This is to certify that the

thesis entitled

EVALUATION OF TRIPLOID INDUCTION IN CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA) USING MICROWAVE RADIATION AND GROWTH COMPARISONS OF DIPLOID AND TRIPLOID CHINOOK SALMON

presented by

Paul Spruell

has been accepted towards fulfillment
of the requirements for

Master of Science degree in Fisheries and Wildlife

Major professor

Date August 4, 1989

EVALUATION OF TRIPLOID INDUCTION IN CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA) USING MICROWAVE RADIATION AND GROWTH COMPARISONS OF DIPLOID AND TRIPLOID CHINOOK SALMON

By

Paul Spruell

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTERS OF SCIENCE

Department of Fisheries and Wildlife

1989

ABSTRACT

EVALUATION OF TRIPLOID INDUCTION IN CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA) USING MICROWAVE RADIATION AND GROWTH COMPARISONS OF DIPLOID AND TRIPLOID CHINOOK SALMON

By

Paul Spruell

As part of a continuing study heat shocks were used to produce 143,607 triploid chinook salmon for stocking in the Great Lakes by the Michigan Department of Natural Resources. Although these heat shocks are effective in producing triploids, they also cause high mortality in treated eggs. Microwave radiation was investigated as a novel approach to induction of triploidy in chinook salmon. Shocks from a 750 watt microwave oven were administered to treatment units of 0.25 L of chinook salmon eggs. Shocks were applied over a range of duration and intensity combinations. Twenty continuous and three intermittent shocks were applied from 10-20 minutes post fertilization. Only three triploids were detected. Microwave radiation did effect mortality, but was not effective in the production of triploids.

During an eight week growth study diploid and triploid chinook salmon reared in 110 L aquaria were fed either 2% or 4% wet body weight daily. Growth in tanks containing 100% diploids, 100% triploids, and a 50% mixture of diploids and triploids was evaluated. No difference ($p>0.05$) in growth was observed with in feeding regimes.

ACKNOWLEDGEMENTS

I would first like to thank my committee, Dr. Donald Garling, Dr. Howard Tanner, and Dr. James Asher for their suggestions in the completion of this degree. Special thanks go to Dr. Garling, my major professor for his help during my work, as well as to Dr. Tanner for working to maintain funding for this project.

I would also like to thank those individuals and companies which have helped fund this project. Specifically, the American Fishing Tackle Manufacturers Association and Bill Cullerton Sr. and his staff at the Chicagoland Sport Fishing, Travel & Outdoor Show. In addition, I would like to thank all contributors and buyers from the Chicagoland auction, whose donations combined to yield the majority of the funding for my project.

I would also like to thank the Michigan Department of Natural Resources, Fish Division for their continued support of the triploid chinook project, despite a less than optimal run in 1989. This includes all personnel at the Little Manistee, and Boardman River Weirs, and the Wolf Lake Hatchery.

Thanks also to Dr. Kathy Brooks of MSU for allowing me to use her lab and flow cytometer for ploidy determination.

In addition to Dr. Brooks, I would like to thank Vonnie VanderPloeg for patiently running the flow cytometer, even when things weren't running smoothly, and Corey Casey for enduring my continual presence in the lab. I would also like to thank Angelo Napolitano for his help in obtaining chicken blood to use as a control.

I would also like to acknowledge Ric Westerhof for his help upon my arrival and all individuals who have voluntarily assisted in carrying out my research, the MDNR production runs, and fund raising activities, Ken Cain, Todd Grischke, Tom Heron, Jay Hesse, Scott Sowa, Chris Starr, and Andy Westmaas. I owe a special debt of gratitude to Roger Glass, Paul Wilbert, and Bill Young. To Roger for his never ending work at our lab, which entailed anything we needed done, to Paul and Bill for providing continual advice, assistance, and support in the design and execution of my project.

Finally, I would like to thank my parents and family for their support throughout my academic career.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

In 1984, Michigan State University, in conjunction with the Michigan Department of Natural Resources (MDNR), began stocking triploid chinook salmon in the Great Lakes. The goal of this project was the production of a trophy fishery for the Great Lakes. Sterile triploids are hoped to reach a larger ultimate size due to their extended life span. Chapter I is a summary of the relavent literature available on induced polyploidy in fishes.

Production runs of 200,000 triploid chinook salmon have been produced using heat shock techniques developed at MSU. Although heat shock reliably produces 95-100 percent triploids, mortality rates are often in excess of 80 percent. This high mortality necessitates shocking two million eggs to obtain the desired 200,000 fish for stocking. The high mortality rate also makes increased care of eggs necessary. If an alternate means to produce triploids could be developed which did not result in high mortality, the cost and effort required to produce triploids could be reduced.

Chapter II details experiments attempting to use microwave radiation as an alternative to heat shock. It was hoped that a short duration microwave shock would induce triploidy without causing the high mortality rates associated

with heat shock. Using both constant and intermittent microwave shocks, a variety of intensities and durations were attempted.

There has been much speculation concerning the performance and behavior of triploids. Researchers have reported triploids to grow faster, slower, and at the same rate as diploids of the same species. Anecdotal reports have also indicated that diploids may have a competitive advantage over triploids under some conditions. Chapter III is a report of a statistically valid comparison of the growth of diploids and triploids grown alone and under competitive conditions.

In 1988 heat shock was used to produce triploids for stocking by the MDNR. A slight modification was made in the procedure used in past years. Survival rates for heat shocked eggs in 1988 were improved by approximately 8 percent over previous years. Although we cannot be certain that this increase in survival is due to the modification, it is a possibility that deserves further attention. A description of the modification and a record of stocking locations and numbers are given in Chapter IV.

CHAPTER I. LITERATURE REVIEW

Natural Polyploids

Polyploidy is usually lethal in animals, but is relatively common in plants (Strickberger, 1985). Only lower vertebrates and more primitive organism appear to survive polyploidy. There are examples of naturally occurring polyploid insects, crustaceans, amphibians, reptiles, and fish (Strickberger, 1985). These organisms produce offspring parthenogenically. Some organisms, such as the amazon molly (*Poecilia formosa*), maintain a natural state of triploidy by gynogenesis (Schultz and Kallman, 1968). The amazon molly is believed to be of hybrid origin. In present populations, sperm from the male serves only to stimulate egg development, but its genetic material is not incorporated into the embryo. Triploidy is maintained by an endomitotic division that results in a hexaploid cell. After meiosis, the resulting egg is triploid. (Schultz, 1967).

Techniques to Induce Polyploidy

Several techniques have been used to induce polyploidy in fishes which are generally based on earlier experiments with amphibians (for review see Fankhauser, 1945). Triploid fish which have been produced by artificial induction have proven to be sterile. Sterile fish have commercial potential, and thus induced polyploidy has been studied

extensively in the past few years.

The earliest attempts to produce polyploid fishes utilized cold shocks to disrupt chromosome migration. Cold shocks have proven to be species specific and variable in their degree of success. Swarup (1959a) reported the occurrence of some mosaics in cold shocked stickleback (Gasterosleus aculeatus). Cold shock has been used to produce triploid plaice (Purdom, 1972), Tilapia aurea (Valenti, 1975), Carp (cyprinus carpio) (Gervai et al., 1980), and channel catfish (Ictalurus punctatus) (Wolters et al., 1981b). Cold shocks did not induce triploidy or tetraploidy when administered to Atlantic salmon (Salmo salar) (Lincoln et al., 1974) and to brook trout (Salvelinus fontinalis) (Lemoine and Smith, 1980).

Researchers have attempted to produce both triploids and tetraploids using chemicals. In past attempts, eggs have been submersed in a chemical solution during part of their development. Cytochalasin B, which blocks cytokinesis has been used to treat eggs of Atlantic salmon (Allen and Stanley, 1979; Refstie et al., 1977) and rainbow trout (Oncorhynchus mykiss) (Refstie et al., 1977; Refstie, 1981). Polyploid mosaics were produced but no pure triploids or tetraploids resulted from these treatments. Similar results were obtained when brook trout eggs were exposed to colchicine (Smith and Lemoine, 1979). Triploidy was successfully induced (>90%) when rainbow trout eggs were

exposed to nitrous oxide coupled with elevated pressure (Shelton et al., 1986). Neither nitrous oxide nor pressure alone resulted in any triploids at the levels used in this study.

Heat shocks have proven to be the most reliable means of producing polyploids in most fishes. Experiments involving heat shock have been summarized by Westerhof, 1988 (Table 1). It has been suggested that the heat shock may denature the spindle fibers during meiosis two (Briggs, 1947; Fankhauser and Godwin, 1948). Rieder and Bajer (1979) have shown heat shocks depolymerize the microtubules in cultures of newt lung cells. The disruption of second polar body extrusion during meiosis is the probable cause of unreduced ova in fishes (Purdom, 1983; Thorgaard, 1986).

The success rate of heat shocks has varied from 13 percent in channel catfish (Bidwell et al., 1985) to 100 percent in rainbow trout (Chourrout, 1982; Lincoln and Scott, 1983; Solar et al., 1984), Atlantic salmon (Benfey and Sutterlin, 1984; Johnstone, 1985), and chinook salmon (Hill et al., 1985; Westerhof et al., 1988). Treatments must be optimized for each species of interest (Thorgaard, 1986). The optimal treatment consists of shock temperature, shock duration, and time after fertilization at which shocks are administered. Johnstone (1985) demonstrated that induction rate and mortality are positively correlated, and suggested that the maximum percentage of triploid induction may not be

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

Species	Heat Shock Temp. (C)	Time After Fert. (min)	Duration (min)	Ploidy %	Surv. %	Primary Author and Year (1900)
sturgeon	34	1-60	3	52.3	---	Vasetskii 67
tilapia	32-38	14	1h	10	50	Valenti 75
trout	27-30	60	10	50	---	Chourrout 80
trout	37-38	10	1	50	10	Thorgaard 81
trout	26	25	20	90-100	63	Chourrout 82
trout	27-28	40	10-15	100	---	Lincoln 83
coho	24-30	10	10	85	47	Utter 83
pink	29	10	10	72	74	Utter 83
chinook	29	10	10	60	88	Utter 83
Atlantic	32	5	5-15	100	80	Benfey 84b
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	38	40	10	90	40	Bye 86
trout	29	10	10	91-96	48-79	Thorgaard 86
tilapia	39.5	3	3.5-4	60	60	Don 88
chinook	28.5	10	10	100	45	Westerhof 88

modified from Westerhof (1988)

the most efficient use of eggs due to the increasing mortality associated with the more extreme treatments. This may be disputed if 100 % sterile fish are necessary for implementation of a successful management scheme.

Hydrostatic pressure is one of the most promising means of blocking the extrusion of the second polar body. It has been used with a great degree of success to produce triploid rainbow trout (Chourrout, 1984; Lou and Purdom, 1984) and Atlantic salmon (Benfey and Sutterlin, 1984a). As well as tetraploid rainbow trout (Chourrout, 1984). It has been suggested that hydrostatic pressure may be a more desirable mechanism for polyploid induction than heat shock since mortality resulting from pressure treatments is usually lower than for heat shocks (Solar et al., 1984). Pressure treatments are limited in the volume of eggs that can be treated at one time, but this limitation may be overcome in time (Thorgaard and Allen, 1987).

Gynogenesis

The primary effect of triploid induction techniques is the production of a diploid ovum. If the eggs were stimulated to continue development after the inducing shock were applied without adding additional genetic material, as is the case if irradiated sperm are used, a gynogenic diploid would be produced (Purdom, 1983; Thorgaard, 1986). This procedure has been used successfully to produce gynogenic

rainbow trout (Chourrout, 1980; Chourrout, 1984) and zebrafish (Brachydanio rerio) (Streisinger et al., 1981). There is increasing interest in gynogenesis in fish species in which the male is the heterogametic sex since all female populations could be produced. These populations could be used for control of reproduction, or the development of inbred lines (Purdom, 1976; 1983; Chourrout, 1982).

Androgenesis

In addition to fish with all maternal inheritance, androgenic fish can be produced in which all genetic material is of paternal origin. These fish result when eggs are irradiated by gamma radiation to inactivate the maternal genome (Purdom, 1969, Parsons and Thorgaard, 1984). After fertilization with normal sperm, first cleavage is blocked (Parsons and Thorgaard, 1985). Androgenic fish could produce all male lines using sex reversed males coupled with a screening procedure to isolate those fish of the appropriate chromosome composition. All male populations produced by androgenesis might be used in a manner similar to gynogenic fish to produce inbred lines for scientific study (Thorgaard, 1986).

Triploid Hybrids

A final possibility in chromosome manipulation involves the production of triploid hybrids. It has been shown that

in some fish crosses the triploid hybrid survives better than the corresponding diploid hybrid (Chevassus et al., 1983; Scheerer and Thorgaard, 1983). Triploid hybrids have three potential aquaculture and fisheries management applications. The higher survival of triploid hybrid fish might make otherwise impractical diploid hybrids feasible (Refstie et al., 1982). Inducing triploidy in hybrids that already show a skewed sex ratio would further reduce the risk of unwanted reproduction (Thorgaard and Allen, 1987). Finally, desirable traits from two species might be combined in one fish (Utter et al., 1983).

Gonadal Development and Sterility

Both male and female triploids generally display a reduction in size of the gonads. The reduction is greater in females than males (Thorgaard, 1986). A gonadal somatic index (GSI) is regularly used to compare development of gonads between fish. The index is calculated by dividing the weight of the gonad by the total weight of the fish (Johnson et al., 1986; Benfey and Sutterlin, 1984a). Benfey and Sutterlin (1984a) found testicular development was reduced by 7.2% while ovarian development was reduced by 52% in Atlantic salmon. In triploid coho salmon GSI in males was 35.7% of normal diploids, while GSI of females was 11.8% of the normal diploid (Johnson et al. 1986). The unequal effect of triploidy on gonadal development has also been observed in

rainbow trout (Thorgaard and Gall, 1979; Lincoln and Scott, 1983;1984; Solar et al. 1984) channel catfish (Wolters et al., 1982), plaice x flounder (Platichthys flesus)(Purdom, 1972) and turbot (Scophthalmus maximus) x brill (Scophthalmus rhombus) hybrids (Lincoln, 1981a; b).

Although gonadal development is usually impaired in triploids it is not completely eliminated. Organisms that have an odd number of chromosome sets are usually sterile (Cassoni et al., 1984; Strickberger, 1985). Thus despite the development of gonads, abnormal development of gametes render the individuals sterile. The presence of a third homologue may prevent proper pairing and migration during meiosis thus preventing the completion of gametogenesis (Solar et al., 1984; Strickberger, 1985). Male triploid rainbow trout produced aneuploid spermatozoa that produced no viable offspring when used to fertilize eggs (Benfey et al., 1986). Allen et al. (1986) induced spermiation in a triploid grass carp by injection of carp pituitary. The resulting sperm was found to be aneuploid, and eggs fertilized with this sperm were not viable.

Artificially induced triploid fishes are functionally sterile. Much of the interest in using triploidy in aquaculture and management depends on the elimination of the secondary sex characteristics, in addition to sterility. It has been shown that elimination of gonadal development by surgical castration prior to maturity eliminated post-

spawning mortality in kokane salmon (Robertson, 1961). The development of secondary sexual characteristics were not exhibited in spontaneous triploid rainbow trout (Thorgaard and Gall, 1979). Based on the sex biased differential reduction in gonadal development, only triploid females may demonstrate all of the traits desired by fisheries scientists (Thorgaard, 1986).

Identification of Triploids

Triploids are usually not identifiable by morphometric observations. In carp, when a female of the scattered scale type was crossed with a male of the normal scale pattern, the resulting triploid offspring displayed a minor disturbance in scale pattern when compared to diploids (Gervai et al., 1980). Swarup (1959b) reported detectable variation in certain body parts in triploid stickleback, but they were identical in most morphometric characteristics. Other researchers have not detected morphometric means to distinguish triploids from diploids based on empirical observation (Wolters et al., 1982b; Cassani et al., 1984; Johnson et al., 1986).

Triploids can be identified by direct observation of chromosome number by karyotyping. Kligerman and Bloom (1977) developed an inexpensive method to make chromosome preparations from solid tissues. Although this method can be used reliably to produce quality chromosome spreads, it takes

considerable practice to become proficient at both the slide preparation, and accurate counting of the spreads (Sweet, 1986). Embryos contain a high percentage of replicating cells. Thus they usually provide quality preparations for karyotyping (Myers, 1986). Tissue cultures can also be used to provide chromosome spreads. Wolters et al. (1981b) found cultured lymphocytes from macerated kidney tissue of channel catfish to provide good chromosome spreads. Most fishes contain a large number of small chromosomes and the exact number may vary in cells from one individual and between fish of the same species (Blaxhall, 1983). It is necessary to do several counts on each specimen to identify variation caused by counting and variation found naturally in the population. The limited number of spreads from each specimen that can be analyzed make detection of mosaics difficult (Thorgaard et al., 1982; Wattendorf, 1986). The number of fish from each treatment which can be tested is also limited, thus the percentage of triploids produced may not be accurately estimated.

Other methods have been developed to identify the ploidy of cells by direct observation under a microscope. Microfluorimetry and microdensitometry can be used to measure the DNA content of individual cells (Gervai et al., 1980; Johnstone, 1985). The number of nucleoli present in each cell can also be used to determine ploidy (Phillips et al., 1986). The nucleoli are silver stained and then counted.

Diploids were found to have either one or two nucleoli, while triploids had one, two, or three nucleoli. Although no diploids possessed cells with three nucleoli, the overlap in number per cell makes observation of many cells necessary to insure accurate estimates of ploidy and there is no way to detect mosaics. Although other "direct observation" methods are an improvement over karyotyping, they are still labor intensive and time consuming.

The nuclear volume of erythrocytes has been found to differ significantly between diploids and triploids (Swarup, 1959; Purdom, 1972; Cimino, 1973; Valenti, 1975; Allen and Stanley, 1978; Lemoine and Smith, 1980). Nuclear volume can be measured and ploidy determined on this basis. The distribution of nuclear volume of diploids and triploids has been found to overlap in some fish species making the detection of mosaics difficult (Purdom, 1972). Variation in nuclear volume within individual fish has also been detected (Allen and Stanley, 1978; Lemoine and Smith, 1980). Thorgaard and Gall (1979) found extensive variation both within individuals and among individuals of each ploidy type. Thus a comparison between individuals of known ploidy level must be done on a species to insure the validity of ploidy determinations using nuclear volumes (Wolters et al., 1982a).

An improvement over direct measures of erythrocyte nuclei is the use of a Coulter Counter and channelizer (Wattendorf, 1986). This method allows measurement of

thousands of cells, and thus is more sensitive to the presence of mosaics. Despite its advantages, Johnson et al. (1984) found that a Coulter Counter gave inconclusive results in 11% of their coho and chinook salmon blood samples.

The use of a flow cytometer allows rapid analysis of ploidy with unequalled precision (Callis and Hoehn, 1976; Thorgaard et al., 1982; Allen, 1983; Johnson et al., 1984). Flow cytometry measures the fluorescence of stained DNA as the cell is passed through a laser (Allen, 1983). The cells are passed through the laser in single file driven by either air pressure or a vacuum (Dowining et al., 1984). As the cells pass through the laser, the amount of fluorescence is converted into an amplified electrical signal, digitized, and displayed. The type of display generated varies depending on the specific information sought by the researcher (Downing et al., 1984). Frequency histograms are usually used to determine ploidy (Thorgaard et al., 1982; Allen, 1983).

CHAPTER II. ATTEMPTED INDUCTION OF TRIPLOIDY IN CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA) USING MICROWAVE RADIATION

ABSTRACT

Methods have been developed to produce large numbers of triploid fish using heat shock. However, these techniques cause high levels of mortality. Microwave radiation was explored as a potential source of shock to induce triploidy. In addition to lowering mortality, we hoped microwave radiation would be less temperature dependent and have application over a broad spectrum of species.

Chinook salmon eggs were shocked using a 750 watt microwave oven over several time-power combinations. Eggs were shocked from 10-20 minutes after fertilization, in a microwave bundt cake pan to provide uniform exposure.

Although mortality rates were increased in some treatments, very few triploids were detected. This would indicate that microwave radiation, while having some effect on the eggs is not specific in depolymerizing microtubules without fatally damaging other components in the egg.

INTRODUCTION

There are many potential advantages to using genetic manipulations to produce sterile fish in aquaculture and fisheries management. One way to produce sterile fish is genetic manipulation to produce triploid individuals

(Lincoln and Scott, 1983, 1984: Benfey and Sutterlin, 1984b). Triploids are sterile due to disrupted gametogenesis caused by the presence of an extra set of chromosomes (Purdom, 1983). Sterile triploid fish could be used to stock waters in which control of reproduction is desirable to protect endemic organisms (Purdom, 1983; Cassani et al., 1984) or to prevent stunting (Allen and Stanley, 1979; Wolters et al., 1982b; Lou and Purdom, 1984). Elimination of sexual maturation in salmonids is of particular interest since secondary sex characteristics, such as reduced growth rate and decreased flesh quality, could be avoided by using sterile triploids (Lincoln et al., 1974; Lemoine and Smith, 1980). Precocial maturation is another problem of economic importance that might be eliminated using triploids (Solar et al., 1984). Finally, as a result of their extended life span, triploid pacific salmon might reach a larger ultimate size than diploids (Thorgaard, 1986). If properly managed, these fish could provide a trophy fishery for the Great Lakes (Westerhof et al., 1988).

Although triploidy is rare in most fishes, some naturally reproducing triploid fish have been described (Schultz, 1967; Schultz and Kallman, 1968; Menzel and Parnell, 1973). Triploidy also occurs spontaneously at a low level in individuals of some species (Cuellar and Uyeno, 1972; Gold and Avise, 1976; Thorgaard and Gall, 1979)

Many fish that are normally diploid have shown little or

no adverse effects when artificially induced to triploids (Purdom, 1972; Wolters et al. 1981b; Chourrout, 1980; Benfey et al. 1984; Johnson et al. 1986). A wide variety of techniques have been used to prevent the extrusion of the second polar body during meiosis, resulting in a triploid fish with two complements of the maternal chromosomes and one complement of the paternal chromosomes (Purdom, 1983). Chemicals that inhibit chromosome migration have been used to produce polyploid mosaic fish, but have rarely produced pure triploids (Refstie et al., 1977; Allen and Stanley, 1979; Smith and Lemoine, 1979). Hydrostatic pressure has been used to produce both triploid (Benfey and Sutterlin, 1984b; Chourrout, 1984; Lou and Purdom, 1984) and tetraploid fishes successfully (Chourrout, 1984). Since only a limited volume of eggs can be treated at one time using pressure it is not applicable to treating large numbers of large eggs (Thorgaard, 1986).

Temperature shock has been the most common method used for triploidy induction in fishes. Cold shocks have been used to induce triploidy in the plaice and its hybrid with the flounder (Purdom, 1972), the channel catfish (Wolters et al., 1981), and tilapia (Don and Avtalion, 1988). Cold shocks have not been used successfully to produce triploid salmonids (Lincoln et al., 1974; Lemoine and Smith, 1980). Triploid salmonids have been successfully produced by heat shocks (Chourrout, 1980; Thorgaard et al., 1981; Benfey and

Sutterlin, 1984b; Johnstone, 1985; Johnson et al. 1986; Westerhof et al., 1988).

Heat shock has been used to produce large numbers of high percentage triploid chinook salmon for the Michigan Department of Natural Resources (MDNR) Great Lakes stocking program. High mortality rates (85-90 percent) have been associated with these treatments (Westerhof, 1988). Producing the number of fish desired for the MDNR production runs is not difficult since excess eggs are usually available. However, if eggs are in short supply, production of 200,000 triploids may not be possible in some years (see Chapter III). The high mortality rate also dictates increased care by MDNR workers. Moribund eggs must be removed or antifungal treatment administered more frequently to prevent fungal growth. Thus, a means to produce triploids that significantly lowered mortality would decrease the number of eggs necessary and the effort required to produce the triploid fish.

In this study, we attempted to induce triploidy in chinook salmon using microwave radiation as the shock. It was hoped that a short duration microwave treatment could be used as the shock, and that mortality would be decreased. The possibility of using microwave shocks was also appealing in that they are easy to administer and would enable triploids to be produced using equipment readily accessible to both fish farmers and management agencies. Microwaves

would also utilize a shock other than temperature which might allow more uniform application over a wide variety of species, adjusting for developmental rate and volume of the eggs being treated.

MATERIALS AND METHODS

Prior to the onset of our experiments with chinook salmon eggs, the uniformity of heating produced by a Sears 750 watt microwave oven was examined. Garden peas were used to simulate salmon eggs and to determine the appropriate procedure to produce consistent heating throughout a treatment group. Salmon eggs were neither available no expendable, thus peas were selected due to their similar size and shape. Our preliminary trials compared heating uniformity in peas heated in 250 ml glass beaker, a 9x9x2 inch glass baking dish, and a microwave safe bundt cake pan. Peas were heated at various settings and temperatures were taken at several points throughout the pan. We concluded that a microwave-safe bundt cake pan produced the most consistent and uniform heating. We also determined that for short treatments ($\leq$ 1 min.), no benefit was gained by using a rotating platform in the oven.

Eggs of salmonids are not easily observed to determine the time of developmental events (Chourrout, 1982). Great Lakes chinook salmon eggs are large, opaque and colored orange making direct observation difficult. Triploidy can be

induced in chinook salmon using heat shocks applied 10 minutes post fertilization as outlined in figure 1 (Hill et al., 1985; Westerhof et al., 1988). We relied on results from the successful induction of triploidy by heat shock to determine the appropriate time to apply the microwave shock.

Our first attempt to induce triploidy using microwave radiation was conducted in October of 1987. Eggs were randomly sampled from female chinook salmon collected by the MDNR and the shocking experiments were conducted at the Little Manistee River Weir near Stronach, Michigan on October 13, 1987. Chinook salmon were spawned using standard procedures (Piper et al., 1982). Eggs were removed from ripe females by injecting compressed air at 2 psi pressure into the body cavity. Eggs from 6 females were mixed in a 5 gallon pail. Milt was obtained by applying pressure to the abdomen of ripe males. Milt from 5 males was collected and mixed in a plastic wash bottle. Treatment units of 0.25 L of eggs were fertilized without water in plastic quart containers. Two minutes after fertilization, river water was added to the quart containers to increase the percent of eggs fertilized (Piper et al., 1982). Immediately prior to shocking, eggs were drained and placed in a bundt cake pan for shocking. At ten minutes post fertilization eggs were shocked (figure 2). Shocks were administered at 25, 50, 75, and 100% power. Three replicates of each power level was tested at 15, 30, 45, and 60 seconds.

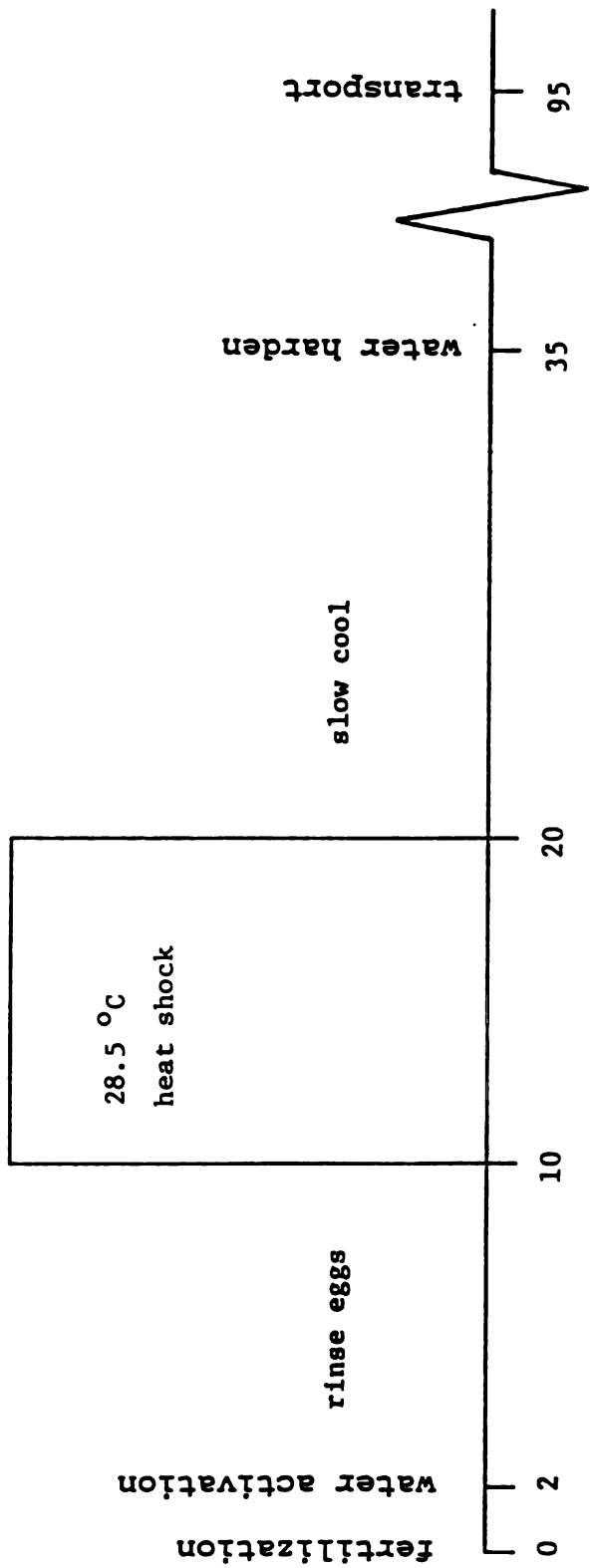

Figure 1. Timing of steps in the production of triploid chinook salmon using heat shocks at the Little Manistee River Weir near Stronach, Michigan.

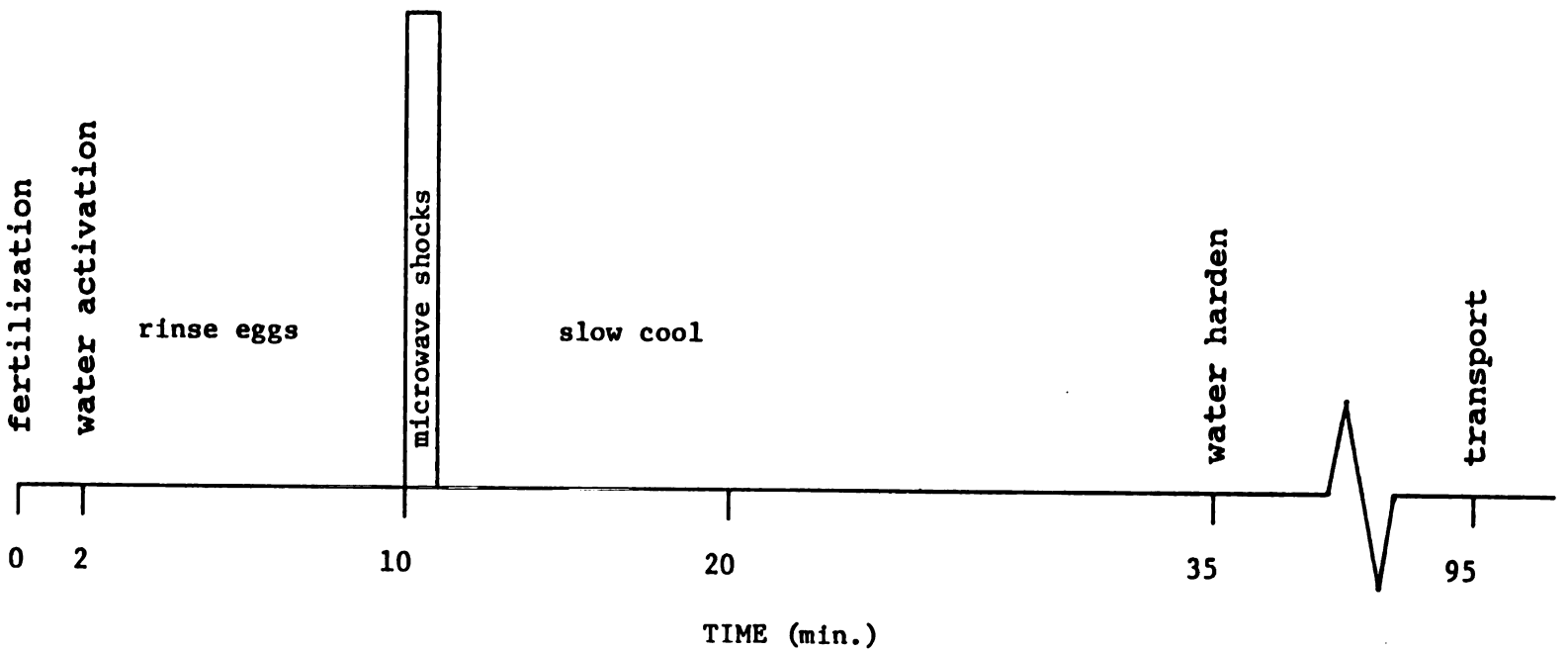

Figure 2. Timing of steps in microwave shocks applied to chinook salmon eggs in 1987 at the Little Manistee Weir near Stronach, Michigan.

After shocking the temperature of each treatment group was determined by placing a thermometer in the bundt cake pan at several places and averaging the recorded temperature. Eggs were returned to the plastic container, slow cooled to ambient temperature of river water over a 15 minute period by addition of cool water, and water hardened for one hour. Water hardening results from the uptake of water by the outer membrane of the egg. This makes eggs more resistant to mortalities due to handling and transport. The plastic containers of eggs were placed in a cooler to maintain constant temperature and transported to the Fisheries Research Lab at MSU.

At the MSU lab, each replicate was randomly placed in a vertical stack incubator (Heath Technica). Incubator tray were divided, so up to four different treatment lots could be reared in one tray. All moribund eggs were removed from the stacks at two to three day intervals to prevent fungal growth. Moribund eggs were counted to calculate mortality rates for each treatment.

Swim up fry were placed in 110 L aquaria. Each aquarium was supplied with 13 ^{o}C well water at a rate of 0.25 liters per minute and equipped with an external stand pipe to maintain constant water level (Garling and Wilson, 1976). Fish were fed to satiation at least three times daily using biodiet salmon starter. Fish were maintained in these tanks until they reached sufficient size for ploidy analysis. Due

to space limitations and the low survival from some treatment groups, some replicates were combined and raised in one tank.

Shortly after all of the yolk sac had disappeared, blood was taken for ploidy analysis using flow cytometry as described in Appendix 1. Five fish from each replicate were sampled, in those treatments which had been combined, 15 fish were sampled. To provide a more reliable estimate of triploid induction rate, an additional 15 fish were tested in those replicates in which triploids were detected.

In the Fall of 1988 a second attempt to induce triploidy using microwave radiation was made. The 750 watt Sears microwave oven was used as the source of the shock. On October 21, 1988 chinook salmon eggs were collected, fertilized, and water activated at the Little Manistee Weir as described for treatments done in 1987.

Based on 1987 results, two variations in shocking were administered. It was hypothesized that a one minute shock did not produce high rates of triploidy because only a low percentage of the eggs were at a susceptible stage of development. Results from heat shock studies indicate that the optimal time for application of shock is from 10-20 minutes post fretilization. A 10 minute microwave treatment to span this entire range of development would have been desirable; however, trials in 1988 proved 10 minute shocks even at 10 percent power caused 100 percent mortality in treatment groups of 0.25 L. If one assumes the percentage of

eggs in the critical developmental stage occurs as a normally distributed function of time, the maximum number of eggs in this stage would occur at 15 minutes post fertilization. To maximaize the number of eggs in the critical period during exposure to microwave radiation the following variations of our initial shocks were applied. In the first variation, eggs were shocked for five minutes, beginning 12.5 minutes after fertilization (Figure 3). Power settings of 5, 10, 15 and 20 percent were used for the continuous shocks. In the second variation power settings of 40, 50, and 60% were used. Eggs were shocked for 30 seconds, at two minute intervals starting at 10 minutes post fertilization, and continuing to 18 minutes post fertilization (Figure 4). This pattern yeilded a total of 2 minutes of shock, over a period of 8 minutes of development. Unlike 1987, eggs in 1988 were kept in water during shocking. This was done to prevent oxygen stress during the longer shock period. A rotating platform was also used in the microwave to insure equal treatment to all eggs during the prolonged exposure.

All eggs were water hardened for one hour and transported to the MSU lab for incubation as described for treatments made in 1987. At swim up, fry were placed in 110 L aquaria and maintained as described above, until ploidy analysis could be conducted. Ploidy analysis was done using flow cytometry as in 1988.

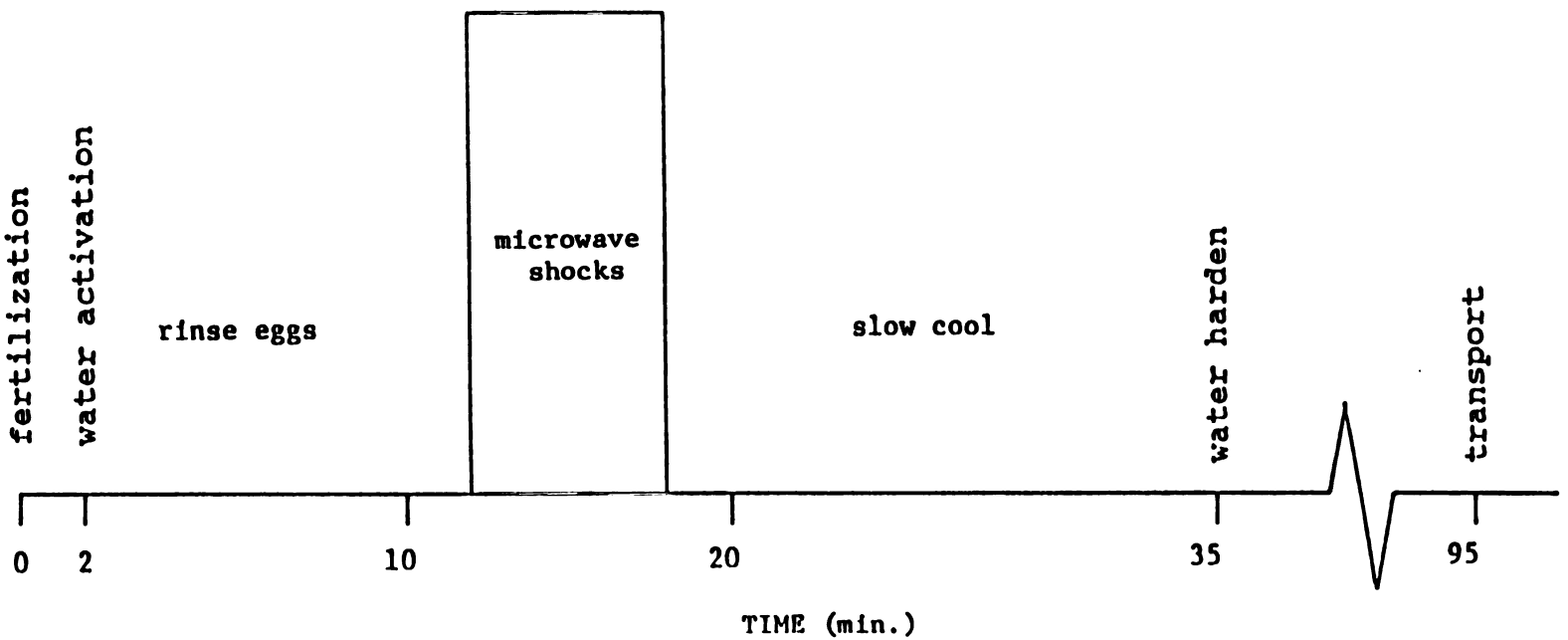

Figure 3. Timing of steps in constant microwave shocks applied to chinook salmon eggs at the Little Manistee River Weir near Stronach, Michigan in 1988.

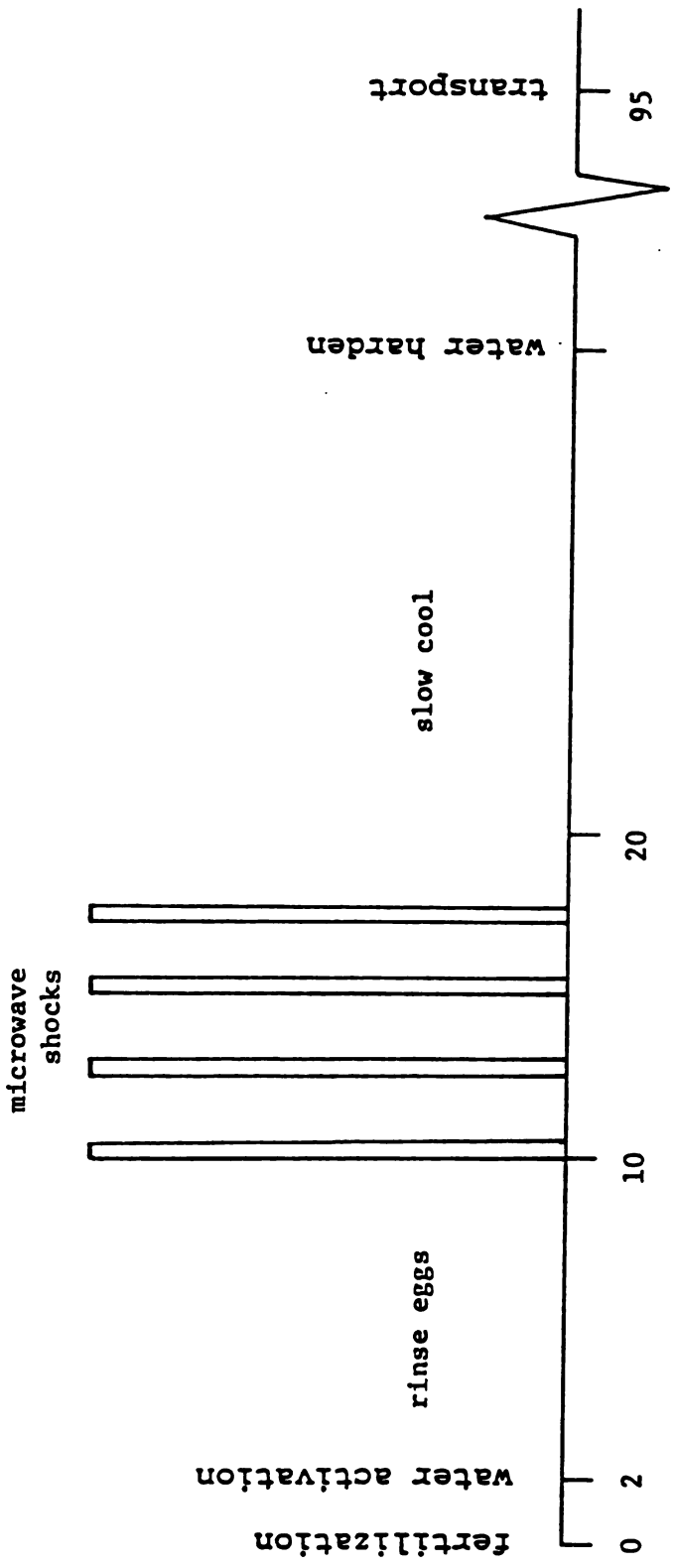

Figure 4. Timing of steps in intermittent microwave shocks applied to chinook salmon eggs at the Little Manistee River Weir near Stronach, Michigan in 1988.

RESULTS

In 1988, two triploid fish were detected, one from a 15 second treatment at 100 percent power and one from a 45 second treatment at 50 percent power. Mortality figures were calculated and are presented in Table 2. Shocks exceeding one minute in duration at full power, and shocks of 10 minute duration at 10 percent power were lethal. Figures 5 and 6 demonstrate the effects of varying time and power setting. In Figure 5, average percent mortality is plotted versus power setting, holding shock duration constant. Figure 6 is a similar graph maintaining power setting as the constant. Mortality increased with increasing percent power and with treatment duration as did the observed temperature (Table 3).

In 1989, one triploid was detected from an intermittent shock, at 50 percent power. Mortality records could not be kept accurately in 1989 because of unexpected problems at the laboratory. On December 10, 1988 a pump failure at the MSU laboratory caused significant mortality in all treatment groups despite our efforts to maintain water flow from an auxiliary source. Due to a ruptured pipe inside of a filter tank we experienced periodic discharges of filter media from a new iron removal system. Although the effect of the media on the eggs is unknown, those eggs in the upper trays of our heath stacks were coated with the media on several occasions. Eggs in the lower trays were not exposed to the media since it was trapped by the screens and eggs in

TABLE 2. Summary of chinook salmon egg mortality after microwave treatments andministered in October, 1987. Treatments are expressed as time in seconds/% power setting. Mortality due to treatment refers to mortalities recorded 24 hrs after exposure to microwave radiation. Mortality after treatment is the fraction of individuals remaining after day one which died prior to transfer from incubator trays to aquaria. (** differs from control $p<0.05$)

TRT	AVE. % MORT.	MORT DUE TO TRT	AVE. % MORT. AFTER TRT
15/25	40	12	32
15/50	40	13	32
15/75	50	41	28
15/100	54	32	34
30/25	53	30	34
30/50	45	20	32
30/75	63	38	41
30/100	72	50	46
45/25	53	25	39
45/50	61	40	37
45/75	85	71 **	55 **
45/100	87	76 **	56 **
60/25	69	41	49
60/50	82	67 **	45
60/75	97	90 **	70 **
60/100	100	100 **	--
IC	45	8	38
EC	45	12	40

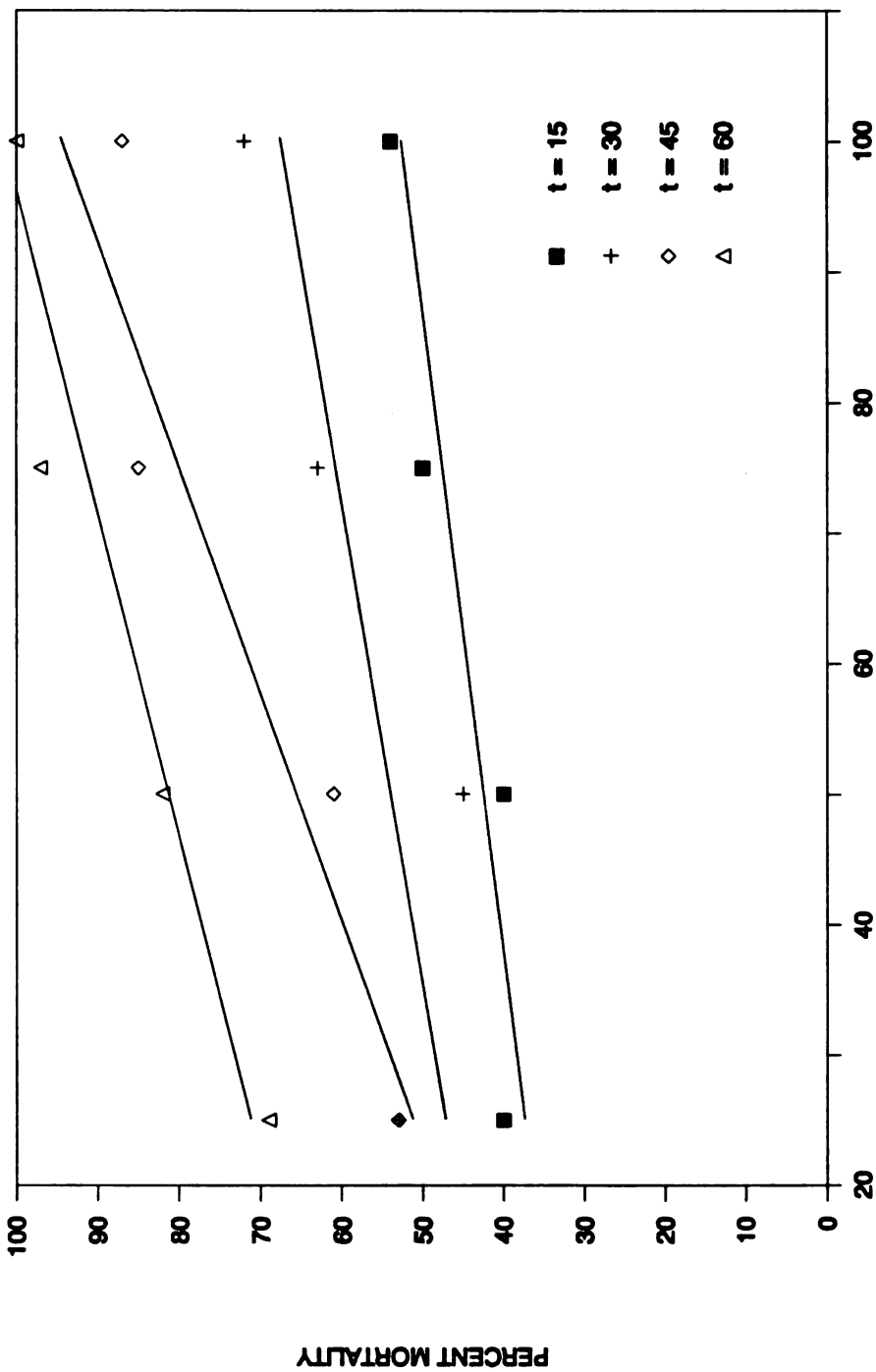

Figure 5. Effect of various microwave power settings on chinook salmon mortality holding shock duration constant (t = shock duration in seconds). Each point represents the mean mortality of three replicates.

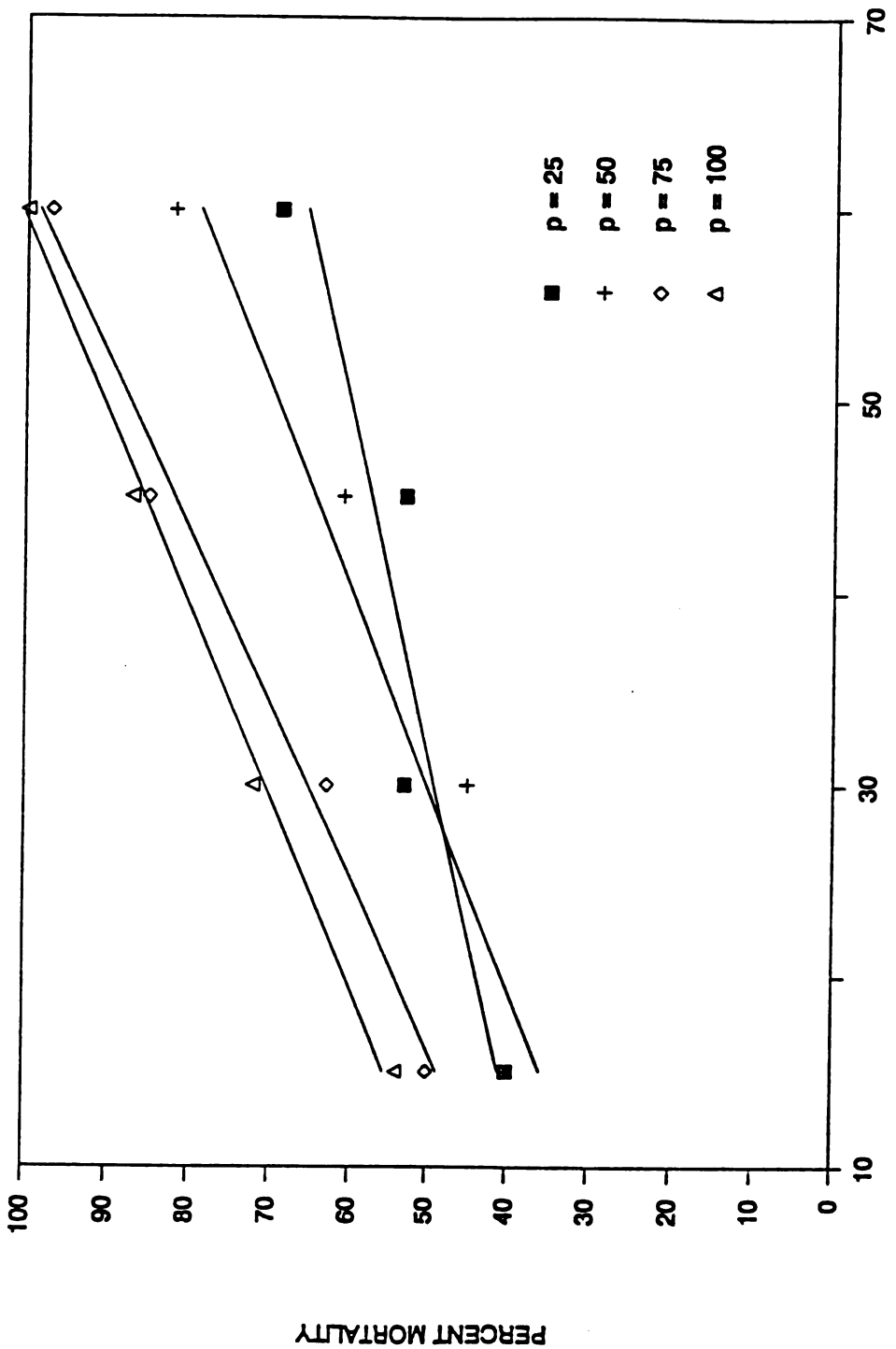

Figure 6. Effect of various microwave shock durations on chinook salmon mortality maintaining a constant power setting (p = percent power). Each point represents the mean of three replicates.

Table 3. Average temperature (°C) resulting from microwave shocks applied to 0.25 L of fertilized chinook salmon eggs in 1987.

	Duration of shock (sec.)			
	15	30	45	60
Power Settings (%)				
25	19	20	27	30
50	22	25	27	37
75	21	27	32	41
100	21	30	40	53

Initial temperature = 12.5 °C

the upper trays. Survival was sufficient for ploidy analysis, but comparison of mortalities is not possible.

DISCUSSION AND CONCLUSIONS

Microwave shock treatments were not effective in induction of triploidy in chinook salmon when applied ten minutes after fertilization. Although three triploids were detected from treatment groups exposed to microwave shocks, we can not be certain they are the result of the microwave treatment. Other researchers have reported triploids in normal fish populations. Gold and Avise (1976) found a spontaneous triploid in a sample of nine California roach Hesperoleucus symmetricus. A single triploid fathead minnow Pimephales promelas was observed from a sample of fifteen fish (Gold, 1986). These are the two reports of viable triploids in natural populations of bisexual teleosts. In hatchery stocks, one triploid rainbow trout was detected from a sample of 18 (Cuellar and Uyeno, 1972). Thorgaard and Gall (1979) found 6 of 11 full sibs, and 1 of 20 cousins of a rainbow trout family to be spontaneous triploids. Detection of triploids in relatively small samples would indicate that triploids occur naturally at some low rate. Thus, our observation of three triploids in over 350 fish sampled may correspond to their frequency in the Great Lakes population. However, in four years of ploidy analysis using flow cytometry, we have never detected any triploids from

approximately 200 control fish tested.

The possibility also exists that the triploids were produced as a result of exposure to microwave radiation. However, their low frequency of occurrence would indicate that microwave radiation is not an effective shock treatment to induce triploidy. Triploid chinook salmon have been produced by our lab for five years using heat shocks yielding 95-100 percent triploids (Westerhof et al, 1988; see chapter IV). Microwave shocks were administered at the same time during development as heat shocks have been. However, it is possible that if administered at an earlier or later stage in development microwaves might produce triploids by a mechanism other than depolymerization of spindle fibers.

It is also possible that the triploids were produced by the heat generated in the microwave oven, instead of the microwave radiation itself. The average temperatures for groups treated for 15 seconds at 100 percent power, and for 45 seconds at 50 percent power were 21 and 27 C respectively. It has been shown that one minute duration heat shocks applied ten minutes post fertilization induced triploidy in rainbow trout, with the incidence of triploidy increasing with shock temperature (Thorgaard et al., 1981). If triploids were being produced by the heat or a heat-microwave interaction, incidence of triploids should increase as a function of average temperature resulting from microwave shocks. This was not observed.

A final possibility is that microwave radiation does inhibit the extrusion of the second polar body; but, also disrupts normal cell structure causing mortality. Data from both 1987 and 1988 demonstrated that some eggs were killed immediately after microwave shock. Mortality data from 1987 indicated that the survival of eggs that were subjected to microwave radiation was decreased beyond the initial deaths due to the shock itself (Table 2). This would indicate that either the microwave shocks or the heat produced by the microwave had some effect on the developmental processes in the egg.

Figures 5 and 6 represent the effect of shock intensity and duration. In Figure 5, mortality increases linearly with a slope of approximately one was expected in our symmetric design. Figure 6 however does not reflect the expected result. Mortality increased at a lower rate than expected. This would indicate that for microwave shocks, the intensity of the shock has a more dramatic effect on egg survival than duration of shock.

We had hoped microwave radiation could be used to induce triploidy. However, our data suggests that it is not effective for polyploid induction when administered ten minutes after fertilization. Based on our experience, 28.5 C heat shock applied for 10 minutes, 10 minutes after fertilization is the optimal method for producing large numbers of triploid chinook salmon (Westerhof et al., 1988).

CHAPTER III EVALUATION OF GROWTH DIFFERNTIALS IN DIPLOID AND TRIPLOID CHINOOK SALMON

ABSTRACT

The use of triploid fish in both aquaculture and management applications has increased dramatically in the past years. As this use continues to increase, more information is needed concerning the differences and similarities between diploid and triploid individuals. Conflicting reports have been submitted regarding the growth of triploid individuals. An experiment was designed to measure the growth of diploids and triploids reared seperately and in competition, at two feed rations.

Twenty fish, either 100 percent diploid, 100 percent triploid or a mixture of 50 percent of each ploidy type, were reared in 110 L aquaria. Three replicates of each ploidy combination were fed rations at either two or four percent body wet weight daily, adjusted at two week intervals. The study continued for eight weeks.

No significant difference ($p<0.05$) in growth rate was detected between treatments within a feeding regime. Feed conversions calculated for tanks containing a single ploidy level did not differ significantly ($p<0.05$) between diploids and triploids or feeding levels.

INTRODUCTION

There is significant potential in using sterile triploid fishes in aquaculture and management (Donaldson and Hunter, 1982; Purdom, 1983; Thorgaard, 1983; Benfey and

Sutterlin, 1984). Sterility which results from induced triploidy could eliminate undesirable traits associated with the onset of sexual maturity, including decreased flesh quality, decreased growth rate, and precocial maturation. However, before triploids can be used on a wide spread basis, more information must be compiled concerning potential differences in performance between diploids and triploids (Thorgaard, 1983).

Theoretically, triploids and diploids should grow at similar rates until the onset of sexual maturation (Purdom, 1983; Thorgaard, 1983). Studies have indicated that there is little or no difference between the growth of juvenile diploid and triploid stickleback (Swarup, 1959), rainbow trout (Thorgaard and Gall, 1979) carp (Gervai et al., 1980), plaice x flounder hybrids (Lincoln, 1981),coho salmon (Johnson et al., 1986) and chinook salmon (Westerhof, 1988). However this may not be the case for all species under all experimental conditions. Triploid Atlantic salmon were found to be significantly longer than diploids but did not differ in weight (Benfey and Sutterlin, 1984).

Other studies have indicated that diploids and triploids may grow at different rates. Rainbow trout triploids reared in fiberglass tanks were smaller than diploids (Solar et al.1984). Cassani and Caton (1986) found triploid grass carp (Ctenopharyngodon idella) grew slower than diploids if reared together in ponds, but no difference if they were

reared separately in indoor greenhouse tanks. Triploid channel catfish have been shown to be heavier than diploids at 8 months of age (Wolters et al., 1982) and triploid tilapia were larger than diploids at 14 weeks of age (Valenti, 1975). Triploid hybrid grass carp (Ctenopharyngodon idella x Hypophthalmicthys nobilis) were shown to grow faster than diploids (Cassani et al., 1984) as were triploid fancy carp produced by crossing two strains (Taniguchi et al., 1986). Both authors however, indicate that the increased growth rate may have been the result of genetic differences in parents. It has been suggested that the sterility of triploids, not the triploid condition is responsible for some of the observed differences in growth (Thorgaard and Allen, 1987).

Comparisons of growth rates between diploids and triploids are based on fish maintained in similar, but not identical environments. Some comparisons that have been reported are based on observations made as part of other studies. In some studies the feeding rates, fish size and age, water temperature, and descriptions of rearing facilities have been omitted making replication of the experimental design impossible. Variation in these conditions between treatments and studies could lead to significantly different results in the same species. Finally, the comparison of fish held in separate environments gives no information concerning diploid-triploid interactions. Although neither diploids or

triploids should have a competitive advantage, anecdotal observations have indicated the possibility of differential performance between diploids and triploids.

Our study was designed to compare the growth of triploids and diploids alone or in equal proportions in the same tank. Fish were fed at fixed rates that were maintained throughout the study and adjusted periodically to compensate for growth. This design allows not only the comparison of growth rates, but also provides the first available information on potential competitive interactions between diploids and triploids.

MATERIALS AND METHODS

In March of 1989, diploid and triploid chinook salmon used in the growth study were randomly sampled from fish reared by the MDNR for the Great Lakes stocking program (see Chapter III) at the Wolf Lake Hatchery. On April 7, 1989 the flow cytometery was used to confirm ploidy (see Appendix 1). The heat-shocked fish tested were 95 percent triploid from a sample of twenty fish. Diploids were marked using a right pectoral clip, while triploids were marked with a left pectoral clip. Prior to fin clipping, fish were anestisized using tricane methane sulfonate (Finquel MS-222, Argent Chemical Laboratories) at 5 grams per gallon.

All fish were reared in 110 L aquaria as described in Chapter I. One of the six ploidy-food ration combinations

was assigned to each of 18 tanks, resulting in three replicates for each combination. Diploids were anestisized, weighed and assigned to the appropriate tank. Ten triploids were then collected at random and weighed. These ten fish were assigned to the mixed tank containing the ten diploids that were most similar in weight. Triploids were then assigned to those tanks designated as all triploid. This process was followed to reduce the possibility of a competitive advantage for either ploidy type due to initial size differences. Fish were fed at either 2 percent or 4 percent wet body weight daily on a wet feed basis (approximately 1.5 percent and 3 percent dry weight basis). Prior to the onset of each day's feeding, tanks were siphoned to remove fecal material, uneaten food, and any other material in the bottom of the tank. A net was placed over all tanks to prevent fish from jumping out of the tanks.

Feeding began the day following the initial weight samples. Fish were fed 14mm biodiet three times a day to total the percent body weight assigned to each tank. A minimum of three hours separated each feeding. Fish were weighed at two week intervals, to adjust the amount of feed being fed. Tanks that included both diploids and triploids were sorted, and weighed separately. To facilitate handling and reduce stress, all fish were anesthesized before weight samples were taken. Fish were not fed on the day they were weighed. After fish had been weighed, they were held in

fresh water while their tanks were scrubbed to remove any algae that had accumulated on the side of the tanks.

The fish used in this study were known to have bacterial kidney disease (BKD) (John Gnath, MDNR fisheries pathologist, pers. comm.). During the study nine fish died exhibiting the signs of BKD. Since BKD is an inherited disease, and the deaths were not associated with the treatments, these fish were replaced by a fish of equal weight which had been held in separate tanks and fed the same diet. Handling and the siphoning of tanks each resulted in the death of one fish, which were also replaced with appropriately sized fish from the axillary tanks. No fish mortalities were observed that were not attributed to handling, tank siphoning, or BKD.

After an eight week period, fish were maintained without feed for two days to insure all feed had been digested and waste expelled. Fish were then separated and weighed as described for biweekly weight samples. Linear regression analysis was used to find the best fit line to describe the growth rate of each tank. The slope of the regression line was used as the growth rate for each tank. A one way analysis of variance was used to test for significant differences of the mean growth rate of each ploidy combination for both feed rations (Gill, 1978).

At the conclusion of the study, feed conversion was calculated for those tanks containing only one ploidy level. Feed conversion is defined as weight gained/weight of feed

fed. It was impossible to calculate feed conversion in mixed tanks since the amount of feed eaten by each ploidy type could not be measured. A two way analysis of variance was used to test for differences between feeding rations, and difference among treatments.

RESULTS

No significant difference in growth was found between ploidy combinations at each feeding ration ($p<0.05$). As expected, fish fed 4 percent body wet weight daily grew significantly faster at all ploidy combinations than fish fed 2 percent body wet weight daily. Feed conversion was calculated for those tanks that contained only one ploidy type. No significant difference ($p<0.05$) was found among treatments at either feed ration nor was there a significant difference between feed rations ($P<0.05$). Figures 7 and 8 are graphs of average daily weight versus time. Most of the growth curves for individual tanks were correlated in a linear response. In no case was r^2 found to be less than 0.920. This would indicate that the average slope of the regression lines for each treatment should be an accurate estimate of the true growth rates.

DISCUSSION

We predicted that juvenile diploid and triploid chinook salmon should grow at similar rates either alone or

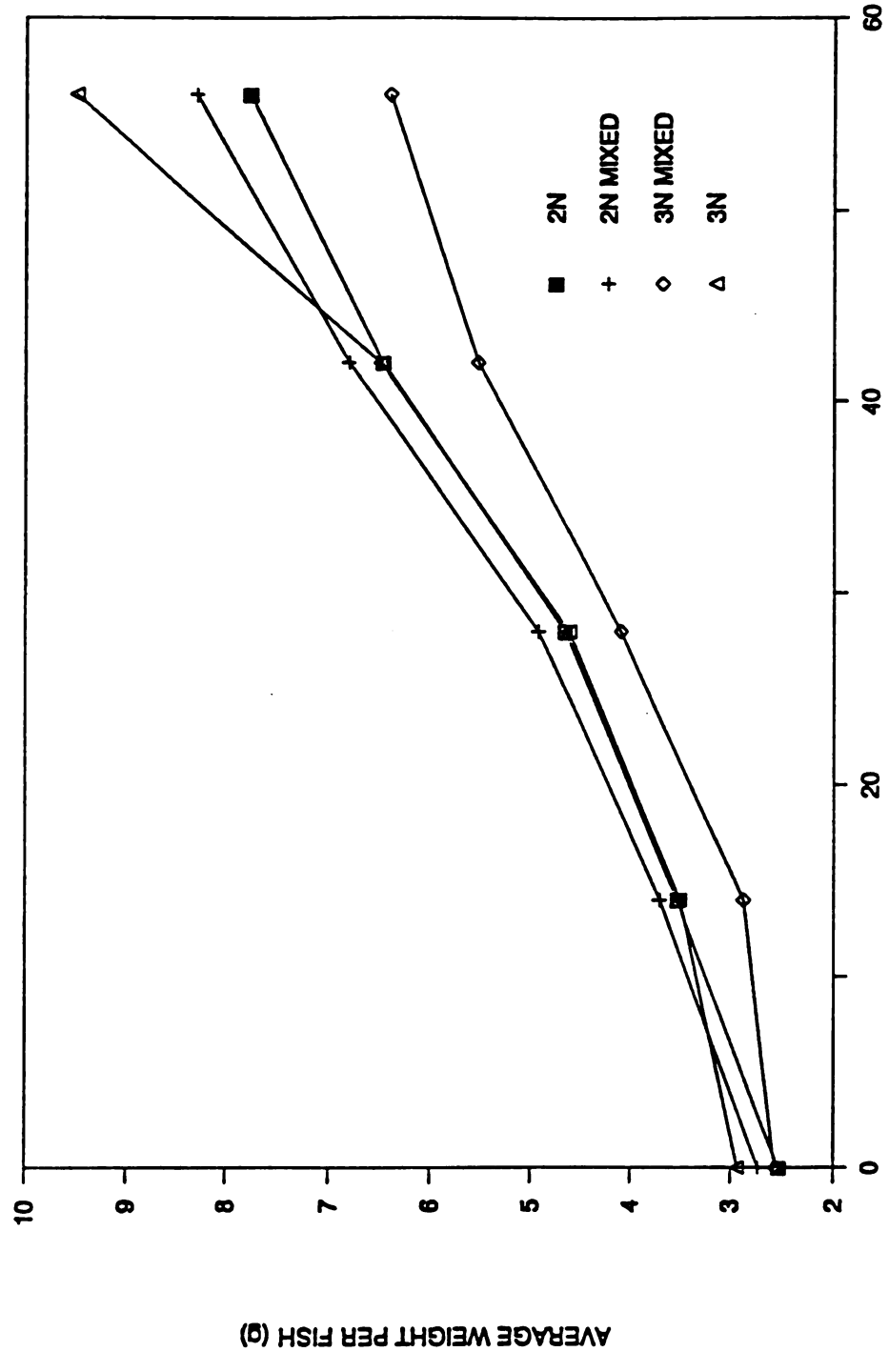

Figure 7. Growth of diploid and triploid chinook salmon fed two percent wet body weight daily grown separately or mixed in a single 110 L aquarium.

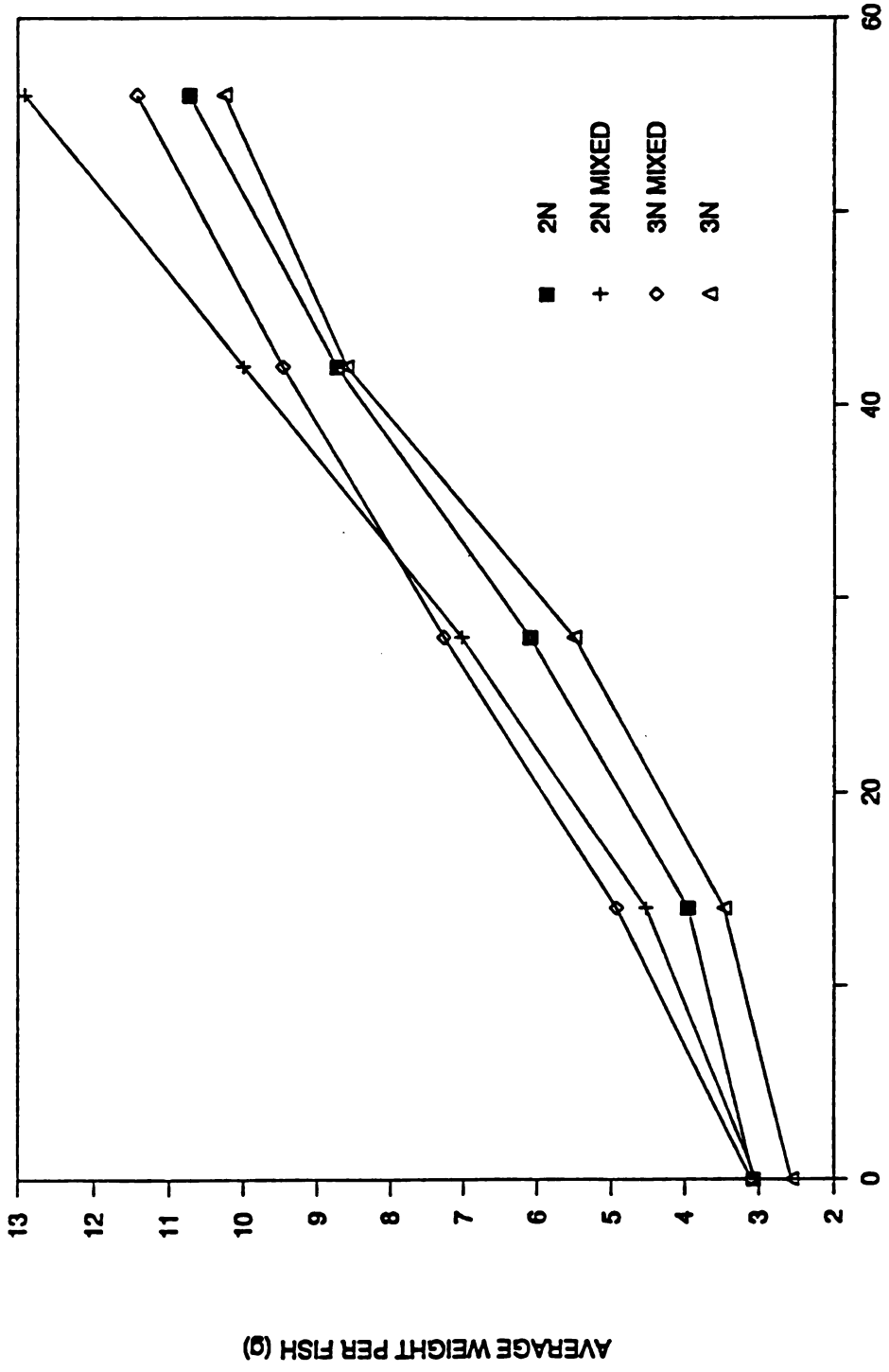

Figure 8. Growth of diploid and triploid chinook salmon fed four percent wet body weight daily grown separately or mixed in a single 110 L aquarium.

together. Spontaneously occuring triploid rainbow trout found in a hatchery stock were of similar size to their diploid counterparts (Thorgaard and Gall, 1979). Diploid and triploid coho salmon reared together in both saltwater netpens and freshwater pools showed no difference in growth (Johnson et al., 1986) Other researchers have observed triploids and diploids to have equal growth rates when grown in separate rearing units (Swarup, 1959; Gervai et al., 1980; Lincoln, 1983; Johnson et al., 1986; Westerhof, 1988). Our findings are consistent with these reports.

Cassani and Caton (1986) found triploid grass carp had lower growth rates than diploids when the two were grown together in ponds. The authors attributed the observed growth differences to a higher consumption rate and a more efficient feed conversion in diploids. Another study found that gut length in triploid grass carp purchased from a commercial source was generally longer than diploids and suggested triploids might digest feed more efficiently as a result (Cassani et al., 1984). Gut length measurements were not taken in the 1986 study. Differences between diploid and triploid length were least pronounced in overstocked, underfed ponds. Maximum length differences were observed when fish were stocked at a moderate rate and fed an abundant supply of food (Cassani and Caton, 1986). This observation seems to conflict with the hypothesis of higher diploid consumption rate and improved feed conversion. In those

ponds that contained few fish and were fed large quantities of feed, it would seem that sufficient nutrition should have been available to sustain near maximum growth in most fish. No difference was observed when diploids and triploids were reared separately in greenhouse ponds (Cassani and Caton, 1986). It is possible that the observed growth differences in diploid and triploid grass carp may be attributable to some factor other than feed ration.

It should also be noted that no plateau was reached during our study, which indicated that no other factors were limiting during our study. Similarly, the absence of a detectable lag in growth in the first two week period would indicate that fish did not suffer from significant stress due to fin clipping or the initial handling involved at the beginning of the study.

Although our findings are only applicable to a short term controlled lab study with chinook salmon, the study provides valuable information on interactions between diploids and triploids. Our results indicated that competition between the diploid and triploid chinook salmon was negligible. This situation may change as diploids reach sexual maturity, as was observed in channel catfish (Wolters et al., 1982). If secondary sex characteristics are eliminated in chinook salmon, their extended life span and maintained growth rates may lead to a fish which reaches a larger ultimate size (Westerhof et al., 1988).

CHAPTER III

1988/89 MICHIGAN DEPARTMENT OF NATURAL RESOURCES TRIPLOID CHINOOK SALMON STOCKING PROGRAM

As part of a continuing study, triploid chinook salmon were produced in 1988 for Great Lakes stocking by the Michigan Department of Natural Resources. In preceding years, about two million eggs were heat shocked over a period of two days to produce 200,000 triploid smolts for stocking. The run in 1988 spanned a greater period of time than normal and fewer fish returned. This was probably due to unusually high fall temperatures and a weak year class, respectively. Fish were shocked on six days spanning a four week period from October 19 to November 13 as a result of the low number of ripe fish on any given day.

Fish were spawned using standard procedures (Piper et al., 1982). Eggs were collected from ripe females by injecting compressed air at 2 psi into the body cavity. Each treatment lot contained approximately 25,000 eggs obtained by spawning four or five females into 5 gallon plastic pails. Milt was collected in plastic wash bottles from approximately five ripe males by applying pressure to the abdomen. Milt was kept chilled until used. The milt was mixed with the eggs in the absence of water. Two minutes after fertilization, river water was added to the fertilized eggs. Eggs were then gently rinsed using river water until time for shocking.

Triploids were produced by heat shocking the fertilized eggs as described by Westerhof et al. (1988). Ten minutes

after fertilization, eggs were drained of excess water and submersed in a 28.5 ^{o}C water bath. The eggs remained in the warm water bath for ten minutes. After removal from the heat shock unit eggs were placed in 15 gallon milk cans, slow cooled to ambient temperature, and water hardened for one hour prior to transport to the Wolf Lake Hatchery. One step of the processes was a modification of the procedure described by Westerhof et al. (1988). In 1987 it was noted that as subsequent batches of eggs were heat shocked, water quality in the warm water baths declined. This decline in water quality was a result of excess milt and material from broken eggs building up over time. In an attempt to maintain water quality in the shocking units, all eggs were continually rinsed from water activation (addition of water 2 minutes post fertilization) to submersion in the warm water.

Approximately 800,000 eggs were shocked in 1988. A total of 143,607 fry survived and were stocked. This marked an increase in survival of approximately 8 percent from previous years (Westerhof et al., 1988). The improved survival observed in 1989 may have been the result of improved water quality. It is also possible that the lower number of eggs shocked enabled the MDNR workers to provide more care for the eggs than was practical when two million eggs were present.

Our efforts in rinsing the eggs were aimed at maintaining water quality in the production runs for MDNR

stocking. It would be desirable to test the effect of declining water quality in subsequent heat shock treatments using a statistically valid study design.

On April 7, 1989 a random sample of 20 heat shocked fish were tested to confirm ploidy. Blood samples were taken by cardiac puncture, and the samples were analyzed using a flow cytometer (see Appendix 1). The heat shocked fish were found to be 95 percent triploid.

Diploid and triploid chinook salmon from 1986 production runs have been held at the MDNR Wolf Lake Hatchery. A sample of these fish had been collected annually and examined for gonadal development. Unfortunately, most of these fish were inadvertently stocked as part of a put-and-take stocking program. Consequently, the annual examination was not done in 1988. It was felt that these few fish remaining should be maintained until the fall of 1989, when the normal diploids should reach sexual maturity.

Triploid chinook salmon were stocked in Lakes Michigan, Huron, and Superior in the spring of 1989. The stocking sites and number of fish stocked is given in Table 4.

TABLE 4. Number and location of triploid chinook salmon stocked by the Michigan Department of Natural Resources in Spring 1989.

Location stocked	Number stocked
Lake Michigan	
Little Manistee River	60,494
Lake Huron	
Van Ettan Creek	21,863
Swan River	21,529
Harbor Beach	20,676
Lake Superior	
Ontonagongan River	10,044
Carp River	9,001

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

CHAPTER V. SUMMARY AND CONCLUSIONS

In continuing the MSU-MDNR triploid chinook stocking program two experiments were conducted. We attempted to induce triploidy using various intensity-duration microwave shocks to reduce the mortality generally associated with heat shocks. We also began to evaluate the performance and behavior of triploids by designing a statistically valid growth study comparing the growth of diploids and triploids reared separately and under competitive conditions. In addition to our experimental work, we continued to use heat shock to produce triploids for stocking by the MDNR.

Microwave radiation treatment was not effective in producing triploid chinook salmon under the conditions used in this study. The developmental time at which microwaves were administered to eggs was based on the timing of heat shocks used successfully to induce triploidy in chinook salmon. We assumed that microwave shocks would produce triploids by the same mechanism as heat shock. Microwave radiation may in fact produce triploids if applied at a different time during development. Microwave shocks may be effective in producing polyploids in other species.

Based on previous studies which have indicated that there may be differences in growth between diploids and triploids, a controlled and statistically valid experiment was designed to detect performance differentials. This study examined growth of diploids and triploids reared separately

in similar tanks and together in the same tank. Three replicates of each tank type were fed at either 2 or 4 percent wet body weight daily. At both feed rations diploids and triploids grew at approximately the same rate regardless of whether reared separately or in tanks containing equal proportion of each ploidy type. Our study indicated that over an eight week period six month old triploid and diploid chinook salmon do not show significant difference in growth.

Using heat shock, 143,000 triploid chinook salmon were produced for stocking in the Great Lakes by the MDNR. These fish were stocked in Lakes Michigan, Huron and Superior (Table 4) as part of a continuing cooperative study between the MDNR and MSU. Procedures to produce triploids by heat shock were modified slightly from past years. Prior to submersion in the warm water bath eggs were rinsed using ambient temperature river water to remove broken egg membranes and other debris that accumulates in the heat shock units as subsequent lots of eggs are shocked. Initial observations indicated that pre-heat shock rinse may have reduced the high mortality rates caused by heat shock.

APPENDIX

APPENDIX A

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

The protocol used to prepare samples (Appendix Table 1) for ploidy analysis by flow cytometry was modified from Westerhof (1988).

Samples of chinook red blood cells were analyzed for ploidy using chicken blood as an internal standard. Pulse-height histograms were generated based on measurements from 10,000 cells per sample. The flow cytometer was adjusted so that peaks resulting from chicken blood usually ran at 27-30. Diploid peaks generally ran at 75-90 while triploid peaks were observed at 120-135. The position of peaks may vary slightly due to sample preparation or machine settings. To insure proper identification of ploidy level, known diploids were run each day to establish the position of the diploid peak. The position of test sample peaks could then be compared to peaks from both the internal standard (chicken blood) and previously analized diploids.

Appendix Table 1. Steps in the cell staining technique used to determine ploidy level in chinook salmon.

Step	Description
1.	Blood was drawn from each fish using a 22 gauge needle and a 5 ml syringe rinsed with sodium citrate buffer solution (CBS)(Appendix Table 2).
1a.	Chicken blood was obtained by drawing blood from the wing of a chicken using a 21 guage needle and a 3 cc syringe rinsed with CBS. Chicken blood was processed in an identical manner to the fish blood with the exception that the amount of blood and reagents used was doubled.
2.	Blood was added to a 12 x 75 mm plastic test tube containing 0.5 ml of CBS by submersing the needle in the buffer and applying slight pressure to the hypodermic. Blood was slowly added until samples were a faint pink color.
3.	Samples were stored on ice or refridgerated until further processing was completed.
4.	Samples were centrifuged at 2500 rpm at 10 C for 5 minutes and the supernant was discarded leaving a cell pellet.
5.	Cells were resuspended in 0.5 mls of the sodium citrate buffer solution and vortexed until all clumps disappeared.
6.	Cells were fixed for approximately 15 minutes using 70 percent ethanol. Ethanol was stored on ice prior to use.
7.	Samples were centrifuged as described in step 4, and the supernant was disgarded.
8.	Cells were resuspended in 1.5 ml of propidium iodide solution (Appendix Table 2).
9.	0.5 ml of RNAse-A solution was added to each tube.
10.	0.5 ml of chicken blood prepared as described in steps 1-9 was added to each tube.
11.	Samples were run using the Ortho Cytofluorograph.

Appendix Table 2. Composition of reagents used in flow cytometric determination of ploidy levels in chinook salmon.

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

REFERENCES

REFERRENCES

Allen, S. K. and J. G. Stanley. 1978. Reproductive sterility in polyploid brook trout, Salvelinus fontinalis. Trans. Am. Fish. Soc., 107(3):473-478.

Allen, S. K. and J. G. Stanley. 1979. Polyploid mosaics induced by cytochalasin B in landlocked Atlantic salmon, Salmo salar. Trans. Am. Fish. Soc., 108:462-466.

Allen, S. K. 1983. Flow cytometry: assaying experimental polyploid fish and shellfish. Aquaculture 33:317-328.

Allen, S. K., R. G. Theiry, and N. T. Hagstrom. 1986. cytological evaluation of the likelihood that the triploid grass carp will reproduce. Trans. Am. Fish. Soc. 115(6):841-848.

Baksi, S. M. and J. C. Means. 1988. Preparation of chromosomes from early stages of fish for cytogenetic analysis. J. Fish. Biol. 32(3):321-325.

Benfey, T. J. and A. M. Sutterlin. 1984a. Growth and gonadal development of triploid landlocked Atlantic salmon (Salmo solar). Can. J. Fish. Aqua. Sci., 41:1387-1392.

Benfey, T. J. and A. M. Sutterlin. 1984b. Triploidy induced by heat shock and hydrostatic pressure in landlocked Atlantic salmon (Salmo salar). Aquaculture, 36:359-367.

Benfey, T. J., I. I. Solar, G. DeJong, and E. M. Donaldson. 1986. Flow-Cytometric Confirmation of aneuploidy in sperm from triploid rainbow trout. Trans. Am. Fish. Soc., 115:838-840.

Bidwell, C. A., C. L. Chrisman, and G. S. Libey. 1985. Polyploidy induced by heat shock in channel catfish. Aquaculture 51:25-32.

Blancher, L.J. 1926. Depenence of secondary sexual characteristics upon testicular hormones in Lebistes reticulatus. Biol. Bull., 50:374-381.

Blaxhall, P. C. 1983. Chromosome karyotyping of fish using conventional and G-banding methods. J. Fish. Biol. 22:417-424.

Briggs, R. 1947. The experimental production and development of triploid frog embryos. J. Exper. Zool.,106:2237-265.

Bye, V. J. and R. F. Lincoln. 1986. Commercial methods for the control of sexual maturation in rainbow trout (Salmo gairdneri). Aquaculture, 57:299-309.

Calis, J. and H. Hoehn. 1976. Flow fluorimetry diagnosis of euploid and aneuploid human lymphocytes. Am. J. Hum. Genet. 28:577-584.

Cassani, J., W. Caton, and B. Clark. 1984. Morphological comparison of diploid and triploid hybrid grass carp, Ctenopharygodon idella female X Hypothalmichthys nobilis male. J. Fish Biol., 25(3):269-278.

Cassini, J. and W. Caton. 1986. Growth comparisons of diploid and triploid grass carp under varying conditions. Prog. Fish Cult., 48(3):184-187.

Chevassus, B., R. Guyomard, D. Chourrout, and E. Quillet. 1983. Production of viable hybrids in salmonids by triploidization. Genet. Sel. Evol. 15:519-532.

Chourrout, D. 1980. Thermal induction of diploid gynogenesis and triploidy in the eggs of the rainbow trout (Salmo gairdneri). Reprod. Nut. Dev., 20:727-733.

Chourrout, D. 1982. Tetraploidy induced by heat shocks in the rainbow trout (Salmo gairneri). Reprod. Nutr. Develop. 22(3):569-574.

Chourrout, D. and E. Quillet. 1982. Induced gynogenesis in the rainbow throut: sex and survival of progenies. Production of all triploid populations. Theor. Appl. Genet.,63:201-205.

Chourrout, D. 1984. Pressure-induced retention of second polar body and suppression of first cleavage in rainbow trout: production of all-triploids, all-tetraploids,and heterozygous and homozygous diploid gynogenesis. Aquaculture, 36:111-126.

Cimino, M. C. 1973. Karyotypes and erythrocyte sizes of some diploid and triploid fishes of the genus Poeciliopsis. J. Fish. Res. Board Can. 30:1736-1737.

Cuellar, O. and T. Uyeno. 1972. Triploidy in rainbow trout. Cytogenetics, 11:508-515.

Don J. and R. R. Avtalion. 1988. Comparitive study on the induction of triploidy in tiilapias using cold and heat shock techniques. J. Fish. Biol., 32(5):665-672.

Downing, J. R. N. A. Benson, and R. C. Braylan. 1984. Flow cytometry: applications in the clinical laboratory. Lab Manage. May 29-37.

Fankhauser, G. 1945. The effectof change in chromosome number on amphibian development. Quart. Rev. Biol., 20:20-78.

Fankhauser, G. and D. Godwin. 1948. The cytological mechanism of the triploidy inducing mechanism effect of heat shock on the eggs of the newts Triturus viridescens. Proc. Nat. Acad. Sci. 34:544-551.

Garling, D. L. Jr. and R. P. Wilson. 1976. Inexpensive constant water level device for flow through aquariums. Prog. Fish. Cult., 38(1):52-53.

Gervai, J., S. Peter, A. Nagy, L. Horvath, and V. Csanyi. 1980. Induced triploidy in carp. J. Fish. Biol., 17:667-671.

Gill, J. L. 1978. Design and Analysis of Experiments in the Animal and Medical Sciences. The Iowa State University Press. Ames.

Goetz, F. E., E. Donaldson, G. Hunter, and H. Dye. 1979. Effeects of estradiol-17 and 17 methyltestosterone on gonadal differentiation in the coho salmon (Oncorhynchus kisutch). Aquaculture, 17:267-278.

Gold, J. R. and J. C. Avise. 1976. Spontaneous triploidy in the California roach hesperoleucus symetricus (Pisces: Cyprinidae). Cytogenet. Cell Genet., 17:144-149.

Hildemann, W. H. 1954. Effects of sex hormones on secondary sex characteristics of Lebistes reticulatus. J. Exptl. Zool., 126:1-15.

Hill, J. M., A. Hickerson, D. L. Sheldon, and K. A. Warren. 1985. Production of triploid chinook salmon (Oncorhynchus tshawytscha) using flow through heat shock and subsequent, gradual cooling. Clatsop Economic Develop. Comm. Fisheries Proj. Astoria, Oregon (unpublished paper).

Johnson, O. W., P. R. Rabinovitch, and F. M. Utter. 1984. Comparison of the reliability of a Coulter Counter with a flow cytometer in determining ploidy levels in pacific salmon. Aquaculture, 43:00-103.

Johnson, O. W., W. W. Dickhoff, and F. M. Utter. 1986. Comparative growth and development of diploid and triploid coho salmon, Oncorhynchus kitutch. Aquaculture, 57:329-336.

Johnstone, R. 1985. Induction of triploidy in Atlantic salmon by heat shock. Aquaculture, 49:133-139.

Johnstone, R. and R. F. Lincoln. 1986. Ploidy estimation using erythrocytes from formalin fixed salmonid fry. Aquaculture 55(2):145-148.

Kligerman, A. D., and S. E. Bloom. 1977. Rapid cfhromosome preparations from solid tissues of fishes. J. Fish. Res. Board Can. 34:266-269.

Lemoine, H. L. and L. T. Smith. 1980. Polyploidy induced in brook trout by cold shock. Trans. Am. Fish. Soc., 109:626-631.

Lincoln, R. F. D. Aulstad, and A. Grammeltvedt. 1974. Attempted triploid induction in Atlantic salmon (salmo salar) using cold shocks. Aquaculture, 4:287-297.

Lincoln, R. F. 1981a. Sexual maturation in triploid male plaice (Pleuronectes platessa) and plaice x flounder (Platichthys flesus) hybrids. J. Fish. Biol., 19:415-426.

Lincoln, R. F. 1981a. Sexual maturation in triploid female plaice (Pleuronectes platessa) and plaice x flounder (Platichthys flesus) hybrids. J. Fish. Biol., 19:499-507.

Lincoln, R. F. and A. P. Scott. 1983. Production of all female triploid rainbow trout. Aquaculture, 4:287-297.

Lincoln, R. F. and A. P. Scott. 1984. Sexual maturation in triploid rainbow trout, Salmo gairdneri Richardson. J. Fish. Biol., 25:385-392.

Lou, Y. D. and C. E. Purdom. 1984. Polyploidy induced by hydrostatic pressure in rainbow trout (Salmo gairdneri) J. Fish. Biol., 25:345-351.

Menzel, B. W. and R. M. Parnell. 1973. Morphology of naturally occuring triploid fish related to Poecilia formosa. Copeia, 350-352.

Myers, J. M. 1986. Tetraploid induction in Oreochromis spp. Aquaculture 57:281-287.

Myers, J. M., W. K. Hershberger, and R. N. Iwamoto. 1986. The induction of tetraploidy in salmonids. J World Aquacult. Soc. 17(1-4):1-7.

Parsons, J. E. and G. H. Thorgaard. 1984. Induced androgenesis in rainbow trout. J. of Exper. Biol., 231:407-412.

Parsons, J. E. and G. H. Thorgaard. 1985. Production of androgenic diploid rainbow trout. J. of Heredity, 76:177-181.

Phillips, R. B., K. D. Zajicek, P.E. Ihssen, and O. Johnson. 1986. Application of silver staining to the identification of triploid fish cells. Aquaculture 54(4):313-319.

Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler, and L. R. Leonard. 1983. Fish Hatchery Management. United States Dept. of the Interior, Fish & Wildlife Service, Washington D. C.

Purdom, C. E. 1969. Radiation induced gynogenesis and androgenesis if fish. Heredity, 24:431-444.

Purdom, C. E. 1972. Induced polyploidy in plaice (Pleuronectes platessa) and its hybrid with the flounder (Platiehthys flesus). Heredity, London, 24:431-444.

Purdom, C. E. 1983. Genetic engineering by the manipulation of chromosomes. Aquaculture, 33:287-300.

Purdom, C. E., D. Thompson, and Y. D. Lou. 1985. Genetic engineering in rainbow trout, salmo gairdneri Richardson, by suppression of meiotic and mitotic metaphase. J. Fish. Biol. 27:73-79.

Refstie, T., V. Vassvik, and T. Gjerdem. 1977. Induction of polyploidy in salmonids by cytochalasin B. Aquaculture, 10:65-74.

Refstie, T. 1981. Tetraploid rainbow trout produced by cytochalasin B. Aquaculture, 25:51-58.

Refstie, T., J. Stoss, and E. M. Donaldson. 1982. Production of all female coho salmon (Oncorhynchus kitsutch) by diploid gynogenesis using irradiated sperm and cold shock. Aquaculture, 29:67-82.

Rieder, C. L. and A. S. Bajer. 1978. Effect of elevated temperatures on spindle microtubules and chromosome movements in cultures newt lung cells. Cytobios, 18:201-234.

Robertson, J. G. 1953. Sex differentiation in the Pacific salmon, Oncorhynchus keta. Can. J. Zool., 31:73-79.

Robertson, O. 1961. Prolongation of the life span of kokanee salmon (Oncorhynchus nerka kennerlyi) by castration before beginning of gonad development. Zoology, 47:609-621.

Scheerer, P. D. and G. H. Thorgaard. 1983. Increased survival in salmonid hybrids by induced triploidy. Can. J. Fish. Aquat. Sci., 40:2040-2044.

Schultz, R. J. 1967. Gynogenesis and triploidy in the viviparous fish Poeciliopsis. Science, 157:1564-1567.

Schultz, R. J. and K. D. Kallman. 1968. Triploid hybrids between the all-female teleost Poecilia formosa and Poecilia sphenops. Nature, 210:280-282.

Schultz, R. J. 1969. Hybridization, unisexuality, and polyploidy in the teleost Poeciliopsis (Poeciliidae) and other vertebrates. Am. Nat., 103:605-619.

Shelton, C. J., A. G. MacDonald, and R. J. Johnstone. 1986. Induction of triploidy in rainbow trout using nitrous oxide. Aquaculture, 58:155-159.

Smith, L. T. and H. L. Lemoine. 1979. Colchicine-induced polyploidy in brook trout. Prog. Fish Cult., 41:86-88.

Solar, I. I., E. M. Donaldson, and G. A. Hunter. 1984. Induction of triploidy in rainbow trout (Salmo gairdneri) by heat shock, and investigation of early growth. Aquaculture, 42:57-67.

Strickberger, M. W. 1985. Genetics. 3rd Edition. MacMillian Pub. Co., New York.

Streisinger, G., C. Walker, N. Dower, D. Knauber, and F. Singer. 1981. Production of clones of homozygous diploid zebrafish. Nature, 291:293-296.

Swarup, H. 1959a. Production of triploidy in Gasterosleus aculeatus. J. Genet., 56:129-146.

Swarup, H. 1959b. Effect of triploidy on the body size, general organization and cellular structure in Gasterosteus aculeatus (L.). J. Genetics, 56:143-155.

Sweet, D. J. 1986. Trial heat shocking to induce triploidy in coho salmon, chinnok salmon and coho x chinook salmon hybrids. Masters Thesis. Michigan State University Dept. of Fish. and Wildlife.

Taniguchi, N., A Kijima, T. Takegami, and I. Yamasaki. 1986. Color, growth and maturation in ploidy-manipulated fancy carp. Aquaculture, 57:321-328.

Thorgaard, G. H. and G. A. Gall. 1979. Adult triploids in a rainbow trout family. Genetics, 93:961-973.

Thorgaard, G. H., M. E. Jazwin, and A. R. Stier. 1981. Polyploidy induced by heat shock in rainbow trout. Trans. Am. Fish. Soc., 110:546-550.

Thorgaard, G. H., P. S. Rabinovitch, M. W. Shen, G. A. E. Gall, J. Propp, and F. M. Utter. 1982. Triploid rainbow trout identified by flow cytometry. Aquaculture 29:305-309.

Thorgaard, G. H. 1986. Ploidy manipulation and performance. Aquaculture, 57:57-64.

Thorgaard, G. H. and S. K. Allen. 1987. Chromosome manipulation and markers in fishery management. In Ryman and Utter eds. Population Genetic & Fishery Management. University of Washington, Seattle.

Utter, F. M., O. W. Johnson, G. H. Thorgaard, and P. S. Rabinovitch. 1983. Measurement and potential applications of induced triploidy in Pacific salmon. Aquaculture, 35:125-135.

Valenti, R. J. 1975. Induction of polyploidy in Tilapia aurea by means of temperature shock. J. Fish Biol., 7:519-528.

Vasetskii, S. G. 1967. Changes in the ploidy of sturgeon larvae induced by heat treatment of eggs at different stages of development. Doklady Akademii Nauk SSSR, 172(5):1234-1237.

Wattendorf, R. J. 1986. Rapid identification of triploid grass carp cells with coulter counter and channelyzer. Pro. Fish. Cult. 48(2):125-132.

Westerhof, R. E. 1988. Development of techniques to produce triploid chinook salmon for the great lakes. Masters Thesis. Michigan State University Dept. Fish. and Wildlife.

Westerhof, R. E., D. L. Garling, and H. A. Tanner. 1988. Development of techniques to produce triploid chinook salmon for the Great Lakes. abstract in J. World Aquacult Soc., 19:73A.

Wolters, W. R., G. S. Libey, and C. L. Chrisman. 1981a. Induction of triploidy in channel catfish. Trans. Am. Fish. Soc., 110:312-314.

Wolters, W. R., C. L. Chrisman, and G. S. Libey. 1981b. Lymphocyte culture for chromosomal analyses of channel catfish. Ictalurus punctatus. Copeia 1981:503-504.

Wolters, W. R., C. L. Chrisman, and G. S. Libey. 1982a. Erythrocyte nuclear measuremtents of diploid and triploid channel catfish, Ictalurus punctatus (Rafinesque). J. Fish Biol. 20:253-258.

Wolters, W. R., S. G. Libey, and C. L. Chrisman. 1982b. Effect of triploidy on growth and gonadal development of channel catfish. Trans. Am. Fish. Soc. 111:102-105.