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Production of Triploid Rainbow Trout for Evaluation in South Dakota Waters

Jeff C. Dillon

PRODUCTION OF TRIPLOID RAINBOW TROUT FOR EVALUATION IN SOUTH DAKOTA WATERS

BY

JEFF C. DILLON

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Charles G. Scalet
Thesis Adviser

Date

Charles G. Scalet, Head,
Department of Wildlife
and Fisheries Sciences

Date

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PRODUCTION OF TRIPLOID RAINBOW TROUT FOR EVALUATION IN SOUTH DAKOTA WATERS

Abstract

Jeff C. Dillon

In September 1986, approximately 50,000 rainbow trout (Salmo gairdneri) eggs were fertilized and subjected to heat shocks to prevent expulsion of the second polar body, theoretically resulting in triploid embryos. Heat shocks were at 26 and 28°C, began 10, 20, 30, or 40 min after fertilization, and lasted for 10 or 20 min for a total of 16 treatments. Another 25,000 eggs served as controls and were handled identically but at a normal water temperature of 12°C. Cumulative hatching rates for eggs that were heat-shocked at 26 and 28°C were 64 and 50%, respectively, compared to control eggs which had a cumulative hatching rate of 76%. At age 7 months 25 fish from each treatment group were individually evaluated for ploidy level by quantifying DNA content of red blood cells using flow cytometry. Rates of triploidy induction ranged from 0-100%, and all variables (temperature, time after fertilization when heat shock began, and duration of heat shock) significantly ($p \leq .01$) affected triploid yield. The most successful treatment under the conditions of this experiment was at 28°C beginning 20 min after fertilization for a duration of 10 min, which resulted in 60.5% survival to feeding and 100% triploidy induction. Four treatment groups

containing the highest percentage of triploids (92-100%), along with an equal number of control diploids, were retained for stocking and further evaluation. Diploid and triploid fish were fin clipped and stocked in equal proportions into six small (1.0-2.5 hectare) impoundments in south-central South Dakota. The relative survival and growth of diploid and triploid fish in a wild situation will be monitored over the next several years. This will allow an assessment of triploid rainbow trout as a fisheries management option in South Dakota.

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INTRODUCTION

Chromosome manipulation to produce polyploid fishes has been actively investigated since the mid-1970's (Thorgaard 1983, 1986). Much of the interest to date has concerned the possible applications of induced polyploidy in fish culture and, to a lesser extent, fisheries management. Research has primarily been focused on the production of triploid salmonids (Thorgaard et al. 1981) and cyprinids, especially grass carp (Ctenopharyngodon idella) (Shireman 1984: Cassani and Caton 1985). Cases of spontaneous viable triploidy have been reported in rainbow trout (Salmo gairdneri)(Cuellar and Uyeno 1972; Thorgaard and Gall 1979) and in brook trout (Salvelinus fontinalis) (Allen and Stanley 1978); it was these early reports which spurred interest in artificial production of triploid salmonids.

CHARACTERISTICS OF TRIPLOID FISH

Triploid fishes exhibit a variety of characteristics which may make induced triploidy useful in both sport fish and commercial fish production. These may include: 1) increased growth rate, 2) increased maximum body size, 3) improved food conversion efficiency, and 4) sterility. The first three characteristics are not directly related to the extra chromosome set, but are primarily a result of the sterility of triploid fish (Thorgaard 1983). Sterility

allows the fish to avoid the growth depression and increased mortality normally associated with maturation in fishes (Scheerer and Thorgaard 1983). The sterility of triploids is due to improper pairing and random separation of trivalent chromosomes during meiosis I, resulting in the production of gametes with aneuploid chromosome numbers (i.e. gametes with incomplete multiples of the haploid chromosome number)(Lincoln 1981; Benfey and Sutterlin 1984a).

Along with this functional sterility, sexual differentiation and gonadal development may also be inhibited. Gervai et al. (1980) found that sex differentiation in 20-month old triploid common carp (Cyprinus carpio) was retarded and that average gonad weights of triploids were 0.7% of body weight compared to 10-25% in normal fish. No motile spermatozoa were found in males and few developing oocytes were found in females. Mature triploid channel catfish (Ictalurus punctatus) exhibited no secondary sex characteristics, making it difficult to sex these fish using external characteristics (Wolters et al. 1982a). It is presumed that because triploid fish allocate less of their energetic and nutritional resources to reproductive activity and gonadal development, they will maintain superior growth rates relative to diploids during and after sexual maturation (Thorgaard 1983).

Increased growth rate and maximum body size in triploid fish compared to diploid fish would make them desirable to both the commercial fish culturist and to the recreational fisheries manager. Purdom (1972) felt that animal species with indeterminate growth patterns are more likely to be larger due to polyploidy. Since fish show indeterminate growth it seems likely that triploid fish would indeed possess some positive growth traits. Further, the sterility of triploids may allow even those organisms with determinate growth patterns to grow at a faster rate even if not reaching a larger maximum size. The actual effects of triploidy on growth rates and maximum body size in fishes is variable and species-dependent. Triploid channel catfish grew significantly ($p \leq .05$) faster than their diploid siblings (Wolters et al. 1982a). Valenti (1975) found that triploid blue tilapia (Tilapia aurea) were significantly ($p \leq .05$) larger than their diploid siblings at 14 weeks of age. Swarup (1959a) reported that triploid threespine sticklebacks (Gasterosteus aculeatus) grew at normal rates and attained a normal adult size. In addition, normal sized triploids were reported in immature Atlantic salmon (Salmo salar) (Benfey and Sutterlin 1984a), in juvenile common carp (Gervai et al. 1980), in juvenile plaice (Pleuronectes platessa) (Purdom 1972), and in two-year old rainbow trout (Thorgaard and Gall 1979). Solar et al. (1984) noted a decreased growth rate in juvenile

triploid rainbow trout compared to diploid controls, apparently due to depressed feeding. In contrast, Thorgaard (1986) reported that adult triploid rainbow trout were larger than same-age diploids. Garling and Tanner (1985a, 1985b) have produced triploid chinook salmon (Onchorhyncus tshawytscha) and speculate that since the fish will not become sexually mature they might live longer and grow larger than diploids which normally spawn and die at age four.

Higher food conversion efficiency in triploids has been documented in channel catfish. Wolters et al. (1982a) found that triploid channel catfish 8-16 months of age consumed the same amount of food as did full sibling diploids but were significantly ($p \leq .05$) heavier due to better conversion rates. Improved food conversion efficiency has not been reported for triploids of other species.

TRIPLOIDY INDUCTION IN FISHES

Triploidy induction is accomplished by disrupting spindle fiber formation at meiosis II of the egg, causing retention of the second polar body and creating an egg with two complements of maternal chromosomes (Thorgaard 1983). Syngamy with the male gametes then results in a triploid zygote. Spindle fiber formation may be disrupted by physical, chemical, or thermal shocks applied to the eggs

after sperm penetration but prior to expulsion of the second polar body. Triploidy has been successfully induced in fish using chemical treatment with colchicine (Smith and Lemoine 1979) or cytochalasin B (Refstie et al. 1977), pressure shock (Benfey and Sutterlin 1984b; Chourrout 1984; Cassani and Caton 1986), cold shock (Swarup 1959a; Purdom 1972; Gervai et al. 1980; Meriwether 1980; Wolters et al. 1981a), and heat shock (e.g. Lincoln and Scott 1983; Scheerer and Thorgaard 1983; Garling and Tanner 1985a, 1985b).

Among salmonids, triploidy has been artificially induced in Atlantic salmon (Benfey and Sutterlin 1984b; Bolla and Refstie 1985; Johnstone 1985), chinook salmon (Utter et al. 1983; Garling and Tanner 1985a, 1985b; Hill et al. 1985), coho (O. kisutch) and pink salmon (O. gorbuscha) (Utter et al. 1983), brown trout (Salmo trutta)(Scheerer and Thorgaard 1983),and rainbow trout (Chourrout 1980, 1984; Thorgaard et al. 1981; Chourrout and Quillet 1982; Lincoln and Scott 1983; Scheerer and Thorgaard 1983; Solar et al. 1984; Bolla and Refstie 1985). Heat shock of fertilized eggs has been shown to be the best method for inducing triploidy in salmonids (Refstie et al. 1982), and rates of up to 100% triploidy induction have been reported for rainbow trout (Chourrout and Quillet 1982; Lincoln and Scott 1983; Scheerer and Thorgaard 1983; Solar et al. 1984).

The timing of heat shock is critical, since it must be applied prior to expulsion of the second polar body, and

the interval between sperm penetration and the second reduction division is most often unknown or at least unreported. Temperature and duration of heat shocks must be sufficient to disrupt spindle fiber formation but not result in excessive mortality. In addition, succeptibility to heat shock appears to vary with species, strain, and even the individual fish from which the eggs are taken (Lincoln and Scott 1983; Solar et al. 1984).

The methods for inducing triploidy in rainbow trout are well known but imprecise; that is, specific heat shock regimes for different strains and under different conditions (e.g. ambient temperature) have not been documented. Furthermore, the potential of triploid rainbow trout as a fisheries management tool has not been documented. Techniques for large-scale production of triploids must first be refined in order to provide adequate numbers for experimental stocking and evaluation.

DETERMINATION OF PLOIDY LEVEL IN FISH

Associated with an increase in ploidy level of an organism is a proportional increase in individual cell size (Swarup 1959b; Purdom 1972), chromosome number, and DNA content; all of these characteristics have been used to identify polyploid fishes. Red blood cell nuclear volume has been used to assess ploidy levels in fish (e.g. Allen and Stanley 1978, 1979; Thorgaard and Gall 1979; Wolters et

al. 1982b; Beck and Biggers 1983; Benfey et al. 1984), as has red blood cell nuclear density (Johnstone 1985). Karyotyping has been done with tail epithelium of embryos and gill epithelium of fry (Chourrout 1982, 1984), kidney tissue and cultured lymphocytes (Wolters et al. 1981b), and cultured leukocytes (Thorgaard and Gall 1979; Gervai et al. 1980).

The fastest and most accurate method reported to date for determining ploidy level in fish is by quantifying nucleated red blood cell DNA content by flow cytometry (Thorgaard et al. 1982; Allen and Stanley 1983). This technique involves staining red blood cell DNA with a fluorescent dye and passing the cells through a flow cytometer (Utter et al. 1983). The instrument measures fluorescence of individual cells and thus indirectly measures DNA content in each cell. A triploid cell will contain 1.5 times the DNA of a normal diploid cell. Flow cytometry is less labor intensive and less subject to experimental error than other methods of assessing ploidy level (Thorgaard et al. 1982; Johnson et al. 1984), but the instrumentation is more costly.

STUDY OBJECTIVES

The objectives of this study were: 1) to refine techniques for efficiently producing and identifying large numbers of triploid rainbow trout, and 2) to stock triploid

rainbow trout and equal numbers of control diploids in South Dakota waters for a subsequent evaluation of relative survival and performance.

STUDY AREA

Fertilization, heat shock, incubation of eggs, and fish rearing took place at Cleghorn Springs State Fish Hatchery in Rapid City, South Dakota. This facility is owned and operated by the South Dakota Department of Game, Fish, and Parks, and annually supplies approximately 65% of the rainbow trout stocked in the state (Larry Ferber, pers. comm. 1988). The hatchery water source is a natural spring with a flow rate of 23 million L/d at a temperature of 11-12°C. High nitrogen content (116-118% saturation) in the water source was present during this experiment and often contributes to production losses in trout and salmon fry at this facility. Ploidy evaluation of rainbow trout fingerlings took place at the Station Biochemistry laboratory at South Dakota State University.

The ponds used for stocking triploid and control diploid rainbow trout were located in Jones County in southcentral South Dakota (Table 1). All are small impoundments created by earthen dams and are filled by runoff. Landowners were relied upon for information regarding their ponds. Each maintained that the ponds were free of other fish species except minnows, and were of adequate depth and surface acreage. Jones County Wildlife Conservation Officer Dennis Mann also felt that the ponds

Table 1. Legal description of ponds in Jones County, South Dakota used for stocking triploid and control diploid rainbow trout (Salmo gairdneri).

Pond owner	Pond size (hectares)	Legal description
Knox	1.0	R27E; T1N; S2; SW1/4
Ray	1.0	R27E; T1N; S9; NE1/4
Matthews	2.5	R29E; T2N; S15; SE1/4
Nix	1.0	R29E; T2S; S33; SW1/4
Kinsley	1.0	R28E; T1S; S9; SW1/4
Iverson	1.5	R29E; T1S; S25; NW1/4

were acceptable for rainbow trout stocking. The Kinsley pond had been stocked with rainbow trout in 1983, but few or none of these fish were believed to remain. The Iverson pond once held rainbow trout, but dam failure in 1980 resulted in the loss of the fishery. The dam was reconstructed in 1982 and reportedly had not been stocked since. The Ray, Knox, Matthews, and Nix ponds were all created from recently built or rebuilt dams (2-4 years old) and none had been stocked. All landowners have agreed to restrict angling in the ponds for the duration of this experiment.

METHODS

HEAT SHOCKING PROCEDURES

Heat shock of fertilized eggs was chosen as the preferred treatment to induce triploidy in rainbow trout. Previous researchers who successfully induced triploidy in rainbow trout did not specify the strain used and the timing of the second reduction division was not known. For these reasons a wide range of heat shocks was selected.

All treatments were completed on 23 Sept 1986. Brood stock for this experiment were 3-4 year old Growth strain rainbow trout which are held at the hatchery year-round. Fish were 1.35-1.80kg in weight and each female yielded approximately 3,000 eggs (Ferber pers. comm. 1986). Control eggs were treated at the ambient temperature of 12^{o}C, while heat-shocked eggs were treated at 26 or 28^{o}C. Treatments began 10, 20, 30, or 40 min after fertilization and lasted 10 or 20 min for a total of 16 heat-shock treatments plus 8 control treatments. It would have been desirable to take all eggs at once, mix them, fertilize them, and then perform all treatments. However, lack of manpower dictated that the experiment be conducted in three steps: controls first, then the 26^{o}C treatments, then the 28^{o}C treatments.

Control treatments and heat shocks took place in a recirculating water bath (Figure 1). The tank was

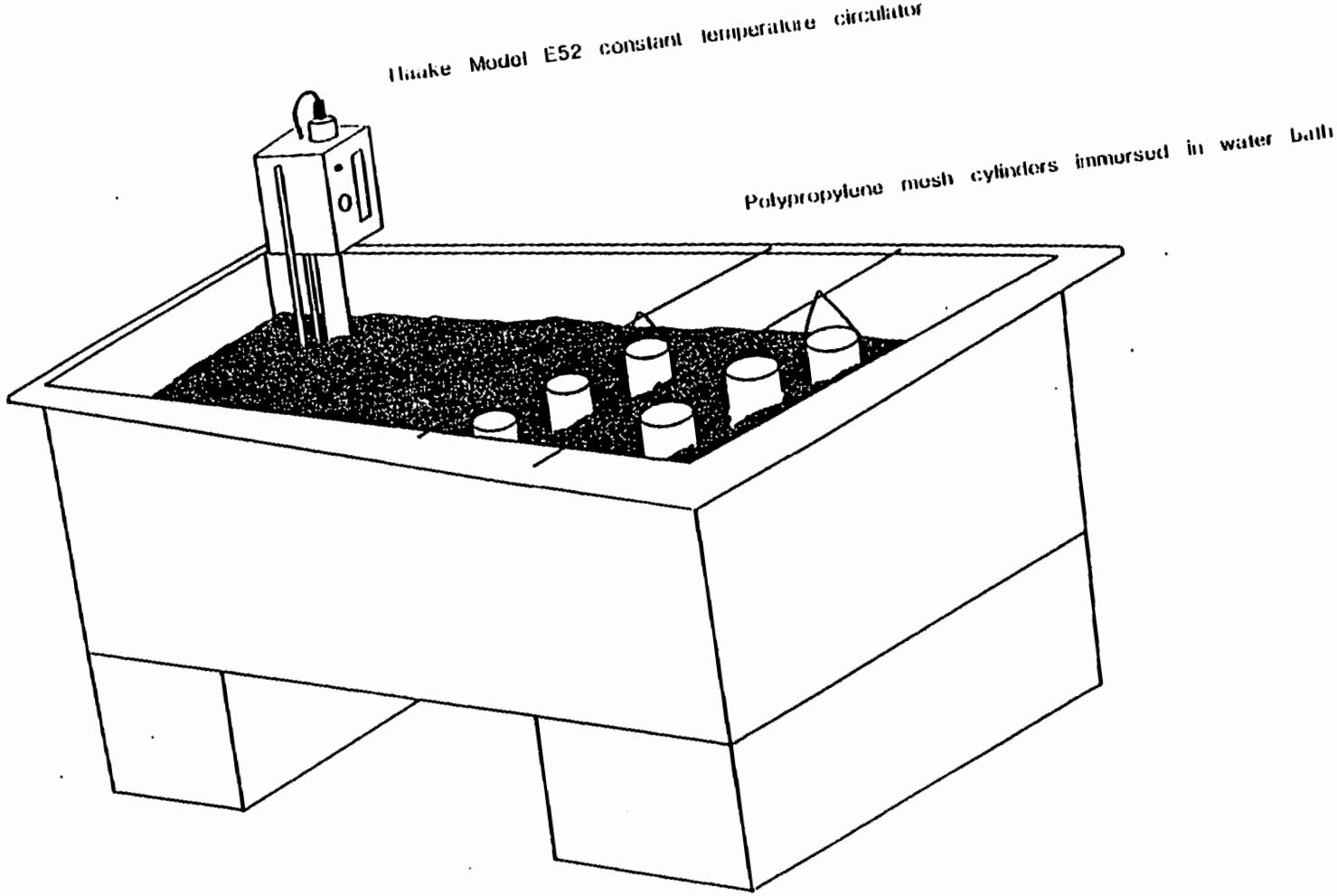

Figure 1. Water bath apparatus used to heat shock rainbow trout (Salmo gairdneri) eggs after fertilization.

constructed of fiberglass and held 150 L. Temperature was regulated by a constant temperature circulator (Haake model E52) attached to the side of the tank.

Control treatments were conducted first. Brood fish were anesthetized with MS-222. Eggs were stripped from ten ripe females into two pans, fertilized with milt from 8 males, water activated simultaneously, and then mixed together. Eggs were then brought to the hatchery building where the water bath had been assembled. Control eggs were not heat-shocked but were subjected to all movement and handling stresses to which the heat-shocked lots were subjected. Eggs were volumetrically counted into lots of 1,000 eggs per replicate, and poured into pre-labeled cylinders fashioned from 3.18 mm polypropylene mesh (Internet Inc., Minneapolis, MN)(Figure 2). The three replicates of each control treatment were simultaneously lowered into and removed from the ambient temperature (12^{o}C) water bath at the appropriate intervals. Temperature was monitored at 5 min intervals throughout the procedure. Eggs were then poured into screen baskets (Figure 3) and placed into Heath tray incubators. Baskets were labeled with temperature, treatment, and replicate, and served to maintain the integrity of all treatments and replicates throughout incubation.

The water bath was then heated to 26^{o}C. Eggs were stripped from nine females, fertilized with milt from 8

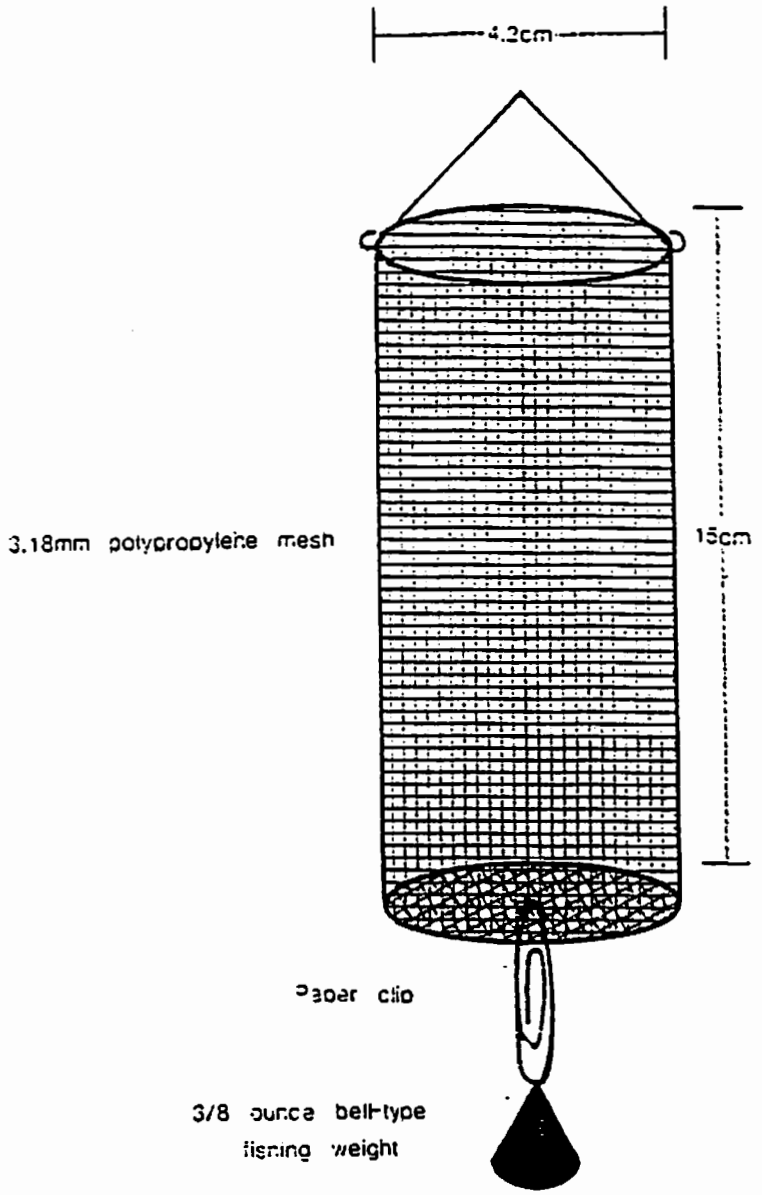

Figure 2. Polypropylene mesh cylinders used to immerse rainbow trout (Salmo gairdneri) eggs into a recirculating water bath.

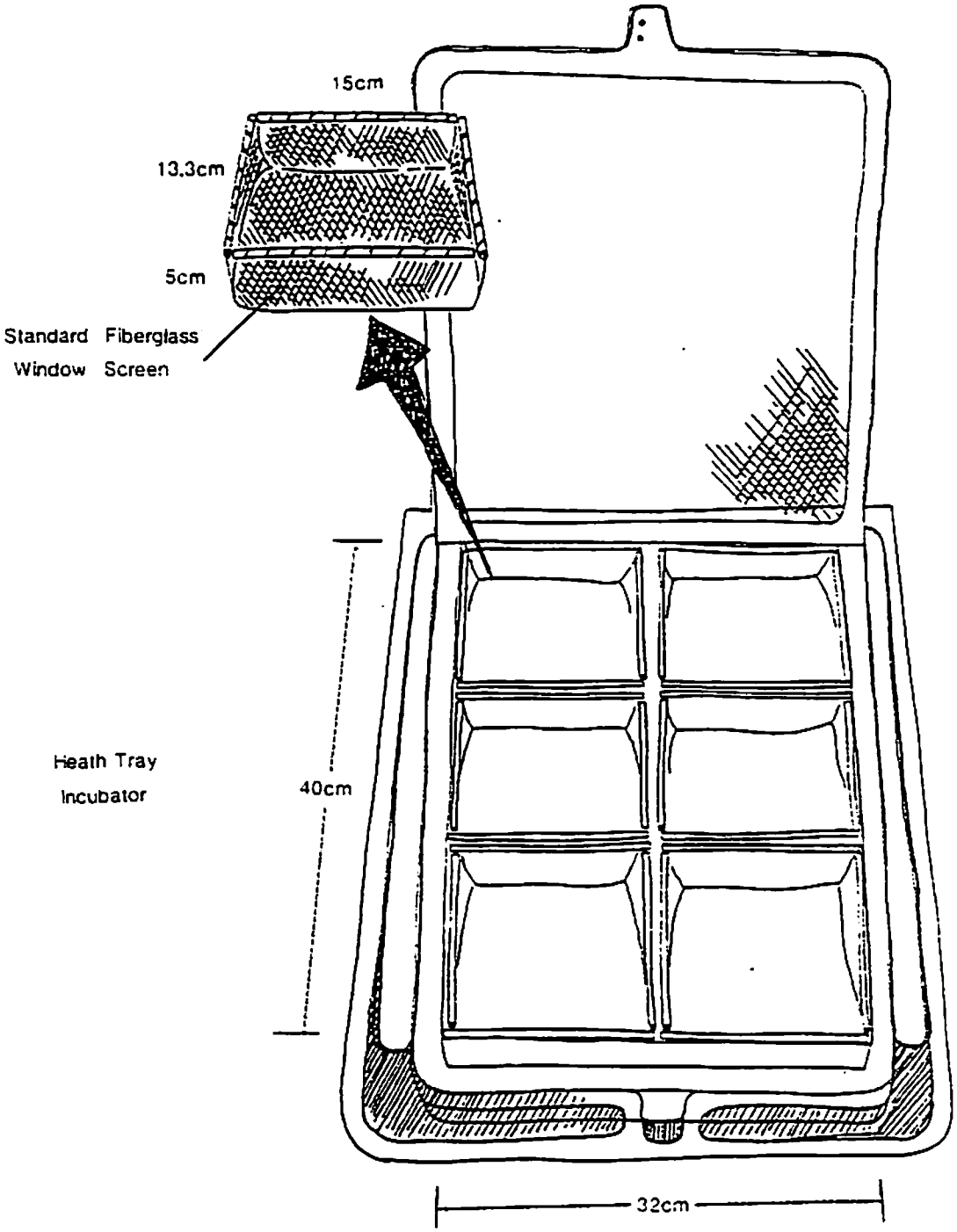

Figure 3. Screen baskets placed within Heath trays to maintain integrity of treatments and replicates of heat-shocked and control rainbow trout (Salmo gairdneri) eggs throughout incubation.

males, water activated, and brought to the hatchery building. Heat shock procedures were identical to the control procedures. Egg cylinders were suspended in an ambient water temperature tank until heat shock, and were returned directly to the ambient temperature after heat shock. Water bath temperature was monitored every 5 min. Eggs were then poured into the screen baskets for incubation. The water was then heated to 28°C, eggs from nine more females were stripped and fertilized with milt from 8 males, and the procedures repeated.

All eggs were incubated at normal temperature (12°C). Six screen incubation baskets fit in each Heath tray, for a total of approximately 6,000 eggs per tray compared to the normal 10,000-12,000 eggs per tray. All dead eggs in each replicate were removed and counted at day one. From day 2-20, eggs were treated every other day with 30 mL saturated copper sulfate solution to control fungus. At day 21, dead eggs were again removed and counted; surviving eggs in each replicate were also counted. This allowed a determination of total number of eggs per replicate. Dead eggs were then removed and counted every 1-2 d for the remainder of incubation. Hatching began on day 28. Remaining dead eggs were removed and counted, permitting a determination of percent hatch per treatment and control replicate.

KARYOLOGICAL EXAM OF EMBRYOS

At day 21 of incubation an attempt was made to assess the ploidy level of 50 heat-shocked embryos by karyological examination using methods described by Chourrout (1982, 1984) and Thorgaard et al. (1981). Live eggs were immersed in a 0.02% colchicine solution for 10 h at 12°C. Colchicine is a mitotic inhibitor which blocks cell division at metaphase (Evenson pers. comm. 1987). Embryos were dissected from the egg membrane, fixed in 3:1 ethanol-acetic acid, and stored at 4°C. For karyological exam, embryos were removed from the fixative and tail epithelial tissue was dissociated on a slide in 50% acetic acid. Cells were squashed with a cover slip. The slide was then moistened with 50% acetic acid and the cover slip removed. Slides were air dried, stained with 4% Giemsa for 10 min, then rinsed and air dried again. Cell preparations were viewed with a light microscope at 1,000X. Most hatchery strains of rainbow trout possess a diploid chromosome number of 60 (Thorgaard and Gall 1979), so triploids would possess 90 chromosomes.

FISH REARING

After hatching all rainbow trout fry were moved to indoor raceway tanks. These are constructed of double-walled fiberglass measuring 4.35 m long x 0.45 m wide x 0.3 m deep, and hold approximately 400 L. All control rainbow

trout fry were combined into one tank, the assumption being that controls contained no triploids. The approximately 18,700 control fry in one raceway is fewer than the 25,000 rainbow trout fry typically placed in such a raceway under normal hatchery procedures (Ferber, pers. comm. 1986). The three replicates of each treatment of rainbow trout fry were combined and placed into separate cages within the fiberglass raceways. These cages had an outer layer of 3.18 mm vexar mesh with an inner lining of standard fiberglass window screen, and fit four to a raceway (Figure 4). The inner screen was to be removed when the fry were of sufficient size to be retained by the 3.18 mm mesh.

Feeding of fry began 16 d after hatch using U.S. Fish and Wildlife Service (USFWS) diet SD9-30 starter (#1 and #2 granules). Almost immediately after the initiation of feeding, the fungus Saprolegnia began growing between the two mesh layers of the cages. Water flow was insufficient and the window screen too fine to allow fecal material and excess food to be flushed from the cages. As a result, it was necessary to transfer all treatment lots to separate tanks. The inner screens were removed and all cages were thoroughly cleaned and dried.

Mortalities in the combined controls and in each of the treatment lots were recorded until most fish were taking feed. Survival to feeding (STF) was calculated using the number of fish surviving at 30 d post-hatch, by which time

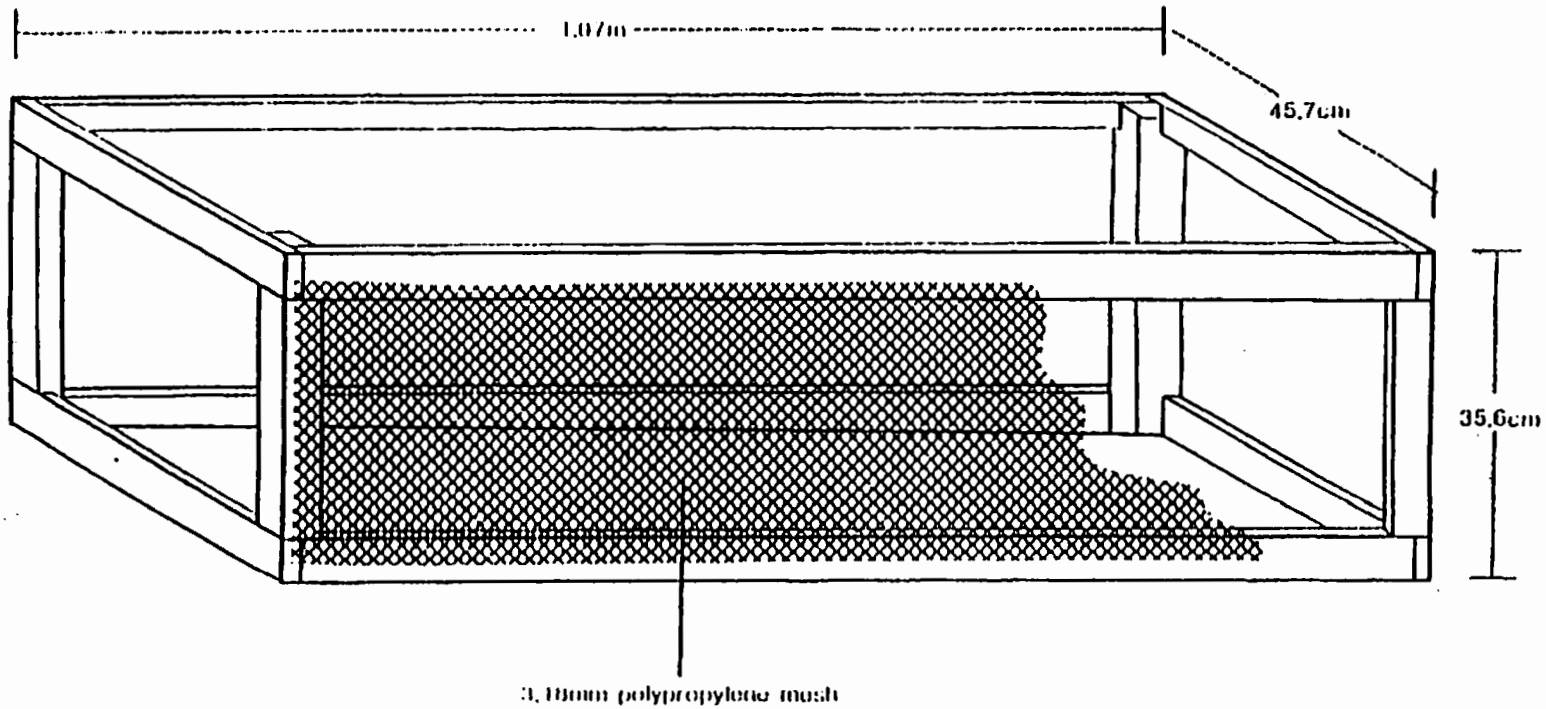

Figure 4. Polypropylene mesh cages used for rearing rainbow trout (Salmo gairdneri) hatched from eggs subjected to various heat shock treatments.

all fish not taking feed had likely died (Ferber, pers. comm. 1986). Analysis of percent hatch and STF data was by maximum likelihood categorical data analysis (Freeman 1987) using values corrected for differences in initial numbers of eggs per treatment.

Treated fish remained in the separate tanks until 13 weeks of age and a mean total length of >60 mm. At this time they were returned to the 3.18 mm mesh cages and fed USFWS GR6-30 (#3 and #4 granules). Fish were held under these conditions for the remainder of the hatchery phase of this experiment (to 200 d post-hatch).

PLOIDY EVALUATION

At 160 d post-hatch, 25 fish from each treatment lot plus 15 fish from the control group were evaluated for ploidy level by flow cytometric analysis of nucleated red blood cell DNA content (Thorgaard et al. 1982; Allen and Stanley 1983; Solar et al. 1984). Blood was taken from each fish by severing the caudal peduncle and drawing blood into a pasteur pipette rinsed with Hank's balanced salt solution (HBSS)(644 mg NaCl + 11 mg KCl + 22 mg CaCl + 12 mg $MgSO_4$ + 7 mg KH_2PO_4 + 10 mg $NaHCO_3$ in 100 mL H_2O) containing 100 IU heparin/mL. Blood was then placed into 0.5 mL of heparinized HBSS, drawn up and aspirated several times to minimize clumping of cells, and placed into a labeled vial containing 2.0 mL of 70% ethanol. Samples were placed on

ice for transport back to the South Dakota State University Station Biochemistry laboratory, where they were stored at 4°C until analysis. Preliminary tests with rainbow trout blood indicated that fixed cells could be stored for two weeks without affecting DNA stainability or cellular integrity.

Ploidy evaluation took place 3, 6, 7, and 8 days after taking the samples, with 57, 110, 124, and 124 samples analyzed per day, respectively. Individual samples were drawn randomly from the labeled vials. Cells were centrifuged out of the fixative for 5 min at 47G, resuspended in 1.0 mL HBSS, then centrifuged again. Cells were then resuspended in 0.5-1.0 mL HBSS to arrive at a final concentration of approximately $2x10^6$ cells/mL. A subsample of 0.2 mL of diluted cells was mixed with 0.4 ml of a solution containing 0.1% Triton X-100 (Sigma Chemical Co., St. Louis, Mo.), 0.08N HCl, and 0.15N NaCl for 30 s. This solution makes the nuclear membrane of the cell permeable to the dye and partially removes the histones from the DNA. Cells were stained with 1.2 mL of a solution containing phosphate-citric acid buffer (pH 6.4) and 6 micrograms/mL chromatographically purified acridine orange (Polysciences, Inc., Warrington, Pa.). Acridine orange intercalates into double-stranded DNA as a monomer and fluoresces green (530 nm) under 488 nm laser light excitation (Lerman 1963) while it associates with single

stranded RNA and fluoresces metachromatically red with maximum emission at 640 nm (Bradley and Wolf 1959).

The fluorescence of individual cells was measured by a Cytofluorograf model 50 flow cytometer (Becton-Dickinson, Westwood, MA). Signals are generated in the instrument as cells pass through a 488 nm argon laser (Darzynkiewicz et al. 1976). The red (F>600) and green (F=530) fluorescence emissions from each cell are separated optically and quantified by separate photomultipliers. Optimum flow rate for this type of staining is 150-200 cells/s (Evenson, pers. comm. 1987). All measurements were filed by computer (Becton-Dickinson 2150 Data Handler) and visual readouts showing fluorescence distribution were produced. This allowed a rapid visual determination of the peak location of red and green fluorescence, which directly relates to DNA and RNA content of the blood cells. Although both DNA and RNA content were recorded, the DNA content value was used to categorize each fish as diploid or triploid.

The number of fish remaining per treatment group at the time of ploidy evaluation was estimated by survival to feeding x 0.8. Analysis of triploid yield data was by maximum likelihood categorical data analysis (Freeman 1987) using corrected values for triploid yield based on percentage triploid induction and surviving fish per treatment. Individual heat shock parameters were also evaluated for significance using maximum likelihood

categorical data analysis. Statistical decisions were made at the 0.01 level of significance unless otherwise stated.

STOCKING PROCEDURES

The four treatment lots with the highest rates of triploidy induction, along with an equal number of control diploids, were retained for stocking and evaluation. In April 1987 both triploids and control diploids were anesthetized with MS-222 and fin clipped. Triploids were left pelvic fin-clipped and controls were right pelvic fin-clipped.

In May 1987 the two treatment lots containing 92% triploids were combined, as were 1,250 each from treatment lots containing 96% and 100% triploids. Thus one treated group was comprised of 92% triploids and the other 98% triploids.

Mean total length (mm) and weight (g) were calculated using approximately 200 fish each from the 98% triploid lot and the controls. Diploids and triploids were loaded into separate hauling tanks on a stocking truck for transport to Jones County, South Dakota, where they were stocked at a rate of approximately 1,000 fish/hectare. At the Knox and Ray ponds, fish were counted into 19.8 L buckets and stocked by hand. These fish were tempered by slowly adding pond water to the bucket of hauling tank water and fish over a period of 3-5 min. Remaining fish were

stocked by 15.2 cm diameter plastic tube directly from the hauling tank into the Matthews pond without tempering. Hauling tank and pond water temperatures were recorded at each site. The following day, mean total length and weight were calculated using approximately 200 fish each from the 92% triploid lot and the remaining controls. Fish were transported to Jones County, hand counted, and stocked by bucket into the Nix and Kinsley ponds. Remaining fish were stocked by plastic tube directly into the Iverson pond. Hauling tank and pond water temperatures were again recorded at each site.

RESULTS AND DISCUSSION

KARYOLOGICAL EXAM OF EMBRYOS

Attempts to conduct chromosome counts from slide preparations of tail epithelial tissue were unsuccessful. Lack of success in producing countable chromosome smears was likely due to inexperience. The proper pressure needed to make squash preparations was unknown and cells were probably ruptured during this process. Examination of cell preparations with a light microscope revealed scattered chromatin material and ruptured cell membranes.

PERCENT HATCH

All heat shock parameters significantly ($p \leq .01$) affected hatch rate (Table 2). Percent hatch for each control and heat shock treatment is given in Table 3. Both treatment temperatures resulted in significantly ($p \leq .01$) lower hatch rates compared to controls. Eggs heat-shocked at 28°C had significantly ($p \leq .01$) lower hatches than eggs heat-shocked at 26°C (Table 4). Eggs heat-shocked 10 min after fertilization (MAF) at 26°C had significantly ($p \leq .01$) lower percent hatches than eggs heat-shocked 20, 30, or 40 MAF, while eggs heat-shocked 10 or 20 MAF at 28°C had significantly ($p \leq .01$) lower hatches than those with heat shocks initiated 30 or 40 MAF. For both temperatures, heat

Table 2. Analysis of variance table for the dependent variable hatch rate of control and heat-shocked rainbow trout (Salmo gairdneri) eggs.[a] TAF = time after fertilization at which heat shocks began.

Source of Variation	Degrees of Freedom	Chi-square
Temperature	2	997.25 **
TAF	3	87.83 **
Duration	1	703.94 **
Temperature * TAF	6	53.18 **
Temperature * Duration	2	631.07 **
TAF * Duration	3	113.75 **
Temperature * TAF * Duration	6	161.84 **

[a] Categorical data analysis used for this and all subsequent statistical analyses.

** Significant at the .01 level of probability.

Table 3. Hatching success and survival to feeding of control rainbow trout (Salmo gairdneri) eggs and eggs subjected to heat shock after fertilization. TAF = time after fertilization at which heat shocks began.

Treatments					
Temp. (°C)	TAF (min)	Duration (min)	Number of eggs	Hatching success (%)	Survival to feeding (%)
11	10	10	3782	75.1	
11	10	20	3601	73.9	
11	20	10	3213	74.5	
11	20	20	3189	75.9	(combined)
11	30	10	3286	75.4	74.4
11	30	20	2839	76.2	
11	40	10	1936	78.4	
11	40	20	2768	80.2	
				mean = 75.9	
26	10	10	4002	70.7	64.5
26	10	20	3721	51.2	46.4
26	20	10	3760	66.5	62.1
26	20	20	3379	62.2	54.8
26	30	10	3314	68.3	62.7
26	30	20	2893	65.5	57.2
26	40	10	3351	72.7	67.8
26	40	20	3284	56.9	52.3
				mean = 64.1	58.5
28	10	10	4085	64.5	59.5
28	10	20	3720	30.2	24.0
28	20	10	3680	67.6	60.5
28	20	20	3577	28.5	21.7
28	30	10	3485	61.1	57.0
28	30	20	3094	46.0	38.8
28	40	10	2892	61.8	55.8
28	40	20	3003	42.7	34.6
				mean = 50.4	44.2

Table 4. Analysis of variance table for the dependent variable hatch rate of control and heat-shocked rainbow trout (Salmo gairdneri) eggs under varying heat shock parameters. MAF = minutes after fertilization at which heat shocks began.

Source of Variance	Degrees of Freedom	Chi-square
26°C vs 28°C	1	595.28 **
Control vs 26 and 28°C	1	457.77 **
20 MAF vs 40 MAF at 26°C	1	0.08 NS
30 MAF vs 20 and 40 MAF at 26°C	1	2.40 NS
10 MAF vs 20, 30, or 40 MAF at 26°C	1	14.45 **
10 MAF vs 20 MAF at 28°C	1	0.34 NS
30 MAF vs 10 and 20 MAF at 28°C	1	31.43 **
40 MAF vs 10, 20, and 30 MAF at 28°C	1	7.40 **
20 MAF vs 30 MAF in Controls	1	0.14 NS
10 MAF vs 40 MAF in Controls	1	9.19 **
10 and 40 MAF vs 20 and 30 MAF in Controls	1	1.51 NS
10 min duration vs 20 in controls	1	0.40 NS
10 min duration vs 20 at 26°C	1	126.87 **
10 min duration vs 20 at 28°C	1	962.66 **

** Significantly different ($p \leq .01$)

shocks of 20 min duration resulted in significantly ($p \leq .01$) lower hatch rates than heat shocks of 10 min duration.

Lower percent hatch in heat shocked eggs compared to controls was expected and has been previously reported (Thorgaard et al. 1981; Scheerer and Thorgaard 1983; Solar et al. 1984). Results also indicated that eggs may be less succeptible to heat-shock induced mortality when shocks are applied further (to 40 min) from the time of fertilization. The average hatch rate of the control lots was 75.9% (Table 2), compared to the approximately 75% hatch of rainbow trout eggs taken the same day and handled under normal hatchery procedures (Larry Ferber, pers. comm. 1986). This indicates that stress from handling and movement probably contributed little to mortality rates, and that decreased hatch rates in treated lots was due primarily to the heat shocks themselves.

SURVIVAL TO FEEDING

All heat shock parameters significantly ($p \leq .01$) affected survival to feeding (STF)(Table 5). STF for each treatment lot is presented in Table 3. Eggs heat-shocked at 28°C had significantly ($p \leq .01$) lower STF than eggs heat-shocked at 26°C (Table 6). At both 26 and 28°C, 20 min heat shocks resulted in significantly ($p \leq .01$) lower STF than 10 min heat shocks. Eggs heat-shocked 10 or 20 MAF at 28°C showed significantly ($p \leq .01$) lower STF than those heat-

Table 5. Analysis of variance table for the dependent variable survival to feeding of rainbow trout (Salmo gairdneri) eggs subjected to heat shocks after fertilization. TAF = time after fertilization at which heat shocks began.

Source of Variance	Degrees of Freedom	Chi-square
Temperature	1	790.86 **
TAF	3	114.26 **
Duration	1	1282.26 **
Temperature * TAF	3	46.63 **
Temperature * Duration	1	391.73 **
TAF * Duration	3	155.00 **
Temperature * TAF * Duration	3	111.21 **

** Significant at the .01 level of probability.

Table 6. Analysis of variance table for the dependent variable survival to feeding of heat-shocked rainbow trout (Salmo gairdneri) eggs under varied heat shock parameters. MAF = minutes after fertilization at which heat shocks began.

Source of Variance	Degrees of Freedom	Chi-square
26°C vs 28°C	1	669.46 **
10 MAF vs 30 MAF at 26°C	1	0.23 NS
20 MAF vs 40 MAF at 26°C	1	1.61 NS
10 and 30 MAF vs 20 and 40 MAF at 26°C	1	14.72 **
10 MAF vs 20 MAF at 28°C	1	0.29 NS
30 MAF vs 40 MAF at 28°C	1	5.17 *
10 and 20 MAF vs 30 and 40 MAF at 28°C	1	40.71 **
10 min duration vs 20 at 26°C	1	220.67 **
10 min duration vs 20 at 28°C	1	1221.58 **

** Significantly different ($p \leq .01$)

* Significantly different ($p \leq .05$)

shocked 30 or 40 MAF, while eggs heat-shocked at 26°C beginning 10 or 30 MAF showed significantly ($p \leq .01$) lower STF than eggs heat-shocked 20 or 40 MAF.

It is unclear why fry from heat-shocked eggs continued to have higher mortality rates than controls. Chourrout and Quillet (1982) reported that heat-shocked and control lots of rainbow trout eggs showed no difference in STF. Solar et al. (1984) found that heat shocking of rainbow trout eggs resulted in significantly ($p \leq .05$) lower STF compared to controls, while Scheerer and Thorgaard (1983) reported improved STF in some heat-shocked rainbow trout eggs and reduced STF in others. Solar et al. (1984) speculated that reduced survival in heat-shocked lots could be due either to a degree of inbreeding associated with the retention of the second polar body, or to the intensity of the treatments themselves. Due to the design of this experiment, no valid statistical analysis could be made to correlate percent triploidy induction with percent hatch and STF. However, no trends were evident that suggested lots with the highest triploidy induction also had the highest mortality. For this reason it seems appropriate to speculate that most of the increased mortality seen in the treated lots was due to the heat shocks themselves and not to the induced triploidy.

FLOW CYTOMETRY

The procedures used in this experiment for flow cytometric analysis of rainbow trout red blood cells differed considerably from previously reported procedures. Allen and Stanley (1983) used heparinized fish physiological saline solution (FPS) in which to collect blood from grass carp x bighead carp (Hypopthalmichthys nobilis) hybrids. Fixed cells were also washed in FPS prior to staining. In this experiment, HBSS was substituted for FPS with no apparent compromise to cellular integrity or DNA stainability. In addition, Allen and Stanley (1983) used 1% formalin to fix blood cell suspensions prior to storage and staining. Formalin has subsequently been found to reduce the amount of fluorescence produced by some stains, and thus may bias comparisons between fixed and unfixed samples (Burns et al. 1986). Still other researchers used only fresh blood samples for flow cytometric analysis (Thorgaard et al. 1982; Utter et al. 1983; Solar et al. 1984). In this experiment, rainbow trout red blood cells were fixed in 70% ethanol and stored for 3-8 days before staining. Fixed cells showed wider variation in stainability with acridine orange, and more clumping than did fresh cells, but peak fluorescence could still be easily distinguished.

Several other fluorescent dyes have been used to assess ploidy level in fish by flow cytometry. Diamidino-2-phenylindole (DAPI) was used successfully (Thorgaard et al.

1982; Utter et al. 1983; Solar et al. 1984), as was propidium iodide (Allen and Stanley 1983; Burns et al. 1986). The use of acridine orange to assess ploidy level in fish has not been previously documented. The results of this experiment indicated that it is an effective alternative for use in quantifying DNA content in rainbow trout blood cells. Because acridine orange differentially stains both DNA and RNA it can simultaneously provide measurements of nuclear DNA and cellular RNA content, whereas DAPI is only DNA-specific (Evenson pers. comm. 1988). Propidium iodide stains both DNA and RNA, but not differentially. Additional treatment with RNA-ase is therefore necessary when using propidium iodide to assess only nuclear DNA content.

TRIPLOID YIELD

None of the 15 control fish tested were triploid. Rates of triploid induction and approximate yield of triploid fish per treatment are given in Table 7. All heat-shock parameters (temperature, TAF, and duration) significantly ($p \leq .01$) contributed to yield of triploids (Table 8). Triploid yield was significantly ($p \leq .01$) higher with 28°C heat shocks than with 26° heat shocks (Table 9). At 26°C, heat shocks of 20 min duration resulted in significantly ($p \leq .01$) higher yield than 10 min heat shocks, while at 28°C heat shocks of 10 min duration resulted in

Table 7. Triploidy induction rates (n = 25 fish per treatment) and yield of triploid rainbow trout (Salmo gairdneri) from eggs subjected to heat shock after fertilization. TAF = time after fertilization at which heat shocks began.

Heat shocks			Incidence of triploidy (%)	Approx. number of fish remaining*	Approx. Yield of triploid fish
Temp. (°C)	TAF (min)	Duration (min)			
26	10	10	0	2000	0
26	10	20	96	1400	1325
26	20	10	8	1860	149
26	20	20	92	1480	1361
26	30	10	8	1660	133
26	30	20	16	1320	211
26	40	10	8	1820	146
26	40	20	12	1370	165
28	10	10	68	1940	1322
28	10	20	96	710	684
28	20	10	100	1780	1780
28	20	20	88	620	546
28	30	10	92	1570	1448
28	30	20	88	960	844
28	40	10	60	1290	775
28	40	20	76	830	631

* Estimated by survival to feeding x 0.8

Table 8. Analysis of variance table for the dependent variable triploid yield from rainbow trout (Salmo gairdneri) eggs subjected to heat shock after fertilization. TAF = time after fertilization at which heat shocks began.

Source of Variation	Degrees of Freedom	Chi-square
Temperature	1	2083.85 **
TAF	3	910.97 **
Duration	1	1386.33 **
Temperature * TAF	3	628.49 **
Temperature * Duration	1	1107.30 **
Temperature * TAF * Duration	3	92.81 **

** Significant at the .01 level of probability.

Table 9. Analysis of variance table for the dependent variable triploid yield from heat-shocked rainbow trout (Salmo gairdneri) eggs under varied heat shock parameters. MAF = minutes after fertilization at which heat shocks began.

Source of Variance	Degrees of Freedom	Chi-square
26°C vs 28°C	1	2214.41 **
10 MAF vs 20 MAF at 26°C	1	2.30 NS
30 MAF vs 40 MAF at 26°C	1	5.75 *
10 and 20 MAF vs 30 and 40 MAF at 26°C	1	1278.23 **
20 MAF vs 30 MAF at 28°C	1	2.06 NS
10 MAF vs 40 MAF at 28°C	1	32.54 **
20 and 30 MAF vs 10 and 40 MAF at 28°C	1	85.46 **
10 min duration vs 20 at 26°C	1	1672.25 **
10 min duration vs 20 at 28°C	1	13.99 **

** Significantly different ($p \leq .01$)

* Significantly different ($p \leq .05$)

significantly ($p \leq .01$) higher triploid yield than 20 min heat shocks.

The effects of TAF on triploid yield were unclear. Heat shocks beginning 10 and 20 MAF at 26°C resulted in significantly ($p \leq .01$) higher triploid yield than heat shocks beginning 30 or 40 MAF (Table 9), while at 28°C, heat shocks beginning 20 or 30 MAF resulted in significantly ($p \leq .01$) higher triploid yield than did heat shocks beginning 10 or 40 MAF. In general for this experiment, heat shocks beginning 40 MAF were not very successful at inducing high rates of triploidy. This may indicate that under the ambient conditions of this experiment, polar body expulsion usually occured before 40 MAF. Higher ambient temperatures would likely increase the metabolic rate of the fertilized egg and therefore reduce the interval between fertilization and the second reduction division (Cassani and Caton 1985). For this reason, ambient temperature should be a consideration when selecting the timing of heat shocks to induce triploidy.

The fact that all heat-shock parameters did affect triploid yield confirms that the correct combination of factors is necessary to maximize yield. Heat shocks at 26°C resulted in higher survival but overall lower triploid yield, while heat shocks at 28°C resulted in lower survival but overall higher triploid yield. Both triploid induction rates and survival must be maximized if large-scale

production of triploids is to be practical. Four treatments in this experiment were the most efficient; i.e. relatively high survival rates and triploidy induction rates (Table 10). It was these four treatment lots which were used for stocking.

Because the eggs used for each heat-shock temperature and for the controls were taken from different lots of 8-10 females each, some difference in viability and succeptibility to heat shock may have been present between lots. Timing of ovulation and egg ripeness differs from female to female, and the stage of egg maturation at the time of shock may also be an important variable in attempts to artificially produce triploid salmonids (Lincoln et al. 1974; Solar et al. 1984). In this experiment it was presumed that any variation in viability or succeptibility to heat shock between the eggs of different females would be diluted by using 8-10 females per treatment temperature. Additionally, all females used as egg sources were of similar age and had a similar culture history which hopefully minimized the variability among females.

The most effective treatments used in this experiment to induce triploidy in Growth strain rainbow trout are probably applicable only in this strain (Scheerer and Thorgaard 1983; Solar et al. 1984). In addition to Growth strain, the State of South Dakota also uses Kamloops, Shasta, and Boulder strains of rainbow trout. If the need

Table 10. Hatching success, survival to feeding, and triploidy induction rates for heat-shocked rainbow trout (Salmo gairdneri) eggs subsequently used for stocking and evaluation. TAF = time after fertilization at which heat shocks began.

Heat shocks					
Temp. (°C)	TAF (min)	Duration (min)	Hatching success (%)	Survival to feeding (%)	Incidence of triploidy (%)
26	10	20	51.2	46.4	96
26	20	20	62.2	54.8	92
28	20	10	67.6	60.5	100
28	30	10	61.1	57.0	92

occurs to produce triploids of these other strains, specific treatments will have to be devised for each.

STOCKING FOR EVALUATION

Water temperatures in the ponds at stocking were considerably higher than that of the hauling tank water in which the fish were transported (Table 11). Stocking rates and mean sizes of diploid and triploid rainbow trout at stocking are presented in Table 12. The short tempering time of 3-5 min probably did little to acclimate the fish to the conditions of the ponds. No mortalities were noted, but ponds were only monitored for 15-20 min after stocking, so initial stocking mortality is unknown. This was not a concern, however, unless 100% mortality occured, since only differential mortality of triploids versus diploids will be assessed at a later date.

All ponds used for stocking were assumed to be both free of other fish species and of adequate depth and water quality to prevent winter and summer kill. The Ray, Kinsley, and Matthews ponds were sampled in late Aug, 1987 and the Matthews pond was found to be turbid and to contain large numbers of black bullheads (Ictalurus melas). Attempts to capture rainbow trout in this pond using a 2.54cm x 38.1m x 2.4m gill net and a 15.2m seine were unsuccessful. The Ray and Kinsley ponds appeared to have better water quality, with an abundance of aquatic

Table 11. Hauling tank and pond water temperatures by location at time of stocking diploid and triploid rainbow trout (Salmo gairdneri) on 11-12 May, 1987.

Pond Owner	Tank Temp. (°C)	Pond Temp. (°C)
Knox	12	23
Ray	14	23
Matthews	14	21
Nix	10	20
Kinsley	13	20
Iverson	14	20

Table 12. Description of stocking sites and mean sizes of diploid and triploid rainbow trout (Salmo gairdneri) stocked in Jones County, South Dakota, May, 1987.

Pond owner	Pond size (hectares)	Number of fish	Ploidy	Mean TL (mm)	Mean wt. (g)
Knox	1.0	500	T	118.9	21.8
		500	D	120.1	21.4
Ray	1.0	500	T	118.9	21.8
		500	D	120.1	21.4
Matthews	2.5	1500	T	118.9	21.8
		1500	D	120.1	21.4
Nix	1.0	500	T	121.7	24.7
		500	D	115.5	23.1
Kinsley	1.0	500	T	121.7	24.7
		500	D	115.5	23.1
Iverson	1.5	1000	T	121.7	24.7
		1000	D	115.5	23.1

macrophytes and invertebrates, especially Gammarus spp. In both of these ponds rainbow trout were caught by gill net. No further sampling has been conducted in these or any of the other ponds since stocking.

SURVIVAL AND PERFORMANCE EVALUATION

The second phase of this project will be an evaluation of the relative survival, growth, and condition of triploid versus diploid rainbow trout in wild conditions over the next several growing seasons. In Great Britain, triploid rainbow trout have been released in small numbers into lakes and reservoirs for recreational angling, but a quantitative evaluation of performance has not been documented (Bye and Lincoln 1986). Because the effects of triploidy on growth rates are due primarily to sterility, differential growth is not expected to arise until the normal fish reach the age of maturation.

The apparently contradictory information available regarding the effects of triploidy on growth rates can be explained in some instances by the ages of fish from which the data are taken. In experiments finding no difference in growth between diploid and triploid common carp (Gervai et al. 1980), Atlantic salmon (Benfey and Sutterlin 1984a), and channel catfish (Wolters et al. 1982a), the data were based on immature fish. In contrast, adult triploid fish are generally heavier than diploids by the time the diploids

have spawned. Thorgaard (1986) reported that two-year-old hatchery-reared triploid and diploid rainbow trout (strain not cited) were the same size, but that the same triploids by age 3.5 years averaged 35% heavier than diploids.

Although the degree of functional sterility associated with triploidy appears to be 100%, the development of secondary sex charateristis may occur in some species, negating to some extent the positive effects of triploidy on growth rates. In adult male triploids, although few functional sperm are produced, testicular development may still progress enough to elevate testosterone levels and cause secondary sex characteristics to arise (Thorgaard and Gall 1979). In rainbow trout, adult male triploids may have testosterone and 11-ketotestosterone levels equal to those of normal fish, while adult female triploids may have testosterone and estradiol levels far below those of normal fish (Lincoln and Scott 1984). Thorgaard and Gall (1979) reported that adult male triploid rainbow trout were normal in external appearance and, like diploids, showed the secondary sex characteristics associated with maturation. Triploid adult females did not attain the spawning coloration of normal fish, had pinker flesh, and continued to gain weight between September and November, unlike the controls which spawned during this time. Benfey and Sutterlin (1984a) used the gonadosomatic index (GSI) to assess gonadal development in immature

Atlantic salmon. They found that the average GSI of triploid females was 7.7% that of normal females, while the average GSI of triploid males was 52% that of normal males. It appears that for salmonids in general, triploid males show greater gonadal development than do triploid females, and thus triploid females are more likely to avoid the deleterious characteristics associated with maturation.

Because the effects of triploidy may be more pronounced in female than in male salmonids, recent research has focused on the production of all-female triploid salmonids for aquacultural applications (Lincoln and Scott 1983; Bye and Lincoln 1986). This technique involves the use of homogametic milt from sex-reversed females to fertilize eggs and then subjecting the eggs to heat shock (Benfey and Sutterlin 1984a). Male rainbow trout mature at an earlier age and smaller size than females, leading to a corresponding earlier loss of flesh quality and asthetic appeal, even in triploids (Thorgaard and Gall 1979). Female rainbow trout mature at a larger size and are more favorably affected by triploidy. Hence, where a larger market-sized fish of high quality is desired, production of all-female triploids may be an option for the commercial aquaculturist.

In Growth strain rainbow trout, sexual maturation occurs at about 1.5 years of age for males and at 2.0 years of age for females (Ferber, pers. comm. 1988). Therefore differential growth rates should begin to become evident in

spring or fall of 1988. In the fall of 1988 monitoring of diploid versus triploid survival and performance will begin. The results of this evaluation will help to assess the potential of triploid rainbow trout as a fisheries management option in South Dakota.

Another possible application of induced triploidy in fishes for aquaculture or management is to increase the survival and performance of interspecific hybrids. Purdom (1976) found that triploid plaice x flounder (Platicthys flesus) hybrids grew faster than normal diploid plaice over age 13-34 weeks. Triploid grass carp x bighead carp hybrids grew faster and had fewer deformities than their diploid hybrid siblings (Cassani et al. 1984). Scheerer and Thorgaard (1983) produced triploid and diploid interspecific crosses with rainbow, brook, and brown trout, and found that in most crosses triploid hybrids exhibited better survival than diploid hybrids. Triploid rainbow trout x coho salmon had better survival and increased resistance to infectious hematopoietic necrosis compared to diploid and triploid pure rainbow trout (Parsons et al. 1986). Triploid interspecific crosses could prove useful in fish culture because hybrid vigor and desirable attributes of two species might be combined in a sterile hybrid (Allen and Stanley 1981).

Swarup (1959b) demonstrated that the body organs of triploid threespine sticklebacks possessed fewer but larger cells than diploid organs of the same size. This increase

in cell size and decrease in cell number of triploids compared to same-size diploids extends to blood cells and as previously noted, red blood cell size has been used to assess ploidy level in fishes. The greater red blood cell volume but lower cell counts may have some physiological implications with respect to oxygen carrying capacity. Holland (1970) speculated that red blood cells of triploids would have a lower oxygenation rate than those of diploids due to a lower surface area to volume ratio. Graham et al. (1985) found that triploid Atlantic salmon had lower red blood cell counts and reduced hemoglobin concentrations, and that the maximum blood oxygen content of the triploids was only 67% that of normal diploid fish. Benfey and Sutterlin (1984c) reported that triploid Atlantic salmon did not differ from diploids in oxygen consumption rate, suggesting that the triploids compensated for reduced blood oxygen capacity by increasing cardiac output. This indicates that triploids may have increased metabolic energy demands compared to diploids. The effects of triploidy on the hematological characteristics of rainbow trout have not been reported but, if similar to those demonstrated in triploid salmon, may lead to physiological stresses which affect performance and as such they should be investigated.

CONCLUSIONS AND RECOMMENDATIONS

The heat shock procedures used in this experiment to produce triploid Growth strain rainbow trout varied widely in triploidy induction rates but several of the treatments produced high rates of triploidy with relatively high survival. The most successful treatment under the conditions of this experiment was heat shock at 28°C initiated 20 min after fertilization for a duration of 10 min. The increase in mortality associated with heat shock is not a major concern since rainbow trout eggs are, in general, readily available. High triploidy induction rates are, however, necessary in large-scale production of triploids so that fish do not need to be individually evaluated for ploidy level. The most successful treatments used in this experiment are likely applicable only in Growth strain rainbow trout and under comparable ambient conditions. Higher ambient temperatures would likely necessitate a shorter interval between fertilization and heat shock, whereas lower ambient temperatures might lengthen the period after fertilization when heat shocks are effective.

The techniques used for flow cytometric analysis differed from those previously reported, but were nonetheless effective. The use of HBSS and acridine orange appears to be a practical alternative in assessing ploidy level in fishes. Additionally, the use of fixed red blood

cells for flow cytometric analysis was shown to be effective, allowing more flexibility with regards to scheduling ploidy evaluation. Fixing blood samples also removes the necessity of bringing live fish to the laboratory for blood analysis.

Because a quantitative evaluation of triploid rainbow trout performance in wild conditions has not been documented, it is difficult to speculate what applications induced triploidy may have in rainbow trout management. Objectives for the evaluation phase of this experiment should include not just a determination of if improved growth rates occur in triploids, but also at what age does differential growth begin and to what degree is growth and performance affected by triploidy. Finally, further research is needed to assess the effects of triploidy on the hematological characteristics of fishes which may adversely affect performance.

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