature (11°-18° C) was different between early, mid and late runs at the weir. However, well water at the laboratory remained fairly constant (11-13° C) throughout the entire experiment. All eggs returned to ambient temperature within 15 minutes.

Fertilized eggs heat shocked at the weir were water hardened for one hour before transport on ice (in coolers) to the Fish Culture Laboratory at Michigan State University. All eggs were slowly acclimated to the well water in the lab before being randomly assigned to a tray in the incubation system. Each treatment was sub-divided into two sub-samples

for statistical purposes. Dead eggs were removed frequently and mortality was recorded through hatching.

After hatching, sac-fry were held in the incubator trays until they reached swim-up stage. Swim-up fry were then transferred to 30 gallon aquariums and reared on a soft pellet feed (Bio Diet #'s 3 & 4) until they were between one and two inches long. Dead sac-fry were removed as often as possible and mortality was recorded until end of hatching.

MDNR Production Runs

All eggs were subjected to a heat shock of 28.5 $\pm$ 1° C, applied at 10 minutes post-fertilization for 10 minutes, followed by a period of slow cooling to produce triploid chinook salmon (Hill, personal communication, 1985). The heat shock system was similar to the system used for the 1986 experimental runs (Figure 2). Except, five gallon pails were used to replace the two and one half gallon buckets to accommodate larger egg volumes per treatment (20,000-30,000) for the MDNR Production Runs. Cooling procedures were identical to the system used for the 1986 experimental runs.

1985 MDNR Production Runs

Two MDNR Production Run lots of 160,000 eggs were heat shocked to induce triploidy by Michigan State University personnel in order to obtain 50,000 fish for stocking.

On October 24, 1985 approximately 20,000 eggs (from five females) were used for each heat shock treatment. Milt was collected from at least five or six ripe males, as needed and

kept in a dry (squirt) bottle on ice until fertilization. Eggs and sperm were mixed by hand using dry fertilization techniques (Piper et al., 1983) in a five gallon plastic pail. Water (12° C river water) was added two minutes after fertilization and the fertilized eggs were rinsed before being heat shocked.

Eight groups of eggs were heat shocked (four per heat shock system) for a total of approximately 160,000 chinook salmon eggs for the first MDNR Production Run. Temperatures were recorded at 0, 1, 3, 6, 9, and 10 minutes during the heat shock to measure temperature fluctuation in the heat shock chamber. The water used for cooling the eggs back to ambient temperature was 12° C river water. Cooling temperatures were recorded at 0, 1, 3, 6, 9, 12, and 15 minutes following the heat shock.

All groups were water hardened in the above system for at least one hour before being transported in 10 gallon milk cans to Wolf Lake State Fish Hatchery (Mattawan, Michigan) by MDNR personnel. Upon arrival at Wolf Lake, eggs were placed in vertical incubation trays and mortalities were recorded on a regular basis by hatchery technicians.

Survival after day one for the first MDNR Production Run was very low (10-15%). Another MDNR Production Run was made on November 1, 1985 to try and reach our goal of 50,000 triploid chinook salmon for stocking in the Great Lakes. This second run used delayed fertilization techniques (Piper, et al., 1983) instead of dry fertilization, in hopes of

increasing survival.

Eggs and sperm were stripped by MDNR personnel at the Little Manistee Weir and packed dry on ice for transport to Wolf Lake State Fish Hatchery. Approximately 160,000 chinook salmon eggs were delayed fertilized (6-8 hours after stripping), heat shocked (8 total) and cooled by methods described for the MDNR Production Run on October 24, 1985 with two modifications. The temperature of the spring water was 11° C at Wolf Lake State Fish Hatchery. Heat shock and cooling temperatures were recorded at the same time intervals used during the October 24 , 1985 MDNR Production Run. Mortalities were recorded from egg until stocking in early spring by MDNR personnel.

All fertilized eggs were placed directly into vertical incubation trays immediately after cooling for water hardening and subsequent incubation. In early spring of 1986 all triploid fry were adipose fin clipped and micro-tagged in a cooperative effort by MSU students and MDNR personnel at Wolf Lake State Fish Hatchery for stocking in the Great Lakes.

1986 MDNR Production Runs

The MDNR requested 150,000 triploid chinook salmon for the 1986-87 Great Lakes stocking program. The procedures used for triploid induction were similar to those during the MDNR Production Runs of 1985, except more eggs were heat shocked (30,000 eggs) per run through the system. A grand total of 2,197,000 eggs were shocked.

The eggs for the MDNR Production Run were heat shocked on October 9, 1986. There were several differences in the conditions and procedures used in 1985. The river water temperature was 13° C. Ten gallon metal milk cans were used to cool the eggs. The heat shocked eggs were transported to Wolf Lake State Fish Hatchery in the 10 gallon cans filled to the top with river water and capped.

A second Production Run was taken on October 16, 1986 under similar conditions and methods as the October 9, 1986 MDNR Production Run. The river temperature was 11° C. Five gallon plastic pails were used to slowly cool the eggs back to ambient temperature, instead of the 10 gallon milk metal milk cans. The heat shocked eggs were transported in egg boxes (Piper, et al., 1983) to Wolf Lake State Fish Hatchery.

1987 Production Runs

A total of 200,000 triploid chinook salmon were requested by the MDNR for stocking in the Great Lakes. The entire procedure was identical to the 1985 MDNR Production Runs. A total of 2,161,680 eggs were taken on October 6 and 7 during the peak run. The first day 1,000,680 eggs were heat shocked. The second day 1,161,000 eggs were heat shocked. The water temperature was a constant 9° C.

Ploidy Analysis of Triploid Chinook Salmon

Ploidy levels for 1985 (experimental and MDNR Production Runs) and 1986 MDNR Production Runs were determined by the use of a flow cytometer at Sparrow Hospital, under the

direction of Martin Oaks, Ph.D. Samples were run on a Coulter (Hialeah, FL) Epics-V Flow Cytometer with a 256-channel analyzer interfaced with a Multiparameter Data Acquisition and Display Computer System (MDADS). A five watt argon ion laser (Coherent, Inc., Palo Alto, CA.) emitting at 488 nm was operated at 500 mW power. A 590 nm longpass absorbance filter was used in monitoring propidium iodide fluorescence.

A single drop of blood was drawn from each fish by cardiac puncture and suspended in a sodium citrate - DMSO buffer solution to prevent clotting (Vindelov et al., 1982). Each sample was frozen in liquid nitrogen (-80° C) for long term storage. The detergent-trypsin method developed by Vindelov and coworkers (1983a) was modified and used for fluorescent staining of nuclear DNA by technicians at Sparrow Hospital (Appendix A). Twenty fish per group were used for ploidy determination. Chicken red blood cells were used as an internal standard to check the daily variation of the flow cytometer (Vindelov et al., 1983b).

The chinook salmon blood samples from 1986 experimental runs and 1987 Production Runs were analyzed on an Ortho Diagnostic Systems, Inc. Model 50-H dual laser Cytofluorograph connected to an Ortho 2150 Computer System. The computer system used Ortho's Cytofluorograph Analysis for Cellular DNA Content of Fixed Cells with DNA Doublet Discrimination to determine ploidy level. The argon-ion laser was tuned to 488 nm with 0.5W output power. The flow

cytometer was located on Michigan State University's campus in Glitner Hall and was under the supervision of Dr. Kathy Brooks.

When a sample was analyzed containing chicken red blood cells (internal reference standard) and salmon red blood cells, a pair of fluorescence pulse-height histograms were produced. Diploid chinook salmon red blood cells were used as a second internal reference standard because samples were run on different days (Vindelov et al., 1983b). A total of 10,000 to 20,000 cells were counted for each sample to insure proper ploidy determination.

A new protocol was used at the MSU facility in preparing and staining the samples with propidium iodide. This method was very simple and more cost effective for analyzing hundreds of samples. The protocol for DNA analysis of chinook salmon red blood cells at Michigan State University using propidium iodide can be outlined as follows:

1. Blood was drawn from each fish by cardiac puncture using a 22 gauge needle and a 5 ml syringe containing about 1 ml of sodium citrate buffer (pH 7.2) to prevent clotting.

2. The mixture was added to a 12 x 75 mm test tube containing 0.5 ml of the buffer solution until a faint pink color was obtained.

3. The cells were centrifuged at 2500 rpm at 10° C for five minutes and the supernant was discarded. The cells were loosened before being fixed in alcohol to prevent clumping.

4. Red blood cells were fixed with alcohol (70% ethanol or methanol).

a. The alcohol was added dropwise at -20° C on vortex at a concentration of 2 parts alcohol to 1 part cells.

b. Cells were fixed on ice for 15 to 30 minutes.

5. Cells were removed from the alcohol by centrifugation at 2500 rpm at 10° C for five minutes and the supernant was discarded.

6. Cells were resuspend in 1.5 ml propidium iodide:triton-X 100:EDTA solution mixed in phosphate buffer solution.

7. 0.5 ml of ribonuclease-A (200 U/ml) was added.

8. The stained sample was run on an Ortho Cytofluorograph at 0.5 watts.

9. Chicken blood and diploid chinook salmon blood were used as internal standards.

1986 Growth Study Between Diploid and Triploid Chinook Salmon

A 10 week controlled growth study was conducted at the MSU Aquaculture Laboratory in the spring of 1986. All fish were held in 110 liter aquaria with flow rates of 250 ml/min of 11° C aerated well water. Eight tanks, four replicates of each group of 20, one to two inch triploid or diploid chinook fingerlings were fed commercial feed (Biodiet followed by and Purina Trout Chow) at 3.5% their wet body weight per day. The feed was divided in two equal feedings adjusted every two weeks. Fish were weighed (Garling and Wilson, 1976) every two weeks and average daily gains were computed for diploid and triploid fish.

Observations of Captive Chinook Salmon Stock 1985-1986

Plans were made to hold five hundred micro-tagged triploid and five hundred diploid chinook salmon produced in 1985 at Wolf Lake State Fish Hatchery for up to five years. Initially, each group of fish was divided into two equal groups and were being held in replicate linear tanks. Fish care was provided by MDNR personnel under the direction of Mr. John Hnath. Fish were reared in 10^{o} C spring water with a dissolved oxygen level between 8-9 ppm with a flow between 8 and 10 liters per minute. They were fed Biodiet, a commercial fish feed, by using automatic feeders at a calculated percentage (3.0%) of their wet body weight per day, which was readjusted monthly. In March of 1986, both groups of fish were moved to outdoor rearing ponds because of space limitations in the lab.

Random monthly observations were made on survival, condition factor (Weight x 10^5/Length^3) and growth of diploid and triploid fish from September 1986 to December 1987. A random sub-sample of 20 one year old fish per group were examined for growth (length and weight), gonadal development and micro-tag retention (triploids only).

Micro-tagging of Triploid Chinook Salmon

Micro-tagging of triploid fish was performed in early spring of 1986, 1987, and 1988 at Wolf Lake State Fish Hatchery as a joint undertaking by MSU and MDNR for the Production Run fish for the Great Lakes. The micro-tagging

system included a tagging unit and a quality control device (Northwest Marine Technology Inc.). The tagging unit implanted a coded wire tag in the snout of the fish. The coded wire was 1 mm long and about the width of a hair. The quality control device magnetizes the tag in the fishes snout and separates the fish into tagged and untagged groups. The device automatically counts both categories and sounds an alarm when an untagged fish passes through the system.

Triploid fish were anesthetized with MS 222 and adipose fin clipped by MDNR workers a few weeks before tagging was to start. The micro-tag was implanted in the snout region and fish were sorted by the quality control device before being held in well oxygenated raceways to enhance recovery.

MDNR Production Runs

In 1985-86, only two micro-tagging machines were available to mark triploid chinook salmon. Nearly 44,000 fish were tagged over an eight day period by five people working eight hours a day. In 1986-87 a total of 145,000 triploid chinook salmon were micro-tagged. It took six days averaging 25,000 fish a day to tag all the fish. Five machines were available to mark the triploid fish. A total of 229,952 triploid chinook salmon were marked in four days for 1987-88. The micro-tagging procedure was identical to 1986-87.

Captive Chinook Salmon Stock

The fish for the captive growth study which began in 1985 were tagged after all the MDNR Production Run fish were tagged. Triploid fish were taken from 1985 experimental groups SM10 and SM15 and diploid fish were taken from NCO held at the Wolf Lake Hatchery Fish Disease Parasitic Laboratory (Table 2). A total of 521 triploids and 647 diploids were tagged

Statistical Analysis

The October 3, 1985 experimental lots were tested for survival and triploid induction rates between slow and rapid cooling of chinook salmon eggs. A 5 x 2 contingency table with dichotomous responses with ordered treatments and unbalanced data was used (Gill, personal communication, 1987; Gill, 1986). The 1985-1986 captive chinook salmon were tested statistically by one-way analysis of variance on length, weight, and condition factor for diploid and triploid chinook salmon held at Wolf Lake State Fish Hatchery (Gill, 1978). The 1986 growth study was tested by one-way analysis of variance for differences in average daily gain between diploid and triploid chinook salmon.

RESULTS

Experimental Runs

1985 Experimental Runs

Eggs were heat shocked at the Little Manistee Weir on October 3, 1985. The eggs used for heat shocking were taken during the "peak" spawning run at the weir. Therefore, the majority of the eggs were assumed to be in optimal condition (ripeness) or quality for fertilization and heat shocking. The eggs were dark orange in color and size was nearly uniform among females.

Slow cooling temperatures were recorded at one minute intervals after the addition of 10° C river water (by hand) for up to 28 minutes (Table 4). Water temperatures for slow cooling remained fairly consistent from one heat shock group to another, with all heat shock groups returning to ambient temperature within 30 $\pm$ 4 minutes. Rapid cooled groups were cooled to ambient temperature within five minutes. Care was taken to avoid excessive agitation caused by the addition of water to the eggs being slowed cooled, which could have increased mortality.

Eggs were incubated at MSU's Fish Culture Laboratory. The average survival for non-heat shock groups of chinook salmon eggs was 76.5% at the time of hatching. Garling and

Table 4. October 3, 1985 slow cooling data for heat shocking chinook salmon eggs at 10 minutes post-fertilization in 28.5 ± 1° C water.

Five Minute Heat Shock

Time (min)	0	3	6	9	12	15	18	21	24	27
Gallons (gal)	1	1	1	1	1	1	1	2	2	2
Reading (min)	1	4	7	10	13	16	19	22	25	28
Temp. (°C)	20.5	18.0	16.0	15.0	14.0	13.5	13.0	11.5	11.0	10.5

Ten Minute Heat Shock

Time (min)	0	3	6	9	12	15	18	21	24	27
Gallons (gal)	1	1	1	1	1	1	1	2	2	2
Reading (min)	1	4	7	10	13	16	19	22	25	28
Temp. (°C)	21.0	18.0	16.5	15.0	14.0	13.5	13.0	12.0	11.5	11.0

Fifteen Minute Heat Shock

Time (min)	0	3	6	9	12	15	18	21	24	27
Gallons (gal)	1	1	1	1	1	1	1	2	2	2
Reading (min)	1	4	7	10	13	16	19	22	25	28
Temp. (°C)	21.0	18.0	16.0	15.0	14.0	14.0	13.0	12.5	12.0	11.0

Twenty Minute Heat Shock

Time (min)	0	3	6	9	12	15	18	21	24	27
Gallons (gal)	1	1	1	1	1	1	1	2	2	2
Reading (min)	1	4	7	10	13	16	19	22	25	28
Temp. (°C)	21.0	18.0	16.0	15.0	14.0	14.0	13.0	12.0	11.0	11.0

Masterson (1985) reported 50% survival rates at hatching for the same facility under similar conditions. Therefore, it can be assumed that incubation conditions were advantageous for development of chinook salmon in the drip incubator trays (Heath Tecna Corporation).

Mortality data was divided into four developmental stages (day one, eyed, hatch and fry) and recorded as cumulative percent (Table 5). The highest mortality usually occurred during day one (24-48 hours after fertilization) and hatching for the majority of the treated groups. Although some groups had a higher mortality rate in the eyed stage. There was no trend in mortality between rapid and slow cooled groups. Some groups had lower survival at one stage and higher survival at the next for the same heat shock. For example, RC15 v.s SC15 and RC20 v.s SC20 in Table 5. RC15 had a higher day one mortality than SC15 but at eyed stage SC15 had more deaths. Conversely, SC20 had higher mortalities at day one and eyed stage than RC20, but at hatching RC20 had more dead sac-fry. After hatching, mortality was primarily attributed to over-crowded conditions (insufficient space) and fish jumping out tanks at night. Fish were found in the morning on the screens, table and floor.

There were no distinguishable morphological differences between larval or juvenile chinook salmon in any groups from October 3, 1985 experimental runs. All triploid groups contained several (1 to 8) deformed individuals that were missing eyes, lower jaws, opercula or had curved vertebral

Table 5. Percent cumulative mortalities or percent during stage of chinook salmon eggs heat shocked (ten minutes post-fertilization, for ten minutes at 28.5 ± 1° C) October 3, 1985.

	DEVELOPMENTAL STAGE			
	Day One (24-48 h)	Eyed (10-17 d)	Hatch (40-48 d)	Fry (152 d)
Groups	%	%	%	%
RCC0	9.2	13.4	18.4	21.3
RC5	18.5	25.4	32.7	36.7
RC10	22.5	40.3	64.7	69.0
RC15	26.7	42.8	67.7	73.7
RC20	29.6	56.7	85.8	91.1
SCC0	14.2	20.9	28.6	31.5
SC5	18.9	31.4	54.7	61.8
SC10	20.2	32.1	46.9	52.0
SC15	25.1	45.6	71.4	77.5
SC20	42.0	60.9	83.0	88.1

SC - slow cooling
RC - rapid cooling
SCC0 - slow cooling control
RCC0 - rapid cooling control

columns (lordosis and scoliosis). Also, a majority of the fish experienced a small hemorrhage on top of the head (origin unknown). However, the hemorrhage did not seem to affect survival. Most deformed fish died prior to fry stage, except some of the one-eyed individuals that survived to the termination of the experiment (150 days).

There was a positive correlation between the duration of the heat shock and increased mortality. As the time of heat shock increased, the number of mortalities for each developmental stage increased. However, for some unknown reason the five minute heat shock with slow cooling (SC5) had a higher mortality rate at hatching (54.7% v.s. 46.9%) and fry stage (61.8% v.s. 52.0%) than the ten minute heat shock (SC10).

The ten (SC10) and fifteen minute (SC15) heat shocks that were followed by a period of slow cooling resulted in production of 100% triploid chinook salmon. Survival rates of 53.1% and 28.6% at hatching were observed (Table 6). The ten (RC10) and fifteen minute (RC15) heat shocks that were cooled rapidly produced only 95% triploids and survival rates of 35.3% and 32.3% (Table 6). The ten minute heat shock followed by a period of slow cooling was the most effective procedure for the production of triploid chinook salmon.

It is interesting to note that the five minute heat shock cooled rapidly (RC5) produced zero triploids (0%), whereas, the five minute heat shock cooled slowly (SC5) produced 55% triploids. This suggests that the prolonged time spent in heated water by slow cooling the eggs effected

Table 6. Ploidy levels and survival to hatch in heat shocked (28.5 ± 1° C, ten minutes post-fertilization) and control groups of chinook salmon from October 3, 1985 experimental runs.

Cooling method	Heat Shock (min.)	Fish sampled	Ploidy 2N	Ploidy 3N	% 3	Surviv. (%)
SC[1]	0	20	20	0	0	71.4
SC	5	20	9	11	55	45.3
SC	10	20	0	20	100	53.1
SC	15	20	0	20	100	28.6
SC	20	20	2	18	90	17.0
RC[2]	0	20	20	0	0	81.6
RC	5	20	20	0	0	67.3
RC	10	20	1	19	95	35.3
RC	15	20	1	19	95	32.3
RC	20	20	5	15	75	14.2

SC1 - slow cooling
RC[2] - rapid cooling

the induction of triploidy (Table 6). Percentages for survival and triploid induction rates of each heat shock can be found in Table 6.

Statistical Analysis

Statistical analysis indicated that the time of shock and type of cooling did affect survival and triploidy rate significantly ($P \leq 0.001$). Time of shock and type of cooling were dependent and interacted significantly in survival and triploidy rate ($P \leq 0.001$). Slow cooling was significantly different from rapid cooling for survival and triploidy rate. Specific heat shock times for slow cooling and rapid cooling were significantly different ($P \leq 0.001$).

1986 Experimental Runs

The main objective of the 1986 experimental runs was to determine the effect of delayed fertilization techniques on eggs taken during early, mid and late spawning season for induction of triploidy and survival. During the fall several unexpected problems were encountered due to heavy flood waters at the Little Manistee Weir. High water allowed an estimated 3,000-5,000 fish to pass upstream of the weir, reducing the number of fish available during the annual egg take. Warm rain water caused the river temperature to vary from the normal 9-10° C up to 18° C from September 19 to October 24, 1986. The fluctuating water temperature resulted in unpredictable fish numbers. This prevented the planned egg collections for experimental purposes at optimal early, mid and late season spawning times. The abnormal

temperatures also caused the magnitude of our heat shock and our cooling rates to change with each experiment and may have been responsible for increased mortality rates throughout the spawning season of both heat shocked and control groups.

1986 Early Run

The early run eggs were taken on September 19, which was earlier than anticipated because of the number of fish that passed upstream. MDNR personnel were worried that they couldn't meet their requests for chinook salmon eggs, and unfortunately, experimental runs were low priority.

Most of the eggs taken were light orange in color and smaller than normal (underripe). Ripe males were very difficult to find. Eventually, some ripe males were found in the outdoor holding ponds. It took nearly two hours to find six ripe males, while the females laid on the spawning table waiting to be stripped of their gametes. Once we stripped the females, they were dead, which probably increased egg mortality for the early season run.

Triploid induction was higher for the weir shocked lots than for the delayed shocked groups (Table 7). Both replicates of the weir shock produced 95% triploids, while the delayed shock groups had induction rates of 85%.

Mortality was extremely high for all groups taken on September 19 (Table 8). Survival (69.1% and 62.7%) of the control groups (at hatching) was much less than expected (Garling and Masterson, 1985) for MSU's Fish Culture Laboratory. Heat shock groups had even higher mortalities ranging from 88.5% to 94.8% at hatching.

Table 7. Ploidy levels and survival for weir and delayed fertilization heat shocked (28.5 ± 1° C, 10 minutes post-fertilization, for 10 minutes) groups of chinook salmon from September 19, 1986 experimental runs.

Groups	Fish sampled	Ploidy 2N	Ploidy 3N	% 3N	Survival (hatch)	Total Fish
WC1	20	20	0	0	31.0	4230
WC2	20	20	0	0	37.4	4325
WS1	20	1	19	95	6.7	7470
WS2	20	1	19	95	6.9	5820
DC1	20	20	0	0	12.3	3876
DC2	20	20	0	0	11.5	3659
DS1	20	3	17	85	5.2	5474
DS2	20	3	17	85	5.4	5245

WS - weir shock
WC - weir control
DS - delayed shock
DC - delayed control

Table 8. Percent cumulative mortalities and number of chinook salmon for the September 19, 1986 weir and delayed fertilization heat shock (10 minutes post-fertilization, for 10 minutes at 28.5 ± 1° C) experimental runs.

	DEVELOPMENTAL STAGE					
	Day One (24-48 h)		Eyed (10-17 d)		Hatch (40-48 d)	
Groups	%	#	%	#	%	#
WC1	20.1	848	46.8	1981	69.1	2921
WC2	9.7	420	30.8	1334	62.6	709
WS1	43.5	3247	89.1	6655	93.3	6971
WS2	40.1	2337	84.8	4937	93.1	5417
DC1	3.5	134	32.0	1241	91.6	3401
DC2	1.8	64	25.1	919	88.5	3239
DS1	29.0	1590	72.7	3983	94.8	5190
DS2	29.7	1558	73.7	3866	94.6	4962

WC - weir control
WS - weir shock
DC - delayed control
DS - delayed shock

Heat shocks groups (weir and delayed) had such high mortality rates that no statistical comparisons were made since the results were not indicative of the heat shock procedure. The groups shocked at the weir (WS1 and WS2) had 93.3% and 93.1% mortality rates at hatching, whereas, the groups delayed shocked had higher mortality rates of 94.8% and 94.6%.

1986 Mid Run

Additional eggs were taken and heat shocked on September 30th (mid run). The quality of the eggs taken from fish of the mid run was better than the egg quality observed during the early run. The eggs were more uniform in size and color (pale orange). Ploidy levels and survival to the end of hatching are summarized in Table 9. Weir shocked groups (WS3 and WS4) produced 95% triploids, whereas, delayed shocked groups (DS3 and DS4) produced 95% and 100%.

Overall mortality was lower for the mid run than the early run eggs (Table 10). Egg mortality from the control groups (WC3 and WC4) eggs fertilized at the weir was 52.4% and 72.5% at hatching. The delayed fertilization control groups had mortality rates of 75.6% and 69.8% respectively.

Eggs shocked at the weir had mortality rates of 90.5% and 89.2%, which was lower than the eggs delayed shocked (97.4% and 95.9%) at the MSU's Fish Culture Laboratory (Table 10). The control and shocked groups at the weir had higher mortality rates for day one than did the groups shocked at laboratory. However, by the eyed stage all the weir shocked groups (except WC4) had lower mortalities than the delayed

Table 9. Ploidy levels and survival for weir and delayed fertilization heat shocked (28.5 ± 1° C, 10 minutes post-fertilization, for 10 minutes) groups of chinook salmon from September 30, 1986 experimental trials.

Groups	Fish sampled	Ploidy 2N	Ploidy 3N	% 3N	Survival (hatch)	Total Fish
WC3	20	20	0	0	47.6	3984
WC4	20	20	0	0	27.5	3100
WS3	20	1	19	95	9.5	3396
WS4	20	1	19	95	10.7	2845
DC3	20	20	0	0	24.4	3762
DC4	20	20	0	0	30.2	4177
DS3	20	1	19	95	2.6	3761
DS	20	0	20	100	4.1	3493

WC = weir control
WS = weir shock
DC = delayed control
DS = delayed shock

Table 10. Percent cumulative mortalities and number of chinook salmon for the September 30, 1986 weir and delayed fertilization heat shock (10 minutes post-fertilization, for 10 minutes at 28.5 ± 1° C) experimental runs.

	DEVELOPMENTAL STAGE					
	Day One (24-48 h)		Eyed (10-17 d)		Hatch (40-48 d)	
Groups	%	#	%	#	%	#
WC3	22.4	891	41.0	1634	52.4	2089
WC4	41.1	1273	62.4	1936	72.5	2249
WS3	71.0	2410	86.7	2944	90.5	3076
WS4	65.3	1857	83.2	2367	89.2	2539
DC3	10.6	348	48.9	1841	75.6	2846
DC4	9.1	382	47.0	1964	69.8	2914
DS3	32.1	1208	95.6	3596	97.4	3663
DS4	59.4	2075	91.2	3186	95.9	3353

WC - weir control
WS - weir shock
DC - delayed control
DS - delayed shock

shocked groups (Table 10).

1986 Late Run Delayed Egg Take

After MDNR Production Run requests had been met, a few remaining fish were held at the weir for seven to ten days before being stripped of their gametes for heat shocking. Less than 10% of all eggs taken survived to the second day after fertilization. Consequently, the eggs were discarded on day three.

MDNR Production Runs

1985 MDNR Production Runs

The October 24, 1985 MDNR Production Run was our first attempt to heat shock large egg numbers (20,000/treatment) for stocking purposes. The heat shocking temperatures were monitored during the ten minute period to see how well the system was operating (Table 11). Most groups were within one or two degrees of the desired temperature for heat shocking (Table 11). However, some of the groups were heat shocked at lower temperatures than anticipated due to our "inexperience" at heat shocking large volumes in a mass production setting.

There was very little variation between groups in cooling temperatures of the October 24, 1985 MDNR Production Run as seen in Table 12. All eight heat shock groups were cooled to ambient temperature within the 15 minute cooling period.

The October 24, 1985 MDNR Production Run resulted in a 100% triploid induction rate. However, egg survival was only 6.2% at the time of stocking. The same heat shock technique

Table 11. October 24, 1985 MDNR Production Run heat shock temperatures starting at 28.5° ± 1° C for 10 minutes, applied at 10 minutes post-fertilization.

	Time (minutes)					
	0	1	3	6	9	10
Temp	(C)	(C)	(C)	(C)	(C)	(C)
Group						
1	28.5	28	27	27	27.5	27.5
2	28.5	27	27	26	26	26
3	24	24	27	25	25	26
4	24	27	27	26	26	26
5	29	27	28	28.5	28.5	28.5
6	29	25.5	27.5	28	28	28
7	28	25.5	27	27.5	27.5	27.5
8	28	25.5	26.5	26.5	27	27

Table 12. October 24, 1985 MDNR Production Run cooling temperatures.

	Time (minutes)								
	0	1	3	5	7	9	11	13	15
Temp	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
Group									
1	27.5	25	20	15	13	13	12.5	12	12
2	26	26	18	14	13	12.5	12	12	12
3	26	22	20	15	14	13	12.5	12.5	12
4	26	26	23	14	14	13	12.5	12.5	12.5
5	28.5	25	20	13.5	12	12	12	12	12
6	28	24	15	14	13.5	13	12.5	12	12
7	27.5	24.5	15.5	14	13.5	13	12	12	12
8	27	23	15	14	13.5	13	12.5	12	12

was used approximately two weeks earlier with survival rates of 53.1%. The only difference was in the number of eggs being shocked. Even though the number of eggs was almost 10 times more than in earlier experiments, it didn't seem to be a major cause of death in the MDNR Production Runs because heat shock temperatures (Table 11) and cooling temperatures (Table 12) remained fairly constant.

A second MDNR Production Run was conducted on November 1, 1985 because of high initial mortalities. This was very late in the spawning season. Heat shock temperatures were observed and recorded to show that the flow through system heats the eggs evenly and approximately at the preferred temperature (28.5° C). This time the temperatures were more uniform for each heat shock and between heat shocks (Table 13). The heaters were more carefully adjusted and more time was allotted between heat shock groups to reduce temperature variations in the heat shock system.

Cooling temperatures were monitored and there was no major differences between heat shocked groups (Table 14). Groups reached ambient temperature approximately 15 minutes after initiation of the slow cooling period. These groups cooled faster than the October 24, 1985 experimental groups within the first few minutes. The eggs were smaller and lighter (light orange) in color than earlier eggs. Triploid induction was zero (0%) for the November 1 MDNR Production Run. Survival was only 5.8% at the time of stocking.

A small sub-sample of both MDNR Production Run groups were kept separate at MSU's Fish Culture Laboratory. Ploidy

Table 13. November 1, 1985 MDNR Production Run heat shock temperatures starting at 28.5° ± 1° C for 10 minutes, applied at 10 minutes post-fertilization.

	Time (minute)					
	0	1	3	6	9	10
Temp	(C)	(C)	(C)	(C)	(C)	(C)
Group						
1	28.5	27	27.5	28	28	28.5
2	28.5	26.5	27	28	29	28.5
3	28.5	26	26.5	27	28	28.5
4	28.5	25.5	26	27.5	29	29
5	28.5	27	28.2	28.2	27.5	28
6	28.5	27	27	28	28.5	28.5
7	28.5	24.5	25	28	28	28
8	28.5	24	25	28.5	29	29

Table 14. November 1, 1985 MDNR Production Run cooling temperatures.

	Time (minutes)				
	0	1	5	10	15
Temp	(C)	(C)	(C)	(C)	(C)
Group					
1	28.5	23	12	12	12
2	28.5	17	13	12	11.5
3	28.5	23	20	13	13
4	29	22	16	14	12
5	28	22	15	14	12
6	28.5	20	15	13	12
7	28	24	17	13	12
8	29	23	17	12	11

analysis of pooled groups resulted in 47.5% triploid induction rates.

Only about half of the 45,000 chinook salmon stocked were triploids (Lake Michigan and Lake Huron). Both MDNR Production Runs were pooled together at Wolf Lake to conserve space and reduce labor before ploidy was determined.

Nearly 44,000 fish (diploid and triploid) were micro-tagged in the spring of 1986. Tagging mortality was only 0.0097% and was primarily caused by anesthetizing the fish before implanting the coded wire. The Little Manistee River (Lake Michigan tributary) was stocked with 24,000 triploid chinook salmon. Swan Creek (Lake Huron tributary) was stocked with 20,000 triploid fish for Lake Huron.

1986 MDNR Production Runs

The MDNR's goal of producing 150,000 triploid chinook salmon for stocking was nearly reached in 1986. About 145,149 sterile individuals were stocked or about 2.4% of the chinook salmon stocked in the Great Lakes by the MDNR in 1986. A total of 2,197,000 eggs were shocked during two days of the "peak" spawning run. Induction of triploidy was 95% and survival was 6.6% at planting for the two MDNR Production Runs combined. Diploid chinook salmon egg take for 1986-1987 was 1,237,000 with 42.0% survival at stocking.

Fish were stocked in seven different streams (Table 15). Lake Michigan and Lake Huron were each stocked with approximately 65,000 triploid fish. Lake Superior was stocked with 15,000 triploid fish. A total of 6,038,265 diploid chinook salmon were stocked into the Great Lakes by

Table 15. Planting Sites for the 1986 MDNR Triploid Chinook Salmon.

PLANTING SITE		
Lake	Tributary	Number
Lake Superior	Dead River	5,056
Lake Superior	Ontonagon River	5,063
Lake Superior	Black River	5,978
Lake Huron	Harbor Beach	20,533
Lake Huron	Van Ettan Creek	21,000
Lake Huron	Swan Creek	24,198
Lake Michigan	Little Manistee	63,321
	Total	145,149

Data courtesy of the Michigan Department of Natural Resources Fish Division, Wolf Lake State Fish Hatchery.

the MDNR for 1986. Triploid chinook were stocked with normal diploid chinook in these areas to minimize any potential effects of predation on the triploids during stocking. Mortality caused by micro-tagging and transport was less than 1%.

1987 MDNR Production Runs

The goal was to produce 200,000 triploid chinook salmon for the 1987-1988 stocking program. A total of 2,161,680 eggs were heat shocked over the a day period. Triploid induction was 96% and numbers at eye-up and plant out was 20% and 10.6%, respectively, of eggs shocked. Survival was higher, but, numbers were reduced at the swim-up stage and before planting because of space availability. A total of 229,952 triploid chinook salmon were produced, but, only 198,904 were stocked into the Great Lakes (about 3.2% of all chinook salmon released into the Great Lakes by MDNR in 1987) because survival was higher than expected and there wasn't enough room to hold that many triploid chinook salmon. Triploid chinook salmon were planted in tributaries of Lake Huron (90,316), Lake Michigan (78,143), and Lake Superior (30,445) (Table 16).

Wolf Lake State Fish Hatchery took their diploid chinook salmon eggs on October 1 and 2 (1,389,000 and 1,272,000) and had survival rates of 45% and 62% at eye-up. Triploids were stocked with 635,427 diploids from Wolf Lake to prevent additional mortalities of the triploid fish. The MDNR released 6,195,091 diploid chinook salmon into the Great Lakes this year from all their hatcheries.

Table 16. Planting Sites for the 1987 MDNR Triploid Chinook Salmon[1].

Planting Site		
Lake	Tributary	Number
Lake Superior	Carp River	10,043
Lake Superior	Ontonagon River	10,200
Lake Superior	Black River	10,202
Lake Huron	Swan Creek	27,968
Lake Huron	Harbor Beach	30,081
Lake Huron	Ausable River	32,267
Lake Michigan	Little Manistee	78,143
	Total	198,904

1 Mr James Copeland (personal communication) Michigan Department of Natural Resources Fish Division, Wolf Lake State Fish Hatchery.

1986 Growth Study Between Diploid and Triploid Chinook Salmon

No significant differences between average daily gains of diploid and triploid chinook salmon were observed (Figure 3) after a 10 week feeding trial conducted at the MSU Fish Culture Laboratory. The average weight for diploid and triploid chinook salmon at the beginning of the study was 4.6g and 4.5g respectively. At the end of ten weeks the diploid fish averaged 13.2g and the triploid fish averaged 12.2g. No differences were observed in their behavior, feeding habits or general appearance.

Captive Chinook Salmon Stock

One-way analysis of variance indicated that length, weight, and condition factor were not significantly different between triploid and diploid chinook salmon from September 1986 to December 1987. Lengths of triploid and diploid chinook were not significantly different through December of 1987 (Figure 4). They peaked during the same months with the triploid fish on the average two inches shorter than the diploid fish in September and December of 1987 (Table 17). Weight data is summarized in Figure 5. The diploids were 60g heavier than the triploids (60g) by December of 1987 (Table 17). The condition factor varied during the 16 month period for diploid and triploid fish with no significant differences being observed (Figure 6 and Table 17).

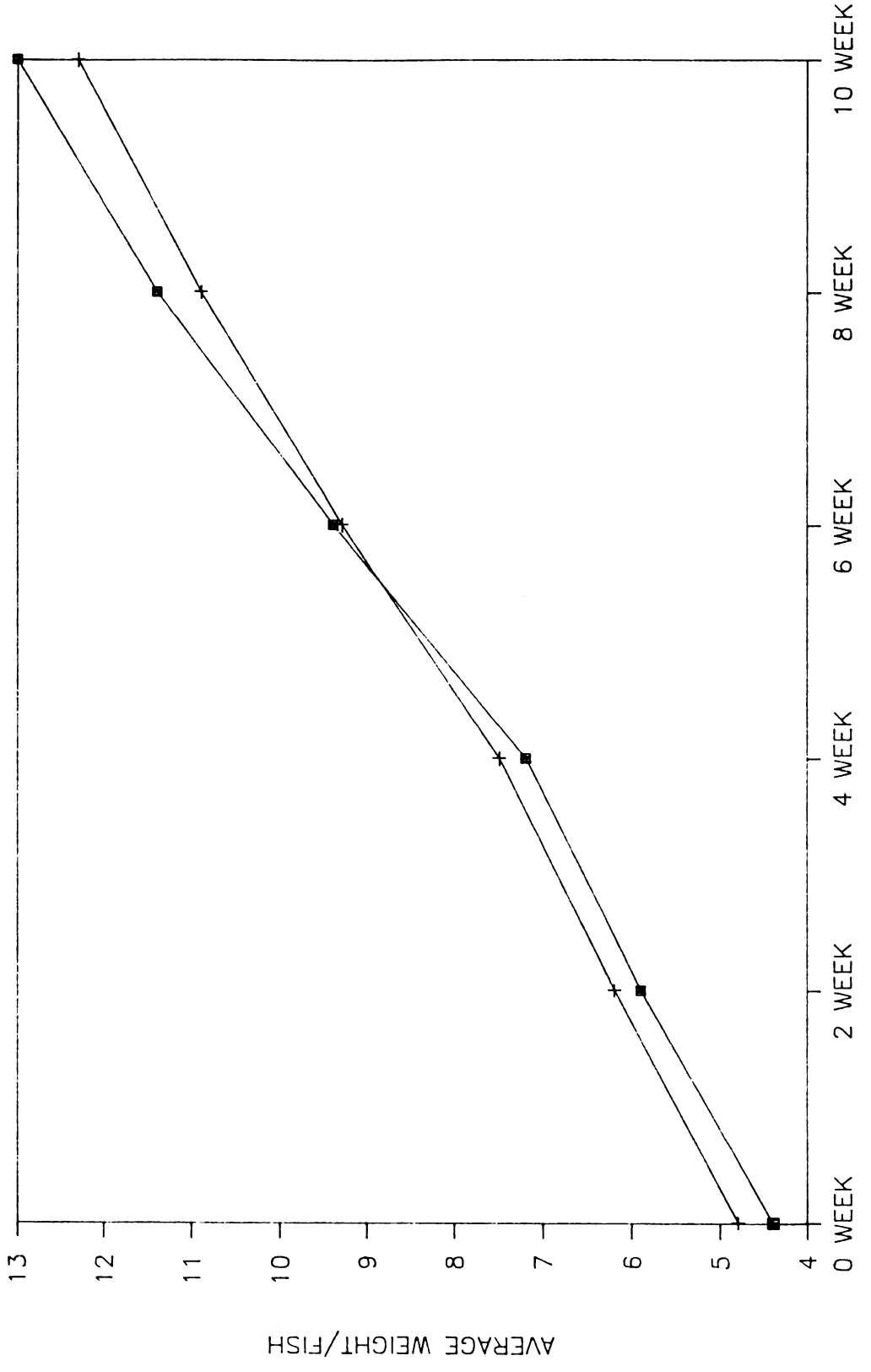


Figure 3. Average individual weight of diploid and triploid chinook salmon over a ten week feeding trial in 1986.

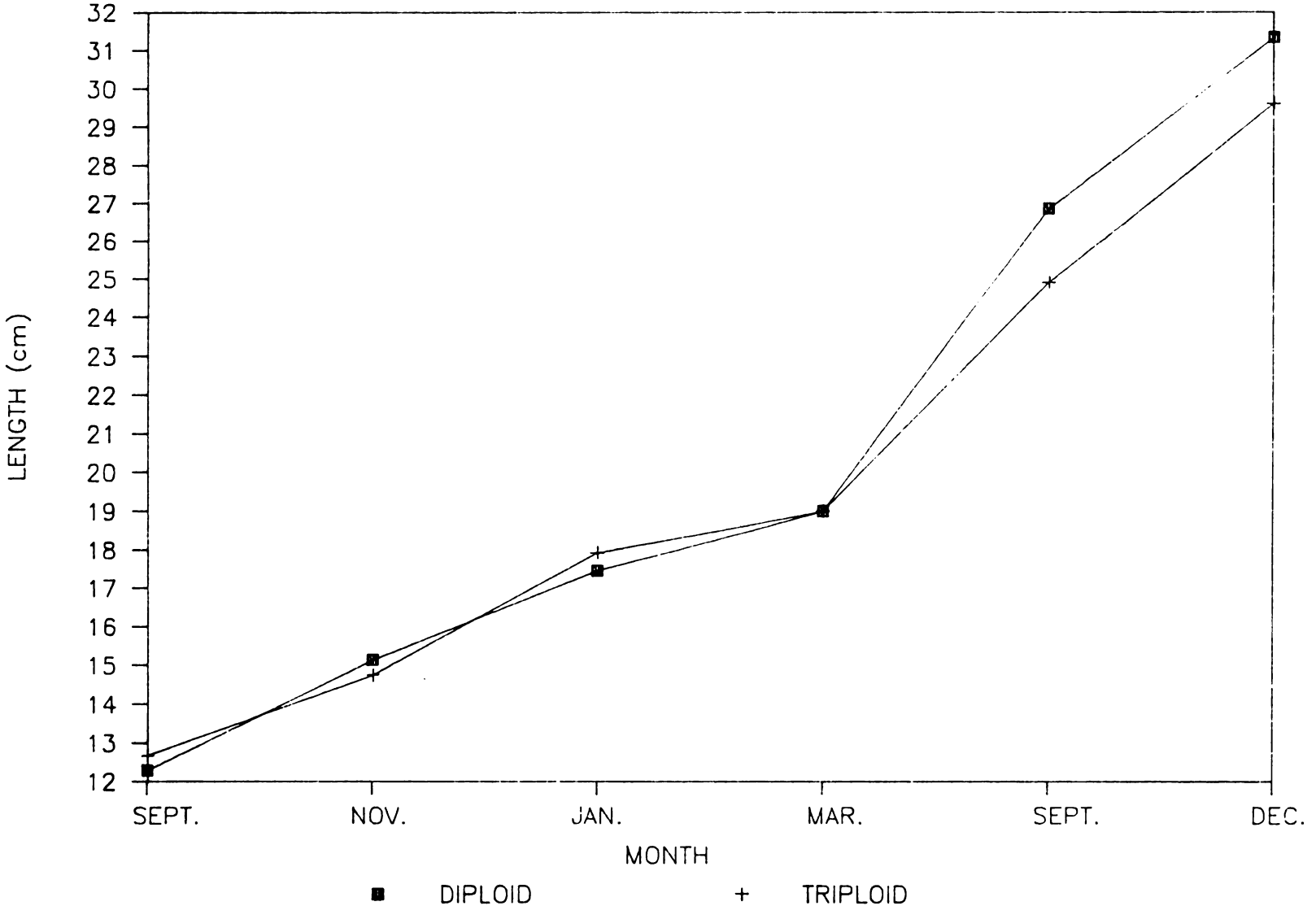


Figure 4. Lengths of captive (diploid and triploid) chinook salmon over a 16 month period (1986–87).

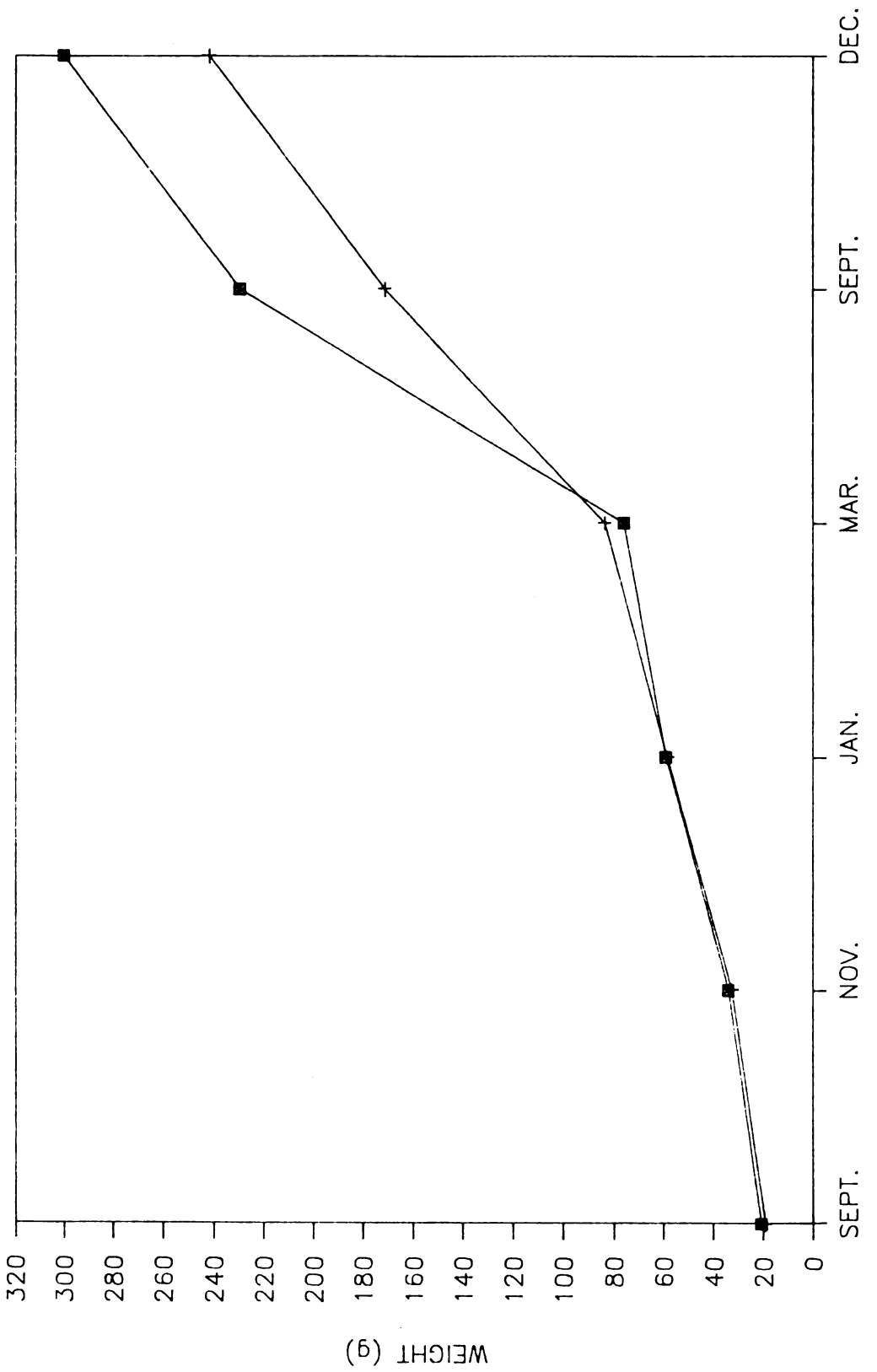


Figure 5. Weights of captive (diploid and triploid) chinook salmon over a 16 month period (1986–87).

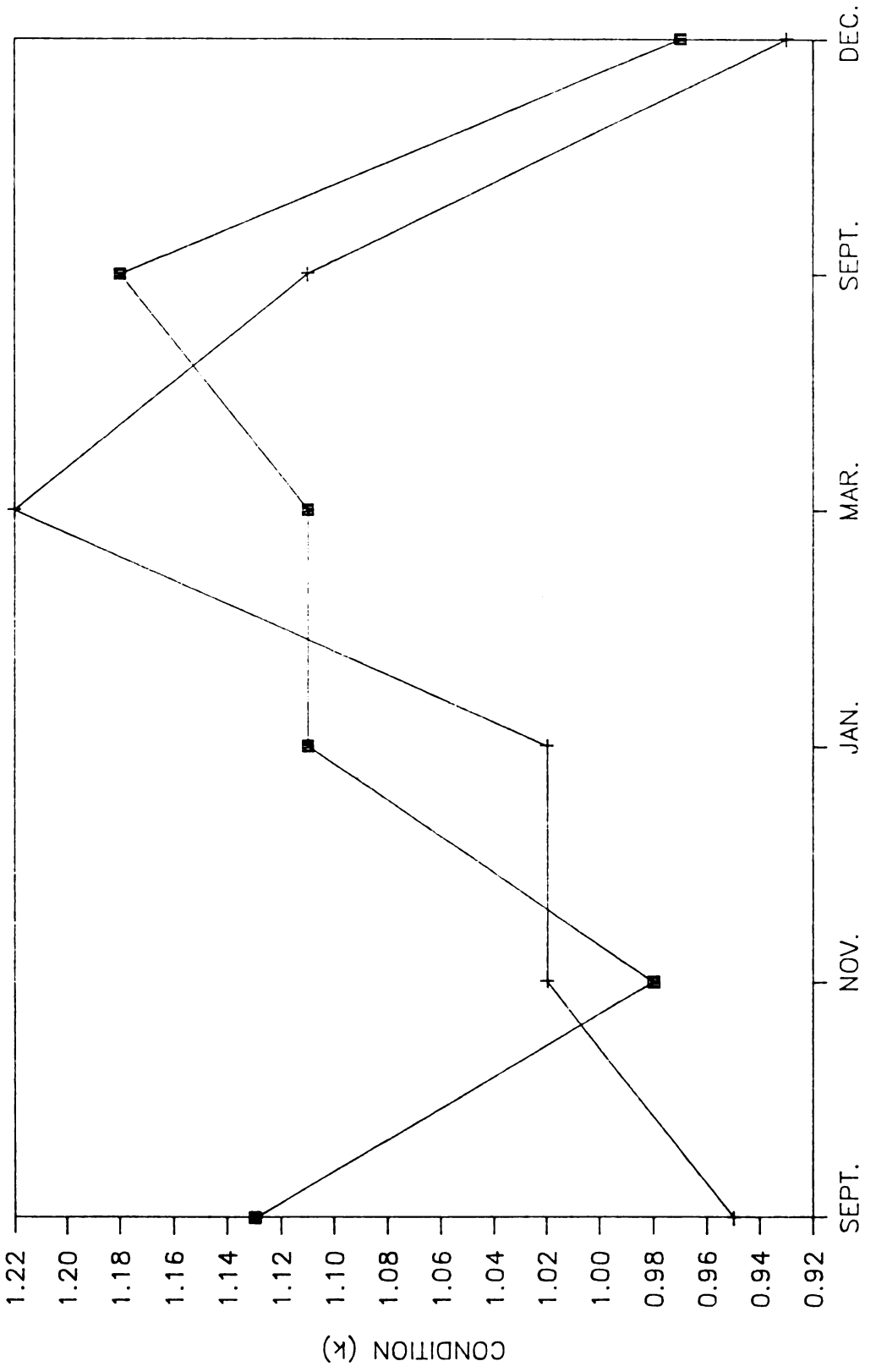


Figure 6. Condition factor of captive chinook (diploid and triploid) salmon over a 16 month period (1986–87).

Table 17. Growth of Captive Chinook Salmon from 1985/86 MDNR Production Runs.

	Diploids	Triploids
Length (cm)		
Sept.	12.29	12.68
Nov.	15.15	14.75
Jan.(1987)	17.47	17.94
Mar.	19.02	19.02
Sept.	26.88	24.92
Dec.	31.40	29.60
Weight (g)		
Sept.	21.04	19.36
Nov.	34.31	32.83
Jan.(1987)	59.26	58.78
Mar.	76.26	84.04
Sept.	229.97	171.75
Dec.	300.04	242.20
Condition (K)		
Sept.	1.13	0.95
Nov.	0.98	1.02
Jan.(1987)	1.11	1.02
Mar.	1.11	1.22
Sept.	1.18	1.11
Dec.	0.97	0.93

$$K = \frac{Wx10^5}{L^3}$$

DISCUSSION

General Factors Affecting Triploid Production

Production of triploid individuals is related to timing of ovulation and egg ripeness (Yamamoto and Ingalls, 1972; Mong et al., 1974; Niebuhr, 1974). Ripening of trout or salmon eggs can be described graphically as a curve with a sharp apex (Leitritz and Lewis, 1980). The peak represents the optimal fertility of a specific lot of eggs. If the eggs are stripped before or after this optimal time, lower fertility will result because ova will either be underripe or overripe (Leitritz and Lewis, 1980).

Several authors have reported that strain, species and degree of ripeness of the egg affect triploidy induction (Lincoln et al., 1974; Thorgaard, 1981; Solar, 1984). Huet (1970) and Lemoine and Smith (1980) have stated that overripe eggs (hard and glassy) increase mortality. Other factors that might affect triploid induction are method of stripping and quality of water and sperm (Bidwell, 1985). For example, Leitritz and Lewis (1980) reported that chinook salmon eggs incubated at a constant temperature below 35° F (1.6° C) resulted in 100% mortality and eggs of rainbow trout and chinook salmon incubated above 56° F (13.3° C) would not develop normally. Some factors are easier to control than

others depending upon the situation but by minimizing these potentially adverse conditions, survival and triploid production should be increased.

Experimental Runs

1985 Experimental Runs

Experiments conducted on October 3, 1985 were designed to determine the best technique to produce triploid chinook salmon. The results indicated that a 28.5 $\pm$ 1° C heat shock for 10 minutes, applied at 10 minutes post-fertilization, followed by a period of slow cooling (15-30 minutes) was the most effective technique (Table 6). Triploid induction rates of 100% were observed with 48% survival over the first 150 days. Survival of controls averaged 69.5% to the same time period. In another similar experiment, 100% triploid chinook salmon were produced with a 10 minute heat shock at 28.5 $\pm$ 1° C administered 10 minutes after fertilization, followed by a 15 minute slow cooling period (Hill personal communication, 1985). Survival of fish in their study for triploids was nearly 70% and 96.5% for controls. Their success was achieved by heat shocking and cooling the eggs at the incubation site in egg incubation trays (Heath Tecna Inc.). Their technique reduced the amount of handling stress on the eggs, which affects survival. We were unable to use this system at the Little Manistee Weir because egg incubation facilities are not available. Table six summarizes our heat shocking data (temperature, time after fertilization, duration of heat shock, ploidy levels and survival to 150

days post fertilization).

The survival values may be misleading since developmental stages are not included. However, the majority of researchers have reported survival at hatching because it is generally considered the last period of high mortality in triploid induced fish.

Slow cooling (SC) significantly increased survival and triploidy rate ($P \leq 0.001$) when compared to rapid cooling (RC). For example, SC10 minute heat shock resulted in higher survival (+ 17%) and triploid (+ 5%) rates than the RC10 minute heat shock (Table 6). Since time of shock and type of cooling were dependent and interacted significantly ($P \leq 0.001$) in survival and triploidy rate, it appeared to be advantageous to use slow cooling.

Johnstone (1985) suggested using triploid yield (triploid induction rate x percent survival at hatch) rather than triploid induction rates to compare heat shock treatments. He speculated that production of 100% triploids may not be optimal because lower survival rates may decrease yield. For example, with triploid induction rates of 94% and 100% and survival rates of 75% and 67% to hatch, respectively, triploid yield would be greater for the lower induction rate because survival was higher. In the Pacific Northwest, stocks of some salmonid species are limited and therefore eggs and diploid individuals are very valuable.

The most effective heat shock treatment for the production of large numbers of triploid chinook salmon spawned at the Little Manistee Weir was a moderate

temperature (28.5° C) lasting 10 to 15 minutes, applied shortly after fertilization (10 to 15 minutes) with a period of slow cooling (approximately 30 minutes). In Michigan the supply of eggs isn't limited since the MDNR has excess fish returning to the weir each year. So 100% triploid induction rates regardless of survival would be optimal for Michigan because fish aren't a problem. However, if egg takes can be increased (as in our production runs) to compensate for higher mortality, then production of near 100% triploids may be more desirable.

1986 Experimental Runs

Experimental runs in 1986 were designed to evaluate delayed fertilization techniques on early, mid, and late season egg take for survival and triploid induction. The results of delayed fertilization on egg take from early, mid, and late season during the spawning run may be misleading. The heavy flooding on the Little Manistee River during the fall of 1986 caused egg quality to vary between early, mid and late season as indicated by poor survival of control and treatment groups (Tables 8 and 10). The high mortality during the early run can be attributed to the eggs being underripe and stripped from dead (for two hours) females.

Triploid induction rates for the early run were 95% for the weir shocked groups WS1 and WS2, and 85% for the delayed shocked groups DS1 and DS2 (Table 7). The mid run had triploid induction rates of 95% for the weir shock groups (WS3 and WS4) and 95% and 100% for the delayed shock groups (DS3 and DS4) at the lab (Table 9). Triploid induction rates

of chinook salmon heat shocked at the weir for early and mid runs were identical (both 95%). However, triploid induction rates of fish heat shocked (at the lab) increased 10-15% between early and mid delayed groups (85% and 95% to 100%). The increased triploid induction in lots with delayed shocks during the mid run could be related to the developmental stage of the eggs. Underripe eggs collected at the weir might not have developed to the proper stage in the second meiotic division to allow the second polar body to be retained and incorporated into the nucleus. Possibly, the eggs were stimulated by some physical means during transportation to the proper stage in development or the extra time after ovulation allowed more eggs to develop to the necessary stage to induce triploidy.

MDNR Production Runs

1985 Production Runs

Poor egg survival was observed for eggs collected October 24 and November 1. The poor survival was probably related to overripe eggs (See section on General Factors Affecting Triploid Production). Overripe eggs subjected to heat shocking and extra handling should have lower survival than eggs in peak condition. Other researchers have reported similar results when overripe eggs were used for triploid induction (Huet, 1970; Lemoine and Smith 1980). Poor egg quality was indicated by high (> 50%) egg mortality within one to three days after applying the heat shock. The eggs were taken late in the spawning season. Even the MDNR's

normal chinook salmon egg take experienced higher than normal mortality rates in 1985 (Jim Copeland, personal communication, 1985).

Triploid induction rates for October 24 and November 1 were 100% and 0%, respectively. Both runs were taken late in the spawning season and heat shocked using the same techniques. However, fertilization was delayed on eggs taken on November 1st, in an attempt to increase survival. These eggs may have developed beyond the extrusion of the second polar preventing the production of triploids.

The second meiotic division could have been stimulated by time after ovulation or by physical shock from being transported for over six hours. However, these factors are unlikely causes of poor triploid induction. Utter et al. (1983), Lincoln and Scott (1983) and Hill (personal communication, 1985) successfully used delayed fertilization techniques (up to six hours after stripping) to induce triploidy. The reason for production of no triploids on the 1st of November is unknown and most likely will be until more is known about the relationship of egg quality and induction of triploidy to the second meiotic division.

1986 Production Runs

Normally the peak spawning run occurs during the first two weeks in October. Eggs taken during this period should result in higher survival and triploid induction rates. However, the spawning run of 1986 was not normal due to heavy flooding. Triploid induction rates were 95%; but, survival was only 5 to 10% for MDNR Production Runs conducted on

October 9 and October 16, 1986, respectively.

The poor survival observed from eggs treated on October 9 might also be attributed to the use of ten gallon milk cans for slow cooling. The metal milk cans retained the heat longer than the five gallon plastic buckets used in the 1985 MDNR Production Runs. This resulted in longer cooling times and increased mortality. Also, the metal milk cans had twice the volume of the five gallon plastic pails, so a flow greater than two gallons per minute would have been needed to cool them at the same rate. This was impossible because the spigots could only maintain one gallon per minute.

Ways to Improve Survival of MDNR Production Runs

Survival of the MDNR Production Runs might be improved by reduced handling of the eggs during the heat shock process and reduced transportation shock. Use of vertical incubation trays may have been the reason for higher survival of heat shocked eggs observed by Hill and coworkers (personal communication, 1985). In our study, vertical incubation trays were impractical because of the design of the Little Manistee Weir. The weir is an egg taking station and is not equipped for incubating and raising fish (Figure 1). Eggs could be transported to the Platte River Hatchery Honor, MI (45 minutes to 1 hour drive, 40 miles) instead of Wolf Lake State Fish Hatchery Mattawan, MI (3-4 hour drive, 180 miles), and the use of the facilities (incubation trays, raceways) there might increased survival of the eggs.

Taking more time to pour the eggs carefully might also improve survival. Changing the heat shocking water more frequently, (Bidwell, 1985) to remove debris and protein would prevent the micropyle from becoming plugged and increase fertilization. Eggs could suffocate if protein (from broken eggs and sperm) coated the outer surface of the egg.

Flow Cytometry

Besides being fast and accurate, the flow cytometer has additional advantages over other ploidy analysis methods (Thorgaard et al., 1982; Allen, 1983). Fish do not need to be sacrificed to determine ploidy since only a small drop of blood or tissue is needed. Most any tissue can be measured. Specimens can be run at almost any age with out complications. Mosaicism can be identified by flow cytometry, while other methods (Coulter Counter) are unable to distinguish between diploid, triploid, and tetraploid cells within the same fish. However, there are some disadvantages to using a flow cytometer. The equipment is very costly to purchase and hospitals with a flow cytometer have high hourly charges to run samples. A person with specialized training in flow cytometry is needed to operate the machine. Plus, whatever is being tested must be in a monodispersed solution. The 1985 experimental and production run samples were analyzed by flow cytometry at Sparrow Hospital. In 1986, Dr. Kathy Brooks became the director of MSU Flow Cytometry Laboratory and made the system available

to other departments. The MSU Flow Cytometer Laboratory was used in 1986 and 1987 because of lower costs for machine time and the staining protocol used at MSU was easier.

Samples run at the MSU flow cytometer laboratory produced frequency histograms with very sharp and distinct peaks. Two peaks were produced because chicken blood was mixed with each salmon blood sample as an internal standard. Peak channel numbers were recorded for each sample. Chicken cells usually peaked around channel numbers 26-33 (DNA fluorescence) and diploid cells between channel numbers 70-88 (DNA fluorescence) depending upon the day analyzed. Fish were classified as triploids when peak channel numbers were between 100 and 145. The ratio of salmon red blood cells to chicken red blood cells was between 2.0 and 3.2 for diploid fish and 3.4 to 5.0 for triploid individuals.

Only enough blood was needed to impart a faint pink color to the test tube. A dark pink color usually indicated that many cells were present which, slowed down the processing speed of the flow cytometer as a result of cells clumping. It is extremely important to prevent the sample aspirator (needle) from becoming clogged. It will slow down analysis of samples and could cause the machine to be shut down, if the technician is unable to correct the situation. Most flow cytometer technicians filter the sample prior to running or have a filter on the end of the aspirator.

As cost of machine time decreases, more researchers will be able to run their samples more rapidly and accurately using a flow cytometer. Between 30 and 50 samples can be run

in one hour.

1986 Growth Study Between Diploid and Triploid Chinook Salmon

The results indicated that there was no significant difference ($P \leq .05$) between diploid fry and triploid fry in average daily gains during a 10 week feeding trial (Figure 3). This was expected since triploids aren't predicted to grow any faster than diploids until after sexual maturity. While diploids are putting energy into the development of gametes, triploids will continue to grow because of their sterility. However, some triploid species develop rudimentary gonadal structures, but are still sterile and continue to use energy for growth.

Benfey and Solar (1986) collected aneuploid (ploidy level between haploid and diploid determined by flow cytometry) sperm from mature (2 year old) triploid rainbow trout males. These fish exhibited secondary sexual characteristics identical to diploids. Triploid males had significantly smaller testes and lower spermatocrit than diploids. Sperm from triploids was watery compared to milt from diploids. Triploid sperm used to fertilize haploid eggs resulted in no viable progeny (Lincoln and Scott, 1984). Therefore, triploid males are truly sterile even if they exhibit secondary sexual characteristics and produce testes. Secondary sexual characteristics and maturation have not been observed in other male triploid salmonids or in females.

Gonadal Development

No gonadal development was detected in diploid or triploid fish from the captive chinook salmon study. This was expected since the chinook salmon strain available in Michigan have a four year life cycle. Gonadal development should occur during 1989. No jacks (precious males) were present in the fish sampled from Wolf Lake. The testes of triploid coho salmon at 30 months were smaller in size than diploid coho salmon (Johnson et al., 1986). Johnson reported the GSI (gonadal somatic index) for triploid males was significantly ($P \leq 0.05$) smaller than the GSI for diploid males. Histologically, the testes of the diploid and triploid coho salmon were identical. Spermatids were not observed in either group.

Ovaries of diploid coho salmon were filled with oocytes while the triploid coho salmon had no oocytes present (Johnson et al., 1986). The mean GSI was significantly ($P \leq 0.05$) higher for diploid females compared to triploid females (Johnson et al., 1986). Benfey and Sutterlin (1984b) also observed differences in oocytes between triploid and diploid rainbow trout. The triploid females had only 1-12 oocytes present compared to diploid females with several hundred. They also reported that triploid rainbow trout males had well developed testes with no spermatozoa and none of the fish reached spermiation. Other studies observed reduced GSI's for triploid fish (Purdom, 1976; Thorgaard, 1979; Gervai, 1980; Lincoln, 1981; Wolters, 1982, Lincoln, 1983).

As mentioned in the introduction, triploid males may still develop gonads to some degree depending upon the species and method of induction. This could be avoided by producing all triploid female populations (Lincoln and Scott, 1983). One way to produce all female triploid populations is to control the sex of fish by adminstration of synthetic sex steroids to sexually undifferentiated fish (Bye and Lincoln, 1986). Masculinization of females with 17 α-methyltestosterone has been accomplished in Atlantic salmon (Simpson et al., 1976; Johnstone et al., 1978), rainbow trout (Johnstone et al., 1978, 1979; Bye and Lincoln, 1981, 1986) coho salmon (Goetz et al., 1979) and chinook salmon (Hunter et al, 1983). Normal female eggs are then fertilized with the sperm from the sex reversed females and heat shocked resulting in all female triploid offspring. Using this technique would avoid problems associated with male triploids.

The exact mechanism causing sterility in triploids is unknown but probably occurs during the random segregation of chromosomes during meiosis (Benfey and Solar, 1986). Either normal bivalents are formed followed by random segregation of a third set of univalents or all the chromosomes segregate randomly. According to Allen et al. (1986) the third homologous chromosome segregates randomly during anaphase I of meiosis in triploid grass carp. This is supported by the fact that both mean and relative variation of DNA content (1.5 haploid, triploid, and hexaploid) fit the theoretical distribution (Allen et al., 1986).

Captive Chinook Salmon Stock

The triploid chinook were not significantly different from diploid chinook in length, weight, or condition factor at the end of 16 months. Other researchers have reported conflicting results when comparing growth parameters of diploid and triploid fish. For example, no significant differences were found between triploid and diploid coho salmon in length, body weight, gut weight or condition factor at 16 months (Johnson et al., 1985). However, Utter et al., (1983) reported that mean weight was significantly different for diploid coho salmon (16.6g) when compared to triploid coho salmon (14.5g) at 17 months. Benfey and Sutterlin (1984b) found that triploid Atlantic salmon were consistently longer than diploids and thus had a lower condition factor.

Mature rainbow trout diploids and triploids had no differences between fork length and dressed body weight; but, condition factor and gut weight of the triploids were both significantly higher than diploids (Lincoln and Scott, 1984). However, Solar et al., (1984) reported average weight for triploid rainbow trout at week 40 and 48 of only 60% and 38% of that of diploid fish. Thorgaard (1986) reported that triploid rainbow had superior growth after sexual maturity (2 years). The triploids weighed 638g at age two, while the diploids weighed 640g. Three and half year old triploids (n = 6) weighed 945g compared to 700g for diploid rainbow trout (n = 17). Growth from 2 years to 3.5 years was 307g for triploids and only 60g for diploids. Five of the six triploids were females.

Growth parameters reported for triploid fish vary greatly between experiments and are not usually based on statistically valid designs (eg. small sample size, unbalanced data and/or no replicates). As can be seen from the above discussion, generalizations can not be made even within a single species because different experiments have produced different results for the same species. The differences reported may have been related to the experimental conditions, type of induction method used or to differences in the strain of fish used. One of the main problems confronting researchers evaluating the growth of triploid fish is availability of rearing or holding space to observe the differences between triploid and diploid fish. For example, Michigan chinook salmon will mature in four years and may reach a size of 25lbs (11.3kg) each.

Diploid and triploid weight data collected in our study at Wolf Lake was not significantly different (P .05) from September 1986 to December 1987. However, by the end of September 1987 to the last sampling period, the diploid fish were nearly 60g heavier than the triploids (not statistical over the 12 month period). The increase in weight for diploids may have been related to the fish (diploid and triploid) being stocked in outdoor ponds at the end of March 1987. The diploids may have adapted to the outdoor conditions better than the triploids or sub-sample error may have occurred.

Graham (1985) reported that triploid Atlantic salmon had only a 68% blood oxygen content when compared to diploids.

He suggested a reduced ability to transport oxygen could hinder the triploids ability to obtain oxygen under conditions that require exertion. For example, triploids trying to compete for food with diploids would be stressed and growth would be reduced. It has been shown that fish subjected to prolonged periods of asphyxiation had reduced growth rates and increased susceptibility to diseases (Stevenson, 1980; Randall et al., 1982). However, Benfey and Sutterlin (1984c) reported little difference in either the efficiency of oxygen uptake by erythrocytes, or in the oxygen carrying capacity of Atlantic salmon blood. There are two main points that support this assumption: one, is that the erythrocyte size is increased only in two dimensions; and second, there is only minor, if any, difference in mean corpuscular haemoglobin concentration (Benfey and Sutterlin, 1984c). In another study, there was no difference in oxygen consumption rates or oxygen partial pressure at asphyxiation for diploid and triploid Atlantic salmon (Benfey and Sutterlin, 1984d). Graham (1985) suggests the reason for no statistical differences observed in oxygen consumption rates or partial pressure was due to increased cardiac output by the triploid fish. This is reasonable because the delivery of oxygen to fish tissues depends upon ambient dissolved oxygen levels, cardiac output, and blood carrying capacity (Cameron and Davis, 1970). The triploid fish could compensate for the lower blood oxygen capacity by increasing the cardiac output. Triploid fish have not been observed to have any respiratory problems in our laboratory or at Wolf

Lake when tanks were cleaned or fish were weighed. Experiments determining the possible physiological limitations of triploid fish should be conducted.

Tetraploid Induction

Results are encouraging for production of tetraploids in some species. However, much work is needed to find the optimal technique (heat, cold, and pressure shocks) and time after fertilization to induce tetraploidy. A tetraploid broodstock to be crossed with normal fish to produce sterile triploids is a very appealing possibility, since the problems associated with triploid males produced by retention of the second polar body could be circumvented.

Eggs shocked from one to five hours after fertilization usually result in tetraploid individuals. Suppression of the first cleavage occurs during mitosis and produces an individual with two sets male and female chromosomes (4N). If tetraploids produce diploid gametes and are crossed with haploid gametes from diploids a triploid zygote should be produced (Myers et al., 1986).

Tetraploidy has been induced in chinook, coho and coho x Atlantic salmon by pressure treatments during first cleavage (Myers et al., 1986). Survival (to hatch) in all treated groups was very low (0.9%, 1.8% and 0.2%), respectively. In another experiment, 100% tetraploid rainbow trout were produced by pressure shocks applied five hours and 50 minutes after fertilization for four minutes (Chourrout, 1984). All offspring were viable and survival rates were nearly 40% at

hatch.

Chourrout (personal communication cited in Myers et al., 1986) then produced triploid rainbow trout by crossing male tetraploids with female diploids. The triploids out performed other triploids produced by second polar body retention and were similar to diploids before maturation. This suggests that induction methods or developmental differences exist between triploids produced from tetraploids crossed with diploids and triploids produced from the retention of second polar body (Myers et al., 1986).

The production of tetraploid chinook salmon would not be as valuable as sterile triploids because they all die after their first spawning. Since it usually takes three to four years for Michigan chinook salmon to mature and spawn the amount of space needed and available to raise tetraploid chinook salmon could be a problem.

SUMMARY AND CONCLUSION

1. Our recirculating heat shock system heated the eggs quickly, evenly and at the desired temperature producing high triploidy induction rates.

2. A heat shock of 28.5 $\pm$ 1^{o} C applied 10 minutes post-fertilization for 10 minutes followed by a 15 to 30 minute period of slow cooling produced 100% triploid chinook salmon with 53.1% survival to hatch.

3. The degree of ripeness of the egg affected egg survival and triploid induction rates. Underripe and overripe eggs have higher mortality than ripe eggs.

4. Evaluation of delayed fertilization on early, mid and late season egg take was affected by abnormal flooding conditions in 1986. However, eggs taken during the peak (mid) spawning season should result in higher survival and triploid induction rates.

5. Mass production of approximately 200,000 triploid chinook salmon can be achieved for the Great Lakes stocking program. However, techniques to reduce handling and stress should be developed to increase

survival.

6. Differences in survival of heat shocked eggs between experimental and MDNR Production Runs may have occurred because of the number of eggs incubated per tray, frequency of removal of dead eggs and transportation.

7. Transporting heat shocked eggs to the Platte River Hatchery instead of Wolf Lake State Fish Hatchery might increase survival rates by reducing transport stress.

8. Growth of diploid and triploid chinook salmon was not significantly different (average daily gains) over a 10 week controlled growth study.

9. Length, weight and condition factor were not significantly different between diploid and triploid chinook salmon at 16 months.

10. Determination of ploidy levels by flow cytometry was fast and accurate. Use of the flow cytometer should increase as machine time costs decrease and new techniques become available to analyze other organisms.

11. Induction of tetraploidy is a promising technique for triploid breeding programs. Crossing tetraploids with diploids can be used to produce triploids with higher survival than production by heat shock. This technique

is not as valuable with Pacific salmon since spawners die after their first spawning.

12. Additional research is needed to identify the physiological limitations of triploids.

APPENDIX A

1. Citrate buffer solution:

   sucrose (85.50 g)
   trisodium citrate, 2 H_2O (11.76 g)
   DMSO (50 ml)

   Sucrose and trisodium citrate were dissolved in approximately 800 ml of distilled water. DMSO and distilled water were added to make a total volume of 1000 ml with a pH of 7.60.

2. Stock buffer solution:

   trisodium citrate, 2 H_2O (2000 mg)
   Nonidet P 40 (2000 ul)
   sperminetetrahydrochloride (1044 mg)
   tris(hydroxymethyl)-aminomethane (121 mg)

   All were dissolved in distilled water to make a final volume of 2000 ml and the pH was adjusted to 7.6. This stock solution was used as the basis for preparing the staining solutions and as the sheath liquid in the flow cytometer.

   Solution A: Trypsin (15 mg) was dissolved in 500 ml of stock solution and pH was adjusted to 7.6.

   Solution B: Trypsin inhibitor (250 mg) and Ribonuclease A (50 mg) were added to 500 ml of stock solution and pH was adjusted to 7.6.

   Solution C: Propidium iodide (208 mg) and spermine tetrayhdrochloride (580 mg) were added to 500 ml of stock solution and pH was adjusted to 7.6. This was protected against light with tinfoil.

Fluorescent cell staining method:

1. Frozen chicken red blood cells (CRBC's) and salmon red blood cells (SRBC's) were thawed before use in a water bath at 37° C but not heated to 37° C. Solutions A and B were kept at room temperature and solution C was kept on ice until use.

2. CRBC's and SRBC's were diluted until a faint pink color was present in the four ml plastic test tube.

3. Solution A (150 ul) was added for one minute.

4. Solution B (150 ul) was added for 10 minutes.

5. Solution C (150 ul) was added for 1-3 hours and kept on ice in a cooler to protect the sample from light.

6. All samples were passed through a 37 u nylon mesh prior to running on the flow cytometer to prevent blockage of aspirater. A total of 20,000 cells were counted for each histogram.

7. Peak channel numbers were recorded for CRBS's and SRBC's for each sample.

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