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Triploid incubation and growth performance: A comparison of meiotic and interploid triploid rainbow trout (*Oncorhynchus mykiss*) inter- and intrastrain crosses

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University of Washington, 1990

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Triploid Incubation and Growth Performance:
A Comparison of Meiotic and Interploid Triploid
Rainbow Trout (*Oncorhynchus mykiss*) Inter- and Intrastrain Crosses.

by

James Miles Myers

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
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1990

Approved by William K. Hershberger
(Chairperson of Supervisory Committee)

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to offer Degree School of Fisheries

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Doctoral Dissertation

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Abstract

Triploid Incubation and Growth Performance:

A Comparison of Meiotic and Interploid Triploid

Rainbow Trout (*Oncorhynchus mykiss*) Inter- and Intrastrain Crosses.

by James Miles Myers

Chairperson of the Supervisory Committee: Professor William K. Hershberger

School of Fisheries

Salmonids undergo major physiological changes during maturation critical for the reproductive success of this group; however, this also causes a marked degradation in the commercial value of the carcass. Aquaculturists have attempted to mitigate the effects of maturation in a variety of ways. Induction of triploidy, the presence of three chromosome sets, has been found to produce sterile fish.

OVerall, the growth of tirploid fish prior to maturation has been signficantly inferior to diploid fish, however, genetic selection may be a potential method to improve the performance of triploids. The relationship between genetic composition and induced polyploidy was examined in several strain hybrids of rainbow trout (*Oncorhynchus mykiss*) during 1988 and 1989. Triploidy was induced via retention of the second polar body using a thermal shock (meiotic triploids) and tetraploid-diploid crosses (interploid

triploids). Comparisons were made between the triploid types and diploid controls with emphasis on incubation and juvenile life history.

Meiotic and interploid triploids exhbited high mortality rates during incubation, but in the case of the interploids this was due to poor fertilization, 5-40% fertilization success. Whereas, in the meiotic triploids this was due to developmental abberations. In general, there were no differences in the embryonic growth rates of diploids and triploid embryos within each cross; however differences between crosses were apparent.

The growth of the different triploid types after ponding varied depending, in part, on the specific cross produced. Meiotic triploids experienced a retardation in growth during the first 60 days after ponding relative to their diploid controls. In the period of 60-120 days, the growth rate of specific meiotic triploid crosses improved considerably such that they were indistinguishable from diploids. Interploid triploids were exhibited growth rates that were indistinguishable from diploid controls, with one exception in 1989 where the interploid cross significantly outperformed its control.

The importance of genetic composition and method of triploid induction strongly suggested that judicious selection of parental strains and individuals within those strains could produce improvements in the growth rate of triploids . Furthermore, the interploid crosses appeared to be the more.effective method of triloid production.

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This study represents more than just my own meager efforts over the past few years. It is a testament; to my friends who supported me physically and spiritually, my supervisory committee who provided for my academic needs and exemplified scientific ethos, and especially, my family without who's love and caring I would never have been able to begin, never mind finish this project. Mom and Dad, thanks for everything.

INTRODUCTION

Every aspect of the physiology of fish is controlled by the genetic composition (genotype) of that organism. Differences in genotypes between organisms may be expressed as differences in physical traits or phenotypes. Agricultural breeders have used this relationship between genotype and phenotype to select and mate individuals exhibiting desirable traits (Falconer 1981). The application of classical selection techniques to aquatic species is a very recent endeavor, but already shows great promise (Kinghorn 1983). Considerable improvements have been made in the growth performance of rainbow trout, *Oncorhynchus mykiss* , (Donaldson and Olson 1955), common carp, *Cyprinus carpio* (Kirpichnikov and Golovinskaya 1966), Atlantic salmon, *Salmo salar* (Gjerde 1986), and channel catfish,*Ictalurus punctatus* (Dunham and Smitherman 1983) through the selection of advantageous genes or gene combinations via their phenotypic expression.

Changes in physical traits can also be produced via changes in the quantity of genetic material present, in the form of additional complete chromosome complements. Polyploid organisms are those with three or more sets of chromosomes (Winkler 1916). Agronomists have utilized induced and natural polyploids in a number of crop species to improve growth and yield characteristics (Müntzing 1951). Research with polyploid vertebrates has been much less extensive than with plants, the majority of the work being done with amphibians (Fischberg 1959). The viability of polyploid higher vertebrates, chickens (Abdel-Hameed and Shoffner 1972) and mice (Snow 1973), is extremely poor.

Triploid fish (those with three complete chromosome complements) have attracted the attention of aquaculturists because they are effectively sterile (Gervai et al. 1980; Wolters et al. 1982; Lincoln and Scott 1984). However, male triploids do exhibit secondary sex characteristics and in some cases produce a few aneuploid spermatozoa (Thorgaard and Gall 1979; Benfey et al. 1986); maturation in female triploids is almost completely suppressed (Solar et al. 1984, Johnson 1988). The inhibition of maturation would prevent the normal degradation in carcass quality during the spawning season (Greene 1926; Askenes et al. 1986) and rechannel energy into somatic rather than gonadal growth. Additionally, precocious maturation and post-spawning mortality (Gjedrem 1985), which are major problems in salmon culture would be averted or minimized. Thus, the production of triploid fish offers an alternative method to selection for improving growth performance.

The sterility imparted by triploidy requires that triploid individuals be produced anew each generation. Two methods currently exist for the induction of triploidy in finfish; (1) inhibition of the second meiotic division and subsequent retention of the second polar body (meiotic triploidy), and (2) the crossing of tetraploid individuals (those with four chromosome complements) with normal diploids (interploid triploidy).

Meiotic inhibition has been extensively studied in a number of species (Thorgaard, 1986); however, the production of tetraploid fish and their use in crosses with diploids has only recently been attempted and primarily among the salmonids (Chourrout et al. 1986; Myers et al. 1987). The paucity of information concerning tetraploid x diploid crosses has prevented determination of the method of choice for triploid induction. Additionally, data concerning the growth of interploid and meiotic triploids is very limited and often contradictory. Our understanding of triploid performance is further complicated by the fact that different stocks of fish respond differently to triploidization (Solar et al. 1984; Guo et al. 1990), and that the two methods of induction produce genetically different triploids (Diter et al, 1988). However, the existence of novel gene interactions in polyploid organisms provides an additional source of genetic variance that may be exploited via classical selection techniques. Thus, polyploidy, in addition to being a method of suppressing maturation, provides a unique avenue for genetic improvement.

The extent to which genotype x polyploid interactions are expressed in the phenotype of triploid finfish remains largely undetermined. Furthermore, the method of triploid induction may directly or indirectly influence these interactions. The specific objectives of this investigation were to:

1) Examine incubation performance, growth, and survival differences among diploids and two types of triploids: meiotic and interploid.

2) Examine the importance of genetic factors and interactions in polyploids on a gross scale via the comparison of intra- and interstrain rainbow trout diploid and triploid hybrids..

LITERATURE REVIEW

Polyploidy has been implicated as an important process in the evolution of species (Ohno 1970), and it also imparts biochemical and physiological attributes to many of these species that makes them desirable as agricultural crops (Stebbins 1950). Natural and induced polyploid plants have been utilized by agronomists since early in this century (Williams 1964). Experimentation with the induction of polyploidy in vertebrates also began in the first half of this century (Fankhauser and Griffiths 1939), although it was not until recently that the use of polyploid animals in agriculture, and aquaculture specifically, has been examined (Purdom 1972). Research has focused on the production of sterile triploid finfish, with particular emphasis on salmonids (Thorgaard 1983).

Triploid induction: Meiotic inhibition

The production of triploid finfish is possible through the disruption of the second meiotic division. The extrusion of the second polar body, which contains a haploid complement, is inhibited. This additional genetic complement fuses with the pronucleus in the oocyte and the paternally derived complement from the sperm, and form a triploid organism (Austin 1965). The retention of the second polar body can be achieved by subjecting a newly fertilized egg to a disruptive shock treatment which can be chemical and physical. The application of this shock is most effective when applied during late metaphase II or anaphase II.

There are several different class of chemicals that have been found to suppress cytokinesis in most organisms. Cytochalasin B, the most commonly used compound, reversibly inhibits the formation of microtubules that pinch off the second polar body (Carter 1967). However, the practical application of chemical induction has been less than satisfactory with finfish. The majority of Atlantic salmon eggs exposed to cytochalasin B were either killed directly by the treatment, aborted during embryogenesis, or resulted in polyploid mosaics (Refstie et al. 1977; Allen and Stanley 1979). Only a small proportion of these treated eggs produced viable triploid fish. The poor induction efficiency of this method combined with the potential for consumer resistance to chemically treated fish severely limits the utility of this approach to finfish aquaculture.

Treating eggs with a physical shock, in the form of hydrostatic pressure, has also been shown to be effective in disrupting the second meiotic division (Streisinger et al. 1981; Onozato 1985). Triploid induction levels of 80% and greater were achieved using pressures of 550 - 650 kg/cm^2 (7000 - 9500 psi) on a number of salmonid species (Benfey and Sutterlin 1984a; Allen and Myers 1985). The treatment intensities necessary to produce high proportions of triploid individuals, unfortunately, also resulted in unacceptably high mortalities. Additionally, the expense of manufacturing a pressure apparatus large enough to meet commercial production requirements would be prohibitively high (Allen 1987). Hydrostatic pressure induction, while of use in experimental studies, is therefore not a viable method for fish breeders.

Triploid induction using a thermal shock, cold or heat, has been widely researched. The ability to treat large numbers of eggs using relatively simple equipment, such as a large temperature regulated water bath, makes this technique especially attractive (Thorgaard 1986). Cold shock treatments, 0-4°C, have been shown to be effective with the common carp (Gervai et al. 1980), tilapia, *Oreochromis aureas* (Valenti 1975), plaice, *Pleuronectes platessa* (Purdom 1972), Atlantic salmon (Lincoln et al. 1974), and brook trout, *Salvelinus fontinalis* (Lemoine and Smith 1980). In general, this technique has been very successful with the warmer water species, but resulted in high mortality levels and poor induction efficiency when employed on salmonids. Alternatively, the heat-induced retention of the second polar body is particularly effective among salmon and trout (Chourrout 1980; Benfey and Sutterlin 1984a; Utter et al. 1983). Treatments vary from 26-40°C depending on the species. Protocols have been developed that induce a high incidence of triploidy with acceptable mortalities on a consistent basis (Chourrout and Quillet 1982; Seeb et al. 1988; Don and Avtalion 1986). Triploid production using the heat-shock technique has proven itself efficient enough that it is currently employed on a commercial basis by salmon aquaculturists (Bye and Lincoln 1986).

Triploid Induction: Interploid production

The crossing of tetraploids, which produce diploid gametes (Chourrout and Nakayama 1987), with diploids having haploid gametes, is an alternate method of producing triploids. Interploid crosses bypass the direct treatment of eggs, as is the case which meiotic inhibition treatments (Refstie et al. 1977). Additionally, the progeny of interploid crosses are 100% triploid, because of the all-diploid composition of tetraploid-derived gametes (Chourrout et aL

1986; Myers and Hershberger, unpublished). The ability to produce large numbers of triploid progeny without directly treating production animals makes the interploid triploid approach very desirable.

The use of interploid crosses as a viable alternative to present heat-shock methodologies is predicated on the production of tetraploid broodstock. Tetraploidy is most commonly induced via inhibition of the first mitotic division (Fischberg 1959). The inhibition can be accomplished by treatments applied at either of two developmental stages prior to cleavage: 1) karyokinesis, the separation of chromatids or, 2) cytokinesis, the separation of cytoplasm and the formation of daughter cells (Chourrout 1984). Fischberg (1959) was able to achieve 93.7% tetraploidy in newts, *Triturus vulgaris* , by applying a heat shock at the time of cleavage (i.e. cytokinesis). Attempts to induce tetraploidy in finfishes using thermal or hydrostatic pressure treatments during cytokinesis have resulted in abnormal tetraploids. These tetraploids proved to be subvital and few survived beyond hatching (Thorgaard et al. 1981; Chourrout 1982; Myers 1986). However, the inhibition of karyokinesis, specifically late metaphase, during the first cleavage interval with hydrostatic pressure does produce a limited number of viable organisms (Steisinger et al. 1981; Chourrout 1984; Onozato 1985; Myers et al. 1987). In salmonids, fertilized eggs are subjected to pressures of $4.8 - 6.2 \times 10^4$ kg/cm^2 (7000 - 9000 psi) for 4 - 6 minutes applied at 65 - 78% of the time to first cleavage. Although this procedure normally results in high mortality levels (in excess of 75%), induced tetraploids can be bred to produce subsequent generations of non-treated tetraploids (Chourrout and Nakayama 1987). Thus, a relatively small number of pressure-induced tetraploids can initiate the development of a true tetraploid broodstock for interploid production on a large scale.

Triploid Growth: Prematuration

The impact of triploidy on the growth and physiology of finfish has only recently been addressed because of the initial emphasis on developing an effective induction methodology. Studies with juvenile fish are of particular interest because triploid genetic effects can be observed separately from maturation related effects. This period in the life history of triploidized fish is also important because of the potential for the expression of latent effects from induction treatments. The commercial utility of different induction protocols may also depend on the subsequent performance of the triploids produced.

Triploids produced via meiotic inhibition generally are either inferior to ,or not-significantly different from, their diploid controls prior to maturation (Quillet et al. 1988). Alternatively, Wolters et al. (1982), with catfish, and Valenti (1975), with tilapia, have reported triploids significantly larger than diploids prior to maturation; however, these studies lacked suitable replicates and experimental control. Also, considerable variability in results is apparent between studies. Solar et al. (1984) reported that heat-shocked triploid rainbow trout were 38% of the weight of their diploid controls at 11 months post-hatching, while Quillet et al. (1988) reported that triploid rainbow trout were only 87% the size of their controls at 18 months post-hatching. Differences in the relative triploid performance between studies may be due to the genetic stock used, the induction protocol, and/or the rearing conditions.

Strains of rainbow trout responded differently to the same thermal shock in the relative survival and growth of triploids compared to diploids (Solar and Donaldson 1985; Guo et al. 1990). Furthermore, diploid and triploid full-sib families within a strain exhibited considerable variability in growth between families (Quillet et al. 1988).

The juvenile growth of triploid finfish also appears to be influenced by induction treatment protocol and husbandry practices. The intensity of the thermal shock used in causing the retention of the second polar body in coho and chinook salmon (*Oncorhynchus kisutch* and *O. tshawytscha*, respectively), also influenced growth for up to a year of age (Johnson, 1988). Additionally, Johnson found that the feeding regime used affected the growth ratio between diploids and triploids. It is apparent that genetic factors influence the performance of meiotic triploids, but these effects cannot be completely isolated from interactions with induction treatment effects.

There is a paucity of information on the growth performance of interploid triploid finfish. Chourrout et al. (1986) first reported the production of interploid triploid rainbow trout. These authors reported contradictory results from growth studies with interploid and heat shocked triploids, and diploid controls. Interploid triploids were reported to be superior to their diploids in one study and slightly inferior to controls in the other. However, in both studies the interploid triploids were larger than heat-shocked triploids at 270 days post-fertilization. These initial results suggested that the induction treatments used to inhibit the second meiotic division significantly retarded early growth.

Chourrout et al. (1986) also found what would be a major potential drawback with the production of interploid triploids using tetraploid males and diploid females. Increases in the DNA content of a cell results in a proportional increase in the volume of the cell to maintain a constant nucleus/cytoplasm ratio (Fankhauser 1941). The induction of tetraploidy produces organisms with cells containing twice the normal amount of DNA and thus twice the cell volume. This also applies to gametes. As a result increased size, diploid sperm is apparently incompatible with the micropyle structure of the egg which allows sperm entry (Chourrout et al. 1986). Fertilization levels for interploid crosses using tettraploid males varied considerably, but were generally poor, 15-47% (Chourrout et al. 1986). Similar results have been obtained in subsequnt studies (Blanc et al. 1987; Myers and Hershberger unpublished).

The performance of both heat-shocked and interploid triploids appears to be influenced by the parental genotype. Blanc et al. (1987) compared the performance of diploid and meiotic and interploid triploid half-sib families. Paternal (sire) effects were important only among the interploid triploids; however, the overall performance of the interploid triploids was significantly inferior to that of their diploid controls at 175 days post-hatching. The presence of paternal effects provides an avenue for the genetic improvement of interploid triploids through selection.

The interaction between genetics and induced polyploidy can take on a myriad of forms. The most pronounced example of this is the induction of interspecfic and intergeneric triploidy (allotriploidy). Many interspecfic and intergeneric hybrids are nonviable or subvital as diploids, but these same crosses produced as triploid hybrids via meiotic inhibition are as viable as or superior to either of the pure species (Purdom 1976; Scheerer and Thorgaard 1983; Johnson 1988). The inclusion of an additional maternal chromosome set from the second polar body stabilizes normally lethal genetic incompatibilities in diploid hybrids and allows more normal gene expression (Seeb et al. 1988).

The doubled maternal genome can be manipulated such that desirable qualities of the two species can be mixed proportionately. Parsons et al. (1986) were able to combine the superior growth rate of rainbow trout with the disease resistance of coho salmon to infectious hematopoietic necrosis virus (IHNV) in a triploid hybrid, while the diploid hybrid was not viable. Similarly, the early seawater entry characteristic of pink (*O. gorbuscha*) and chum salmon (*O. keta*) has been combined with the growth and market qualities of chinook and coho salmon in a series of triploid hybrids (Johnson 1988; Seeb et al. 1988). Novel gene combinations can also be produced via triploid inductions in intraspecies strain hybrids. The growth of strain hybrids of rainbow trout produced as triploids were quantitatively different from their diploid counterparts (Guo et al. 1990). This study suggested that trigenic effects in triploids were not trivial. In fact, a triploid rainbow trout hybrid outperformed not only its diploid counterpart but both pure lines at 120 days post-hatch. Thus, the ability to improve growth performance in heat-shocked fish prior to the onset of any maturation effects through strain selection appears feasible. Although the mechanisms involved are somewhat different, interstrain or interspecfic triploid hybrids could be of considerable utility to fish culturists.

Triploid Growth: Maturation Effects

The use of induced triploidy was initially envisioned as a technique to obtain sterile fish for culture. This would be especially useful in salmonid culture where sexual maturation has a detrimental impact on growth, survival, and market quality (Lincoln and Scott 1984). Flesh quality characteristics such as protein content, fat level, and pigmentation in salmonids are markedly reduced during maturation (Askenes et al. 1986). The market size for fish is, in part, limited by the age of maturity because the fish must be harvested prior to flesh and skin color deterioration (Gjedrem 1985). Furthermore, the price of fish is positively correlated with size. Limitations in growth due to early maturation may prevent the commercial farmer from optimizing his return on investment. Triploidy could significantly improve the efficiency of salmon culture and provide a constant high quality product to the consumer.

The gonadal development of induced triploid finfish was first described by Swarup (1959) in the stickleback, *Gasterosteus aculeatus* . Female sticklebacks produced rudimentary ovaries, while testicular tissues were much more fully developed in males. Triploid male plaice were capable of producing a limited number of spermatozoa, although crosses between triploid males and diploid females resulted in aborted embryos (Lincoln 1981). Studies with salmonids [rainbow trout (Lincoln and Scott 1984; Solar and Donaldson 1985), Atlantic salmon (Benfey et al. 1985), and Pacific salmon

(Johnson 1988)] similarly report that triploid males develop nearly normal testes and exhibit secondary sexual characteristics. Maturation in triploid males is extensive enough that there is no growth advantage over diploid males during and after spawning (Lincoln and Scott 1984; Quillet et al. 1988). Furthermore, Benfey et al. (1986) reported that triploid derived sperm is aneuploid, containing a 1 1/2 N DNA complement. The fertilization of normal rainbow trout diploid derived eggs with triploid derived sperm results in aneuploid, 2 1/2 N, embryos that abort early in development (Myers and Johnson, unpublished). Triploid males, therefore, are functionally sterile in that they cannot successfully reproduce. However, the presence of secondary sexual characteristics in triploid males results in a degradation in market quality (Chevassus et al. 1985; Johnson 1988), and reduces their usefulness in commercial culture.

The degree of sexual development is much less extensive in female triploids than in male triploids. Ovarian development is almost completely inhibited in triploid plaice x flounder, *Platichthys flesus* , hybrids (Purdom 1972), catfish (Wolters et al. 1982), and salmon and trout (Thorgaard and Gall 1979; Benfey and Sutterlin 1984b; Johnson 1988). Although a small number of primary oocytes have been observed in triploid females, hormone levels appear to be too low to initiate vitellogenesis (egg maturation) or secondary sexual development (Lincoln and Scott 1984; Johnson 1988). Female triploids, therefore, circumvent much of the physiological stress and growth loss associated with maturation. In fact, all-female triploid production is currently being employed on a commercial basis (Bye and Lincoln 1986; Y. Harache personal communication, 1989).

Proposed Study: Justification and Objectives

The use of induced triploidy as a method to provide aquaculturists with sterile fish appears to be promising, specifically with female triploids. Technology is currently available to produce all-female progeny on a commercial basis (Shelton 1986; Bye and Lincoln 1986). However, the extent to which genotype influences the growth of polyploid organisms is still unclear.

The increase in the size of the genetic template in triploids provides additional sources of variation in the form of bi- and trigenic interactions (Kempthorne 1969). The performance of interstrain triploid rainbow trout hybrids suggests that these interactions are not trivial, and that heterotic effects can be magnified in triploids (Guo et al. 1990). Crossbreeding studies with rainbow trout (Hörstgen-Schwark et al. 1986) and other finfish (Moav et al. 1975; Dunham and Smitherman 1983; Gjerde and Refstie 1984) indicate that specific strain crosses may show considerable heterosis. In general, strain cross performance is due to two types of effects; general and specific combining abilities. The underlying genetic bases for these effects are additive and non-additive genetic variation, respectively (Falconer 1981). Additive genetic effects in polyploids may not be operationally different from diploids since the protein(s) produced from a "A" gene would, biochemically, be the same in AA diploids and AAA triploids. Non-additive, primarily dominance, effects would differ in diploids and triploids because of the two heterozygote states possible in the triploid, AAa and Aaa, rather than the one heterozygote possible in the diploid, Aa. Furthermore, the proportion of homozygotes present in triploids could be considerably less than that found in diploids depending on the gene frequencies (Kempthorne 1969). However, the mechanisms controlling dominance expression in AAa and Aaa genotypes relative to Aa genotypes are not well understood. It is possible that this increase in heterozygosity in triploid organisms may be partially responsible for the performance of interstrain hybrids produced by Guo et al. (1990).

The relative utility of meiotic vs. interploid triploid organisms has still not been fully investigated. The poor performance of juvenile meiotic triploids relative to their diploid counterparts indicates that induction techniques produce a latent growth retardation (Solar et al. 1984; Happe et al. 1988; Guo et al. 1990). Triploid induction via interploid crosses avoids the need to directly treat production fish (Chourrout 1984). Additionally, the genetic contributions from the ovum and second polar body (meiotic induction) and the diploid gamete (interploid induction) are quantitatively equal, but may differ in the gene content of the diploid complement. The second polar body contains the sister chromosomes of those found in the pronucleus; these chromosomes are identical in the absence of recombination events during the first stages of meiosis (Stanley and Sneed 1974). The recombination frequencies in finfish are surprisingly high, approximately 60% (Thorgaard et al. 1983; Guyomard 1984; Allendorf et al. 1986). However, tetraploid gametes undergo more random assortment and tetraploid rainbow trout have yielded triploids with heterozygosity levels 45% higher than those found in triploids produced via polar body retention (Diter et al. 1988). If dominance effects are important in triploid performance then the progeny of interploid crosses should contain substantially higher levels of heterozygosity and subsequently exhibit improved performance over their meiotic triploid and diploid counterparts.

The purpose of this study was to investigate the extent of genotype x polyploid interactions in finfish. Specifically, the use of strain crosses to improve the growth and survival of juvenile meiotic and interploid triploid rainbow trout was evaluated. Non-related strains of rainbow trout were used to produce potentially high levels of heterozygosity in all progeny types. If the major benefit of polyploidy is the presence of novel bi- and tri-genic interactions, such crosses should accentuate this effect. The benefits of triploidy, in regards to maturation effects, have been well established. Therefore the focus of this study was embryonic and juvenile performance, specifically survival and growth. If the growth of triploids prior to maturation can be improved then any subsequent effects due to non-maturation would be further exaggerated. Rainbow trout, *O. mykiss* , is the only species that has been successfully tetraploidized and subsequently spawned, and is the obligatory species of choice. Additionally, a considerable data base has been amassed concerning the performance of polyploid rainbow trout. Information from this project may provide fish culturists with a more definitive comparison of meiotic and interploid triploid survival and growth, as well as suggest future avenues for selective breeding that can possibly be employed in conjunction with polyploidization.

MATERIALS AND METHODS

Experimental Organisms;

Crosses of rainbow trout strains were made in 1988 and 1989. In 1988, three strains; the University of Washington's Donaldson trout strain, the Kamloops strain, and the US Fish and Wildlife 's Ennis strain, strain register # 23 (Kincaid 1981), were crossed. The Donaldson trout strain (UW) has been subject to a selective breeding program for growth and fecundity since 1932 (Donaldson and Olson 1955). The Kamloops strain (TL) was obtained from Trout Lodge Inc., McMillin, Washington, a commercial trout egg supplier. The Ennis strain (USDA) has been maintained by US Fish and Wildlife for sport fishery enhancement, and has been subject to a limited amount of selection (Kincaid 1981). In 1989 only the UW and TL strains were used.

UW and Ennis broodstock used to produce gametes for the study were maintained on site at the School of Fisheries' Hatchery at the University of Washington, Seattle Washington. The Kamloops strain was obtained as unfertilized gametes from Trout Lodge Inc.

Tetraploid fish were produced via inhibition of the first mitotic division using hydrostatic pressure (Myers et al. 1987). Tetraploidized fish from the UW strain, were used in the 1988 crosses. Tetraploid fish for the 1989 crosses were produced by crossing tetraploid UW males with diploid UW females and then subjecting the fertilized eggs to a heat-shock to suppress the second meiotic division (Myers and Hershberger unpublished). The eggs from these crosses are initially triploid and with the inclusion of the second polar body become tetraploid (Chourrout et al. 1986). In both years there were insufficient numbers of spawning female tetraploids available for incorporation into the study and only male tetraploids were used. Male broodstock for the interploid crosses in both years consisted of induced tetraploids and their untreated diploid sibs or cousins.

Experimental Design;

1988 Study; A partial factorial mating design was used to create diploid, meiotic and paternal interploid triploid groups in each of the strain crosses, UW x TL and UW x USDA, and the pure strain standard, UW x UW (Figure 1). In order to produce triploids with equivalent genetic contributions using the two induction methods reciprocal strain crosses were utilized. TL(♂) X UW(♀) matings were divided into untreated control (TL UW) and heat-shocked treatment triploid groups (TL UW^2), while UW (♂) X TL(♀) matings used tetraploid and diploid males to produce triploid (UW^2 TL) and diploid offspring (UW TL), respectively. Crosses between UW diploid and tetraploid fish and USDA fish were similarly made.

1989 Study: The mating design provided for the production of pure UW as well as hybrid UW x TL diploids, meiotic and paternal interploid triploids. A TL x TL diploid pure line was provided as a reference for heterosis calculations (Figure 2). The TL x UW cross was repeated on the basis of data collected from the 1988 study. Additionally, the UW (♂) x TL (♀) reciprocal

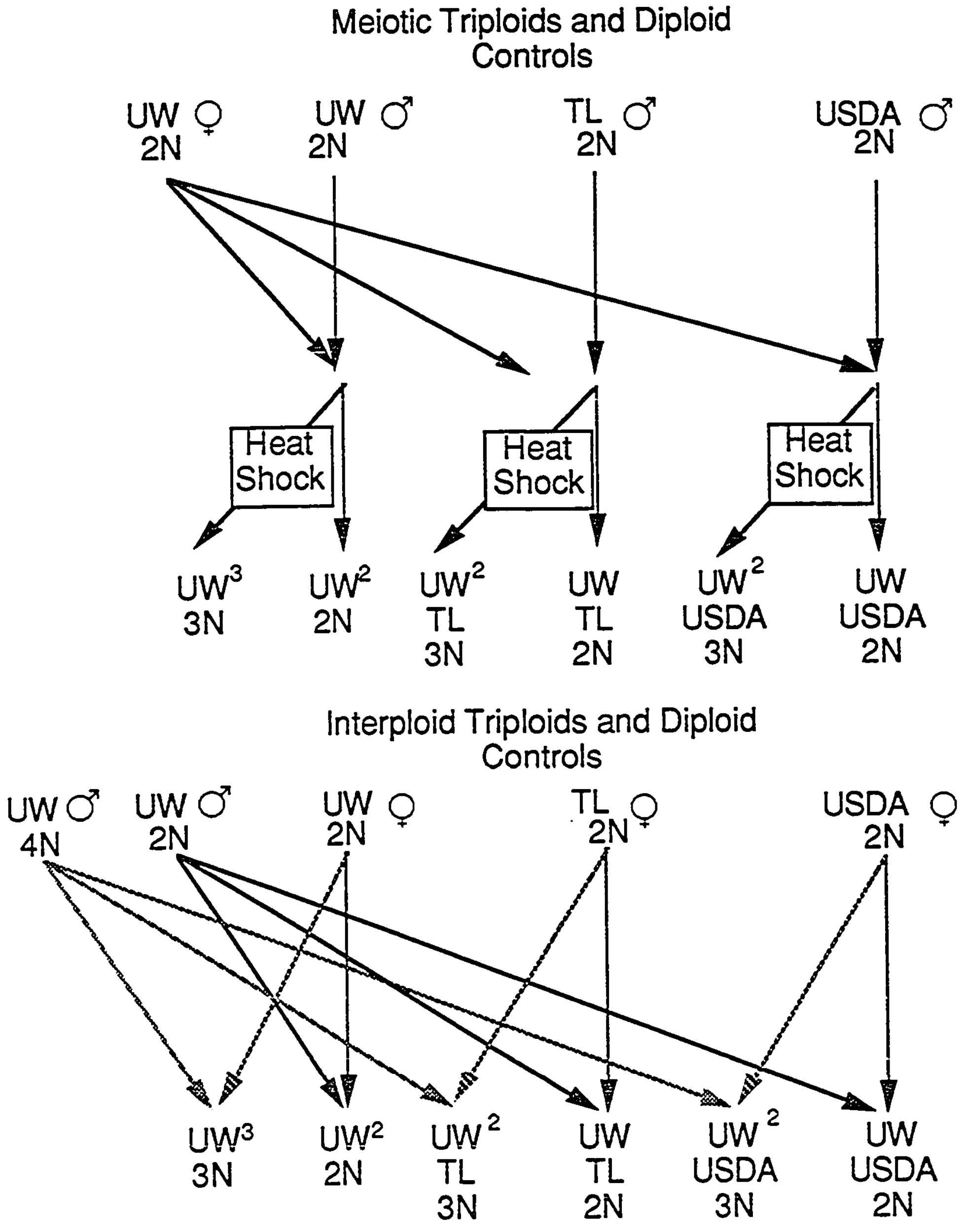
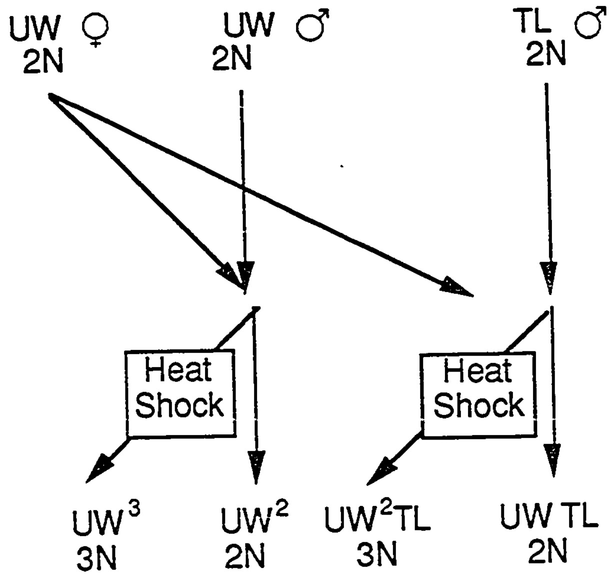


Figure 1. Mating and treatment design used to produce diploid and triploid inter- and intrastrain crosses of rainbow trout for the 1988 study

Meiotic Triploids and Diploid Controls



Interploid Triploids and Diploid Controls

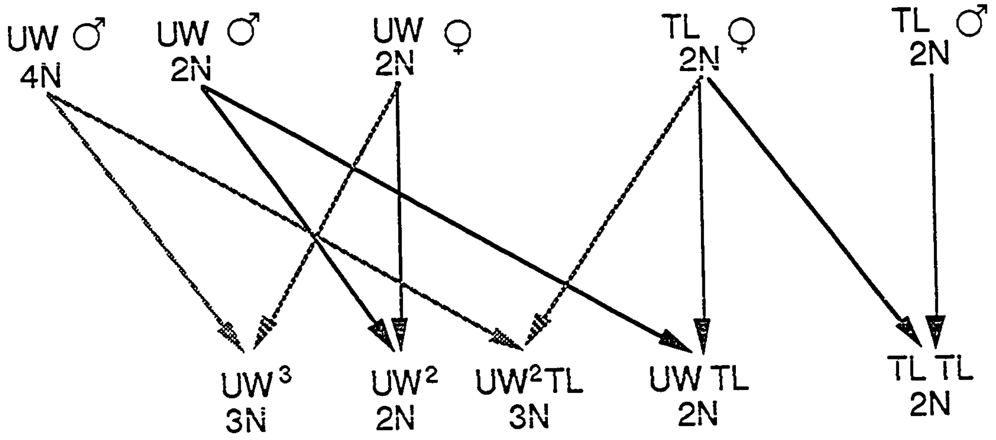


Figure 2. Mating and treatment design used to produce diploid and triploid inter- and intrastrain crosses of rainbow trout for the 1989 study.

diploid cross was eliminated, based on 1988 data, and due to rearing space restrictions.

Spawning Protocol;

Standard hatchery spawning procedures were utilized for UW and USDA (1988 only) strains maintained at the University of Washington. Broodstock were conditioned for spawning using luteinizing hormone-releasing hormone analog, LH-RHa (Sigma Chemical Co., St. Louis, Mo), injections of 6 µg/kg body weight (Sower et al. 1984). TL gametes were collected by employees of Trout Lodge Inc., packed on ice, and driven to the University of Washington for use the same day. In 1988 gametes were taken from 5 tetraploid and 4 diploid UW males, 6 UW diploid females, 4 male and 4 female diploid USDA fish, and 8 male and 8 female diploid TL broodstock. In 1989 8 tetraploid and 8 diploid UW males, 8 diploid UW females, and 8 diploid male and 6 diploid female TL parents, were used to make crosses. Female contributions were standardized by mixing equal volumes of eggs from each female prior to mass fertilization with pooled sperm (1988). This procedure was further refined in 1989, by subdividing each female lot into equal portions and separately fertilizing these portions with milt from a single male. In this way the contribution of each male and each female within a cross was initially equal. Milt was mixed with a saline diluent (Billard 1977) prior to fertilization in 1988 and 1989. Following fertilization egg lots for each cross were pooled. Prior to incubation the eggs were water hardened in 150 ppm Argentyne (Argent Chemical Lab., Redmond, WA) as a prophylactic treatment for virus infection (Wood 1979). The eggs were then transferred to isolation incubator buckets supplied with dechlorinated city water.

Meiotic Inhibition Treatment;

Pooled lots from crosses with diploid UW females were divided by volume into two groups; control and heat-shock treatment. To compensate for higher mortalities in the heat-shock treatment group the volume of eggs in the treatment group was twice that of the control group. Prior to treatment the eggs were held at 10° C for 25 minutes. They were then immersed in a warmwater bath at 27.0 ± 0.2° C for 25 minutes (Guo et al. 1990). Following treatment the eggs were incubated normally at ambient temperatures (8-11°C).

Rearing;

Sac-fry were transferred from incubators to grow-out tanks two weeks after hatching. Each experimental group was divided into lots of 500 ± 25 fry and put into each of three separate 200 L circular tanks supplied with ambient temperature water from the Lake Washington ship canal and provided with constant aeration. During the first two weeks of feeding in 1988, fish were fed dry salmon meal to excess six or seven times daily. Following this initial period the fish were fed a moist diet prepared at the UW hatchery (Donaldson 1989) at 150% of the daily recommended feeding levels adjusted for water temperature and fish size (Leitritz and Lewis 1976). The initial *ad libitum* feeding was extended to three weeks in 1989, and the fish were fed a commercial diet (Bioproducts Inc., Warrenton, OR) for the first 65 days post ponding, after which the UW hatchery diet was used. The fish were fed daily until 50 days post-ponding after which they were fed six out of seven days (1989 only). For both

1988 and 1989 rearing densities were equalized across tanks when the density of any one tank exceeded 18 kg/m^3 (1.2 lbs/ft^3). Water flow in each tank was approximately 10L/min. Tanks were cleaned daily with a siphon hose to remove feces and uneaten food. Rearing and feeding conditions were regulated to enable the fish to grow at or near their maximum potential.

Antibiotics and therapeutic chemicals were applied in response to epizootics and not as prophylactics. Treatment dosages and durations were applied following Piper et al. (1982). Outbreaks of protozoans, *Ichthyophthirius* and *Trichodina* were treated with formalin. Topical bacterial infections of *Columnaris* were treated via a bath treatment with nitrofurazone (Sigma Chemical, St. Louis, MO), while systemic infections required feeding food mixed with oxytetracycline and oxolinic acid (Sigma Chemical Co., St. Loius, MO). Maintaining clean tanks at relatively low rearing densities minimized the need to use chemotherapeutic agents.

Sampling Procedures;

During the incubation period mortalities were removed 24 hours after fertilization, after the eggs had developed eye pigmentation (approx. 18 days post-fertilization), and periodically during incubation until the fry were ponded. Egg mortalities were enumerated and cleared (1988 only) in Stockard's solution (see Guo 1988). Clearing the eggs allowed the determination of the stage of development at the time of death. These eggs were then divided into four categories: (1) blank eggs, showing no development, (2) primitive streak, showing formation of the neural folds and somites, (3) primary embryos, eyed embryos usually showing retarded growth or developmental abnormalities, and (4) secondary and tertiary embryos, large normal individuals with well pigmented eyes and body formation. When all the lots were completely hatched, samples of sac-fry were taken and stored in 10% neutral-buffered formaldehyde, 10 fish per group in 1988 and 15 fish per group in 1989. A second sample was taken when the groups were transferred to the rearing tanks. Preserved fry were dissected and the embryo and yolk sac weighed separately as wet samples, and again after drying for 12 hours in an oven at 100° C. Three samples of 25 live sac fry were taken from each group at the time of transfer to rearing tanks (ponding), and these weights were used calculate the appropriate weight of fry necessary to stock 500 fish per tank. This completed the incubation phase of the study.

The rearing phase of the study was designed to monitor individual growth and group mortality. Fish were sampled on a biweekly basis. In 1988, three pools of 25 fish each were taken from each tank and weighed biweekly for the first 47 days post-ponding. After this , 25 fish from each tank were anesthetized in 3-aminobenzoic acid ethyl ester, MS-222 (Argent Chemical Lab., Redmond, WA), and individually weighted and measured . In order to minimize stress·to the fish in the 1989 study, the first sampling was delayed until 22 days post-ponding; when 25 fish from each tank were individually sampled. Total biomass weights for the tanks were initially taken on a monthly basis, and biweekly during the last 48 days of the study, in order to adjust densities. Each study was initially projected to run for 120 days post-ponding; however, in 1989 water temperatures rose unexpectedly high and the study was terminated at 117 days (see Figure 3 for sampling schedule).

Ploidy analysis;

The DNA content of tetraploid broodstock and meiotic and interploid triploid fish used in the study was verified via flow cytometry (Allen 1983). Tetraploid fish were anesthetized in MS-222 and marked with an identifying numbered anchor tag prior to sampling. Blood samples from presumptive tetraploid broodstock were obtained via the caudal vein with a heparinized syringe. A drop of blood was then immediately transferred to a test tube containing one ml of DAPI, 4,6-diamidine-2-phenylindole, a DNA specific fluorescent dye. Semen was manually expressed and a drop suspended in DAPI stain. Approximately 100 days post-ponding, in both 1988 and 1989, twenty fish were taken from each tank containing presumptive triploids. Fish were sacrificed and the the caudal fin removed. Blood was collected with a heparinized capillary tube from the severed caudal vein and a drop immediately transferred to a test tube with DAPI stain. All samples were kept on ice prior to analysis. Polyploid level was determined by the sample cell fluorescence relative to semen and blood samples from known diploid trout using an ICP-22 flow cytometer (Ortho Diagnostic Systems, Westwood, MA) at the University of Washington's Department of Pathology.

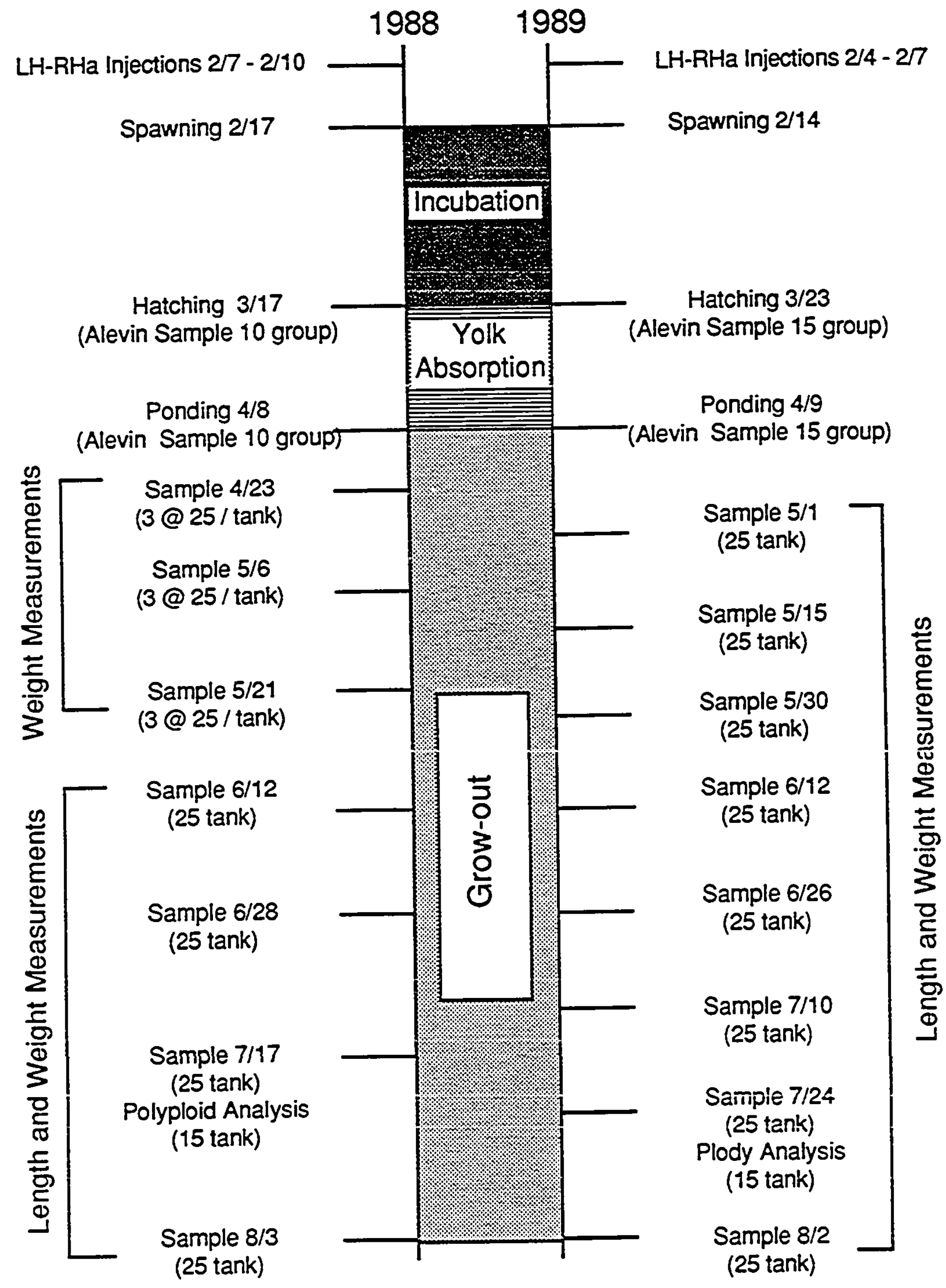


Figure 3. Activity and sampling schedule for the 1988 and 1989 studies.

Statistical Analysis;

Triploid Induction ; Each experimental triploid group was treated *en masse* prior to rearing in separate tanks. Therefore, the triploid induction data for each group from replicate tanks was pooled prior to analysis. Pairwise comparisons were made using Fisher's exact test (Zar 1974) at the 0.05 significance level.

Mortalities ; Data for each incubation unit and rearing tank were recorded and averaged over periods of approximately 30 days. Percent daily mortality (PDM) was computed by:

$$\mathrm{PDM} = \left(\frac{M_i}{N_i \times D_i}\right)$$

where M_i = mortalities during period i

N_i = number of fish at the beginning of period i

D_i = number of days in period i.

For statistical analysis PDMs were transformed using the square root-arcsin transformation (Zar 1974).

Within each cross, mortality type compositions were compared using pairwise chi-square tests at the 0.05 significance level.

Yolk absorption ; Yolk conversion efficiency, YC, was calculated using:

$$\mathrm{YC} = \left(\frac{Ewt_f - Ewt_i}{Ywt_i - Ywt_f}\right)$$

where Ewt_f = final embryo weight
Ewt_i =initial embryo weight
Ywt_f =final yolk weight
Ywt_i =initial yolk weight.

The terminal nature of the sampling required that fish during the two time periods be randomly paired within each group. This procedure does not affect between group variance estimates, but does potentially bias within group variance estimates (Turner and Young 1969).

Growth;: Embryonic growth rates were described as instantaneous growth in weight, IGRw, and were calculated according to:

$$IGRw = \frac{(\mathrm{Log}_e\, wt_f - \mathrm{Log}_e\, wt_i)}{\text{Days in Period}}$$

where wt_f = final weight
wt_i = initial weight

All length and weight measurements were converted using the $\log_e$ transformation prior to statistical analysis to correct for nonnormality in the data distribution. Weight measurements were emphasized in the results section because of the economic importance of this trait. Furthermore, weight measurements tend to be more variable than length measurements (Refstie and Steine, 1978).

Changes in rank among groups for any observed trait between sample dates were examined via the Student-Newman-Kuels means test. If the sign of the difference between groups changed and if the differences at both sample dates was significant ($P < 0.05$), a significant change in rank had occurred.

Correlations between egg size and embryo size and growth rates were calculated using the group means. The between class correlation eliminates variability due to the random pairing of data within a group from different sampling periods. Comparisons of correlations from 1988 and 1989 were done using Fisher's t-test (Zar 1974).

The performance of hybrids was measured in terms of relative heterosis, H. Two methods were used to measure this value for triploid hybrids, each measures hybrid growth relative to the parental contribution in different ways The standard formula used for both diploids and triploids is:

$$H_1\% = \frac{H - \left(\frac{Pm + Pp}{2}\right)}{\frac{Pm + Pp}{2}} \times 100$$

where $H_1\%$ = percentage heterosis relative to midparent for weight (wt) or for length (ln)

H = wt or ln of hybrid line

Pm = wt or ln of maternal parental line

Pp = wt or ln of paternal parental line.

The second method was used to analyze triploid hybrids only. H_1 estimates reflected the proportional contribution of the parental lines:

$$H\% = \frac{3H - \left(\frac{2Pd + Ph}{3}\right)}{\frac{2Pd + Ph}{3}} \times 100$$

where H% = percentage heterosis relative to midparent
H = wt or ln of hybrid line
Pd = wt or ln of parental line contributing diploid complement
Ph = wt or ln of parental line contributing haploid complement.

Lastly, triploid performance was expressed relative to its diploid counterpart:

$$T\% = \frac{T - D}{D} \times 100$$

where T% = percentage triploid heterosis
T = wt or ln of triploid group
D = wt or ln of diploid group.

Condition factor : The weight to length relationship for each group was expressed in terms of the condition factor. This value was calculated as:

$$K.F. = \frac{Wt}{Ln^3} \times 100$$

where K.F. = condition factor (grams/cm^3 x 100)
Wt = weight of fish in grams
ln = length of fish in centimeters.

Growth rate : Differences in the growth rates of experimental groups were calculated by using the $\log_e$-transformed mean weight and length for each tank and comparing these values in the analysis of variance designs with time (days) as a main effect. The significance of the interaction term group x time would be indicative of significantly different growth rates between groups (Baker and Gebeyehou 1982).

Analysis of variance:

The differences in experimental design between the studies in 1988 and 1989 required the use of several different ANOVA models. Additionally, in some cases only mean values were available (e.g. survival and incubation data) and specific ANOVA models were necessitated for these instances. In general, strains, groups, and ploidy state were treated as fixed effects, while tanks were nested randomly within groups and individual fish were measured in each tank.

Model I ANOVA was used in cases where strain and polyploid factors were ignored and each treatment group was treated separately. Variation between tanks was used as the error term to test group effects.

$$Y_{ij} = \mu + G_i + E_{ij}$$

where μ = overall mean
G_i = effect of the ith group
E_{ij} = random differences between fish within a group

In cases where individual fish data were available the residual effects component was further partitioned into tank effects, nested within groups, and individual (residual) effects (Model II).

$$Y_{ijk} = \mu + G_i + T_{j(i)} + E_{j(i)k}$$

where μ = overall mean
G_i = effect of the ith group
$T_{j(i)}$ = effect of the jth tank within the ith group
$E_{j(i)k}$ = random differences between fish within group

Genotype x polyploid interactions were tested by partitioning the group effects according to the specific strain cross, S, and the polyploid type (diploid and meiotic and interploid triploids), P (Model III).

$$Y_{ijk} = \mu + S_i + P_j + T_{k(ij)} + E_{k(ij)m}$$

where μ = overall mean
S_i = effect of the ith ploidy
P_j = effect of reciprocal cross
S_iP_j = effect of the interaction between the ith ploidy type and the jth strain cross
$T_{k(ij)}$ = effect of the kth tank within the ith ploidy type and the jth cross
$E_{k(ij)m}$ = random differences between fish within a group

Model III was further modified for the 1988 study. Due to the loss of interploid and meiotic triploid groups among the strain crosses the group effect was partitioned into ploidy, 2N vs. 3N, rather than ploidy type and strain cross, S.

$$Y_{ijk} = \mu + N_i + S_j + NS_{ij} + T_{k(ij)} + E_{k(ij)m}$$

where μ = overall mean

N_i = effect of the ith ploidy

S_j = effect of jth strain cross

NS_{ij} = effect of the interaction between the ith ploidy and the jth reciprocal strain cross

$T_{k(ij)}$ = effect of the kth tank within the ith ploidy and jth strain cross

$E_{k(ij)m}$ = random differences between fish within a group

The growth rate of each of the groups was analyzed using both the Model II and III designs with the incorporation of Time, TM, as a main fixed effect. Growth rate was compared using the interactions of the other effects with time.

Analyses employed the SAS statistical package (SAS Institute Inc., 1985) using the general linear model, GLM, procedure.

RESULTS

1988 Study

Spawning and Incubation;

Eggs obtained from the three strains of rainbow trout used in the study varied significantly in size ($p < 0.05$)[1] . Trout lodge females produced the largest eggs on the basis of egg diameter, 5.61 ± 0.04 mm, and USDA females the smallest, 4.60 ± 0.03 mm. The UW females produced eggs of intermediate size with the reciprocal crosses varying slightly in egg size, 4.75 and 4.88 ± 0.04 mm.

Fertilization levels varied considerably within and between groups. The control group for the UW interploid cross had the highest fertilization rate, 86.7 ± 5.7%, while the USDA interploid control group had the lowest, 28.4 ± 14.1%. Egg lots taken from the USDA females contained a large proportion of overripe ova. The egg lots from Trout lodge females exhibited variable levels of fertilization, 71.9 ± 14.09 %, in the control spawn and the UW eggs used in the heat-shock treatment control groups had similar but more consistent fertilization levels, 66.5 ± 3.0%, with no significant differences in the fertilization ability of the males from the three strains. Heat-shock treated eggs did not differ significantly from their control groups or between one another in fertilization level, 62.7 ± 4.7%. Interploid crosses experienced poor fertilization success. USDA and UW interploid cross fertilization levels, 3.9 ±

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[1] Significance will be at the $P<0.05$ level unless otherwise noted.

2.7% and 5.6 ± 2.2%, respectively, were significantly different from their control crosses and the TL interploid cross, 41.9 ± 4.0%. The TL interploid cross and control were, however, not significantly different.

Analysis of mortalities removed during incubation revealed that the majority of dead eggs showed no development (Figure 4). Clearing techniques did not allow the differentiation of eggs that were overripe, unfertilized, or fertilized but expired prior to first cleavage (e.g. heat-shock treatment related). The heat-shock treated groups, however, did show a large proportion of aborted embryos, 20-30% of all mortalities from those groups. The majority of these embryos were highly deformed relative to the few visually normal dead embryos found in the other groups.

Fry weight, minus yolk sac, at hatch was strongly related to egg size. Maternal strain effects, which may be related to egg size, was the most significant factor influencing fry weight, $P < 0.001$, accounting for 75.2% of the total variance. Correcting for egg size would tend to remove strain differences. Analysis of variance indicated that polyploid level was a highly significant factor with the triploids, overall, being smaller than diploids at hatch (Table 1). However, within each cross there was no difference between diploid and triploid embryos, except in the USDA x UW heat-shocked group (Figure 5). Wet weights (ww) and dry weights (dw) were highly correlated, $r = 0.914$ (Table 2), and there was no significant change in rank between groups when compared on a wet or dry basis. Similarly, fry weights at ponding, two-weeks after hatch, appeared to be influenced by egg size, the maternal component accounted for 53.4% of the total variation. Polyploid level was not

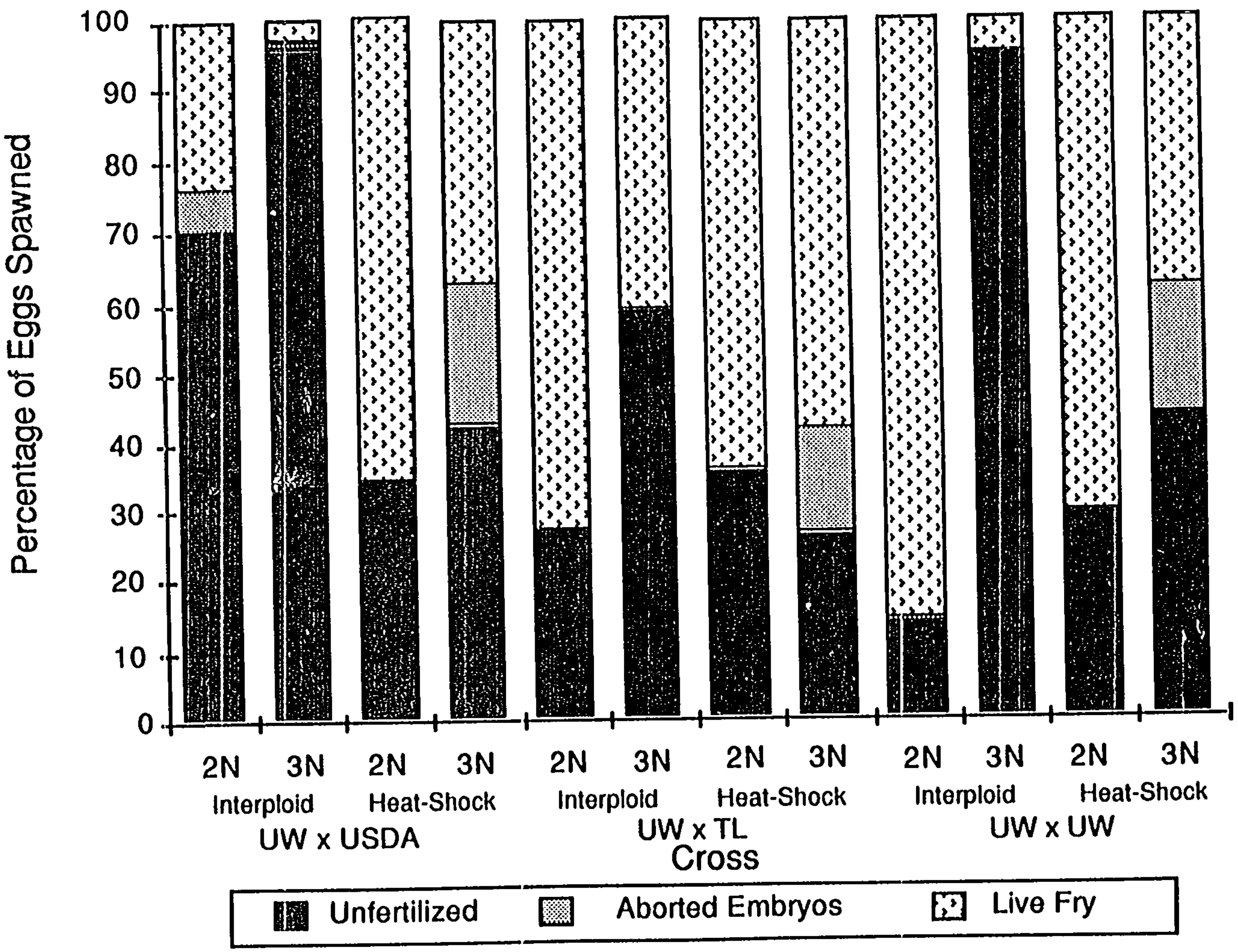


Figure 4. Fate of eggs from diploid and meiotic (heat-shocked) and interploid triploid inter- and intrastrain crosses of rainbow trout. N = 4000 - 10,000 for each group.

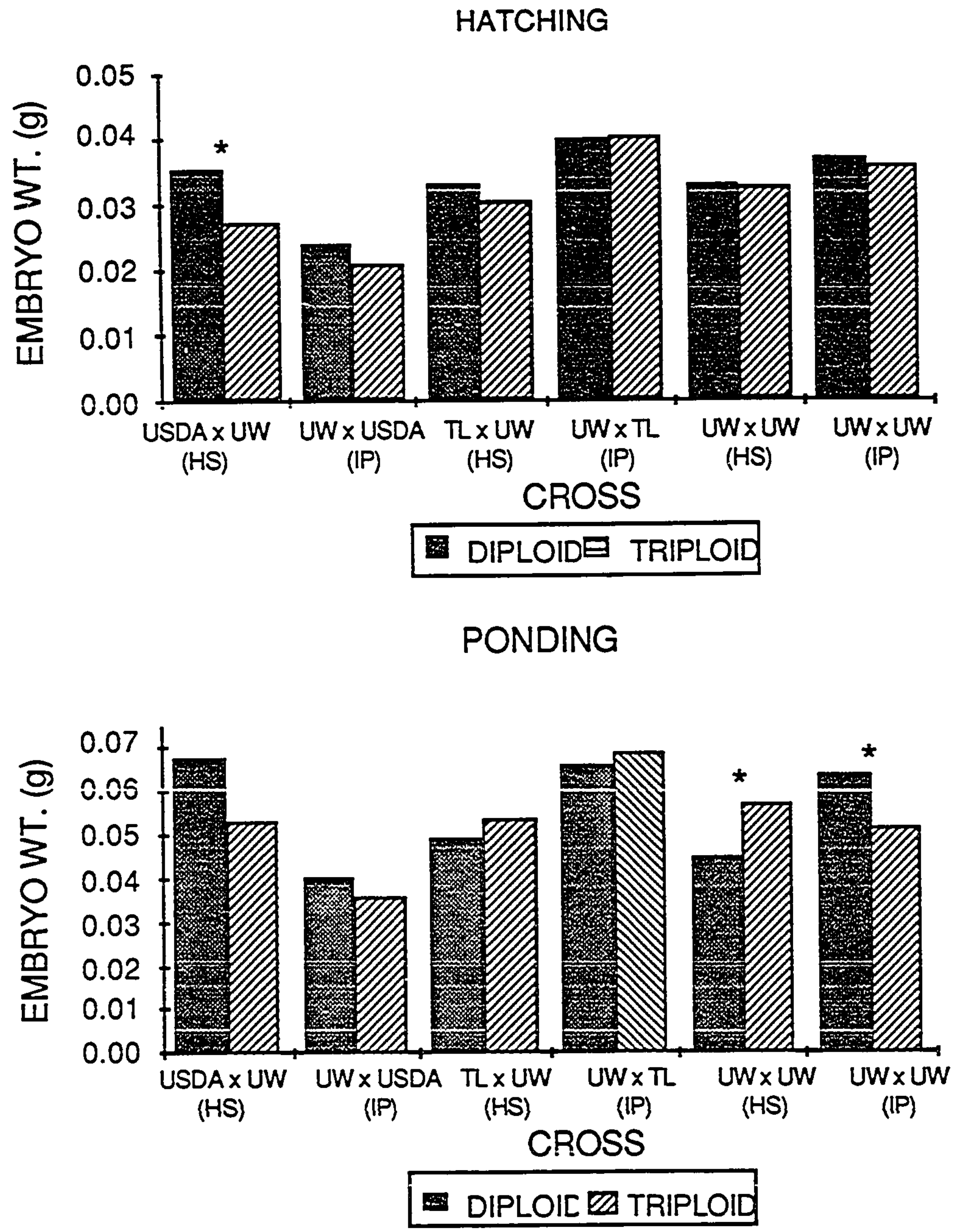
Table 1. Analysis of variance for aspects of diploid and triploid rainbow trout development from hatching (1) and ponding (2) during the 1988 study. ; IGR - instantaneous growth rate, YC - yolk conversion.

		Embryo Wet Wt. 1	Embryo Dry Wt. 1	Embryo Wet Wt. 2	Embryo Dry Wt. 2
Source	df	MS (x 10^6)	MS (x 10^6)	MS (x 10^6)	MS (x 10^6)
Group	11	377 **	10.7 **	1070 **	58.7 **
Error	108	14.1	0.58	85.3	3.40
Strain (S)	3	1230 **	29.7 **	2980 **	185 **
Ploid Type (P)	2	125**	7.76 **	211	33.3 **
S x P	2	14.1	0.157	270	1.79
Error	112	15.2		98.1	3.46

		IGR Wet	YC Wet
Source	df	MS (x 10^2)	MS
Group	11	36.2 **	16.1
Error	108	3.22	48.6
Strain (S)	3	101 **	4.18
Ploid Type (P)	2	8.95	23.11
M x P	2	7.64	1.02
Error	112	3.66	47.86

* $P < 0.05$

** $P < 0.001$



HATCHING

PONDING

Figure 5. Embryo weight minus yolk sac for newly hatched and ponded diploid and triploid rainbow trout fry in the 1988 study.(*) indicates a significant difference at $P < 0.05$

Table 2. Correlations for diploid and triploid rainbow trout sac fry development traits from post-hatching (1) to ponding (2) during the 1988 study. Correlations are based on between-group estimates. W - wet weight, D - dry weight, IGR - instantaneous growth rate, YC - yolk conversion. (*) indicates the correlation is significantly different from zero.

Variable	Emb W1	Emb D1	Emb W2	Emb D2	IGR WET	IGR DRY	YC WET	YC DRY
Egg Dia.	0.73*	0.77*	0.72*	0.87*	0.68*	0.78*	0.02	0.51
Emb W1	1.00	0.95*	0.86*	0.90*	0.84*	0.92*	0.14	0.66
Emb D1	-	1.00	0.82*	0.85*	0.78*	0.84*	0.03	0.51
Emb W2	-	-	1.00	0.86*	0.99*	0.87*	0.27	0.32
Emb D2	-	-	-	1.00	0.83*	0.99*	0.14	0.65*
IGR WET	-	-	-	-	1.00	0.86*	0.31	0.32
IGR DRY	-	-	-	-	-	1.00	0.18	0.65
YC WET	-	-	-	-	-	-	1.00	0.25
YC DRY	-	-	-	-	-	-	-	1.00

* significantly different from 0 ($P < 0.05$)

a significant factor, although the maternal x polyploid interaction was significant,and in pairwise comparisons significant differences were found in the UW x UW heat-shock and interploid crosses. Significant differences were found in the instantaneous growth rate, IGR, of the different crosses, although this appeared to be most related to the absolute weight of the embryos, the correlation between IGR and fry weight being 0.99. The ability of embryos to convert yolk material to tissue was similar in all crosses, as no significant main factors or interactions were detected in the ANOVA.

At the time of ponding insufficient numbers of USDA x UW individuals were present to justify their inclusion in the growth study. This shortfall was primarily related to the poor quality of the USDA eggs. Poor fertilization levels in the UW x UW interploid triploid group provided only enough fish to stock one tank along with one tank of controls.

Triploid Induction

The heat-shock treatment and tetraploid x diploid cross were almost equally effective in producing triploids. Analysis of the meiotic triploids sampled revealed 96.7% (29/30) and 100% (30/30) triploidy in the TL x UW and USDA x UW triploid groups, respectively. The UW x UW meiotic triploid group was discarded prior to ploidy sampling because of a disease outbreak. All the interploid triploids sampled, UW x UW (30/30) and UW x TL (30/30), were triploid.

Growth 0-63 Days Post-ponding;

The early growout period of the study was marked by distinct performance differences between heat-shocked and interploid triploids relative to their controls. All three heat-shocked triploid and UW x UW interploid triploids were not significantly different in weight from their controls at the time of ponding, while the TL x UW interploid triploids were significantly heavier than their controls (Figure 6). After the initial sample, the heat-shocked triploids' growth rate slowed considerably relative to their diploid controls . Following the first sampling period, 17 days post-ponding, the UW x UW heat-shock triploid fish experienced an epizootic of Infectious Hematopoietic Necrosis, IHN, and were destroyed. The other heat-shocked triploid groups continued to exhibit inferior growth such that 30 days after ponding the TL x UW and USDA x UW heat-shocked triploids were 29.7 and 24.1% smaller than their controls, respectively. This relationship was relatively unchanged up to 63 days post-ponding. Interploid triploids also showed a decline in growth rate relative to their controls, although this was not as marked as with the heat-shocked triploids.

Several factors exerted a significant influence on the ANOVA for weight during this period. Between group effects were strong throughout the fry phase (Table 3), although initially this was partially due to egg size differences rather than growth differences. Ploidy and strain effects, but not their interaction, emerged during the later part of the period as important factors.

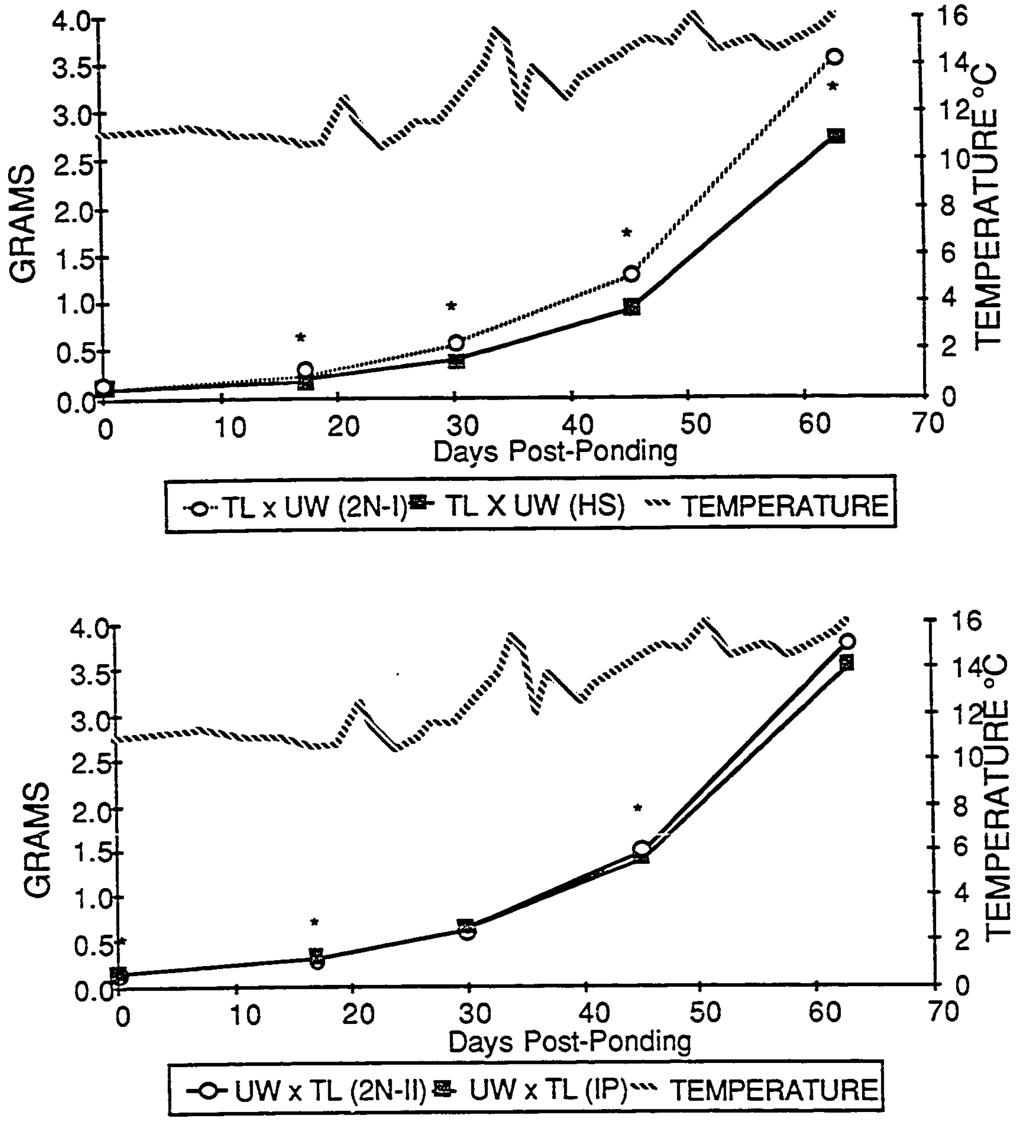


Figure 6. Weight of rainbow trout triploid and diploid hybrids during the first 63 days post-ponding in 1988. (*)indicates significance at the P < 0.05 level.

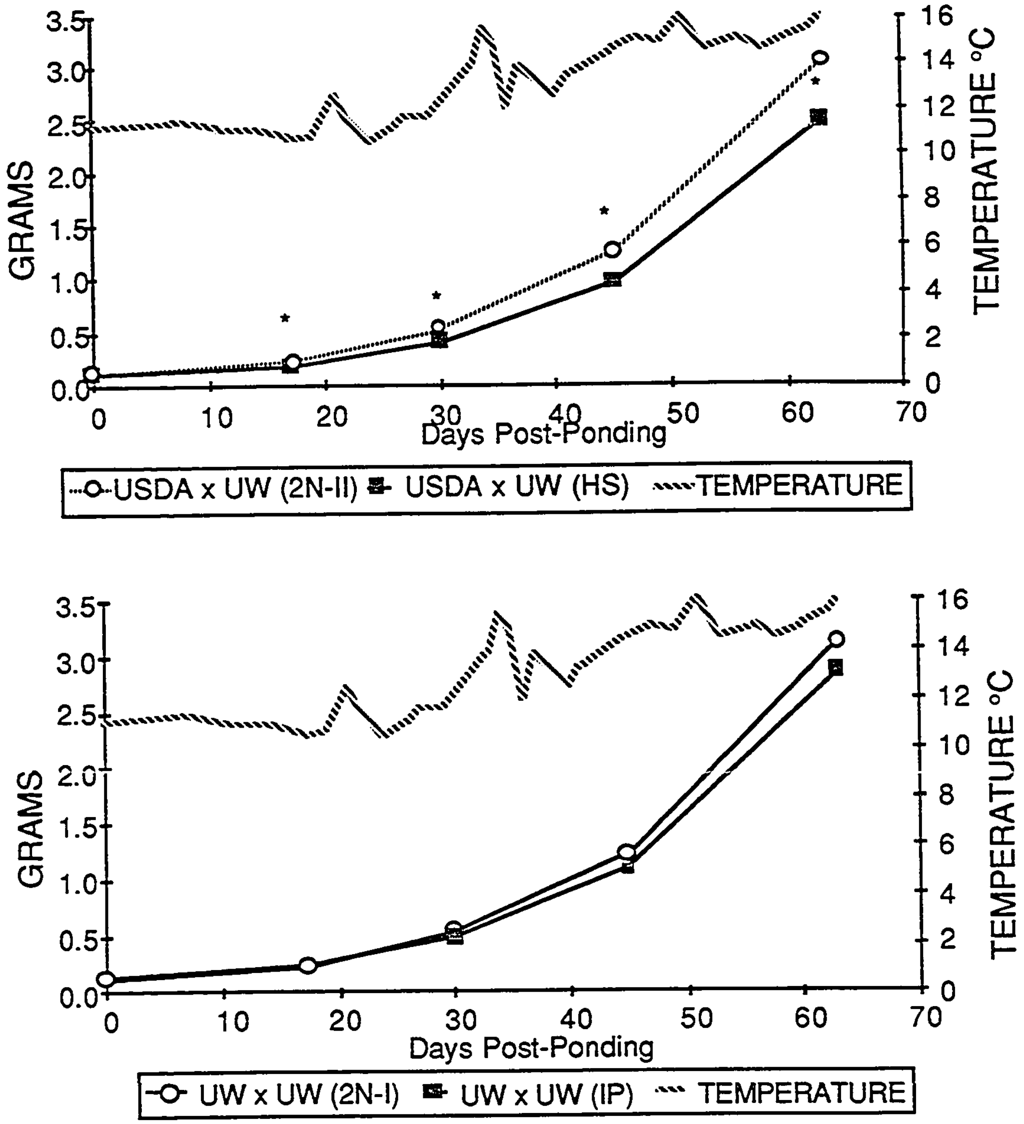


Figure 6. (cont.). (*) indicates significance at the $P < 0.05$ level.

Table 3. Analysis of variance for the weight of triploid and diploid strain hybrids and pure strain rainbow trout from ponding to Day 63 of the 1988 study. Mean squares (MS) have been corrected for unequal sample size.

	Weight Day 1		Weight Day 16		Weight Day 30	
Source	df	MS (x 10^3)	df	MS (x 10^3)	df	MS (x 10^3)
Group	8	101 **	9	374 **	8	285 **
Tank/Group	NA		16	5.02	12	5.01
Error	18	2.59	52	3.16	42	3.04
Ploid (P)	1	697 **	1	228	1	450
Strain (S)	2	2.77	2	313	2	99.2
P x S	2	99.9 **	2	6.57	2	7.63
Tank / P x S	NA		20	122 **	15	109 **
Error	22	2.47	52	3.16	42	3.04

	Weight Day 45		Weight Day 63	
Source	df	MS (x 10^3)	df	MS (x 10^3)
Group	8	212 **	8	1410 **
Tank/Group	12	7.17 *	12	152 *
Error	42	3.44	504	79.3
Ploid (P)	1	487 *	1	3740 **
Strain (S)	2	65.3	2	2290 **
P x S	2	23.0	2	62.5
Tank / P x S	15	76.8 **	15	312 **
Error	42	3.44	504	79.3

* $P < 0.05$

** $P < 0.001$

Growth 63 - 120 Days Post-Ponding;

The latter half of the growth study was marked by the rapid change in the size of the TL x UW heat-shocked triploids. At 63 days post-ponding this triploid group was 23.1 % lighter and 8.4 % shorter than their control group, but at 83 days this difference had diminished to 0.7 % lighter and 0.4 % longer (Figure 7). In contrast, the relative growth between the other triploid groups and their controls was unchanged. Water temperatures during this period did not appear to impact growth, despite level well above 15°C, (average of 18.4 °C with a maximum of 22.5 °C). At the conclusion of the study the weights and lengths for all groups were comparable, except for the heat-shocked USDA x UW and interploid UW x UW triploids which were significantly smaller than their controls, as well as the other groups.

Several trends were evident in the analysis of causative factors in growth. Ploidy was a significant factor in ANOVA for weight and length (Tables 4 and 5) in all except the day 83 sampling. The strains used to produce the crosses, irrespective of ploidy, were generally not a major source of variation other than at day 63 for weight and length and day 102 for weight. However, the ploidy x strain interaction was significant for length at the Day 102 sample and for weight and length at the Day 120 sample. Reciprocal effects, primarily due to egg sizes, diminished as the study progressed and were not a significant component during the last sampling periods, Days 83 -120.

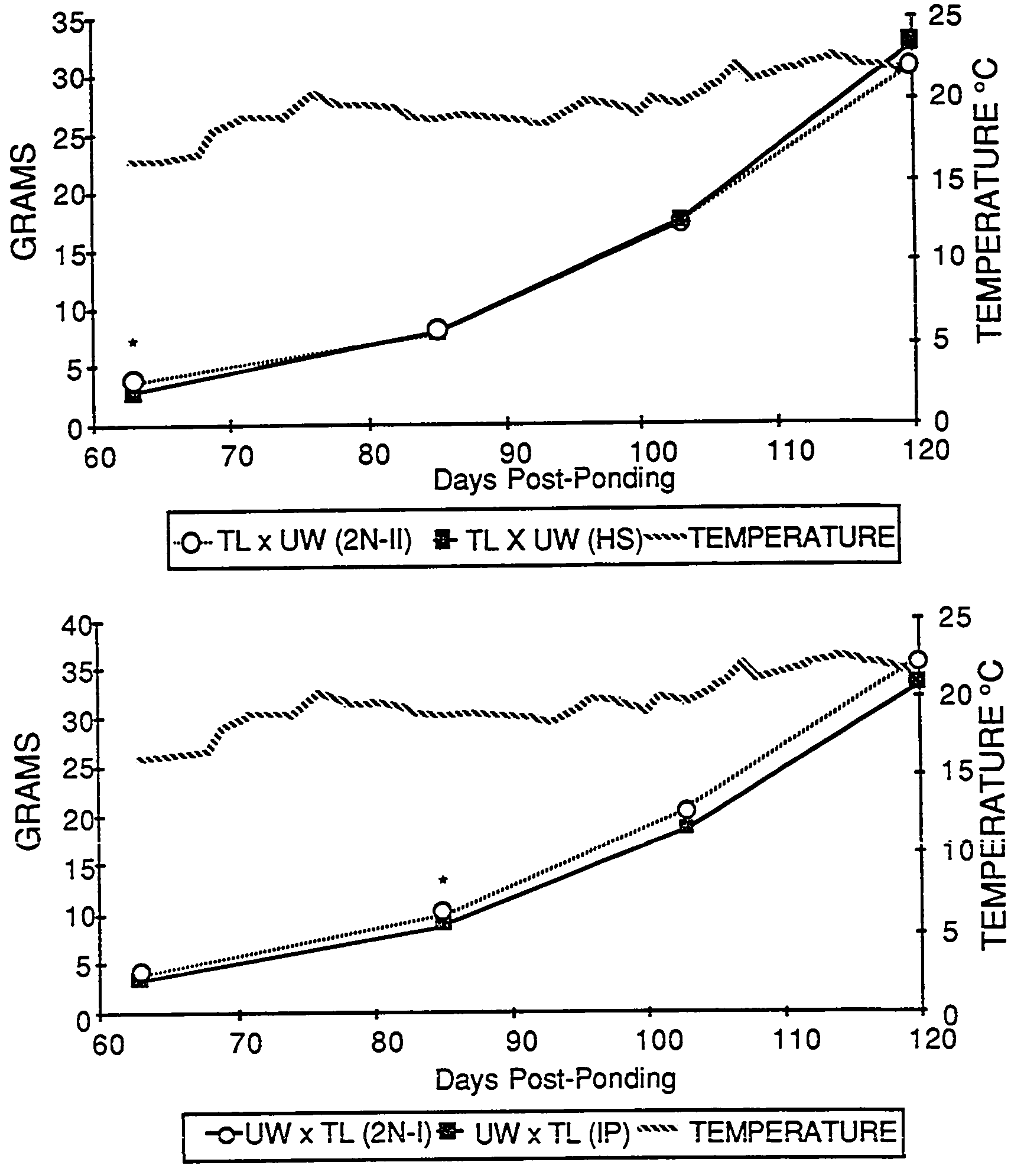


Figure 7. Weight of rainbow trout triploid and diploid hybrids during the first 63 days post-ponding in 1988. (*) indicates significance at the $P < 0.05$ level.

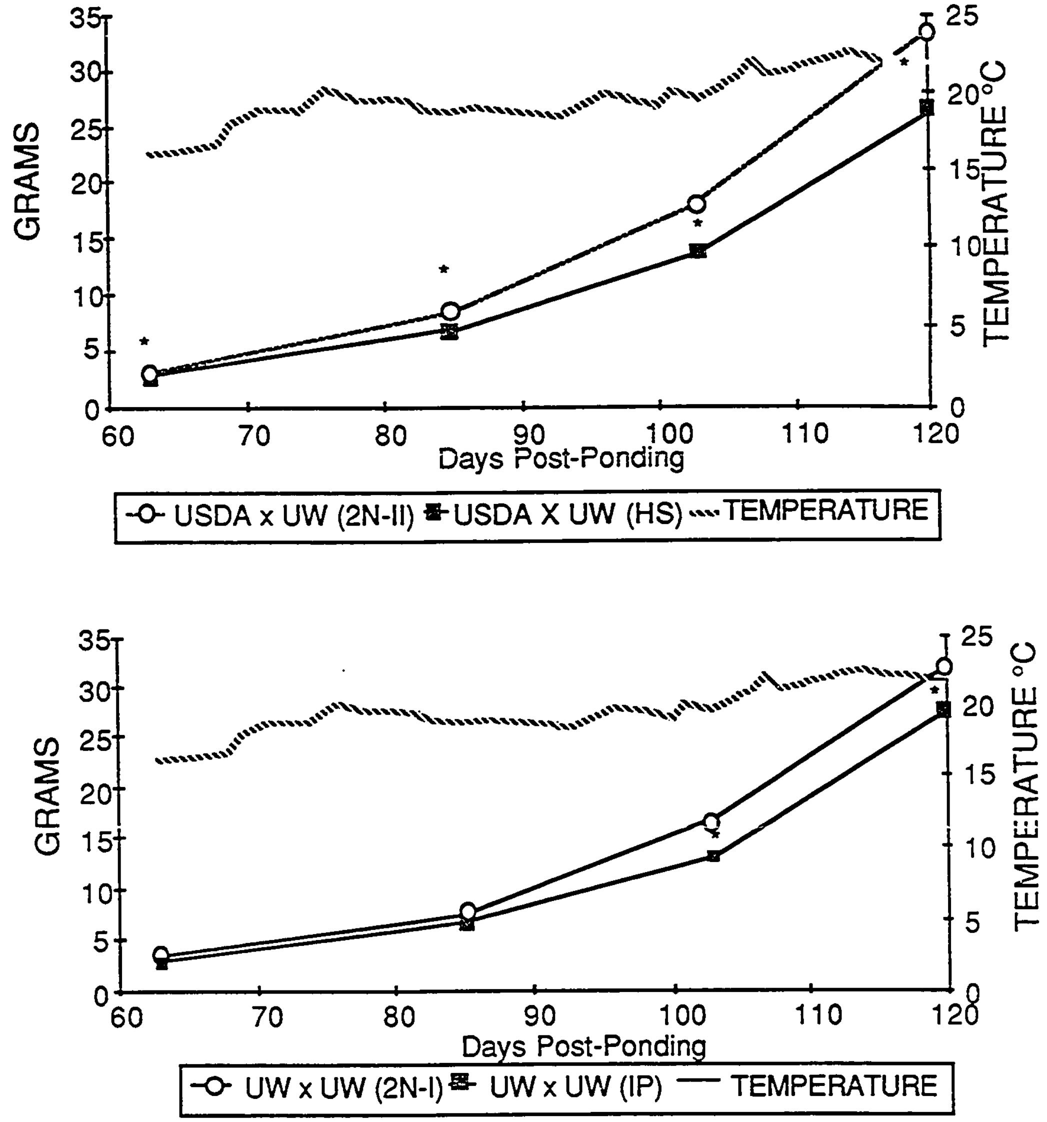


Figure 7 (cont.). (*) indicates significance at the P < 0.05 level.

Table 4. Analysis of variance for the weight of triploid and diploid strain hybrids and pure strain rainbow trout from Day 63 to Day 120 of the 1988 study. Mean squares (MS) have been weighted for unequal sample size.

		Weight Day 63	Weight Day 83	Weight Day 102	Weight Day 120
Source	df	MS (x 10^2)	MS (x 10^2)	MS (x 10^2)	MS (x 10^2)
Group	8	141 **	136	143 *	72.5
Tank/Group	12	15.2 *	54.7 **	48.2 **	27.9 **
Error	504	7.93	89.6	9.58	8.57
Ploid (P)	1	374 **	274	382 *	189 *
Strain (S)	2	229 **	93.0	189 *	48.2
P x S	2	6.25	58.7	108	104 *
Tank /P x S	15	31.2 **	76.2 **	49.3 **	28.0 **
Error	504	7.93	8.96	9.58	8.57

* P < 0.05

** P < 0.001

Table 5. Analysis of variance for the length of triploid and diploid strain hybrids and pure strain rainbow trout from Day 63 to Day 120 of the 1988 study. Mean squares (MS) have been weighted for unequal sample size.

		Length Day 63	Length Day 83	Length Day 102	Length Day 120
Source	df	MS (x 10^3)	MS (x 10^3)	MS (x 10^3)	MS (x 10^3)
Group	8	107 **	115	150 *	82.2 *
Tank/Group	12	10.6	48.5 **	39.5 **	26.9 **
Error	504	6.17	8.91	9.14	9.49
Ploid (P)	1	360.*	266	430 **	207
Strain (S)	2	124 *	39.5	104	138
P x S	2	7.57	78.2	231 *	174 **
Tank /P x S	15	24.4 **	65.8 **	38.5 **	26.5 **
Error	504	6.17	8.91	9.14	9.49

* $P < 0.05$

** $P < 0.001$

One potential factor in the poor growth of the USDA x UW heat-shock triploid group was the presence of a congenital caudal peduncle deformity. Several vertebrae in the caudal peduncle were misshapen or simply missing. This condition existed in varying levels of severity (Figure 8). At the conclusion of the study fish from the USDA x UW heat-shock triploid group were examined and the number of deformities scored. Of 313 fish from the three replicate tanks, 181 (57.8%) exhibited no deformity, 92 (29.4%) showed some vertebral compression, and 40 (12.8%) exhibited severe caudal peduncle malformation. The condition was not seen in either the USDA x UW controls or in any of the other groups.

Strain and Triploid Heterosis;

The growth performance of triploid groups relative to their diploid controls varied depending on genetic and temporal factors. All the heat-shocked triploids exhibited poor growth on both a length and a weight basis during the early phase of the study, while the interploid triploids were not distinct from their controls in this period (Figure 9). The growth pattern for UW x UW interploid triploids and TL x UW heat-shocked triploids changed dramatically during the second part of the study. The size of the UW x UW interploid triploids relative to diploids decreased dramatically while the TL x UW meiotic triploids increased in weight and length relative to their diploid counterparts.

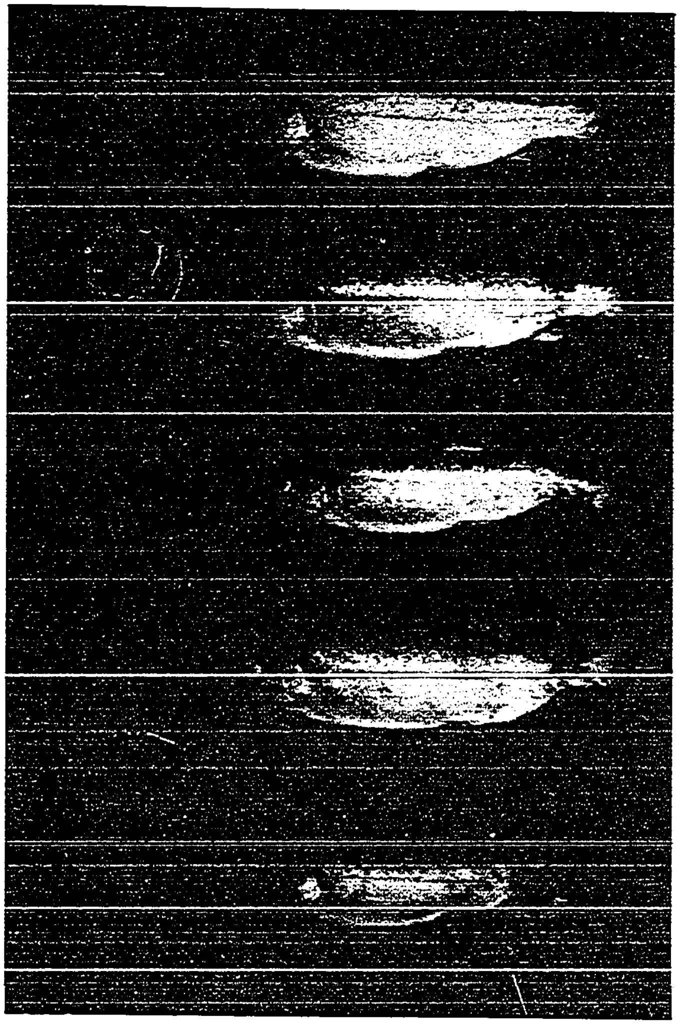


Figure 8. Caudal fin deformities in triploid heat-shocked USDA x UW hybrids. The extent of the deformities increases from top (normal) to bottom (extreme).

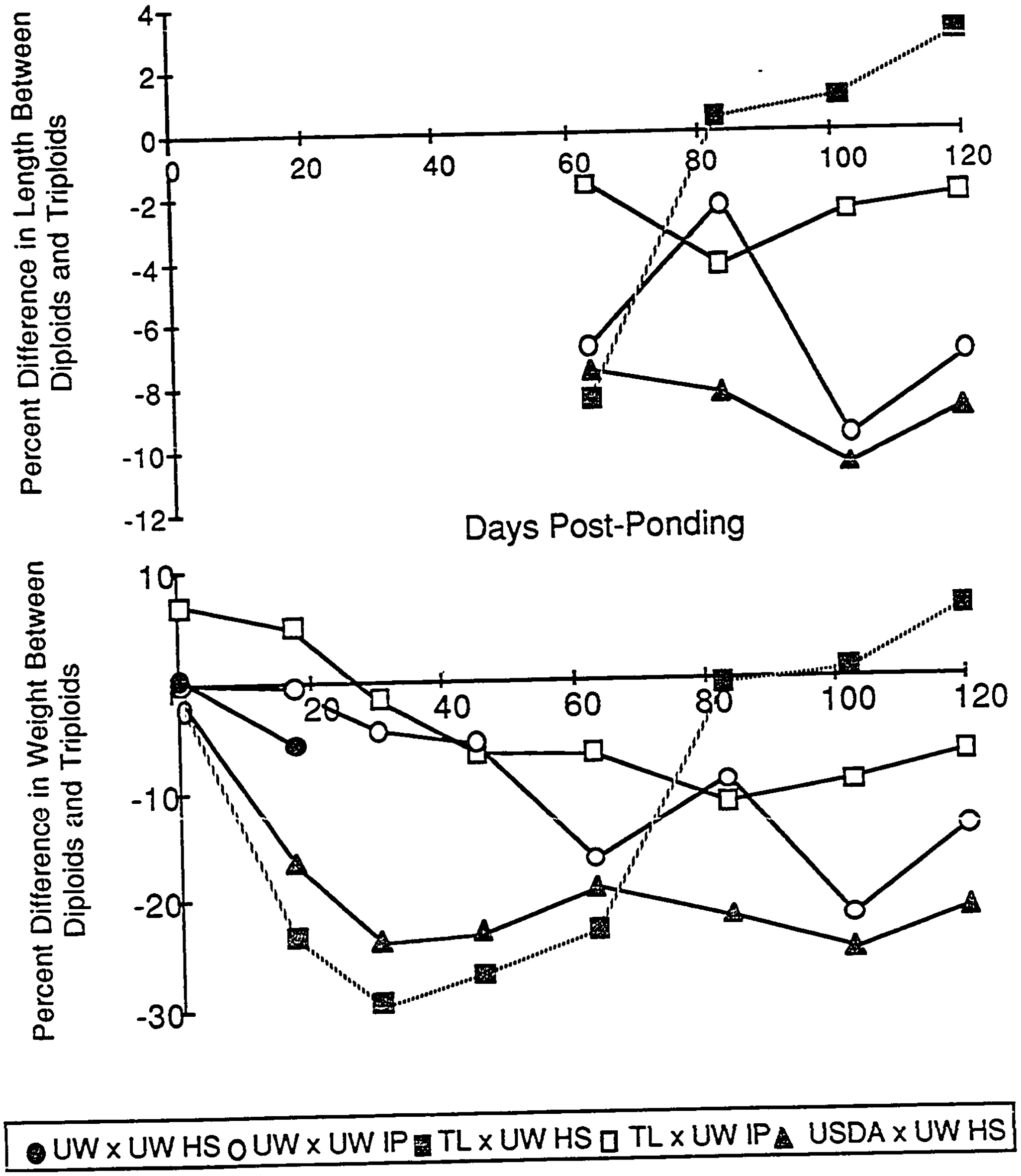


Figure 9. Percent difference in length and weight of triploid inter- and intrastrain hybrids of rainbow trout relative to their diploid controls. (HS) heat-shock (IP) interploid.

Growth Rate;

The pattern of growth for each group, as analyzed via the ANOVA group x time interaction, did not vary significantly (Table 6). Further analysis was performed with the UW x UW interploid group removed, since only one treatment and control tank were reared. However, the results of the analysis were unchanged and the group x time interaction was still not significant..

Condition Factor;

The condition factor (K.F.) varied considerably (see Appendix 2) across the different crosses, as well as increasing significantly ($P < 0.05$) over the course of the study. The main causative factor for this variation was the strain composition of the cross during all the sample periods that both weight and length data were recorded, Day 83-120 (Table 7). Additionally, the ploidy x strain interaction was significant during the last two samples, Day 102 -120. Ploidy was never a significant causative factor in the analyses of condition factors.

Mortalities 0-120 Days Post-Ponding;

The occurrence of mortalities in tanks was due to a wide variety of factors and showed considerable change over time (Figure 10). Mortalities during the first sampling period were due primarily to developmental abnormalities rather than any obvious pathogen; this was especially true of the heat-shocked triploid groups. Within and between group variation in mortality was quite high during

Table 6. Analysis of variance for the change in weight over time for diploid and triploid rainbow trout groups during the 1988 study.

	Weight 1988	
Source	df	MS (x 10^2)
Group (G)	7	243 **
Time(T)	6	51800 **
G x T	36	21.1
Tank (GxT)	93	21.1 **
Error	2068	8.03

* P < 0.05

** P < 0.001

Table 7. Analysis of variance for condition factor of diploid and triploid inter- and intrastrain rainbow trout crosses in the 1988 study from Day 63 to 120. Mean squares have been weighted for unequal sample size.

		K.F. Day 63	K.F. Day 83	K.F. Day 102	K.F. Day 120
Source	df	MS ($\times 10^1$)	MS ($\times 10^3$)	MS ($\times 10^3$)	MS ($\times 10^4$)
Group	8	11.5	152 **	296 **	2230 **
Tank/Group	12	10.8	18.8 **	18.8 *	153 *
Error	504	9.12	7.85	9.47	87.3
Ploid (P)	1	7.35	196	3.06	5.43
Strain (S)	2	12.7	27.5 *	372	3340 **
P x S	2	6.30	13.1	290	2000 *
Tank /P x S	15	11.7	54.6 **	76.6 **	544 **
Error	504	9.12	7.85	9.47	87.3

* $P < 0.05$

** $P < 0.001$

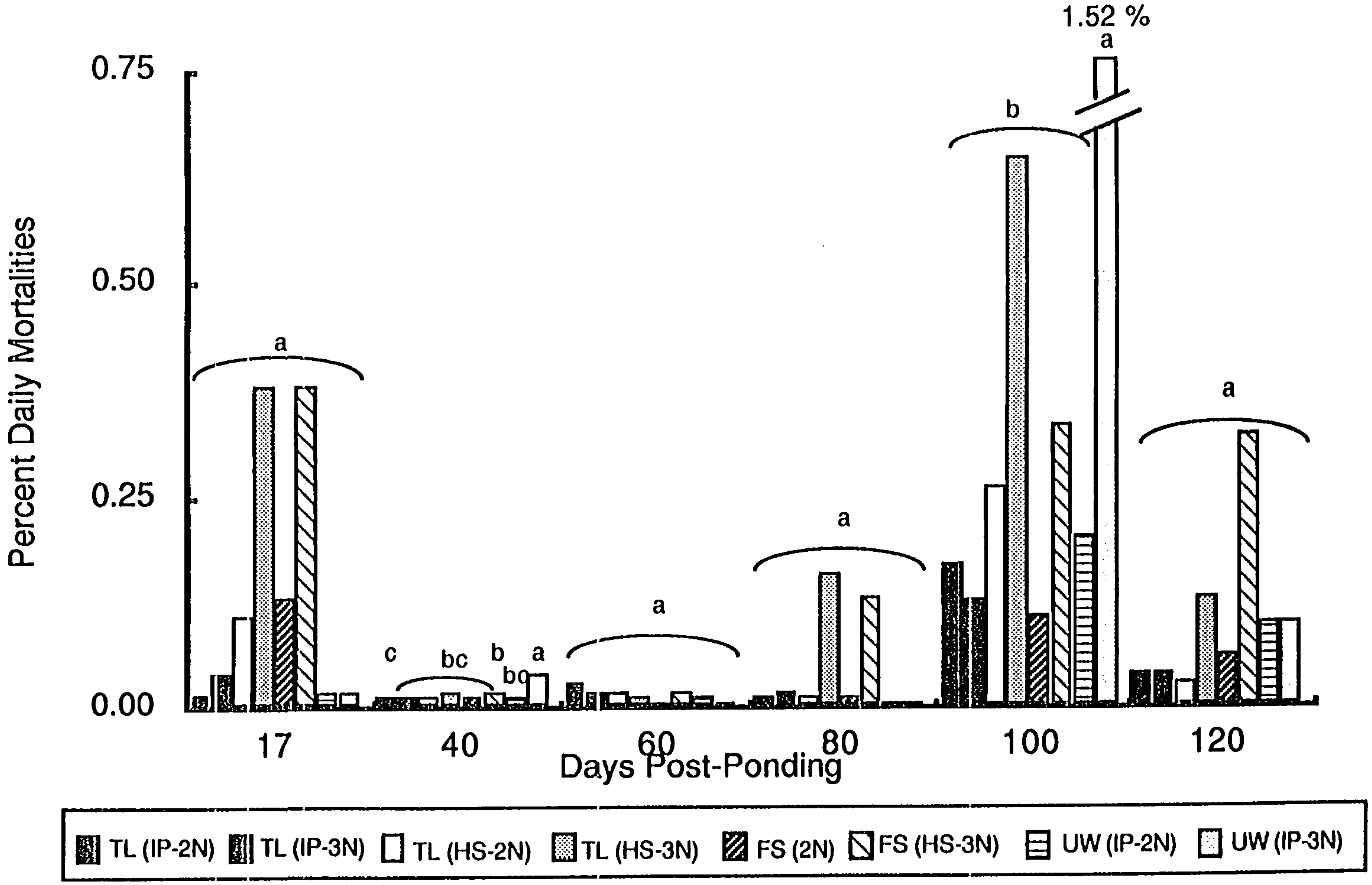


Figure 10. Percent daily mortalities for diploid and triploid inter- and intrastrain crosses of rainbow trout during the 1988 study. Letters denote groups that are not significantly different at the P > 0.05 level for each time phase of the study.

this period. No significant differences were detected between any of the groups. There was a second period of high mortality during the last 30 days of the study for all groups, corresponding to summer rise in temperatures above 20° C. In general, the heat-shocked triploids were more sensitive ($P < 0.05$) to this rise in temperature. Fish examined during this later period exhibited clinical signs of protozoan, *Ichthyophthirius* spp., and bacterial,*Columnaris* spp., inflections common to fish at the School of Fisheries hatchery. The single tank of interploid UW x UW triploids suffered an unusually severe epizootic of *Ichthyophthirius* spp. during the day 80-100 growout period.

The large variability found in mortality among replicate tanks obscured differences between groups. However, significant between cross differences were found for the Day 17-30 and Day 63-85 period (Table 8). When group effects were separated into strain and ploidy effects, ploidy level (diploid vs. triploid) was significant during the Day 17-30 and Day 83-120 periods. In both cases the triploids experienced higher levels of mortality.

Table 8. Analysis of variance for percent daily mortalities incurred by diploid and triploid rainbow trout inter- and intrastrain crosses during the 1988 study.

		Mortality Day 1-17	Mortality Day 17-30	Mortality Day 30-45	Mortality Day 45-63
Source	df	MS (x 10^3)	MS (x 10^6)	MS (x 10^5)	MS (x 10^4)
Group	6	60.1	74.9 **	24.6	1.23
Tank/Group	11	47.0	10.6	36.4	91.8
Error	1	1.80	0.00	45.0	4.50
Ploid (P)	1	244	267 **	3.02	313
Strain (S)	2	3.35	44.0	18.4	7.43
P x S	1	2.21	4.10	45.2	15.6
Tank /P x S	8	84.9 **	14.6	32.7	66.4
Error	6	4.19	27.8	37.5	134

		Mortality Day 63-83	Mortality Day 83-120
Source	df	MS (x 10^3)	MS (x 10^3)
Group	6	113 *	39.9
Tank/Group	11	25.8	17.8
Error	1	1.80	7.02
Ploid (P)	1	232 *	170 *
Strain (S)	2	4.28	88.5
P x S	1	6.90	52.6
Tank /P x S	8	20.6	25.6 **
Error	6	88.2	1.64

* $P < 0.05$

** $P < 0.001$

1989 Study

Spawning and Incubation;

Female broodstock from UW and Trout Lodge strains produced eggs of varying size and quality. Trout Lodge eggs were significantly larger than UW eggs with egg diameters of 5.11 ± 0.03 mm and 4.82 ± 0.04 mm, respectively. Eggs taken from UW females for use in the UW x UW interploid cross were larger, 5.07 ± 0.03 mm, than eggs used in the heat-shock and control treatments, 4.75 ± 0.04 mm. The treatment groups varied initially from between 3000 and 5000 eggs.

Incubation performance was dependant on a number of factors (Fig. 11). Untreated eggs from Trout Lodge females exhibited higher fertilization levels, 70.4 ± 1.7 %, than did the UW eggs, 57.7 ± 2.5 %. There was no difference between fertilization levels in heat-shocked and control groups, 58.8 ± 1.8 % and 53.5 ± 4.8 %, respectively, but significant differences existed between the control groups and interploid crosses, 19.2 ± 3.6 %. Mortality among developing embryos was most marked in the heat-shocked triploid groups with an average of 44.8 % of fertilized eggs dying during incubation relative to 22.6 % of control eggs and 2.3 % of interploid eggs. Control groups had the highest survival to hatch, 45.5 ± 0.1 %, which was significantly higher than heat-shocked triploid groups and interploid triploid groups, 29.2 ± 2.1 and 18.7 ± 4.1 %, respectively. The difference in survival to hatch between the two triploid types was not significant.

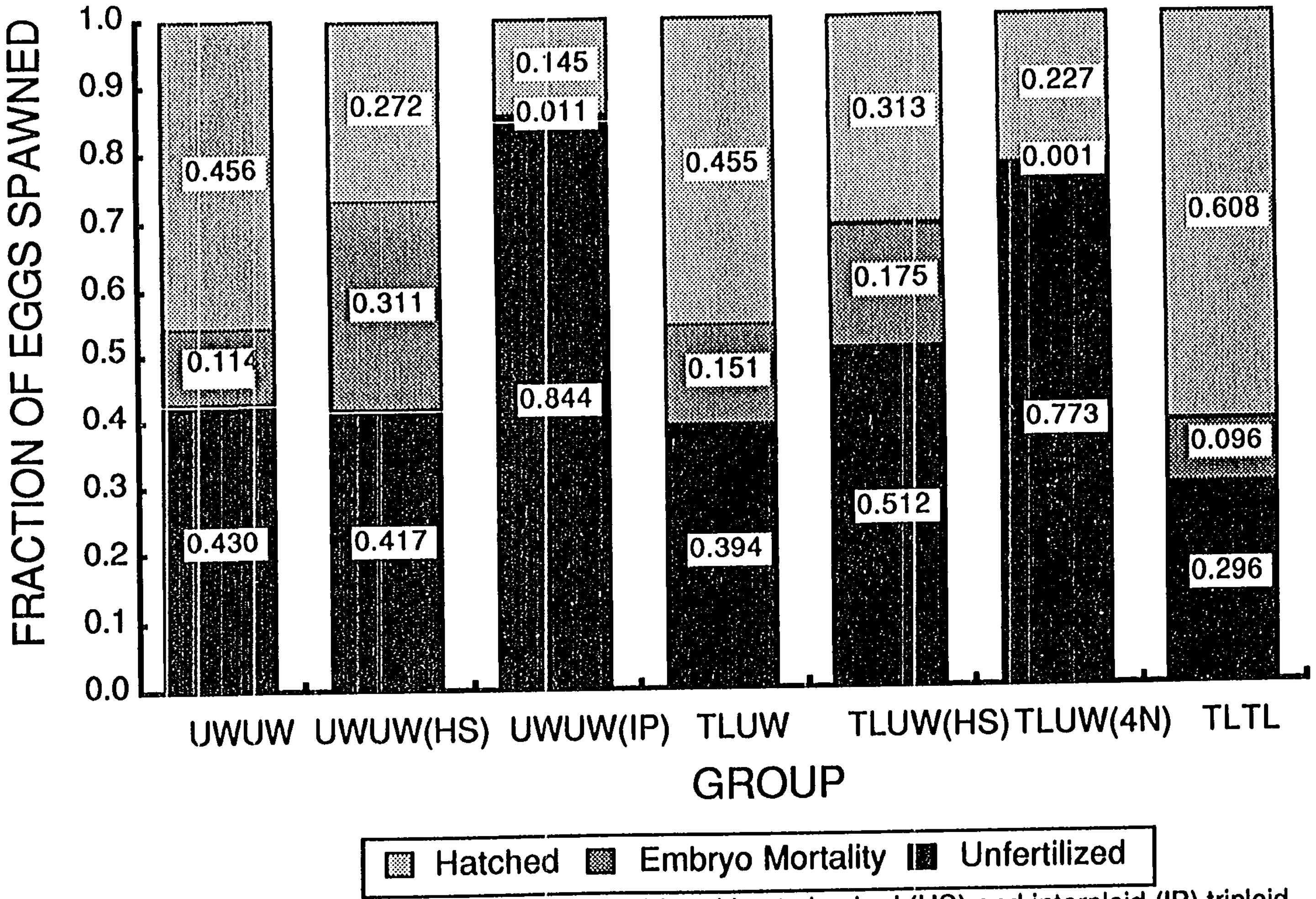


Figure 11. The fate of eggs from diploid and heat-shocked (HS) and interploid (IP) triploid inter- and intrastrain crossess of rainbow trout during the 1989 study.

The wet weight of newly hatched fry (Figure 12), minus yolk sac, was significantly correlated to egg size, r = 0.88. The progeny of Trout Lodge females ranked as the largest embryos, although in most cases this was not significant. The UW interploid triploids were intermediate in weight, and the heat-shock triploids and their controls were the smallest. Dry weights were similarly disposed, and there were no significant changes in rankings among groups on a dry or wet weight basis. This degree of similarity is reflected in the significant correlation between individual wet and dry weights, r = 0.801 (Table 9). Paternal strain and ploid type were identified by ANOVA as being significant factors; however, fry weights at hatching may have been influenced by differences in egg size (Table 10).

At the time of ponding, 18 days post-hatching, the wet weight of the embryos had increased an average 204 % and the dry weight 177 %. The gain in weight between groups differed and there were some significant changes in rank between groups. Specifically, the UW interploid triploid group was the largest on a wet weight basis at ponding (Figure 12), and the UW diploid control the smallest. There was no significant difference in the group rankings on a wet or dry basis, due, in part, to the part-whole relationship between wet and dry weights, r = 0.93 . The correlation between egg size and size at ponding decreased, r = 0.61 , as the differences in the growth rates of the groups expressed themselves. As with the fry weights at hatching, ANOVA indicated that paternal genotype and ploid type were significant factors, but these were still probably related to egg size. Analysis using maternal strain and ploidy as major effects resulted in a significant

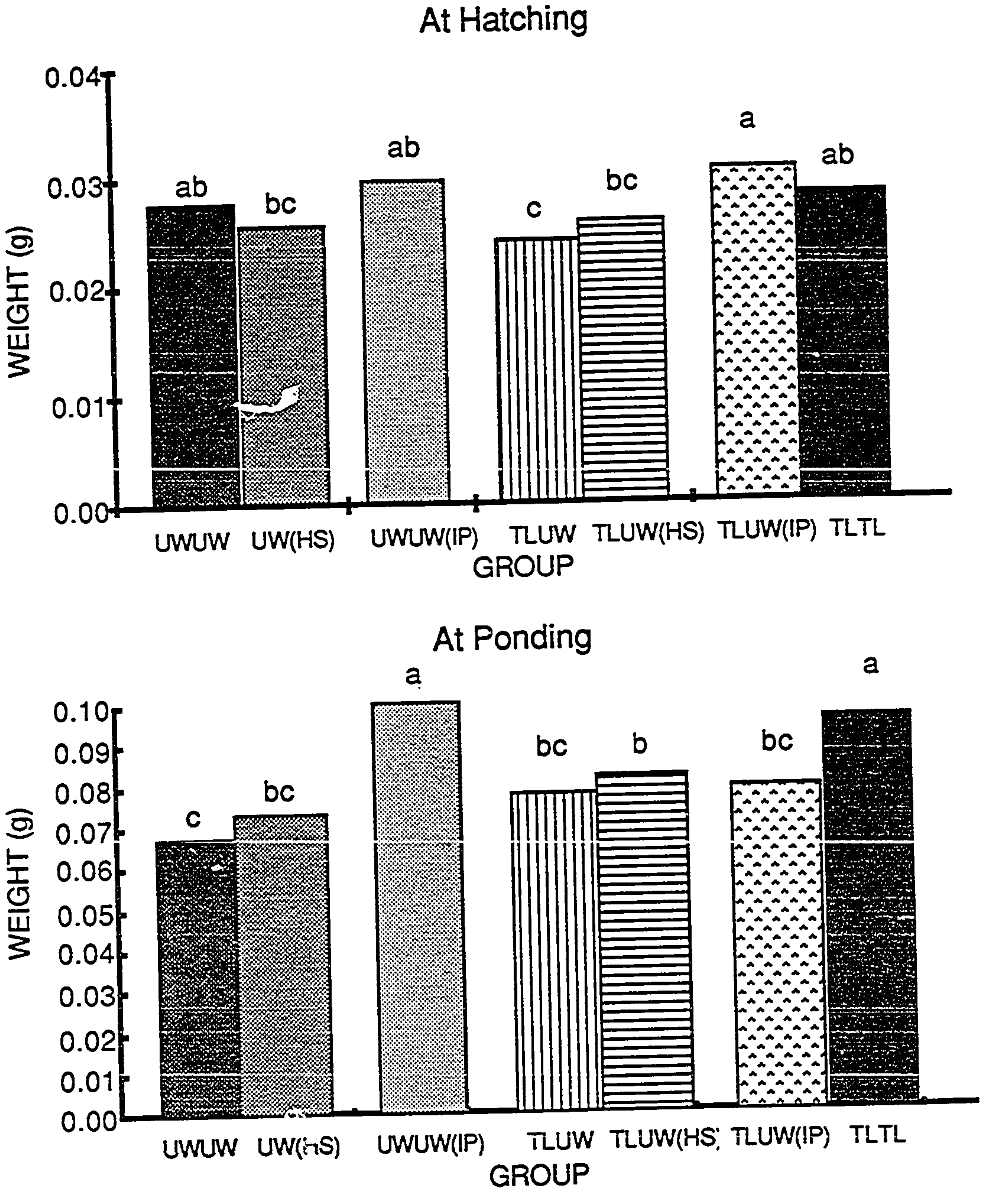


Figure 12. Wet weights (in grams) of embryos, minus yolk-sac at hatch and at the time of ponding during the 1989 study. Common letters designate groups not significantly different at the P < 0.05 level.

Table 9. Correlations for diploid and triploid rainbow trout sac fry development traits from post-hatching (1) to ponding (2) during the 1989 study. W - wet weight, D - dry weight, IGR - instantaneous growth rate, YC - yolk conversion. Correlations are based on between-group estimates.

Variable	Emb W1	Emb D1	Emb W2	Emb D2	IGR WET	IGR DRY	YC WET	YC DRY
Egg Dia.	0.88*	0.86*	0.61*	0.78*	0.53*	0.71*	-0.66*	-0.66*
Emb W1	1.00	0.80*	0.25	0.51*	0.15	0.32	-0.67*	-0.50
Emb D1	-	1.00	0.32	0.48	0.24	0.41	-0.88*	-0.79*
Emb W2	-	-	1.00	0.93*	0.99*	0.99*	-0.10	-0.34
Emb D2	-	-	-	1.00	0.89*	0.98*	-0.16	-0.31
IGR WET	-	-	-	-	1.00	0.96*	-0.04	-0.29
IGR DRY	-	-	-	-	-	1.00	-0.15	-0.35
YC WET	-	-	-	-	-	-	1.00	0.90*
YC DRY	-	-	-	-	-	-	-	1.00

* significantly different from 0 (P <0.05)

Table 10. Analysis of variance for aspects of diploid and triploid rainbow trout development from hatching (1) and ponding (2) during the 1989 study.

		Embryo Wet Wt. 1	Embryo Dry Wt. 1	Embryo Wet Wt. 2	Embryo Dry Wt. 2
Source	df	MS (x 10^5)	MS (x 10^7)	MS (x 10^4)	MS (x 10^5)
Group	6	6.80 **	21.9 **	19.1 **	2.75 **
Error	98	1.05	7.40	1.38	1.09
Ploid (P)	1	1.34	4.90	7.46	3.02
Strain (S)	1	1.44	4.90	8.17	1.23
P x S	1	5.10	5.40	32.2 **	3.15
Error	101	1.38	8.30	2.10	1.17

		Embryo IGR Wet	Embryo IGR Dry	Yolk Conversion Wet	Yolk Conversion Dry
Source	df	MS (x 10^2)	MS (x 10^2)	MS (x 10^1)	MS (x 10^2)
Group	6	27.6 **	16.8 *	29.4 **	37.3 **
Error	98	3.05	4.13	6.45	10.1
Ploid (P)	1	8.98	11.4	58.0 **	61.1 *
Strain (S)	1	12.7	8.82	5.88	1.18
P x S	1	51.5 **	20.6 *	34.3 *	20.8
Error	101	4.03	4.71	7.24	13.0

* $P < 0.05$

** $P < 0.001$

maternal strain x ploidy interaction influencing the wet weight ($P < 0.002$) at ponding, but not the dry weight ($P < 0.104$). The weight of the fry at ponding was strongly linked to the instantaneous growth rate, IGR, from hatching to ponding, $r = 0.99$ (ww) and $r = 0.98$ (dw). Differences in growth rate were evident between groups of the same maternal origin (egg size). Maternal strain and ploidy interactions significantly influenced the instantaneous growth rate of the fry from hatch to ponding on both a wet and dry basis.

The efficiency with which fry in each of the groups converted yolk to body tissue after hatching was dependent on a number of factors. The two heat-shocked triploid groups exhibited the best conversion rates on both a wet and dry basis (Figure 13). There was also a negative correlation between conversion rates and egg size, $r = -0.68$ (ww) and $r = -0.66$ (dw), as well as between fry weights and yolk conversion . Analysis of variance identified ploidy as a significant factor influencing yolk conversion on a wet and dry basis, subsequent testing showed that triploids had a significantly better yolk conversion efficiency than diploids on both a wet and dry basis; 2.28 vs. 1.88 and 0.71 vs. 0.56, respectively. Furthermore, the ploidy type was a significant factor in ANOVA ($P < 0.001$) for wet and dry weight yolk conversion efficiency. Efficiencies were significantly higher for heat-shock triploids, 2.67 (ww) and 0.85 (dw), than for interploid triploids, 1.90 (ww) and 0.56 (dw), or diploids, 1.88 (ww) and 0.56 (dw).

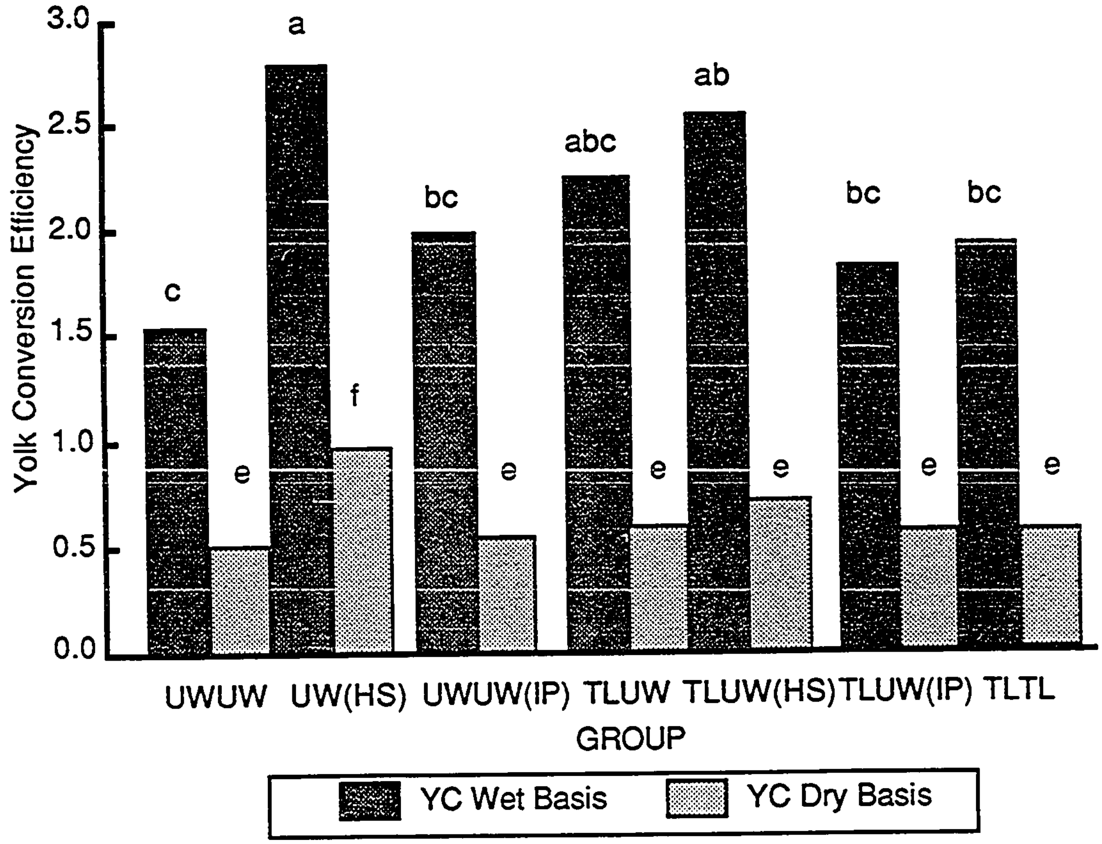


Figure 13. Yolk conversion efficiencies (YC) based on wet weight and dry weight for diploid and triploid inter- and intrastrain hybrids of rainbow trout. Groups that share a common letter are not significant at the P < 0.05 level

Triploid Induction

High triploid induction levels were observed in both the meiotic and interploid triploid groups. Analysis of the meiotic triploids sampled revealed 96.7% (29/30) and 100% (30/30) triploidy in the UW x UW and TL x UW triploid groups, respectively. The all-diploid nature of tetraploid derived sperm was verified via flow cytometry, and confirmed by the 100% triploid composition (30/30) of the UW x TL and UW x UW groups.

Growth 0-63 Days Post-ponding;

The initial grow-out period of the study was marked by degradation in the growth performance of heat-shock triploids relative to their diploid controls, while the interploids maintained a constant advantage over their controls. Water temperatures during this period rose considerably from 10.4 °C at the time of ponding to 19.2 °C at the Day 63 sampling, with an average of 15.0 ± 0.7 °C.

There were no significant differences in weight between groups at the time of ponding (Figure 14), although groups were a significant factor in one-way ANOVA ($P < 0.001$). None of the other ANOVA models were able to detect significant factors or interactions affecting weight during the first sample. However, from Day 21 to Day 63 the interploid triploids were significantly heavier than their diploid controls (Figure 15). The UW x TL interploids were also longer than controls during this period , although only the Day 21 sample were the UW interploids significantly longer than controls.

Both heat-shock triploid groups were smaller ($P < 0.05$) than controls throughout the first growth period . Between cross differences were significant for both weight and length throughout the first growth period (Tables 11 and 12). The ploidy type was also significant throughout the Day 21 - 63 period, while strain type (pure vs. hybrid) was only significant at the Day 63 sample and their interaction was not significant . Comparisons of ploidy types during this period indicated that heat-shock triploids were significantly shorter and lighter than either the diploids or interploid triploids from Day 21 - 63, and the interploids were heavier than the diploids from Day 50 -63, and longer only on the Day 63 sampling.

Growth Day 63 -117;

Water temperatures ranged from 19.2 °C to a high of 21.8 °C with an average of 19.9 ± 0.3°C during the latter period of the study in the late spring and early summer of 1989. In general, the triploid groups' growth improved relative to diploid controls; however, this was more marked in some groups.

The UW x TL interploid triploids were the only triploid groups to exhibit a decline in growth relative to the diploid hybrid cross. Up to Day 91 this triploid group was significantly longer (4.9%) and heavier (22.7%) than its control, but after that there was no significant difference between the UW x TL interploids and their controls (Figure 16). Alternatively, the TL x UW heat-shock triploid group growth improved relative to its control, such that at the Day 105 and 117 samplings there were no differences in weight or length between them.

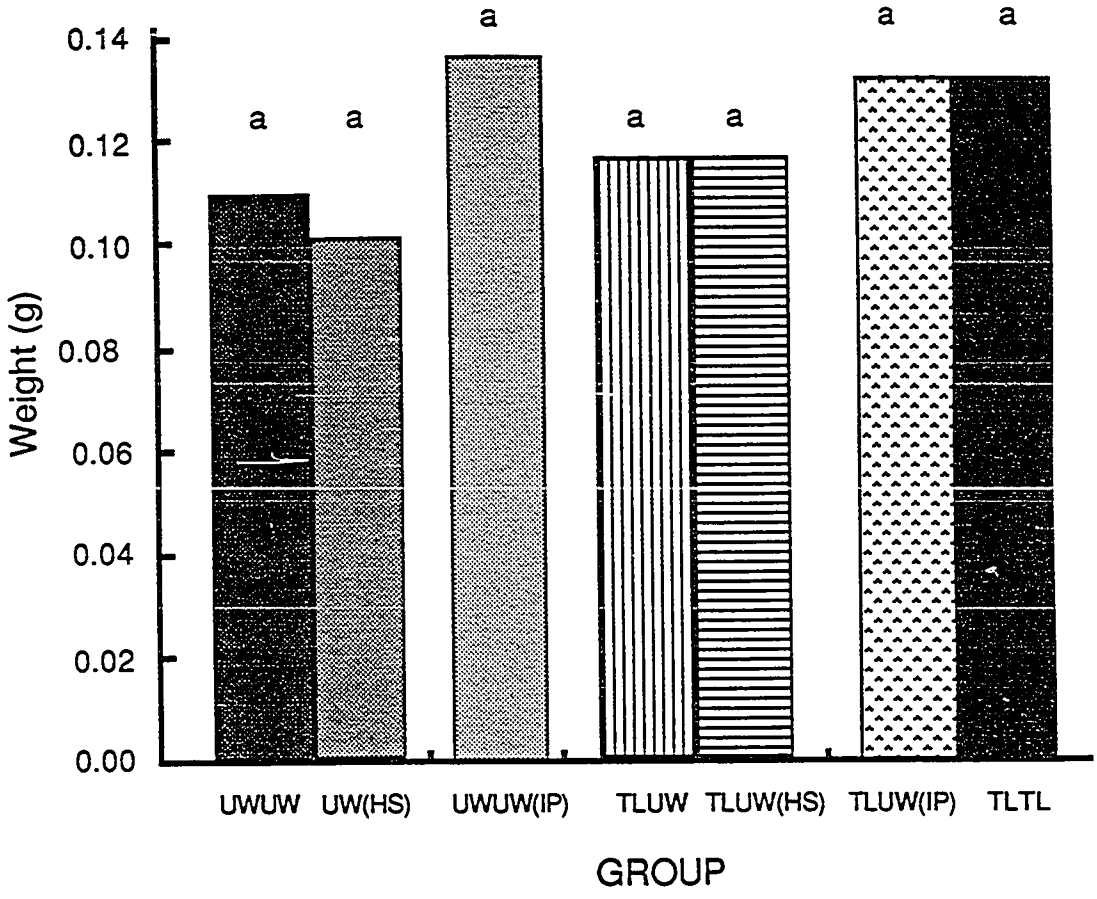


Figure 14. Weight of diploid and triploid rainbow trout fry at the time of ponding. Groups that share a common letter are not significant at the $P < 0.05$ level.

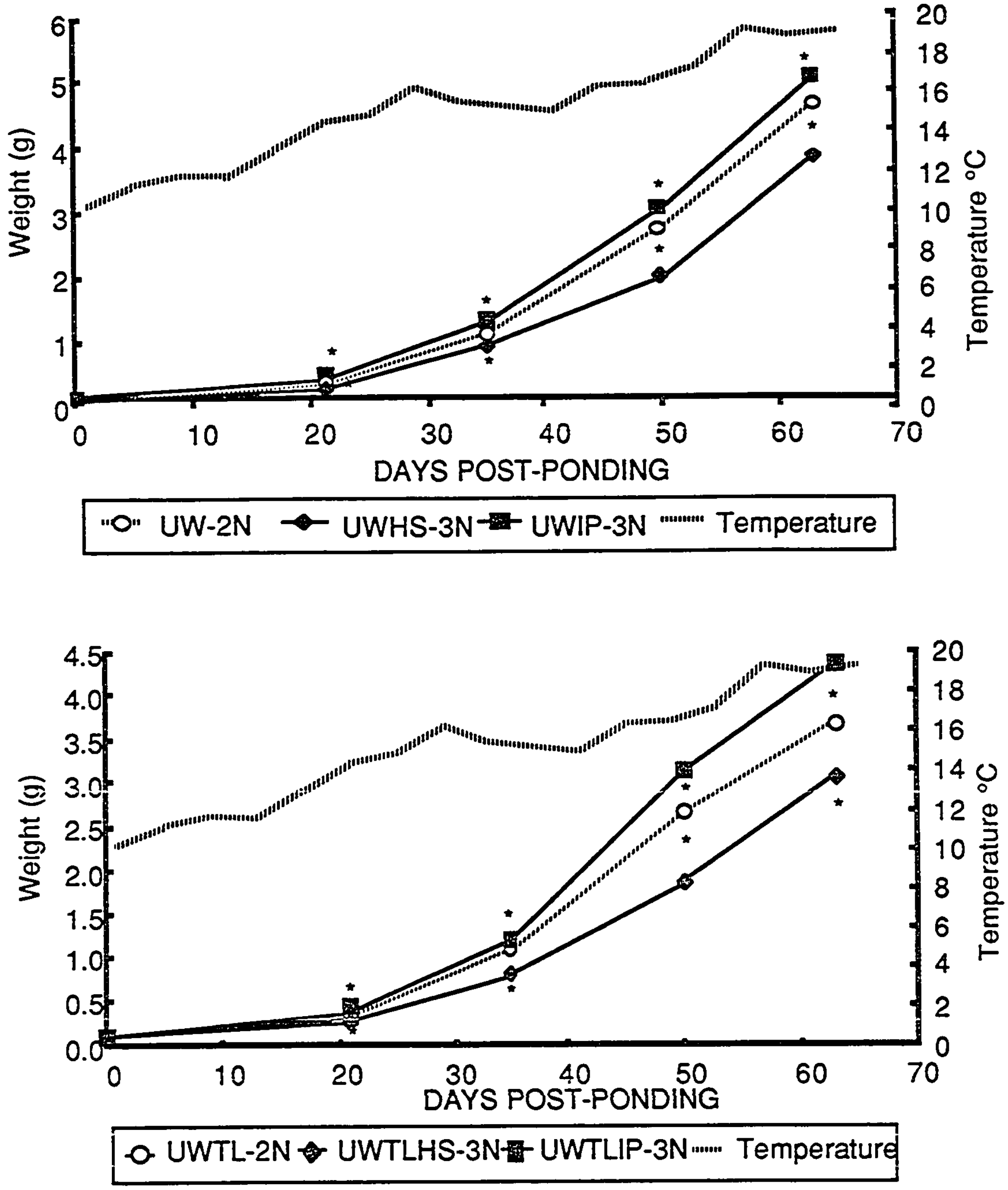


Figure 15. Weight of rainbow trout triploid and diploid hybrids during the first 63 days post-ponding in 1989. (*) indicates a significant difference between the triploid and control group at the $P < 0.05$ level.

Table 11. Analysis of variance for the weight of triploid and diploid strain hybrids and pure strain rainbow trout from ponding to Day 63 of the 1989 study.

	Weight Day 1		Weight Day 21		Weight Day 35	Weight Day 50
Source	df	MS (x 10^4)	df	MS (x 10^2)	MS (x 10^2)	MS (x 10^2)
Group	5	1640 **	5	322 **	494 **	538 **
Tank/Group			12	11.3 *	29.0 **	36.7 **
Error	12	138	432	6.19	8.09	9.30
Ploid (P)	2	190	2	416 **	717**	1072 **
Strain (S)	1	6.70	1	2.15	125	5.51
P x S	2	30.1	2	1.47	53.9	11.4
Tank /P x S			12	11.3 *	29.0 **	36.7 **
Error	12	138	432	6.18	8.09	9.30

	Weight Day 63	
Source	df	MS (x 10^1)
Group	5	27.1 **
Tank/Group	12	2.58 **
Error	432	1.02
Ploid (P)	2	39.5 **
Strain (S)	1	74.4 **
P x S	2	1.57
Tank /P x S	12	2.58 **
Error	432	1.02

* $P < 0.05$

** $P < 0.001$

Table 12. Analysis of variance for the length of triploid and diploid strain hybrids and pure strain rainbow trout from Day 21 to Day 63 of the 1989 study

		Length Day 21	Length Day 35	Length Day 50	Length Day 63
Source	df	MS (x 10^3)	MS (x 10^3)	MS (x 10^3)	MS (x 10^3)
Group	5	299 **	474 **	482 **	264 **
Tank/Group	12	14.7	48.2 **	43.6 **	20.6 *
Error	432	6.96	7.76	9.65	9.70
Ploid (P)	2	373 **	736 **	958 **	351 **
Strain (S)	1	18.2	163	13.1	571 **
P x S	2	6.02	24.6	26.2	38.9
Tank /P x S	12	14.7	48.2 **	43.6 **	20.6 *
Error	432	6.96	7.76	9.65	9.70

* $P < 0.05$

** $P < 0.001$

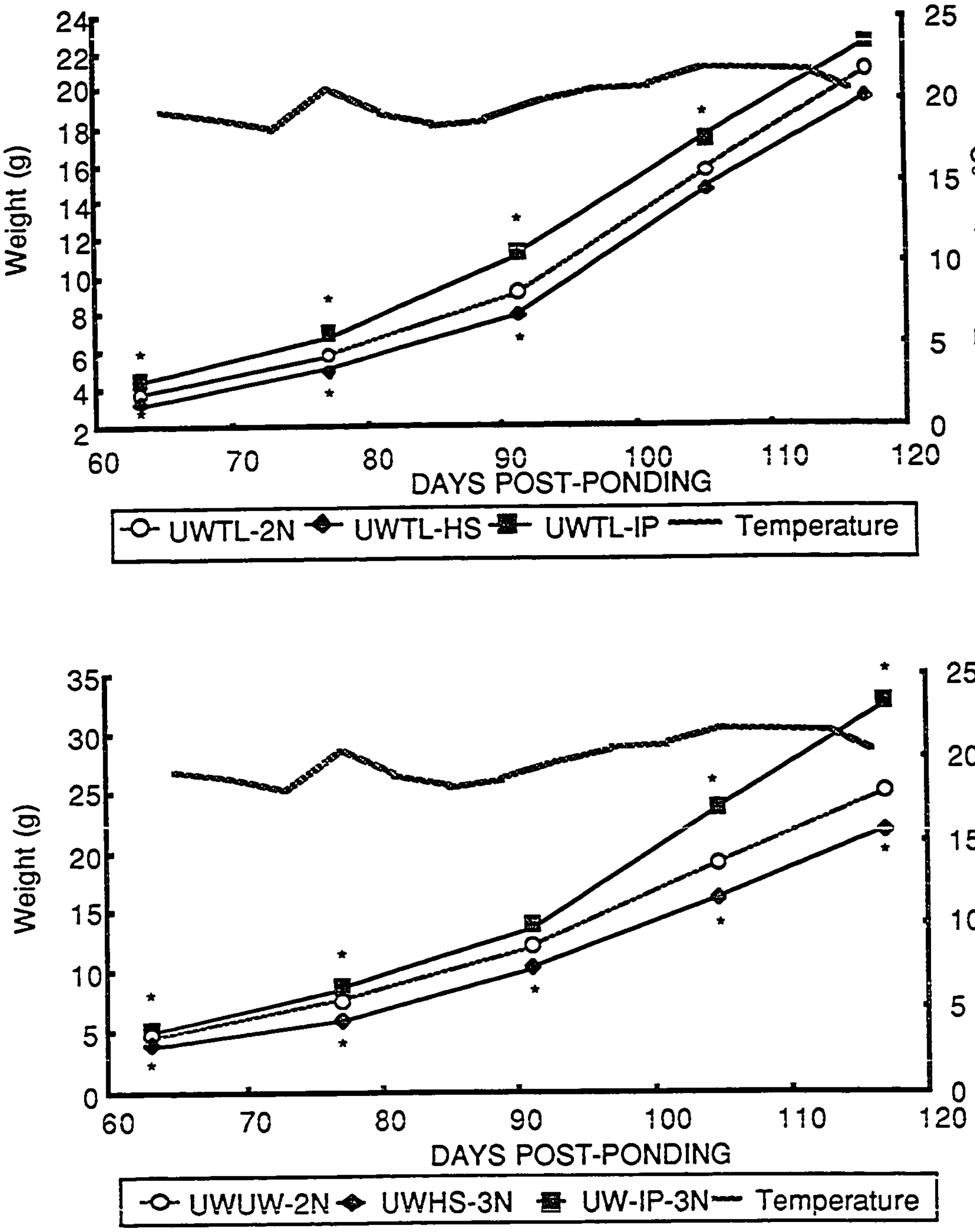


Figure 16.Weight of rainbow trout triploid and diploid hybrids during the first 63 days post-ponding in the 1989 study. (*) indicates a significant difference between the triploid and control group at the $P < 0.05$ level.

The UW interploid triploid and heat-shock triploid groups both show an improved performance relative to their control during the second growth period. The UW heat-shock group remained significantly smaller than the control, but the difference in length and weight had decreased to 3.5 % and 12.2 % of the diploid group, respectively. The difference in size between the UW interploid group and its control increased considerably. At the Day 77 sample there was no difference in length or weight between the interploids and diploids; however, at the end of the study the interploids were 6.0 % longer ($P < 0.05$) and 30.4 % heavier ($P < 0.05$) than controls. The UW interploids were also the the largest of any of the groups on both a length and weight basis at the end of the study.

Analysis of variance indicated that ploidy type and strain type were significant main effects ($P < 0.001$) for both weight and length, except during the last sampling when the ploidy type effect was significant at $P < 0.01$ (Tables 13 and 14). Comparisons of the three ploidy types indicated that interploids were significantly larger than either diploids or heat-shock triploids. Diploids, in turn, were heavier ($P < 0.05$) than heat-shocked triploids throughout the second period, and significantly longer for all but the last two samples, Day 105 and 117, when the difference was nonsignificant.

Strain and Triploid Heterosis;

The relative growth, on a weight basis, of triploids relative to their controls depended on the method of triploid induction. Triploids produced via

Table 13. Analysis of variance for the length of triploid and diploid strain hybrids and pure strain rainbow trout from Day 63 to Day 117 of the 1989 study.

		Length Day 63	Length Day 77	Length Day 91	Length Day 105
Source	df	MS (x 10^3)	MS (x 10^3)	MS (x 10^2)	MS (x 10^3)
Group	5	264 **	292 **	23.4 **	249 **
Tank/Group	12	20.6 *	26.1 **	1.90	15.1
Error	432	9.70	1.14	1.24	9.60
Ploid (P)	2	351 **	437 **	27.1 **	220 **
Strain (S)	1	571 **	581 **	71.6 **	646 **
P x S	2	38.9	6.27	2.01	31.7
Tank /P x S	12	20.6 *	26.1 **	1.90	15.1
Error	432	9.70	1.14	1.24	9.60

		Length Day 117
Source	df	MS (x 10^2)
Group	5	22.1 **
Tank/Group	12	2.53 *
Error	432	1.23
Ploid (P)	2	17.0 *
Strain (S)	1	68.9 **
P x S	2	2.61
Tank /P x S	12	2.53 *
Error	432	1.23

* $P < 0.05$

** $P < 0.001$

Table 14.Analysis of variance for the weight of triploid and diploid strain hybrids and pure strain rainbow trout from Day 21 to Day 63 of the 1989 study.

		Weight Day 63	Weight Day 77	Weight Day 91	Weight Day 105
Source	df	MS (x 10^1)	MS (x 10^1)	MS (x 10^1)	MS (x 10^2)
Group	5	27.1 **	35.7 **	27.9 **	299 **
Tank/Group	12	2.58 **	2.53 **	1.86	19.5 *
Error	432	1.02	1.11	1.29	9.89
Ploid (P)	2	39.5 **	47.1**	36.8 **	361**
Strain (S)	1	74.4 **	66.6**	75.1 **	632**
P x S	2	1.57	1.67	1.73	27.3
Tank /P x S	12	2.58 **	2.53 **	1.86	19.5 *
Error	432	1.02	1.11	1.29	9.89

		Weight Day 117
Source	df	MS (x 10^2)
Group	5	26.1 **
Tank/Group	12	1.97 *
Error	432	1.05
Ploid (P)	2	30.2 **
Strain (S)	1	64.5 **
P x S	2	5.09
Tank /P x S	12	1.97 *
Error	432	1.05

* $P < 0.05$

** $P < 0.001$

interploid crosses exhibited positive "triploid" heterosis, while the reverse was true for heat-shocked triploids (Figure 17). The comparison of triploid hybrids to controls was effected considerably by the use of different reference lines. When both diploid and triploid hybrids were compared to the weighted means of the two pure lines the diploid TL x UW hybrid was an average 11.3 ± 1.4% smaller ($P < 0.05$) than the reference line, similarly the heat-shock triploids exhibited considerable negative heterosis throughout the study, while the interploid triploids were undistinguishable from the reference line (Figure 18). The UW triploids were unaffected by this change in reference line.

Growth rate;

At the conclusion of the study significant differences existed in the weight, length, and condition factor of the different groups raised, additionally, differences were found in the growth rate of the groups. The group x time interaction for weight and length was significant (Table 15). Analysis using strain type with time also indicated that a significant interaction existed for both length and weight data ($P < 0.001$). No other significant interactions with time were detected.

Condition Factor;

Throughout the study the condition factor of all groups increased steadily. At the Day 21 sample K.F. averaged 1.05 ± 0.01, by the end of the first growth period it had increased to 1.33 ± 0.02, and at the conclusion of the study the overall K.F. was 1.53 ± 0.02.

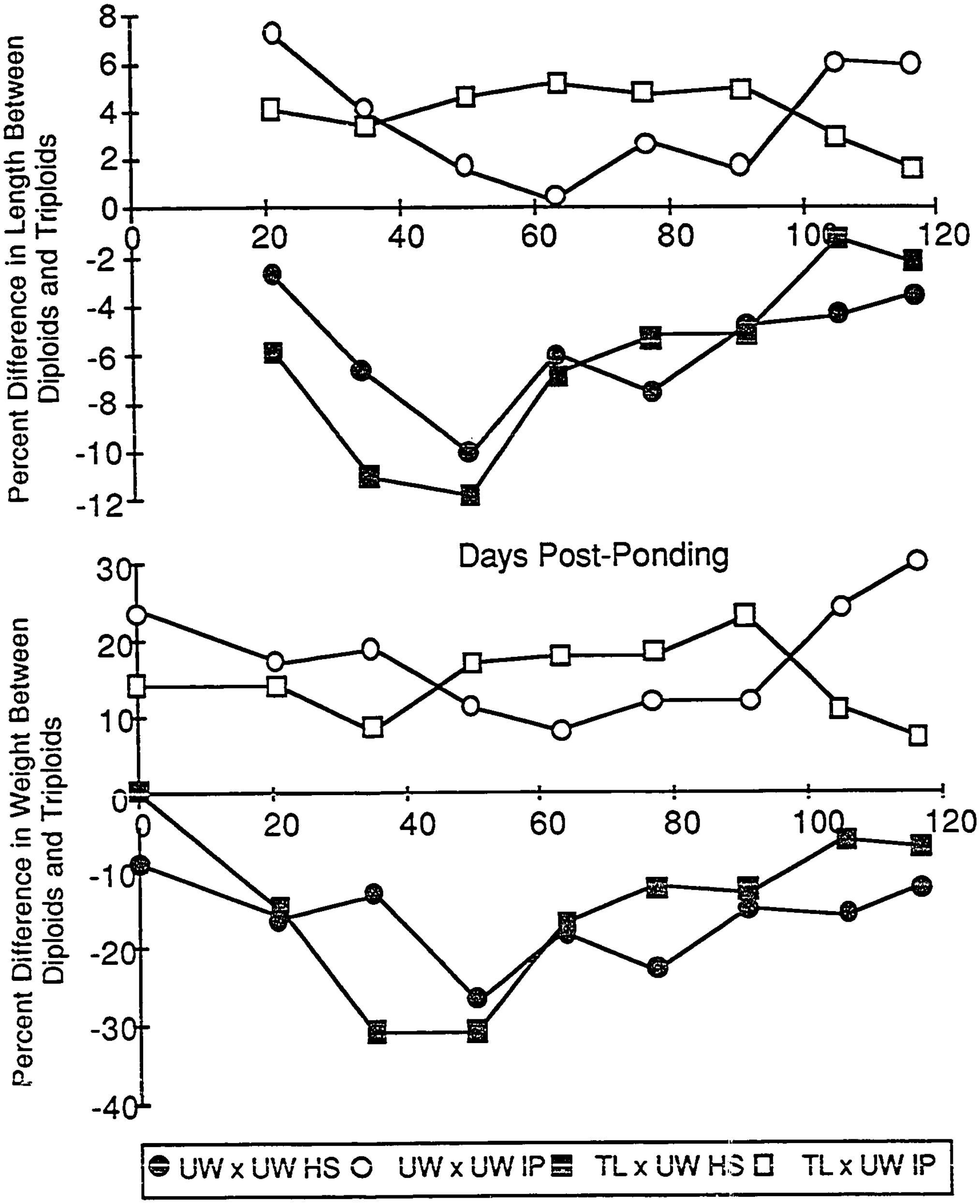


Figure 17. Percent difference in length and weight of triploid inter- and intrastrain hybrids of rainbow trout relative to their diploid controls. (HS) heat-shock (IP) interploid.

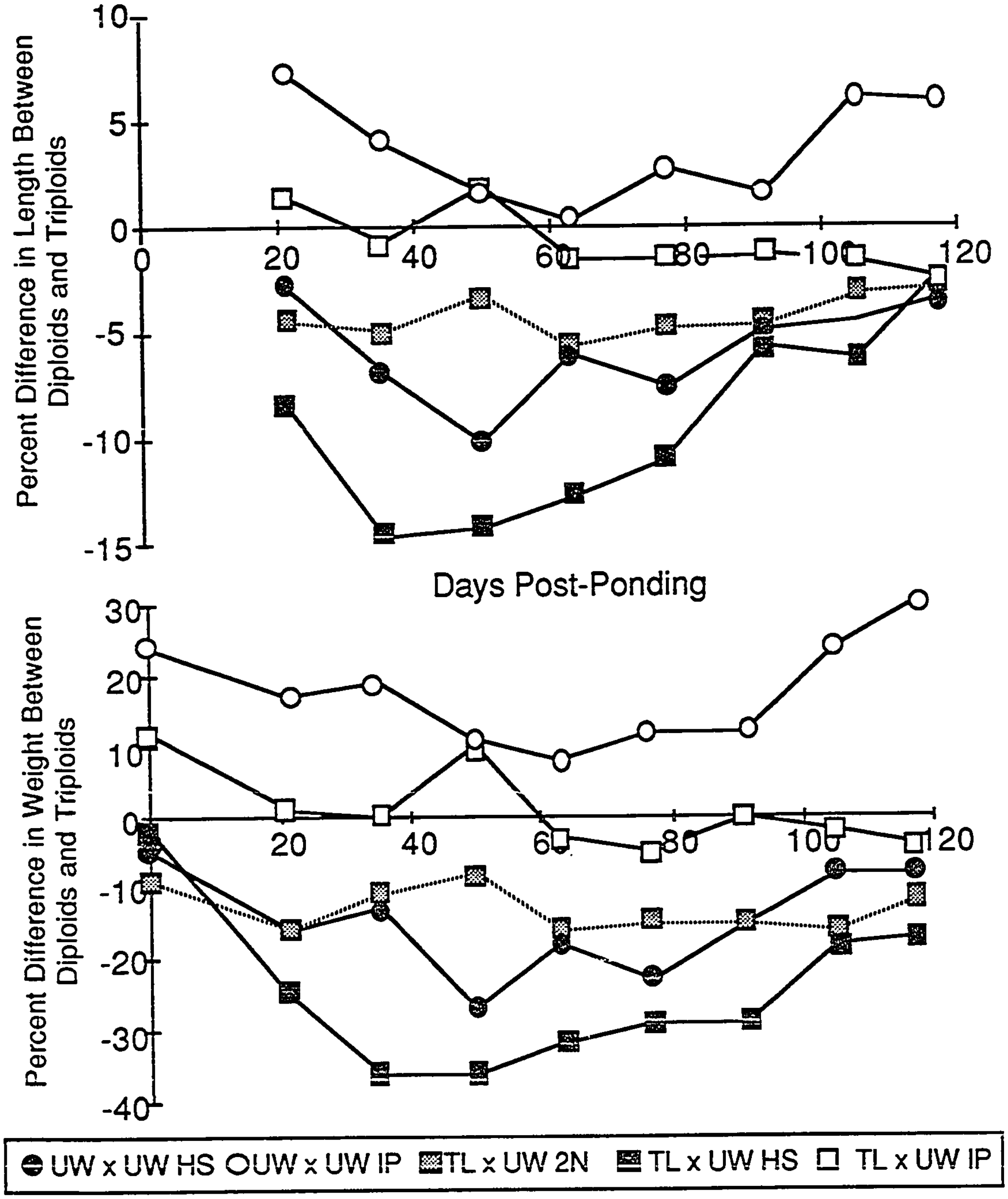


Figure 18.Percent difference between the length and weight of triploid inter- and intrastrain hybrids of rainbow trout and the weighted mean of their diploid parental lines. (HS) heat-shock (IP) interploid.

Table 15.Analysis of variance for the change in length and weight over time for diploid and triploid rainbow trout groups during the 1989 study;

	Length 1989	
Source		MS
Time (T)	7	3880 **
Ploid Type (P)	2	139 **
Strain (S)	1	187 **
S x P	2	0.030
S x T	7	12.4 **
P x T	14	1.73
S x P x T	14	1.86
Tank (S x P x T)	96	1.32 **
Error	3456	0.60

	Weight 1989	
Source		MS
Time (T)	7	921 **
Ploid Type (P)	2	38.4 **
Strain (S)	1	25.7 **
S x P	2	0.001
S x T	7	1.26 **
P x T	14	0.342
S x P x T	14	0.271
Tank (S x P x T)	96	0.231 **
Error	3456	0.098

* $P < 0.05$

** $P < 0.001$

During the first growth period of the study, Day 0-63, there was little variability in K.F. on a group, strain type, or ploidy type basis (Table 16). With the sole exception of ploidy type on the Day 50 sample, $P < 0.0142$, none of the effects was significant. Comparisons of K.F. for the ploidy types on Day 50 indicated that the interploids had a significantly higher K.F. than either the diploids or heat-shock triploids.

Differences in K.F. became more apparent in the latter phase. In general, the heat-shock triploids had the poorest condition factor, although they were not significantly different from their respective controls until the last sample, Day 117 (Table 17). Ploidy had a significant effect on condition factors during the last three samples, Day 91, 105, and 117, with interploid triploids having the highest K.F., heat-shock triploids the lowest, and diploids were intermediate.

Mortalities Day 0 - 120;

The mortalities experienced by the groups varied considerably over the course of the study (Figure 19). Variability among replicates tanks within groups was very high with coefficients of variance in excess of 100% for many groups. These random differences between tanks obscured differences between groups.

Table 16. Analysis of variance of condition factor (K.F.) for diploid and triploid rainbow trout inter- and intrastrain crosses from Day 21 to 63 in the 1989 study.;

		K.F. Day 21	K.F. Day 35	K.F. Day 50	K.F. Day 63
Source	df	MS (x 10^2)	MS (x 10^2)	MS (x 10^2)	MS (x 10^2)
Group	5	6.77 **	6.95	11.1	9.25
Tank/Group	12	1.82	7.02 **	4.84	9.64 **
Error	432	1.10	1.61	4.28	2.03
Ploid (P)	2	5.05	2.39	29.9 *	11.6
Strain (S)	1	7.97	1.83	0.339	7.62
P x S	2	4.95	14.6	8.27	8.97
Tank /P x S	12	1.82	7.02 **	4.84	2.03 **
Error	432	1.10	1.61	4.28	

* P < 0.05

** P < 0.001

Table 17.Analysis of variance of condition factor (K.F.) for diploid and triploid rainbow trout inter- and intrastrain crosses from Day 77 to 117 in the 1989 study.

		K.F. Day 77	K.F. Day 91	K.F. Day 105	K.F. Day 117
Source	df	MS (x 10^2)	MS (x 10^2)	MS (x 10^2)	MS (x 10^2)
Group	5	7.94	13.3 *	14.8 **	27.1 **
Tank/Group	12	6.30 **	4.41 **	1.96	4.19 *
Error	432	2.21	1.49	1.78	2.24
Ploid (P)	2	3.91	2.96 *	40.9 **	55.0 **
Strain (S)	1	22.0	8.20	0.51	0.50
P x S	2	7.18	1.01	0.99	13.9
Tank /P x S	12	6.30 **	4.41 **	1.96	4.19 *
Error	432	2.21	1.49	1.78	2.24

* $P < 0.05$

** $P < 0.001$

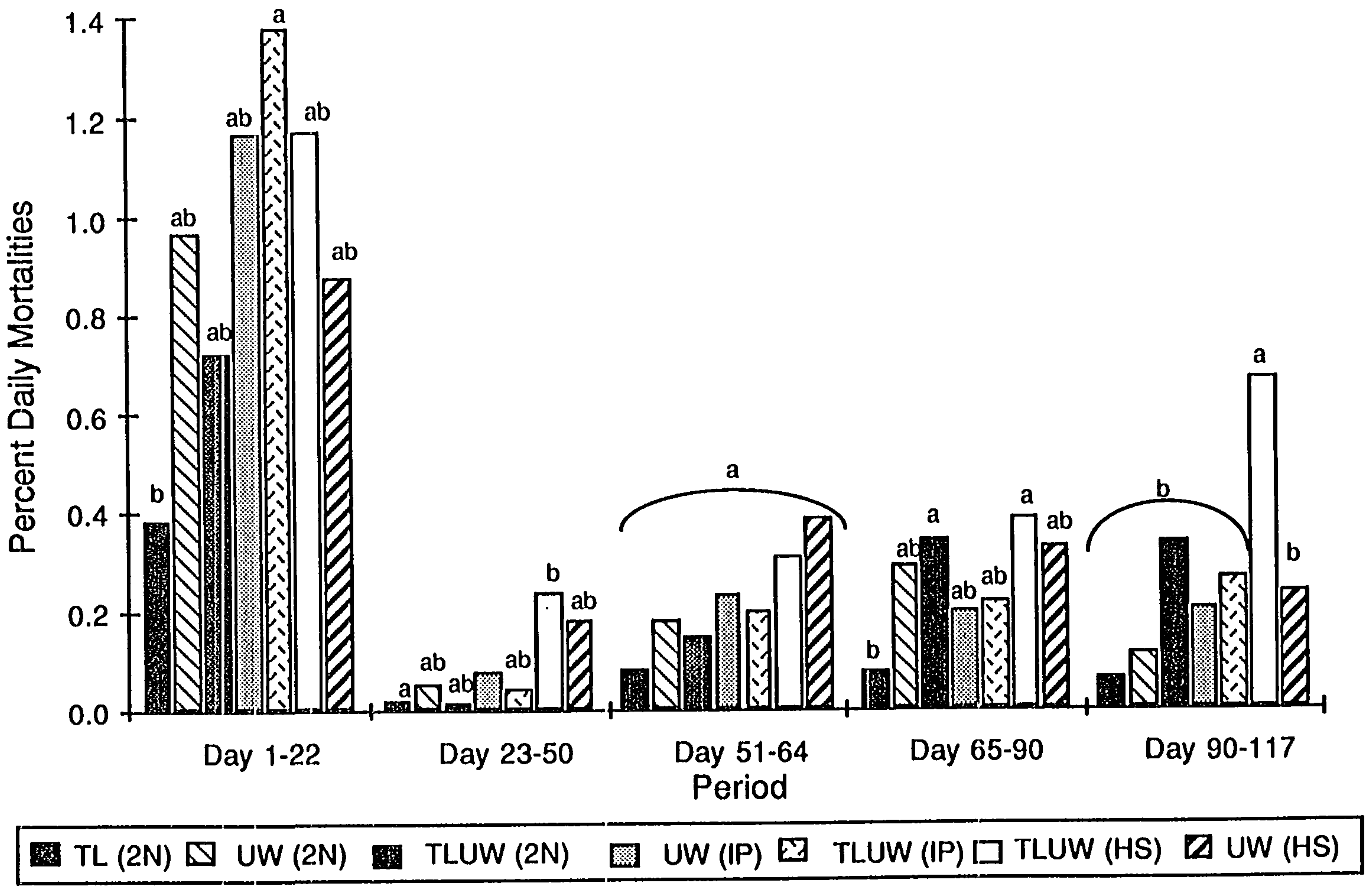


Figure 19. Average percent daily mortalities for diploid and triploid inter- and intrastrain crosses of rainbow trout during the 1989 study. Letters denote groups that are not signficantly different at the $P < 0.05$ level for each time phase of the study.

Daily mortalities during the first three weeks averaged 1.05 ± 0.13 %, after which mortalities were maintained below an average 0.30 % per day. Early mortalities were associated with a myriad of causal factors. IHNV was detected in mortalities from every tank; in addition, numerous deformed embryos were removed. Fish examined during the later periods exhibited clinical signs of bacterial inflections, primarily *Columnaris* spp. Protozoans were not as common in 1989 as in 1988.

Within each phase of the experiment there was no difference between group means, however, variability between replicate tanks was quite high. Only during the last three week period were group effects significant (Table 18). Polyploid level was never identified as being a significant source of variation. When mortalities were analyzed on a strain and ploidy type basis, type was a significant effect during the Day 23-50 and 91-117 sampling periods, while strain was significant only during the last period. Heat-shock triploids had higher mortalities throughout the growout period, but only during Day 23-50 was this significant. Similarly, UW x TL hybrids were significantly inferior to pure UW fish in period Day 91-117 alone, while showing higher, but not significantly higher, mortalities during the rest of the experiment.

Table 18. Analysis of variance for percent daily mortalities incurred by diploid and triploid rainbow trout inter- and intrastrain crosses during the 1989 study.

		Mortalities Day 1-22	Mortalities Day 23-50	Mortalities Day 51-64	Mortalities Day 65-90
Source	df	MS (x 10^2)	MS (x 10^2)	MS (x 10^2)	MS (x 10^2)
Group	5	16.8	2.38	2.42	1.69
Tank/Group	12	8.89	1.03	1.57	1.71
Error	0	0.00	0.00	0.00	0.00
Ploid (P)	1	3.21	0.001	1.03	0.889
Strain (S)	2	27.9	5.48 *	5.44	3.69
P x S	2	12.6	0.452	0.094	0.087
Tank /P x S	12	8.93	1.03	1.57	1.70
Error	0	0.00	0.00	0.00	0.00

		Mortalities Day 91-117
Source	df	MS (x 10^2)
Group	5	11.1 *
Tank/Group	12	2.45
Error	0	0.00
Ploid (P)	2	25.9 **
Strain (S)	1	9.76 *
P x S	2	5.05
Tank /P x S	12	2.45
Error	0	0.00

* $P < 0.05$

** $P < 0.001$

DISCUSSION

The performance of triploid organisms relative to diploid organisms is influenced by a myriad of factors. The range of effects for each factor is quite extensive, perhaps more so than in diploids. This study attempted to better elucidate the importance of two such factors, method of triploid induction and genetic composition. Emphasis was placed on embryonic and juvenile life history stages to avoid the confounding effects of sexual maturation. The results indicate that the impact of induction method and parental strain composition was significant, but the magnitude and direction of this impact differed over the course of the study.

Incubation;

Results from the incubation phase of the study revealed differences between the ploidy types; diploids and meiotic and interploid triploids. In both 1988 and 1989 the interploid triploid crosses had the lowest levels of fertilization of any group. Tetraploid males gave fertilization levels one-twentieth to one-third that of their diploid counterparts. These results agree with those of Chourrout et al. (1986), who concluded that the larger size of tetraploid-derived sperm was incompatible with the diameter of the egg micropyle. Chourrout found average fertilization levels for tetraploid males to be 40% of controls, but values for individual males varied from 0 to 97 %. The pooling of egg lots after fertilization prevented analysis of between male contributions in the present study. However, in other studies at the University of Washington variability in male fertilization appears to be minimal (Myers and

Hershberger, unpublished). In both years of this study egg size appeared to be positively correlated with fertilization level, but these effects are partially confounded with strain differences. The potential exists for egg size to be correlated the micropyle diameter, although Chourrout et. al. (1986) dispute this assumption. The depression in fertilization levels between heat-shocked groups and their controls was not as marked as in the interploid crosses (92% of controls in 1988 and 91% in 1989). This would suggest that the immediate impact of the heat treatment is rather mild, since mortalities caused directly by the heat-treatment would have been scored as undeveloped. Similarly high fertilization levels, 97% of controls, were observed in another study with heat-treated eggs from the UW and USDA strains using the same protocol (Guo 1987). Happe et al. (1988) found survival to the eyed stage in heat-treated groups of rainbow trout to be 87% of controls, while Blanc et al. (1987) actually observed higher survival to the eyed stage in heat-treated groups. The two latter studies used relatively mild heat treatment 26 - 26.5 °C. Treatments over 30°C for short durations have been attempted, but resulted in high levels of initial mortalities in rainbow trout (Chourrout 1980, Thorgaard et al. 1981, Lincoln and Bye 1984), but less so in Atlantic Salmon (Benfey and Sutterlin 1984a). Additionally, temperatures less than 26° C produce low levels of polar-body retention (Chourrout 1980). Thus, the temperatures used in this study for thermal treatment minimized initial mortalities, but maintained high level of triploid induction (>90%).

Mortality differences emerged between the heat-shocked groups during the latter part of the incubation period. Large numbers of malformed embryos were found among the heat-shocked groups in both years. These latent mortalities from the thermal treatment have also been observed in other studies with rainbow trout (Lincoln and Bye 1984; Guo 1987). Prior analysis of similar mortalities via flow cytometry indicated that they were euploid, and that if the treatment damage was genetic it was not large enough to be detectable (Myers and Hershberger, unpublished). While treatment temperatures of 26-27.5°C are of sufficient intensity to inhibit the second meiosis, they could also produce other deleterious physical changes in the newly fertilized eggs. Interestingly, the proportion of abnormal embryos in the interploid triploid groups was extremely low, less so than in their controls and much less than in heat-treated groups. This effect was more marked in 1989 than in 1988, but without replication it is difficult to separate random incubation effects from genetic or physiology effects. However, the low number of mortalities among fertilized eggs in the interploid triploid groups suggests that triploidy alone is not deleterious and that the mortalities in heat-shocked groups are either treatment side-effects or indicate differences inherent in the method of treatment. Furthermore, it is not clear whether the observed developmental and growth deficiencies found in heat-shocked triploids are genetic and/or physiological in nature. The developmental stage of the fish at the time of death, however, may indicate the probable factor responsible (Figure 20).

Differences in the genetic constitution of meiotic and interploid triploids are related to the source of the additional chromosome set. In meiotic triploid fish the contribution of the second polar body provides this extra genetic material. There is a certain amount of inbreeding created by this process, depending on the level of recombination. The presence of deleterious recessive alleles in the maternal genome should not, however, pose a problem

Theoretical Factors Influencing Mortalities

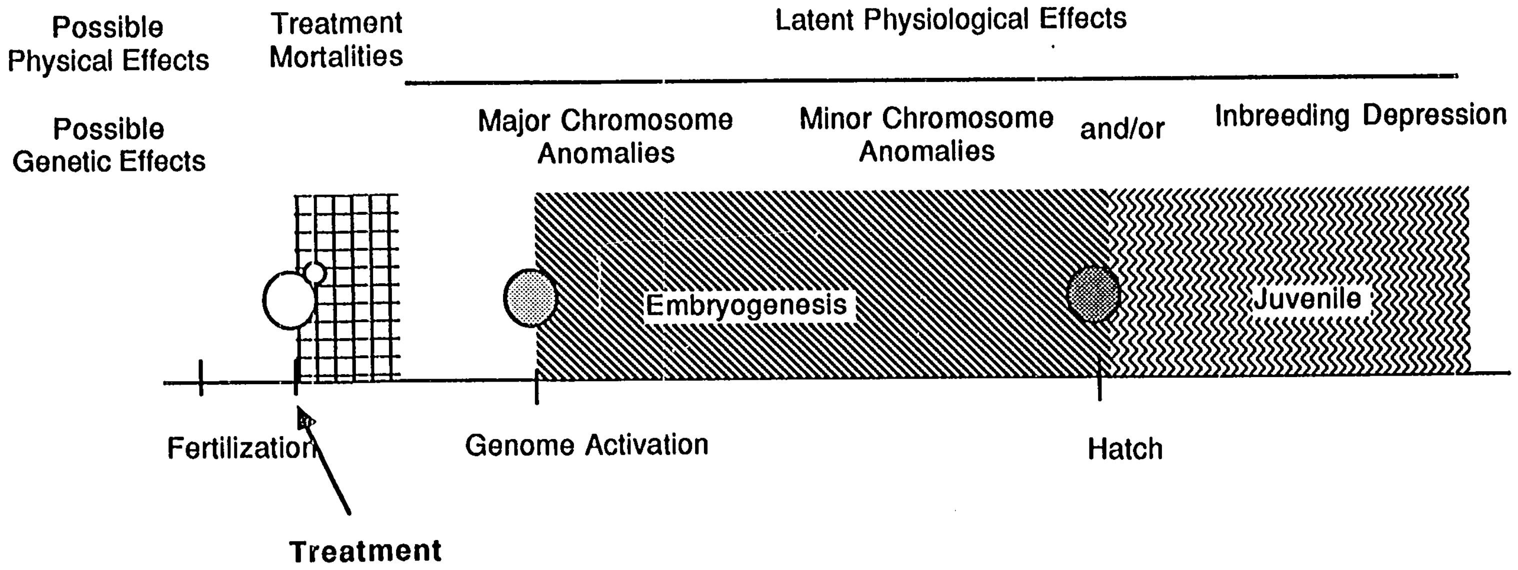


Figure 20. The early life history of heat-shocked triploids and potential genetic and physiological factors that may influence their development and growth.

because the paternal genetic contribution would mitigate any recessive effects. In fact, meiotic triploids should exhibit higher levels of heterozygosity relative to controls and thus be less impacted by any recessive deleterious alleles There is some evidence that meiotic divisions are not random and the chromosome complement present in the polar body may not be complete. Recombination during meiosis in genomes that contain chromosome inversions produce polar bodies with gene deletions or chromosome fragments (White 1973). Genetic aberrations of this type could cause the mortalities observed during incubation and would probably be undetectable with flow cytometry. Alternatively, meiosis appears to proceed normally in tetraploids (Diter et al. 1988), and aside form fertilization difficulties tetraploids produce vital triploid progeny.

The size and development rate of alevins during the yolk-sac absorption period varied considerably between groups. There were significant differences among correlations between yolk-sac absorption and other developmental traits and among factors analyzed by ANOVAs between the 1988 and 1989 studies. The 1989 study is probably the more accurate of the two owing to the larger number of fish sampled per group and the need to sample from a small number of surviving fry in 1988. Egg size was a principal factor influencing both embryo size and growth rate. This relationship between egg size and fry weight has been well established in salmonids (Gray 1928; Svärdson 1949; Thorpe et al. 1984). Analysis of variance main effects were influenced by this relationship, but, comparison among ploid types within a cross would be unaffected. Maternal strain and ploidy interactions (1989) were significant for embryo wet weight at ponding and for both instantaneous growth rate and yolk conversion efficiency (wet basis). The high yolk-sac absorption efficiency found in the heat-shocked triploids in 1989 was also observed by Guo (1987). However, Happe et al. (1988) found no difference between heat-shocked triploids and controls. The yolk-sac absorption efficiencies may be related to the lower level of activity observed among heat-shocked groups during the subsequent growout period. If this behavior was also present during incubation it would reduce metabolic requirements and provide more yolk energy for growth. In general, there were no significant differences in yolk conversion efficiencies between diploid and triploid embryos in this study prior to ponding. In 1988 the heat-shocked UW x UW triploids were larger than their controls while the reverse was true for the interploid UW x UW triploid cross. These differences may simply be related to the small numbers of embryos sampled. Additionally, Guo (1987) found no significant differences between diploids and heat-shock triploids during yolk-sac absorption. The absence of consistent developmental differences between diploids and meiotic and interploid triploid sac-fry during incubation may be due to the relatively undemanding environment of artificial incubation systems, but under more stressful conditions differences in performance may have expressed themselves.

Growth;

The post-ponding growth of diploids and triploids in both 1988 and 1989 could be separated into two distinct phases; (1) fry phase: 0-63 days post-ponding, and (2) fingerling phase: from 63 days post-ponding to the conclusion of the experiment. On a seasonal basis the fry phase occurred in early spring when water temperatures were in an ideal range for trout culture, 10-17°C, while the fingerling phase occurred during the late spring and summer when water conditions were more extreme, often in excess of 20°C. This progressive change in rearing conditions presented the fish with an increasing environmental challenge and growth differences must be viewed in the context of these changes.

The growth of interploid and meiotic triploids and their controls during the fry phase was very consistent during both years of the study. Meiotic triploids exhibited a significantly slower growth rate than their respective diploids, while interploid triploid growth was equal or superior to diploid growth. Weight based and length based growth estimates yielded similar conclusions, although variability between fishes was much lower on a length basis.

In spite of the absence of significant differences in weight at ponding the heat-shocked triploid fish were significantly smaller (20-35%) than their controls at the conclusion of the fry rearing phase. This tendency for meiotic triploids to exhibit inferior growth performance has been reported by other researchers working with rainbow trout (Solar et al. 1984; Guo 1987; Happe et al. 1988). Other studies, however, have found no significant differences between meiotic triploids and diploid controls during the first few weeks of rearing (Blanc et al. 1987; Quillet et al. 1988). Differences in the performance by heat-shocked triploids reported in these studies are probably due to a myriad of factors. Factors such as husbandry techniques, induction protocol, and specific strain used may play some role in determining the response to triploidization. Furthermore, the response of the UW strain and its hybrids may be unique, given the strain's highly inbred nature. Therefore, some caution should be used in the comparison of these results with other studies.

In general, there appears to be a chronic response to the thermal treatment which may be physiological or genetic in nature. The absence of significant strain x ploidy interactions for length or weight during the fry phase in this study indicates that the treatment impact is similar for different strain hybrids. Growth differences have been observed in different strains of rainbow trout subjected to identical treatment protocols (Solar et al. 1984; Guo 1987). It should be emphasized that all groups in this study had a common UW maternal component and strain-related differences in egg size or other physical characteristics may still be important overall. The magnitude of the latent impact of the thermal treatment can also be affected by the husbandry protocol employed. An aggressive feeding regime combined with low rearing densities may provide the diploid controls an opportunity to maximize their growth while the heat-shocked triploids may not be physiologically able to take advantage of these conditions. Alternatively, under a more conservative rearing protocol the two groups may both be limited by environmental conditions. The impact of husbandry is far from trivial. In comparing the results from different studies, the weights for fish at 60-80 days post-ponding, the end of the fry phase, differ by more than an order of magnitude. The protocols employed in this study and by Guo (1987) perhaps more fully illustrate the impact of the heat-shock on subsequent growth early in the fishes' life.

The growth performance of interploid triploids during the fry phase further illustrates the latent effects of the heat-shock on meiotic triploids. Throughout the fry phase the interploids expressed equivalent or superior growth relative to controls. In one case, specifically the UW x TL interploids in 1989, this could be related to differences in egg size. These results are comparable with the only other extensive study using interploid triploid fish (Blanc et al. 1987), and highlight the differences between interploid and meiotic triploids. In comparing triploid groups in this study there were a relatively small number of potential effects that could produce these differences. The use of treated and untreated sibs as parents to produce both types of triploids in this study reduced the potential for within strain effects to confound the between ploid type comparisons. Some factor inherent in the thermal treatment or subsequent retention of the polar body would appear to be involved.

The fingerling phase of the study provided a more accurate measure of the growth potential of the different groups. Maternal influences, primarily egg size, are greatly reduced during this period and at the conclusion of the study (120 days post-ponding) should be negligible (Kincaid 1975; Iwamoto 1982). Furthermore, the heat-shocked groups appeared to overcome non-genetic damage caused by the induction treatment. Interploid triploid growth was relatively constant over the course of both the fry and fingerling phases, remaining equal or superior to their respective diploid controls Significant differences were still observable between the two triploid types.

The performance of the heat-shocked groups early in the fingerling phase differed greatly between crosses. During the 1988 study the TL x UW meiotic triploids exhibited a rapid growth increase in the Day 63-85 sampling period, while the USDA x UW group maintained a constant growth rate, remaining about 25% smaller than their controls. This growth increase was also observed in other studies using heat-shocked rainbow trout hybrids (Guo 1987; Myers and Hershberger unpublished). However, the growth retardation experienced by heat-shocked groups in 1989 during the fry rearing phase (20-30% smaller ($P < 0.05$) relative to controls) was gradually reduced such that by the end of the fingerling phase the TL x UW triploids were not significantly different from controls and the UW x UW triploids were only 12 % lighter ($P < 0.05$) and 3.5% shorter ($P < 0.05$) than controls. The results for heat-shocked triploids in the 1989 study were similar to the findings of other studies with meiotic triploids (Johnson et al. 1986; Blanc et al. 1987; Happe et al. 1988).

Parental strain differences appear to be important in influencing the growth of meiotic triploids. The growth rate of the USDA x UW triploid cross was significantly slower relative to its control and to the other groups, while the diploid USDA x UW cross was very similar to the other diploid groups, TL x UW and UW x UW. The caudal peduncle deformities observed in the USDA x UW triploid group may have been important in reducing their growth potential. The fact that these deformities did not appear in the diploid controls nor in any other heat-shock group which shared a common maternal component is indicative of a variable heat-labile response, perhaps similar to certain environmentally induced deformities in *Drosophila* (Waddington, 1953). The TL x UW crosses exhibited different relative growth rates for the meiotic and interploid triploids vs. diploids during both years of the study. The selection of parents from different year classes within each of the strains may be partially responsible for this, although the relative growth should be largely unaffected.

Moreover, there were slightly different numbers of broodstock used from each strain during the two years, although the magnitude of these differences is probably not enough to influence the results. One additional source for this variation was identified after the study. The Trout Lodge broodstock consists of three "distinct" spawning groups of fish (Kristina Seabolt, personal communications 1989). It was not possible to verify which of the groups was sampled in either 1988 or 1989, but if different spawning groups could have been used. Variation in the specific combing ability between these spawning groups could be responsible for the observed differences. The relative performance of the UW x UW diploid and heat-shocked triploid groups in this study agreed with that observed by Guo (1987). The highly inbred nature of the UW "Donaldson" strain of rainbow trout (Allendorf 1973) limits the potential for within strain variability, for meiotic triploids as well as diploids.

A very interesting aspect of the performance of many of the heat-shocked triploid groups was the improvement in growth rate observed in the 60-80 day period post-ponding. The TL x UW meiotic triploids in the 1988 study clearly exhibited this growth rate improvement, but it was also evident in 1989 to a lesser degree. Furthermore, this phenomenon was observed by Guo (1987) and in the pilot study for this investigation (Myers and Hershberger, unpublished). In all these cases the inflection point in growth rate coincided with a rapid warming in spring water temperatures, although the accumulated temperature units at this point varied by as much as 35% between years. Additionally, the average temperature during this period was quite different for each year of the study. The weakness of temperature correlations between years reduces the likelihood that this growth rate change is environmentally driven, rather similarities in the size and age of fish at this time suggest a physiological explanation. The heat-shocked triploids may have repaired or compensated for the deleterious effects of the thermal treatment or achieved a sufficient body size to buffer the effects of the damage via homeostasis. If these effects were solely responsible for the improvement in growth rate then, at best, the heat-shock triploids would parallel their controls and not exhibit superior growth. The ability of certain groups to surpass controls may be indicative of genetic differences between meiotic triploids and diploids, differences that should also exist between interploid triploids and their controls.

Interploid triploid growth during the fingerling phase was generally similar to diploid controls. Other researchers also found no significant difference between interploid triploids and their controls at ages corresponding to the end of the fingerling phase (Chourrout et al. 1986; Blanc et al. 1987). At the conclusion of the 1988 study the TL x UW interploid cross was not significantly smaller than the diploid cross, while the UW x UW interploids were significantly smaller than their controls. However, only one tank of UW x UW interploids was reared in 1988, and during the Day 80-100 period they suffered a severe epizootic of "ich" which certainly retarded their growth. In the 1989 study the situation was reversed at 117 days post-ponding. The UW x UW interploids were significantly larger than controls in both length and weight, while the TL x UW interploids were not significantly larger than controls. The contrast between the two years in this study may be due to random selection of parents, as with the heat-shocked group, or may be due to differences in the nature of the tetraploid parents utilized in the two years. The tetraploid rainbow trout used in 1988 were produced by inhibition of first cleavage via hydrostatic pressure, while the parents for the 1989 study were produced by crossing a tetraploid male with a diploid female and then inducing polar body retention with a thermal shock (Figure 21). The genetic composition of the two tetraploid types would differ in the degree of heterozygosity present. In the case of the former "mitotic tetraploid" the genotype of the tetraploid would be that of a doubled diploid, with only one possible heterozygote class (AAaa), while in the "meiotic tetraploid" case the male tetraploid contribution would undergo random assortment and the female contribution would contain some variability depending on the recombination frequency. The meiotic tetraploids and their gametes would possess a higher level of heterozygosity, while on average having the same gene frequencies, as the mitotic tetraploids. Guo (1987) suggested that heterosis is accentuated in triploids, if this is the case, then the tetraploids used in 1989 would produce progeny with higher levels of heterozygosity and therefore higher growth rates than the interploid progeny produced in 1988.

Growth during the fingerling phase was influenced by several factors. Ploidy or ploidy type were important main effects during both the 1988 and 1989 studies, while strain composition was significant only in 1989. Interactions between strain composition and ploidy were important only in the later part of the 1988 study, however, interploids and meiotic triploids were pooled for analysis because of the loss of entire groups to an IHN virus epizootic. Triploid strain hybrids did not show the response range reported by Guo (1987), although reciprocal hybrids were not produced in this study and the genetic differences between crosses were comparatively small.

Interploid crosses generally did better than their meiotic counterparts,

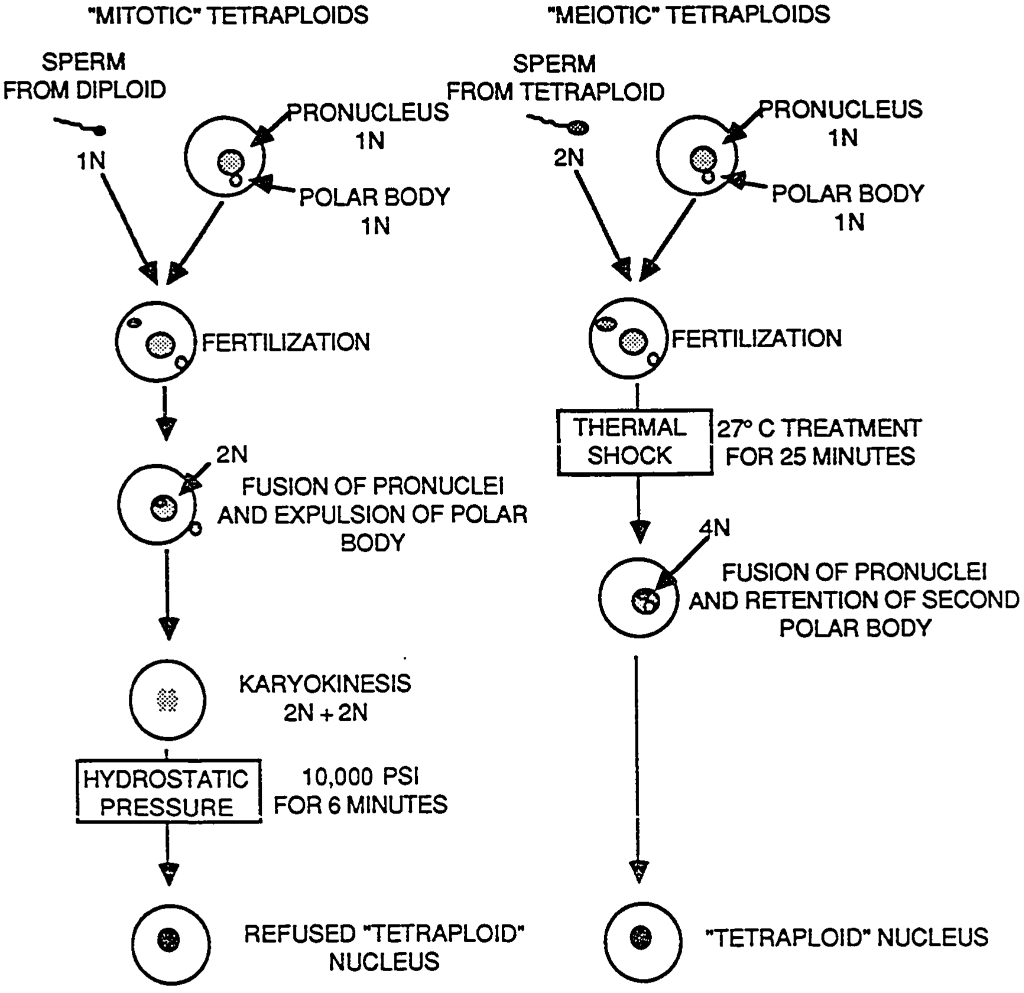


Figure 21. Methods of producing tetraploids via mitotic and meiotic inhibition for the 1988 and 1989 studies, respectively.

and were equivalent to controls. Heat-shocked triploids, in turn, were able to overcome the deleterious effects of induction experienced during the fry period and achieve sizes equivalent to controls. Both triploid types performed well under the suboptimal water temperatures (>20°C) present during the fingerling phase. Factors influencing between hybrid differences did not appear to be as important as differences among ploidy types within a hybrid cross.

The higher levels of heterozygosity produced by interploid crosses as compared to polar body retention may be responsible for the differences in growth between the two groups. Empirical data suggests that tetraploid-derived gametes are 45% more heterozygous than the diploid complement present in a normal ovum following polar body retention (Allendorf et al. 1986; Diter et al. 1987). The genotypic magnitude of these differences varies according to the gene frequency, recombination frequency, and the nature of dominance effects (complete, partial, etc.). However, if the mechanisms suggested for explaining differences between interploid and meiotic triploids are solely responsible for these effects then meiotic triploids should, in turn, outperform their diploid controls. The observed growth performance of heat-shocked triploids would indicate that other factors, such as continuing thermal treatment effects limit the growth of these groups. The exceptional growth of the UW x UW interploids in the 1989 study suggests that additive genetic variance still has an important role in determining growth, especially in light of the low heterozygosity levels, about 20% that of wild trout (Allendorf 1973), present in this inbred strain. There still remains a paucity of information concerning the interactions of alleles in a triploid, or tetraploid, genetic template.

Condition Factor;

There were considerable differences between the two years in the significance of causal factors influencing condition factor. In 1988, cross effects were significant throughout most of the study, while in 1989 ploidy type effects and not cross effects were the most important source of variation. These differences between years probably reflect differences in experimental design rather than genetic differences. The 1988 study utilized more strains but lacked a balanced representation of triploid types. Alternatively, the 1989 study effectively tested ploidy types but employed only one strain hybrid. Condition factor in salmonids has been shown to have little within population variability, with heritabilities not significantly different from 0 (Gunnes and Gjedrem 1978; Gunnes and Gjedrem 1981), but differences in condition factor between populations are larger (Iwamoto et al. 1985). Condition factor would be expected to vary some between strains used in this study, given that body conformation was one of the traits selected in the UW "Donaldson" strain (Donaldson 1971). Interploid triploids tended to have higher condition factors than diploids, while heat-shocked triploids tended to have lower condition factors than diploid controls. This finding contrasts with those of Benfey and Sutterlin (1984b), who found triploids to be longer than diploids but equal in weight, thus giving them a lower condition factor. Guo (1987) found that the maternal genotype influenced both the sign and magnitude of differences between the condition factors of diploids and triploids. Furthermore, the difference in condition factors was not due to triploids being longer. Similarly, in this study the meiotic triploids were generally shorter than their controls in addition to having lower condition factors. It is difficult to compare condition factors from different studies because husbandry practices, specifically feeding levels, have an obvious impact on the length-weight relationship. Johnson (1988) found that while feeding level significantly influenced overall condition factor there was no difference between diploid and meiotic triploid chinook salmon. It is apparent that both strain and polyploidy do affect condition factor, but the relative importance of these two factors and their interaction is still unclear.

Mortality;

The mortality differences between ploidy types and crosses were more subtle during the growth phases of the study, relative to incubation mortalities. Several factors were responsible for mortalities during the fry phase of the study; congenital defects, incubation effects not related to treatments or cross (chlorine poisoning, fungal growth, etc.), or pathogens. The separation of mortalities due to each of these effects is almost impossible because of the cumulative interactions among them. Mortality rate must be considered as a general indication of resistance to stress, regardless of its source.

The meiotic triploids experienced higher mortalities than either interploids or diploids throughout the first month of the fry phase. These differences were not always significant owing to the large between tank variability with each group. Mortality levels correlate well with the growth retardation seen in meiotic triploids during this period, and both are undoubtedly related to the thermal induction treatment. Heat-shock treatments have been widely shown to reduce the survival of salmonid juveniles (Thorgaard et al. 1981; Solar et al. 1984; Quillet et al. 1988). Alternatively, interploid triploids were indistinguishable from their diploid counterparts throughout the study, with two exceptions. Firstly, during 1988 the sole UW x UW interploid tank suffered from a heavy infestation of ich related more to water flow problems in that specific tank than genetic problems. Secondly, the TL x UW interploids experienced heavy initial mortalities from a latent response to chlorine contamination during incubation. Chourrout et al. (1986) and Blanc et al. (1987) found equivalent mortality levels in interploid triploids and diploids throughout the juvenile phase.

The physiological challenge to the experimental groups during the fingerling phase was much more intense than in the fry phase. The water conditions in Lake Washington are such that summer temperatures in excess of 22°C are not uncommon, and the subsequent algal bloom in response to these temperatures results in a high incidence of bacterial and protozoan pathogens. Mortality levels were significantly higher for the meiotic triploids than for interploid triploids or diploids in 1989 during the last 30 days of the study. Similar, although less conclusive, results were found in 1988. Guo (1987), however, found mortality levels in meiotic triploids tended to be lower than in diploids during the similar summer conditions. The mortality levels observed may also be related to size differences rather than solely to immune capacity. The presence of significant sources of variation in both years of the study is remarkable given the random exposure of groups to pathogens via the water system and the large between tank variability. Under more controlled conditions it may be possible to further identify the degree to which strain and/or polyploid type influence survival.

Future Endeavors: Polyploid Selection;

There is extensive literature available that indicates triploidy retards maturation, especially in females. Triploid fish did not experiance the growth depression and carcass quality degradation associated with reproduction. This and other studies, however, indicate that triploid growth can be improved prior to the onset of any maturation effects. Judicious selection of strains used in interploid crosses can produce significant changes in growth relative to diploids within a few months after hatching. Studies with plant crops have shown that interploid breeding schemes are an effective approach to genetic improvement (De Jong and Tai 1977; Gordei and Gordei 1983).

A selection program for interploid triploids would require the maintenance of both tetraploid and diploid broodstock lines. The selection of parents from each of these lines would be based on one of two possible criteria. Parents could be selected on the basis of their own performance. In this system diploid and tetraploid lines would be independently selected for growth performance. An alternate system would be based on the performance of the interploid progeny themselves, and diploid and tetraploid parents subsequently selected. The base populations for each of these lines would be selected on the basis of pilot studies with pure strains and strain crosses. The two selection schemes would target different types of genetic interactions. The parallel line selection scheme would be targeting mostly additive effects, while the progeny selection scheme would concentrate on dominance and epistatic effects.

Of the two schemes, the parallel line scheme is the best suited for salmonid aquaculture. Although progeny selection would directly test the performance of interploid triploids the seasonal nature of salmon spawning would require a year of testing before parents could be identified for interploid production and broodstock propagation. Additionally, Pacific salmon are semelparous and a progeny selection scheme would be impractical. Furthermore, the parallel line strategy has the advantage of eliminating inbreeding in the interploid triploids since the diploid and tetraploid lines would be maintained as distinct entities. Selection within each of the lines would be based on family selection, the most effective method based on reported heritabilities for most salmonid species (Gjerde 1986). The estimation of expected gains from this type of selection scheme is difficult given the absence of empirical data, but gains should be similar to those expected with a normal diploid selection scheme.

CONCLUSIONS

The growth and survival of meiotic and interploid triploids relative to their diploid controls was influenced by several factors, which varied in importance throughout the course of the study.

1) Fertilization levels were uniformly poor for the interploid triploids, but the subsequent embryonic development and survival were similar, and in some cases superior, to that of their diploid counterparts.

2) Meiotic triploids suffered from acute and latent trauma incurred during the heat-shock induction procedure. The induction treatment did not directly cause mortalities, however, developmental abnormalities and aborted embryos were more common. This trauma was also expressed in the retarded early post-emergence growth of meiotic triploids relative to controls. This appears to be a vestige of the thermal treatment rather than any strain/genotype effect.

3) The growth and survival of interploid triploids during the fry period was not markedly different from controls whereas the growth of meiotic triploids was significantly better or worse than controls depending on the specific cross. in general, meiotic triploids exhibited a growth rate recovery during the later fingerling phase, except where congenital deformities limited growth, such that they were not significantly different from controls. One specific cross of interploid triploids, UW x UW, did show a significantly better growth rate than its diploid and meiotic counterpart at the end of the study.

4) Genetic factors significantly influenced the performance of the groups in a variety of ways. The existence of additive effects is suggested by parallel differences in strain crosses observed within each of the ploidy types in 1989. Dominance and/or epistatic effects may be implicated in the disparity between the relative size of interploid triploids in 1988 and 1989 and the growth of meiotic triploids in 1988 relative to controls. The importance of these genetic effectssuggests that selective breeding could be an efficacious method of further improving triploid growth.

5) Interploid triploids appear to be the 'triploid of choice" for commercial culture. The progeny of interploid crosses are all-triploid and do not need to be exposed to potentially deleterious treatments. Additionally, the growth of interploids was equal to or superior to that of their diploid controls prior to the onset of sexual maturation, while, the relatively slow early growth and fragility of meiotic triploids during the fry phase may be magnified under more intensive commercial conditions.

REFERENCES

Abdel-Hameed, F., and Shoffner, R.N., 1972. Intersexes and sex determination in chickens. Poultry Science 50: 962-964.

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

Allen, S.K. Jr., 1987. Reproductive sterility of triploid shellfish and fish. Ph.D. Dissertation, University of Washington, Seattle, Washington.

Allen, S.K. Jr. and Myers, J.M., 1985. Chromosome set manipulation in three species of salmonids using hydrostatic pressure. pp 64. in Iwamoto R.N. and Sower, S. (eds). Salmonid reproduction: An international symposium. Washington Sea Grant Program University of Washington, Seattle, Washington. (abstract)

Allen, S.K. Jr., and Stanley, J.G., 1979. Polyploid mosaics induced by cytochalasin B in landlocked Atlantic salmon *Salmo salar*. Transactions of the American Fisheries Society, 108: 462-466.

Allendorf, F.W., 1973. Genetic variation, inheritance, and preliminary population distribution of some proteins of Salmo gairdneri. M.S. Thesis, Univ. of Washington. 61 pp.

Allendorf, F.W., Seeb, J.E., Knudsen, K.L., Thorgaard, G.H., Leary, R.F., 1986. Gene-centromere mapping of 25 loci in rainbow trout. Journal of Heredity 77: 307-312.

Askenes, A., Gjerde, B., and Roald, S.O., 1986. Biological, chemical, and organoleptic changes during maturation of farmed Atlantic salmon, *Salmo salar*. Aquaculture, 53: 7-20.

Austin, C.R., 1965. Fertilization. Prentice-Hall, Inc. Englewood Cliffs, NJ.

Baker, R.J. and Gebeyehou, G., 1982. Comparative growth analysis of two spring wheats and one spring barley. Crop Science 22: 1225-1229.

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

Benfey, T.J. and Sutterlin, A.M., 1984b. Growth and gonadal development in triploid landlocked Atlantic salmon (*Salmo salar* L.). Canadian Journal of Fisheries and Aquatic Science 41: 1387-1392.

Benfey, T.J., Solar, I.I., DeJong, G., and Donaldson, E.M., 1986. Flow cytometric confirmation of aneuploidy in sperm from triploid rainbow trout. Transactions of the American Fisheries Society, 115: 838-840.

Billard, R., 1977. A new technique of artificial insemination for salmonids using a sperm diluent. Fisheries 1: 24-25.

Blanc, J.M., Chourrout, D., and Krieg, F., 1987. Evaluation of juvenile rainbow trout survival and growth in half-sib families from diploid and tetraploid sires. Aquaculture, 65: 215-220.

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

Carter, S.B., 1967. Effects of cytochalasin on mammalian cells. Nature, 213: 261-264.

Chevassus, B., Quillet, E., and Chourrout, D., 1985. La production de truites steriles par voie génétique. La pisciculture française. 78: 10-19.

Chourrout, D., 1980. Thermal induction of diploid gynogenesis and triploidy in the eggs of the rainbow trout (*Salmo gairdneri* Richardson). Reproduction, Nutrition and Development, 20: 727-733.

Chourrout, D., 1982. Tetraploidy induced by heat shock in the rainbow trout (*Salmo gairdneri* R.). Reproduction, Nutrition and Development 22: 569-574.

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 gynogenetics. Aquaculture, 36: 111-126.

Chourrout, D., and Quillet, E., 1982. Induced gynogenesis in rainbow trout: sex and survival of progenies. Production of all-triploid populations. Theoretical and Applied Genetics, 63: 201-205.

Chourrout, D., Chevassus, B., Krieg, F., Happe, A., Burger, G., and Renard, P., 1986. Production of second generation triploid and tetraploid rainbow trout by mating tetraploid males and diploid females. Potential of tetraploid fish. Theoretical and Applied Genetics, 72: 193-206.

Chourrout, D., and Nakayama, I., 1987. Chromosome studies of progenies of tetraploid female rainbow trout. Theoretical Applied Genetics, 74: 687-692.

De Jong, H., and Tai, G.C.C., 1977. Analysis of tetraploid-diploid hybrids in cultivated potatoes. Potato Research 20: 111-121.

Diter, A., Guymard, R., and, Chourrout, D., 1988. Gene segregation in induced tetraploid rainbow trout: genetic evidence of preferential pairing of homologous chromosomes. Genome, 30: 547-553.

Don, J., and Avtalion, R.R. 1986. The induction of triploidy in *Oreochromis aureas* by heat shock. Theoretical and Applied Genetics, 72: 186-192.

Donaldson, L.R., 1971. Development of select strains of salmonid fishes for use in management. pp. 19-32 in: W.M. Carter (ed). Atlantic Salmon Workshop-1971. The International Atlantic Salmon Foundation, NY, NY.

Donaldson, L.R., 1989. Evolution of salmonid diets used at the school of fisheries, University of Washington. University of Washington School of Fisheries Publication No. 790.

Donaldson, L.R., and Olson, P.R., 1955. Development of rainbow trout broodstock by selective breeding. Transactions of the American Fisheries Society, 85: 93-101.

Dunham, R.A., Smitherman, R.O., 1983. Response to selection and realized heritability for body weight in three strains of channel catfish, *Ictalurus punctatus*, grown in earthen ponds. Aquaculture 33: 89-96.

Falconer, D.S., 1981. Introduction to quantitative genetics, 2nd edition. Longman Inc., New York, NY.

Fankhauser, G., 1941. Cell size, organ and body size in triploid newts (*Triturus viridescens*). Journal of Morphology 68: 161-177.

Fankhauser, G. and Griffiths, R.B. 1939. Induction of triploidy and haploidy in the newt, *Triturus viridescens*, by cold treatment of unsegmented eggs. Proceedings of the National Academy of Science, 25:233-238.

Fischberg, J., 1959. Experimental tetraploidy in newts. Morphology 6: 392-402.

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

Gjerde, B., 1986. Growth and reproduction in fish and shellfish. Aquaculture. 57: 37-55.

Gjerde, B., and Refstie, T., 1984. Complete diallel cross between five strains of Atlantic salmon. Livestock Production Science 11: 207-226.

Gjedrem, T., 1985. Genetic variation in age at maturity and its relation to growth rate. pp. 62-61 in: R.N. Iwamoto and S.W. Sower (eds). Salmonid Reproduction: Papers presented at the international symposium on salmonid reproduction. Washington Sea Grant, Seattle, WA.

Gray, J., 1928. The growth of fish. II. The growth rate of the embryo of *Salmo fario* . J. Exp. Biology., 6: 110-124.

Gordei, I., and Gordei, G.M., 1983. Genetic principals of triticale production. I. Inheritance of quantitative characters of wheat and rye in wheat-rye hybrids and triticale. Genetika 21: 464-471.

Greene, C.W., 1926;. The physiology of spawning migration. Physiological Review, 6: 201-241.

Gunnes, K., and Gjedrem, T., 1978. Selection experiments with salmon IV. Growth of Atlantic salmon during two years in the sea. Aquaculture, 15: 19-33.

Gunnes, K., and Gjedrem, T., 1981. A genetic analysis of body weight and length in rainbow trout reared in seawater for 18 months. Aquaculture, 24: 161-174.

Guo, X., 1987. Early growth and development of intrastrain and interstrain triploids of rainbow trout (*Salmo gairdneri*). Master's thesis. University of Washington, Seattle, Washington.

Guo, X., Hershberger, W.K., and Myers, J.M., 1990. Growth and survival of intrastrain and interstrain triploids of rainbow trout (Oncorhynchus mykiss). J. World. Aqua. Soc. 00:000 (accepted for publication).

Guyomard, R. 1984., High levels of residual heterozygosity in gynogenetic rainbow trout, *Salmo gairdneri* Richardson. Theoretical Applied Genetics 67: 307-316.

Happe, A., Quillet, E., and Chevassus, B., 1988. Early life history of triploid rainbow trout (*Salmo gairdneri* Richardson). Aquaculture, 71: 107-118.

Hörstgen-Schwark, H., Fricke, H., Langholz, H.J., 1986. The effect of strain crossing on the production performance in rainbow trout. Aquaculture 57: 141-152.

Iwamoto, R.N., 1982. Strain-photoperiod-temperature interactions in coho salmon: freshwater growth, smoltification, and seawater adaptation. Ph.D. dissertation. University of Washington., Seattle, Washington.

Iwamoto, R.N., Myers, J.M., Hershberger, W.K., 1985. Genotype-environment interactions in rainbow trout production traits. Final report. Grant No. 82-CRSR-2-1064. 147 pp.

Johnson, O.W., 1988. Induced triploidy in Pacific salmon. Ph.D. dissertation. University of Washington, Seattle, Washington.

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

Kempthorne, O., 1969. An introduction to genetic statistics. Iowa State Univ. Press. Ames, Iowa.

Kincaid, H.L., 1975. A preliminary report on the genetic aspects of 150-day family weights in hatchery rainbow trout. Proc. 52nd. Ann. Conf. of West. Assoc. State Game and Fish comm., Portland July 16-19, 1975. pp 562-565.

Kincaid, H.L., 1981. Trout strain registry. National Fisheries Center - Leetown. U.S. Fish and Wildlife Service, Kearneysvile, W.V. 118 pp.

Kinghorn, B.P., 1983. A review of quantitative genetics in fish breeding. Aquaculture 31: 283-304.

Kirpichnikov, V.S., and Golovinskaya, K.A., 1966. Kharakteristicka priozvoditelei osnovnykh porodnykh grupp karpa, razvodimykh v SSSR (Characterization of spawners of the major breeds of carp in the USSR). - Izvestiya GosNIORKh, Vol 61.

Leitritz, E., and Lewis, R.C., 1976. Trout and salmon culture (Hatchery Methods). California Department of Fish and Game. Fish bulletin 164.

Lemoine, H.L. Jr., and Smith, L.T., 1980. Polyploidy induced in brook trout by cold shock. Transaction of the American Fisheries Society. 109: 626-631.

Lincoln, R.F., 1981. Sexual maturation in triploid male plaice (*Pleuronectes platessa*) and plaice x founder (*Platichthys flesus*) hybrids. Journal of Fish Biology, 19: 415-426.

Lincoln, R.F., Aulstad, D., and Grammeltved, T., 1974. Attempted triploid induction in Atlantic salmon (*Salmo salar*) using cold shock. Aquaculture, 4: 287-297.

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

Moav, R., Hulata, G., and Wohlfarth, G., 1975. Genetic differences between the Chinese and european races of the common carp. I. Analysis of genotype-environment interaction for growth rate. Heredity 34: 323-340.

Müntzing, A., 1951. Genetics and plant breeding. pp 473-492. in Genetics in the 20th. Century. Macmillan Co., NY NY.

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

Myers, J.M., Iwamoto, R.N., and Hershberger, W.K. 1987. Tetraploid induction in salmonids. Journal of the World Aquaculture Society. 17: 1-7.

Ohno, S., 1970. Evolution by gene duplication. Springer Verlag Publishing Co., New York.

Onozato, H. 1985. Diploidization of gynogenetically activated salmonids eggs using hydrostatic pressure. Aquaculture 43: 91-98.

Parsons, J.E., Busch, R.A., Thorgaard, G.H., and Scheerer, P.D. 1986. Increased resistance of triploid rainbow trout x coho salmon hybrids to infectious hematopoietic necrosis virus. Aquaculture 57: 337-343.

Piper, R.G., McElwain, I.B., Orme, L.E., McCraren, J.P., Fowler, L.G., and Leonard, J.R. 1982. Fish hatchery management. U.S. Department of the Interior. Washington D.C.

Purdom, C.E., 1972. Induced polyploid in plaice (*Pleuronectes platessa*) and its hybrid with the flounder (*Platichthys flesus*). Heredity, 29: 11-24.

Purdom, C.E., 1976. Genetics techniques in flatfish culture. Journal of Fisheries Research Board of Canada 33: 1088-1093.

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

Quillet, E.B., Chevassus, B., Blanc, J.M., Krieg, F., and, Chourrout, D., 1988. Performances of auto and allotriploids in salmonids I. Survival and growth in freshwater farming. Aquatic Living Resources. 1: 29-43.

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

Refstie, T. and Steine, T.A., 1978. Selection experiments with salmon III. Genetic and environmental sources of variation in length and weight of Atlantic salmon in the freshwater phase. Aquaculture, 14: 221-234.

SAS Institute Inc., 1987. SAS User's Guide: Statistics. SAS Institute Inc. Gary, NC.

Scheerer, P.D., and Thorgaard, G.H., 1983. Increased survival in salmonids hybrids by induced triploidy. Canadian Journal of Fisheries and Aquatic Science, 40: 2040-2044.

Seeb, J.E., Thorgaard, G.H., and, Utter, F.M., 1988. Survival and allozyme expression in diploid and triploid hybrids between chum, chinook, and coho salmon. Aquaculture, 72: 31-48.

Shelton, W.L., 1986. Broodstock development for monosex production of grass carp. Aquaculture, 57: 311-319.

Snow, M.H.L., 1973. Tetraploid mouse embryos produced by cytochalasin B during cleavage. Nature, 244: 513-514.

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

Solar, I.I., and Donaldson, E.M., 1985. Studies on genetic and hormonal sex control in domesticated rainbow trout. I. The effect of heat shock treatment for induction of triploidy in cultured rainbow trout (*Salmo gairdneri* Richardson). Canadian Technical Report of Fisheries and Aquatic Sciences, 1379: 10-15.

Sower, S.A., Iwamoto, R.N., Dickhoff, R.N., and Gorbman, A., 1984. Ovulatory and steroidal responses in coho salmon and steelhead trout following administration of salmon gonadotropin and D-Ala^6, des Gly^{10} Gonadotropin-releasing hormone ethylamide (GnRHa). Aquaculture, 43:35-46.

Stanley, J.G., and Sneed, K.E., 1974. Artificial gynogenesis and its application in genetics and selective breeding of fishes. pp. 527-537. In Blaxter, J.H.S. (ed.) Early life history of fish. Springer-Veliger, New York.

Stebbins, G.L. 1950., Variation and evolution in plants. Columbia Univ. Press. New York.

Steel, R.G.D., and Torrie, J.H., 1980. Principles and procedures of statistics. Second Edition. McGraw-Hill, New York.

Streisinger, G., Walker, C., Dower, N., Knauber, D., and Singer, F., 1981. Production of clones of homozygous diploid zebra fish (*Brachydanio rerio*). Nature, 291: 293-296.

Svärdson, G., 1949. Natural selection and egg number in fish. Rep. Inst. Freshwater Res. Drottingholm. 29:115-122.

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

Thorgaard, G.H., 1983. Chromosome set manipulation and sex control in fish. In Hoar, W.S., Randall, D.J., and Donaldson, E.M. (eds). Fish Physiology, Vol 9B. Academic Press, New York.

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

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

Thorgaard, G.H., Jazwin, M.E., and Stier, A.R., 1981. Polyploidy induced by heat shock in the rainbow trout. Transactions of the American Fisheries Society, 110: 546-550.

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

Thorgaard, G.H., Allendorf, F.W., and Knudsen, K.L., 1983. Gene centromere mapping in rainbow trout: high interference over long map distances. Genetics 103: 771-783.

Thorpe, J.E., Miles, M.S., Keay, D.S., 1984. Development rate, fecundity, and egg size in Atlantic salmon, *Salmo salar.* Aquaculture, 43: 289-305.

Turner, H.N. and Young, S.S.Y., 1969. Quantitative genetics in sheep breeding. Cornell Univ. Press, Ithaca, NY.

Utter, F.M., Johnson, O.W., Thorgaard, G.H., and, Rabinovitch,P., 1983. Measurement and potential applications of induced triploidy in Pacific salmon. Aquaculture, 35: 125-133.

Valenti, R.J., 1975. Induced polyploidy in *Tilapia aurea* (Steindachner) by means of temperature shock treatment. Journal of Fish Biology, 7: 519-528.

Waddington,C.H. 1953. Genetic assimilation of an acquired character. Evolution 7:118-126.

White,M.J.D. 1973. Animal cytology and evolution. Cambridge Uinv. Press. NY. 961 pp.

Williams, W. 1964. Genetic principles and plant breeding. Blackwell Scientific Publ. Oxford.

Winkler, H., 1916. Uber die experimentelle Erzeugung von Pflanzen mit abweichenden Chromosomenzahlan. Z. Bot. 17. 417-531.

Wood, J.W., 1979. Diseases of Pacific Salmon. Their prevention and treatment State of Washington Department of Fisheries, Seattle.

Wolters, W.R., Libey, G.S., and Chrisman, C.L., 1982. Effect of triploidy on growth and gonad development of channel catfish. Transactions of the American Fisheries Society, 111: 102-105.

Zar, H.J., 1974. Biostatistical analysis. Prentice-Hall, Inc. Englewood Cliffs, NJ.

Appendix 1. Incubation and developmental characteristics of diploid and triploid groups during the 1988 study.

Group	N	Egg Dia. (mm)	N	Emb Wt. 1 Wet (g)	Emb. Wt. 2 Wet (g)
USDA UW					
2N H.S. Con	3	4.60±0.01d	10	0.035±0.002bc	0.061±0.004abcd
3N H.S.	3	4.60±0.01d	10	0.027±0.001de	0.053±0.003cdef
2N I.P. Con	3	4.71±0.02c	10	0.024±0.001ef	0.040±0.003gh
3N I.P.	3	4.71±0.02c	10	0.021±0.001f	0.036±0.002h
TL UW					
2N H.S. Con	3	4.76±0.01c	10	0.033±0.001bc	0.049±0.002efg
3N H.S.	3	4.76±0.01c	10	0.030±0.001cd	0.053±0.004bcdef
2N I.P. Con	3	5.61±0.01a	10	0.040±0.001a	0.066±0.003ab
3N I.P.	3	5.61±0.01a	10	0.040±0.001a	0.068±0.003a
UW UW					
2N H.S. Con	3	4.76±0.01c	10	0.033±0.002bc	0.044±0.003fgh
3N H.S.	3	4.76±0.01c	10	0.032±0.001bc	0.057±0.003bcde
2N I.P. Con	3	4.88±0.02b	10	0.037±0.001ab	0.063±0.003bcd
3N I.P.	3	4.88±0.02b	10	0.036±0.001a	0.051±0.003def

Appendix 1 (cont).

Group	N	Emb. Wt. 1 Dry (g)	Emb. Wt 2 Dry (g)
USDA UW			
2N H.S. Con	10	0.0068±0.0003bc	0.0097±0.0005c
3N H.S.	10	0.0065±0.0002f	0.0084±0.0005c
2N I.P. Con	10	0.0046±0.0002f	0.0080±0.0005c
3N I.P.	10	0.0044±0.0002f	0.0061±0.0006d
TL UW			
2N H.S. Con	10	0.0058±0.0001cde	0.0099±0.0005c
3N H.S.	10	0.0052±0.0002ef	0.0089±0.0007c
2N I.P. Con	10	0.0070±0.0003b	0.0150±0.0009a
3N I.P.	10	0.0078±0.0003a	0.0135±0.0006ab
UW UW			
2N H.S. Con	10	0.0059±0.0003cde	0.0096±0.0006c
3N H.S.	10	0.0056±0.0003de	0.0103±0.0004c
2N I.P. Con	10	0.0064±0.0002bcd	0.0121±0.0003b
3N I.P.	10	0.0063±0.0002bcd	0.0094±0.0006c

Appendix 1 (cont).

Group	N	IGR Wet	YC Wet
USDA UW			
2N H.S. Con	10	-2.56±0.06abcd	2.24±0.67ab
3N H.S.	10	-2.69±0.05bcde	1.76±0.36ab
2N I.P. Con	10	-2.97±0.05f	1.08±0.24b
3N I.P.	10	-3.07±0.04f	1.24±0.13b
TL UW			
2N H.S. Con	10	-2.78±0.04de	1.89±0.69ab
3N H.S.	10	-2.71±0.07abcd	2.06±0.35a
2N I.P. Con	10	-2.50±0.05ab	2.28±0.72ab
3N I.P.	10	-2.46±0.05a	1.54±0.38b
UW UW			
2N H.S. Con	10	-2.90±0.06ef	1.74±1.02ab
3N H.S.	10	-2.64±0.06abcd	1.45±0.26b
2N I.P. Con	10	-2.54±0.06abc	1.77±0.33ab
3N I.P.	10	-2.75±0.06cde	1.51±0.39b

Appendix 2. Weight, length, and condition factor for diploid and triploid groups from ponding to Day 120 of the 1988 study.

Group	N	Day 1 (4/10/88)	N	Day 17 (4/23/88)
		Weight (g)		Weight (g)
USDA UW				
2N H.S. Con	3	0.109±0.002d	9	0.220±0.004de
3N H.S.	3	0.107±0.004d	9	0.183±0.004f
2N I.P. Con	-	-	-	-
3N I.P.	-	-	-	-
TL UW				
2N H.S. Con	3	0.104±0.003d	9	0.232±0.008d
3N H.S.	3	0.103±0.002d	9	0.177±0.004f
2N I.P. Con	3	0.157±0.003b	9	0.313±0.004b
3N I.P.	3	0.168±0.001a	9	0.328±0.003a
UW UW				
2N H.S. Con	3	0.112±0.006d	9	0.214±0.005de
3N H.S.	3	0.113±0.004d	9	0.202±0.004e
2N I.P. Con	3	0.132±0.002c	3	0.256±0.006c
3N I.P.	3	0.128±0.003c	3	0.219±0.004c

Appendix 2 (cont).

Group	N	Day 30 (5/6/88)	N	Day 45 (5/21/88)
		Weight (g)		Weight (g)
USDA UW				
2N H.S. Con	9	0.53±0.01bc	9	1.25±0.02c
3N H.S.	9	0.40±0.01d	6	0.96±0.04e
2N I.P. Con	-	-	-	-
3N I.P.	-	-	-	-
TL UW				
2N H.S. Con	9	0.54±0.01b	9	1.26±0.03c
3N H.S.	9	0.38±0.01d	9	0.92±0.03e
2N I.P. Con	9	0.66±0.01a	9	1.50±0.02a
3N I.P.	9	0.64±0.01a	9	1.340±0.02b
UW UW				
2N H.S. Con	6	0.54±0.01b	6	1.24±0.02c
3N H.S.	-	-	-	-
2N I.P. Con	3	0.52±0.01bc	3	1.20±0.02cd
3N I.P.	3	0.50±0.01c	3	1.12±0.03d

Appendix 2 (cont).

Group	N	Day 63 (6/8/88)		
		Length (cm)	Weight (g)	K.F. (g/cm^3)
USDA UW				
2N H.S. Con	75	6.25±0.06ab	3.09±0.09bcd	1.23±0.01a
3N H.S.	50	5.78±0.08c	2.50±0.09e	1.26±0.01a
TL UW				
2N H.S. Con	75	6.47±0.05a	3.58±0.08a	1.31±0.01a
3N H.S.	75	5.93±0.06c	2.75±0.08de	1.29±0.01a
2N I.P. Con	75	6.50±0.04a	3.79±0.08a	1.37±0.01a
3N I.P.	75	6.40±0.06ab	3.53±0.11a	1.32±0.01a
UW UW				
2N H.S. Con	50	6.19±0.07b	3.14±0.11bc	1.30±0.01a
2N I.P. Con	25	6.38±0.07ab	3.43±0.12ab	1.31±0.02a
3N I.P.	25	5.95±0.08c	2.86±0.13cd	1.34±0.02a

Group	N	Day 83 (6/28/88)		
		Length (cm)	Weight (g)	K.F. (g/cm^3)
USDA UW				
2N H.S. Con	75	8.51±0.10ab	8.34±0.27cd	1.31±0.01de
3N H.S.	50	7.80±0.11d	6.51±0.28f	1.33±0.01e
TL UW				
2N H.S. Con	75	8.18±0.09bc	7.80±0.28cde	1.38±0.01bc
3N H.S.	75	8.23±0.10bc	7.75±0.30cde	1.34±0.01de
2N I.P. Con	75	8.83±0.10a	10.02±0.29a	1.43±0.01a
3N I.P.	75	8.45±0.09ab	8.87±0.29bc	1.43±0.01a
UW UW				
2N H.S. Con	75	8.79±0.08a	9.38±0.24ab	1.36±0.01cd
2N I.P. Con	25	7.97±0.13cd	7.37±0.38def	1.42±0.02ab
3N I.P.	25	7.78±0.14d	6.69±0.36ef	1.38±0.02bc

Appendix 2 (cont).

Group	N	Day 102 (7/17/88)		
		Length (cm)	Weight (g)	K.F. (g/cm^3)
USDA UW				
2N H.S. Con	75	10.86±0.10a	18.12±0.52b	1.384±0.010e
3N H.S.	75	9.71±0.14c	13.59±0.57c	1.414±0.011de
TL UW				
2N H.S. Con	75	10.54±0.11ab	17.42±0.53b	1.454±0.010c
3N H.S.	75	10.66±0.15ab	17.52±0.67b	1.384±0.009e
2N I.P. Con	75	10.90±0.11a	20.52±0.60a	1.551±0.009a
3N I.P.	75	10.63±0.11ab	18.58±0.58ab	1.511±0.009b
UW UW				
2N H.S. Con	75	10.93±0.10a	18.93±0.47ab	1.426±0.008cd
2N I.P. Con	25	10.34±0.14b	16.69±0.67b	1.494±0.015b
3N I.P.	25	9.34±0.18d	13.05±0.71c	1.560±0.016a

Group	N	Day 120 (8/3/88)		
		Length (cm)	Weight (g)	K.F. (g/cm^3)
USDA UW				
2N H.S. Con	75	12.98±0.10a	33.48±0.77a	1.51±0.01f
3N H.S.	75	11.85±0.15b	26.41±0.94b	1.54±0.02ef
TL UW				
2N H.S. Con	75	12.37±0.15a	30.86±1.04a	1.59±0.01cd
3N H.S.	75	12.76±0.16a	32.81±1.15a	1.53±0.01ef
2N I.P. Con	75	12.79±0.15a	35.64±1.18a	1.66±0.01b
3N I.P.	75	12.54±0.14a	33.23±1.00a	1.64±0.01b
UW UW				
2N H.S. Con	75	12.98±0.12a	34.67±0.84a	1.56±0.01ef
2N I.P. Con	25	12.52±0.17a	31.92±1.13a	1.61±0.02bc
3N I.P.	25	11.64±0.24b	27.57±1.45b	1.70±0.02a

Appendix 3. Percent daily mortality for diploid and triploid groups during the 1988 study.

Group	N	Daily Mortality Day 0 - 17 (%)	Day 17-40	Day 41-60
USDA UW				
2N H.S. Con	3	0.13±0.04a	0.01±0.00bc	0.00±0.00a
3N H.S.	2	0.38±0.11a	0.02±0.00b	0.02±0.02a
TL UW				
2N H.S. Con	3	0.11±0.04a	0.01±0.00bc	0.02±0.01a
3N H.S.	3	0.38±0.28a	0.02±0.00b	0.01±0.00a
2N I.P. Con	3	0.02±0.01a	0.01±0.00c	0.03±0.02a
3N I.P.	3	0.04±0.02a	0.01±0.00bc	0.02±0.01a
UW UW				
2N H.S. Con	2	0.08±0.02a	0.02±0.01	0.01±0.00a
2N I.P. Con	1	0.02±-.---a	0.01±-.---bc	0.01±-.---a
3N I.P.	1	0.02±-.---a	0.04±-.---a	0.00±-.---a

Group	N	Day 61-80	Day 81-100	Day 100-120
USDA UW				
2N H.S. Con	3	0.01±0.00a	0.11±0.04b	0.03±0.03a
3N H.S.	2	0.13±0.10a	0.42±0.17b	0.40±0.20a
TL UW				
2N H.S. Con	3	0.02±0.01a	0.21±0.11b	0.00±0.00a
3N H.S.	3	0.16±0.12a	0.65±0.09b	0.10±0.10a
2N I.P. Con	3	0.01±0.01a	0.17±0.06b	0.00±0.00a
3N I.P.	3	0.02±0.01a	0.13±0.05b	0.03±0.03a
UW UW				
2N H.S. Con	2	0.01±0.01a	0.20±0.17b	0.10±0.00a
2N I.P. Con	1	0.17±-.---a	0.19±-.---b	0.00±-.---a
3N I.P.	1	0.00±-.---a	1.52±-.---a	0.10±-.---a

Appendix 4. Incubation and developmental characteristics of diploid and triploid groups during the 1989 study.

Group	N	Egg Dia. mm	N	Emb Wt. 1 Wet (g)	Emb. Wt. 2 Wet (g)
UW-2N	3*	4.89 ± 0.03b	15	0.028±0.001ab	0.069±0.002c
UWHS-3N	3	4.68 ± 0.03c	15	0.026±0.001bc	0.073±0.003bc
UWIP-3N	3	5.07 ± 0.03a	15	0.028±0.001ab	0.100±0.003a
UWTL-2N	3	4.74 ± 0.03c	15	0.024±0.001c	0.078±0.003bc
UWTLHS-3N	3	4.74 ± 0.03c	15	0.026±0.001bc	0.083±0.002b
UWTLIP-3N	3	5.09 ± 0.04a	15	0.031±0.001a	0.079±0.004bc
TLTL-2N	3	5.13 ± 0.04a	15	0.028±0.001ab	0.096±0.003a

Group	N	Emb. Wt. 1 Dry (g)	Emb. Wt 2 Dry (g)	IGR Wet
UW-2N	15	0.0050±0.0001a	0.0114±0.0004b	-2.48±0.03c
UWHS-3N	15	0.0043±0.0001a	0.0122±0.0011ab	-2.42±0.04bc
UWIP-3N	15	0.0051±0.0001a	0.0151±0.0004a	-2.11±0.03a
UWTL-2N	15	0.0046±0.0002a	0.0121±0.0005ab	-2.35±0.04bc
UWTLHS-3N	15	0.0043±0.0001a	0.0128±0.0004ab	-2.29±0.03b
UWTLIP-3N	15	0.0051±0.0002a	0.0137±0.0017ab	-2.39±0.09bc
TLTL-2N	15	0.0048±0.0004a	0.0144±0.0004ab	-2.16±0.03a

Appendix 4 (cont).

Group	N	IGR Dry	YC Wet	YC Dry
UW-2N	15	-4.19±0.04c	1.52±0.15c	0.51±0.06b
UWHS-3N	15	-4.15±0.08bc	2.79±0.30a	0.96±0.15a
UWIP-3N	15	-3.90±0.03a	1.99±0.07bc	0.56±0.02b
UWTL-2N	15	-4.12±0.04bc	2.23±0.29abc	0.61±0.09b
UWTLHS-3N	15	-4.06±0.03abc	2.54±0.13ab	0.73±0.03b
UWTLIP-3N	15	-4.06±0.08abc	1.80±0.24bc	0.57±0.09b
TLTL-2N	15	-3.95±0.03ab	1.88±0.14bc	0.56±0.06b

Appendix 5. Weight, length, and condition factor for diploid and triploid groups from ponding to Day 117 of the 1989 study.

Group	N*	Day 1 (4/9/89)	N	Day 21 (5/1/89)		
		Weight (g)		Length (cm)	Weight (g)	K.F. (g/cm^3)
UW-2N	3	0.11±0.00a	75	3.16±0.03c	0.35±0.01c	1.06±0.01a
UWHS-3N	3	0.10±0.01a	75	3.07±0.03d	0.29±0.01d	0.99±0.01b
UWIP-3N	3	0.14±0.00a	75	3.39±0.03b	0.41±0.01b	1.04±0.01a
UWTL-2N	3	0.12±0.01a	75	3.20±0.02c	0.34±0.01c	1.05±0.01a
UWTLHS-3N	3	0.12±0.00a	75	3.01±0.03d	0.30±0.01d	1.06±0.02a
UWTLIP-3N	3	0.13±0.00a	75	3.33±0.03b	0.40±0.01b	1.07±0.01
TLTL-2N	3	0.13±0.01a	75	3.54±0.02a	0.48±0.01a	1.07±0.01a

Group	N	Day 35 (5/15/89)		
		Length (cm)	Weight (g)	K.F. (g/cm^3)
UW-2N	75	4.54±0.03cd	1.11±0.02c	1.17±0.01a
UWHS-3N	75	4.23±0.06e	0.97±0.03d	1.24±0.02a
UWIP-3N	75	4.72±0.04b	1.32±0.03a	1.24±0.01a
UWTL-2N	75	4.45±0.04f	1.12±0.03c	1.25±0.02a
UWTLHS-3N	75	3.96±0.06d	0.77±0.03e	1.20±0.02a
UWTLIP-3N	75	4.60±0.03c	1.22±0.03b	1.23±0.01a
TLTL-2N	75	4.83±0.03a	1.40±0.03a	1.23±0.01a

Appendix 5 (cont).

Group	N	Day 50 (5/30/89)		
		Length (cm)	Weight (g)	K.F. (g/cm^3)
UW-2N	75	5.85±0.05ab	2.77±0.07b	1.36±0.01a
UWHS-3N	75	5.26±0.08c	2.01±0.08c	1.32±0.02a
UWIP-3N	75	594±0.07ab	3.07±0.10a	1.46±0.05a
UWTL-2N	75	5.76±0.04b	2.69±0.07b	1.29±0.01a
UWTLHS-3N	75	5.08±0.08c	1.86±0.08c	1.36±0.02a
UWTLIP-3N	75	6.03±0.07a	3.16±0.09a	1.41±0.01a
TLTL-2N	75	6.05±0.04a	3.11±0.07a	1.39±0.01a

Group	N	Day 63 (6/12/89)		
		Length (cm)	Weight (g)	K.F. (g/cm^3)
UW-2N	75	7.05±0.05a	4.70±0.11b	1.33±0.02a
UWHS-3N	75	6.63±0.07bc	3.88±0.13d	1.30±0.02a
UWIP-3N	75	7.07±0.08a	5.09±0.16a	1.40±0.02a
UWTL-2N	75	6.50±0.08d	3.70±0.14d	1.30±0.01a
UWTLHS-3N	75	6.06±0.09b	3.07±0.13e	1.33±0.02a
UWTLIP-3N	75	6.84±0.08	4.36±0.16bc	1.33±0.02a
TLTL-2N	75	6.73±0.05bcd	4.06±0.08cd	1.32±0.01a

Appendix 5 (cont).

Group	N	Day 77 (6/26/89)		
		Length (cm)	Weight (g)	K.F. (g/cm^3)
UW-2N	75	8.32±0.07a	7.89±0.18b	1.37±0.02a
UWHS-3N	75	7.69±0.10c	6.08±0.23d	1.30±0.02a
UWIP-3N	75	8.55±0.10a	8.80±0.30a	1.35±0.02a
UWTL-2N	75	7.64±0.08d	5.87±0.19d	1.28±0.01a
UWTLHS-3N	75	7.24±0.11c	5.15±0.22e	1.30±0.02a
UWTLIP-3N	75	8.00±0.09b	6.91±0.24c	1.31±0.01a
TLTL-2N	75	7.72±0.07c	5.99±0.15d	1.30±0.03a

Group	N	Day 91 (7/10/89)		
		Length (cm)	Weight (g)	K.F. (g/cm^3)
UW-2N	75	9.48±0.09a	12.13±0.35ab	1.39±0.01bc
UWHS-3N	75	9.02±0.11bc	10.30±0.36cd	1.36±0.02c
UWIP-3N	75	9.62±0.13a	13.62±0.53a	1.46±0.02
UWTL-2N	75	8.67±0.09c	9.08±0.30d	1.36±0.01c
UWTLHS-3N	75	8.22±0.12d	7.90±0.40c	1.35±0.02c
UWTLIP-3N	75	9.10±0.11b	11.20±0.42bc	1.42±0.01ab
TLTL-2N	75	8.70±0.09c	9.32±0.26d	1.39±0.01bc

Appendix 5 (cont).

Group	N	Day 105 (7/24/89)		
		Length (cm)	Weight (g)	K.F. (g/cm^3)
UW-2N	75	10.90±0.09b	19.39±0.48b	1.48±0.01a
UWHS-3N	75	10.42±0.11c	16.27±0.46cd	1.42±0.01b
UWIP-3N	75	11.57±0.11a	24.13±0.65a	1.53±0.02a
UWTL-2N	75	10.13±0.10cd	15.82±0.50cd	1.49±0.01a
UWTLHS-3N	75	10.00±0.10d	15.16±0.44e	1.48±0.01b
UWTLIP-3N	75	10.43±0.12c	17.57±0.60c	1.51±0.01a
TLTL-2N	75	10.01±0.10cd	15.16±0.44de	1.48±0.01a

Group	N	Day 117 (8/2/89)		
		Length (cm)	Weight (g)	K.F. (g/cm^3)
UW-2N	75	11.81±0.12b	25.22±0.75b	1.50±0.01b
UWHS-3N	75	11.40±0.12c	22.13±0.69c	1.46±0.02b
UWIP-3N	75	12.51±0.15a	32.89±1.07a	1.64±0.02a
UWTL-2N	75	11.06±0.13cd	21.19±0.73cd	1.53±0.02b
UWTLHS-3N	75	10.80±0.17d	19.70±0.90d	1.50±0.02b
UWTLIP-3N	75	11.24±0.15cd	22.74±0.90c	1.53±0.02b
TLTL-2N	75	10.95±0.10cd	20.72±0.56cd	1.55±0.01b

Appendix 6.Percent daily mortality for diploid and triploid groups during the 1988 study.

Group	N	Daily Mortality (%)		
		Day 0-22	Day 23-50	Day 51-64
UW-2N	3	0.97±0.05ab	0.05±0.01a	0.18±0.06a
UWHS-3N	3	0.88±0.07ab	0.18±0.10a	0.39±0.08a
UWIP-3N	3	1.17±0.14ab	0.08±0.03a	0.23±0.09a
UWTL-2N	3	0.72±0.26ab	0.02±0.02a	0.15±0.06a
UWTLHS-3N	3	1.17±0.26ab	0.24±0.09a	0.31±0.03a
UWTLIP-3N	3	1.38±0.12a	0.04±0.02a	0.20±0.10a
TLTL-2N	3	0.38±0.03b	0.02±0.01a	0.08±0.05a

Group	N	Day 65-90	Day 91-117
UW-2N	3	0.29±0.03ab	0.11±0.05b
UWHS-3N	3	0.33±0.11ab	0.24±0.10b
UWIP-3N	3	0.20±0.11ab	0.20±0.11b
UWTL-2N	3	0.35±0.12a	0.34±0.11b
UWTLHS-3N	3	0.39±0.03a	0.67±0.11b
UWTLIP-3N	3	0.22±0.07ab	0.27±0.05a
TLTL-2N	3	0.07±0.01b	0.06±0.38b

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Hershberger, W.K., Myers, J.M., Iwamoto, R.N., McAuley, W.C., and Saxton, A.M. 1990. Genetic changes in growth of coho salmon (*Oncorhynchus kisutch*) produced by ten years of selection. Aquaculture, 00:000 (in press).

Iwamoto, R.N., Myers, J.M., and Hershberger, W.K. 1990. Heritability and genetic correlations of flesh coloration in pen-reared salmon. Aquaculture, 00:000 (in press).

Hershberger, W.K. Myers, J.M., Iwamoto, R.N., and McAuley, W.C. 1990. Assessment of inbreeding and its implication for salmon broodstock development. Proceeding to the Sixteenth U.S.-Japan Natural Resources (UNJR) Panel on Aquaculture, Charleston, S.C., October 19-22, 1987. (in press).

Guo, X., Hershberger, W.K., Myers, J.M. 1990. Growth and survival of intrastrain and interstrain triploids of rainbow trout (*Oncorhynchus mykiss*). Journal of the World Aquaculture Society, 00:000 (in press).

Thorgaard, G.H., Scheerer, P.D., Hershberger, W.K., and Myers, J.M. 1990. Androgenetic rainbow trout produced using sperm from tetraploid males show improved survival. Aquaculture, 00:000 (in press).

Myers, J.M., Hershberger, W.K., and Iwamoto, R.N. 1986. The induction of tetraploidy in salmonids. Journal of the World Aquaculture Society, 17: 1-7.

Iwamoto, R.N., Myers, J.M., and Hershberger, W.K. 1986. Genotype-environment interactions for growth of rainbow trout. Aquaculture, 57 153-162.

Myers, J.M. 1986. Tetraploid induction in *Oreochromis* spp. Aquaculture, 57: 281-288.

Myers, J.M., and Iwamoto, R.N. 1986. Evaluation of thermal and chemical marking techniques for tilapia. The Progressive Fish-Culturist 48:288-289.

Allen, S.K. Jr., and Myers, J.M. 1985. Chromosome set manipulation in three species of salmonids using hydrostatic pressure. in Salmonid Reproduction. Iwamoto, R.N., and Sower, S.A. eds. Washington Sea Grant Program Seattle, Washington. 167 pp. (abstract).