SALMONIDS[14]

Project Component Termination Report for the Period
June 1, 1990 to August 31, 1996

NCRAC FUNDING LEVEL: $79,799 (June 1, 1990 to February 28, 1994)

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REASON FOR TERMINATION

The objective for this work on Salmonids was completed.

PROJECT OBJECTIVE

Evaluate all–female diploids and all–female triploids, and use brood stock developed in the region to produce all–female diploid and all–female triploid trout populations.

PRINCIPAL ACCOMPLISHMENTS

Efforts culminated in a 265–day grow–out trial at Southern Illinois University–Carbondale (SIUC) in which the performance of all–female triploid, all–female diploid, and mixed–sex diploid rainbow trout were compared. The results of the grow–out trial vindicated the NCRAC interest in all–female and all–female triploid rainbow trout.

The grow–out trial was initiated with approximately 100–g (3.53-oz) fish. Progeny from three families of all–female triploid and progeny from three corresponding full–sib families of all–female diploid trout were used in the trial. The mixed–sex diploid trout were progeny of three families that were half–sibs of the corresponding all–female diploid and all-female triploid families. Trout used in the grow–out trial were from crosses made at the University of Minnesota (UM), where they were also reared to 10 to 20 g (0.35 to 0.71 oz) prior to shipping to SIUC for the grow–out trial.

A water re–use system and twelve raceways were used in the grow–out trial, four raceways per treatment. Each raceway was stocked with 25 trout, but stocking densities were reduced to 15 trout per raceway on day 180 of the trial. Mean initial weights were 93.5, 84.2 and 111.6 g (3.30, 2.97 and 3.94 oz) for the mixed–sex diploid, all–female diploid, and all–female triploid, respectively. Mean initial lengths and weights did not differ among the three groups.

Growth was linear during the grow–out trial. Absolute growth rate was highest for the all–female triploid, intermediate for the all-female diploid, and lowest for the mixed–sex diploid, 2.38, 1.78, and 1.58 g/day (0.08, 0.06, and 0.05 oz/day), respectively ($P < 0.025$). Mean final weights were 520.5 g (1.15 lb) for the mixed–sex diploids, 567.5 g (1.25 lb) for the all–female diploids, and 748.9 g (1.65 lb) for the all–female triploids.

No significant differences ($P > 0.05$) were found in survival, food conversion ratios, condition factor, liver somatic index, visceral fat weight or dress–out percentage yield among treatments. By day 180 of the growth trial, most of the males in the mixed–sex diploid group were sexually mature, while the mixed–sex diploid females and the all–female diploids were still maturing. Based on subsamples of trout sacrificed at that time; mean gonadosomatic index for mixed–sex diploid males was 3.13, while values for the mixed–sex diploid females, all–female diploid and all–female triploid trout were 1.13, 1.86 and 0.38, respectively.

All–female diploid and all–female triploid trout show promise for practical trout farming. All–female trout production eliminates the problem of early maturation in males which leads to poor flesh quality and undesirable appearance, and results indicate that all–female diploid trout grow better than mixed–sex diploid trout. All–female triploid trout, however, grew the fastest. Farmers should consider all–female triploid trout production, especially those targeting markets utilizing larger trout.

The all–female triploid trout grew faster than the all–female diploids and mixed–sex diploids in growth trials, but aquaculturists also need to know how triploids perform in other respects to make decisions regarding their production and use. Many culturists produce food fish, but fingerling production for recreational fish stocking programs provides another market outlet for cultured fish. Harvest, crowding, handling, and hauling are problems inherent to fish–farming as well as to fish stocking programs.

Survival of triploids was evaluated during simulated transportation in one experiment with 33–g (1.16-oz) chinook salmon, another with 14–g (0.49-oz) coho salmon, and a third with 1.5–g (0.05-oz) rainbow trout. Triploids were produced via heat shocks. Both diploids and triploids were stocked into replicate containers in each experiment at densities recommended for transporting salmonids. Mortality was recorded every 30 min. Diploids had been exposed to heat shocks in the chinook salmon experiment, but not in the other two experiments.

Triploid chinook salmon died faster than diploids ($P < 0.005$). The maximum difference between mortality distributions (D_{max}) was 21.7%. Coho salmon triploids also died faster than diploids ($P < 0.005$). D_{max} occurred at 660 to 690 min, when 74% of the triploids were dead but only 47% of the diploids were dead. D_{max} in the rainbow trout experiment was only 6.7% ($P > 0.05$), indicating no difference. These results indicate that triploid rainbow trout can tolerate extreme environmental conditions about as well as diploids.

The reduced survival found for triploid chinook and coho salmon indicates that survival may be lower for triploids of these two species under some aquacultural conditions, and survival may also be reduced after stocking. Diminished survival, however, does not necessarily preclude the use of triploids in situations where natural stock protection is an important consideration in stocking programs or in site–selection for an aquaculture installation.

Early growth and survival was also examined for mixed–sex diploid, all–female diploid, and all–female triploid rainbow trout at UM and SIUC during earlier periods of this research. Mixed–sex diploids and all–female diploids early growth and survival was also examined by Seeb at the Fort Richardson State Fish Hatchery, Anchorage, Alaska, under practical fish culture conditions.

Survival through the eyed stage was relatively high (83 to 97%) for mixed–sex diploid, all–female diploid, and all–female triploid eggs in the UM study. Survival through the eyed stage was significantly lower for all–female triploids, in comparison to mixed–sex diploids, but only by approximately 13%. All–female triploids survived as well or better than mixed–sex diploids and all–female diploids after hatching, and growth through 14 weeks did not differ between mixed–sex diploid, all–female diploid, and all–female triploid trout ($P > 0.05$).

Cumulative mortality from fertilization through hatching, yolk–sac absorption, and up to 0.5 g (0.02 oz) was about 30% higher ($P < 0.05$) for all–female triploids, as compared to mixed–sex diploid and all–female diploid trout in the SIUC trial. Differences in mortality appeared to primarily occur prior to hatching and, secondarily, during yolk–sac absorption. These results, in conjunction with those obtained at UM, suggest that the triploidy induction procedure, the heat shock, was the primary factor responsible for the increased mortality. Mortality did not differ ($P < 0.05$) after hatching through growth to 0.5 g (0.02 oz) at SIUC.

A 240–day growth trial initiated with the mixed–sex diploid, all–female diploid, and all–female triploid trout once they reached 0.5 g (0.02 oz) was then conducted at SIUC. There were no mortalities in mixed–sex diploid, all–female diploid, and all–female triploid trout during the 240–day growth trial. This pattern of similar survival after yolk–sac absorption between diploid and triploid rainbow trout was confirmed in the UM study, in the simulated transportation experiment (above) and in the grow–out trial. The trout grew from 0.5 g (0.02 oz) to approximately 2.7 to 3.0 g (0.10 to 0.11 oz) during the trial. Growth did not differ between mixed–sex diploid, all–female diploid, and all–female triploid trout ($P < 0.05$). Feed conversion efficiencies (wet weight of fish/dry weight of feed) did not differ during the 240–day growth trial; they ranged from 93% for the mixed–sex diploids to 99% for the all–female diploids. Feed conversion efficiency was 96% for the all–female triploid trout.

Findings in the UM and SIUC studies show that survival in triploid trout is somewhat diminished prior to the onset of exogenous feeding. However, the economic loss associated with this additional mortality in all–female triploid trout prior to exogenous feeding is minor, since numbers of eggs generally are not limiting in rainbow trout culture, and relatively little investment in rearing costs occurs prior to feeding. The additional production costs associated with this early mortality is more than offset by the better growth during grow–out. All–female triploid trout do not undergo sexual maturation, so the retention of good flesh quality and appearance is reason enough for producing them.

The Fort Richardson State Fish Hatchery study confirmed no differences in survival between mixed–sex diploid and all–female diploid trout following yolk–sac absorption and through 349 days of age; survival for mixed–sex diploid and all–female diploid trout exceeded 90% during the trial. The growth trial was divided into three phases (83, 148 and 349 days) because numbers of trout per replicate were reduced twice as they grew. Mean weights for the mixed–sex diploid and all–female diploid trout, respectively, were 4.1 g (0.14 oz) and 3.7 g (0.13 oz) at 83 days; 32.4 g (1.14 oz) and 27.8 g (0.98 oz) at 148 days; and 81.0 g (2.86 oz) and 67.8 g (2.39 oz) at 349 days of age. Growth did not statistically differ between mixed–sex diploid and all–female diploid trout. Food conversion efficiency also did not differ between mixed–sex diploid and all–female diploid trout; it ranged from 65.3 to 73.7% during the first two phases.

The results of the Fort Richardson State Fish Hatchery study were consistent with findings in the UM and SIUC pre–maturation trials with rainbow trout. Prior to the onset of sexual maturation, mixed–sex diploid and all–female diploid trout differ little in survival and growth.

To determine why all–female diploid and triploid rainbow trout grow faster than mixed–sex trout during grow out, two lines of investigation were pursued at SIUC. One investigation examined daily activity patterns and activity intensity in mixed–sex diploid and all–female diploid trout, and the other studied muscle cell growth dynamics in diploid and triploid trout.

Although adult all–female diploid trout showed activity levels higher than mixed–sex diploids at lower water temperatures, the reverse was true at the higher temperatures (above 12.5°C; 54.5°F) at which this species is typically cultured. This means that all-female diploid trout have more dietary energy available for growth at culture temperatures, because they waste less energy on nonessential swimming. Another, and perhaps the most important, reason why all–female diploids outgrow mixed–sex diploids is that rainbow trout males mature and slow their growth earlier than females, due to the investment of energy into gonadal tissues and development of secondary sexual characteristics.

Muscle fiber growth dynamics in triploids is of interest, because whole–body growth occurs via two processes: (1) increased size of muscle fibers or hypertrophy, and (2) increased numbers of fibers or hyperplasia. Fish show what has been referred to as indeterminate growth; i.e., they are capable of hyperplastic and hypertrophic growth even after adulthood, whereas postnatal growth occurs only by hypertrophy in other vertebrates. In fish, however, hyperplastic growth eventually ceases, but the longer a species is capable of hyperplastic growth, the larger its ultimate size and the faster its growth.

Muscle fiber growth dynamics were examined in triploid rainbow trout using both biochemical (RNA, DNA, and protein measurements) and histological (muscle fiber diameter sizes) approaches. It is believed that this is the first time that muscle cell growth dynamics has been investigated in any triploid animal.

Triploid trout less than 30 cm (11.8 in) in total length showed muscle fiber size distributions which differed from diploids. Specifically, triploid hyperplastic muscle fibers were larger than those of diploids. However, the difference in fiber size distributions diminished as the trout grew, and it disappeared in larger trout where hyperplasia plays only a small role in growth. This increase in hyperplastic muscle fiber size results in a decrease in the cellular surface area to volume ratio which may be unfavorable to metabolic exchanges between the cell and its external milieu. Poorer survival in triploids during early life may be linked to the increase in hyperplastic muscle fiber size. Another potential disadvantage for triploids is that their muscle cells (which are multinucleate) appear to have fewer nuclei per muscle cell. This study also showed that larger diploid and triploid rainbow trout have similar growth capacities; i.e., they are capable of growing to the same maximal size and at the same rate, all else being equal. This suggests that the superior growth in all–female triploid trout is not due to any inherent differences in growth capacity. Rather, it is probably because triploid female rainbow trout do not direct dietary energy into gonadal growth and the development of secondary sexual characteristics.

SIUC researchers also found that RNA concentrations did not differ between diploids and triploids growing at the same rate and that protein concentrations did not differ in diploid and triploid muscle tissues. This indicates that the rate of protein synthesis does not differ between diploids and triploids, despite the latter having fewer nuclei per cell. This further suggests that genes of the third set of chromosomes in triploids are expressed to compensate for the reduced number of nuclei in triploid muscle cells. Meiotic gynogenesis, followed by sex reversal, is an important initial step in the production of brood stock for all–female rainbow trout production, because it ensures all XX progeny. However, meiotic gynogens exhibit poor viability and growth, because they are highly inbred; a level of inbreeding roughly equivalent to several generations of full–sib matings. Gynogenesis followed by sex–reversal does ensure the production of 100% all–female progeny, but it is inefficient to use gynogenesis for the continuing production of brood stock. A far better approach is to sex–reverse all–female progeny produced from an outcross between an XX sex–reversed male gynogen and a normal XX female, because the outcross eliminates inbreeding depression. The progeny can thus be much more successfully and efficiently raised to sexual maturation.

SIUC researchers shipped about 500, 5–cm (2.0-in) sex–reversed XX males to the Seven Pines Trout Hatchery. Since these were the progeny from an outcross between XX Isle of Mann males and XX Seven Pines females, their viability should be excellent. However, only about 20 pairs of trout were used to produce the 500 progeny, substantially lower than the number of brood stock required to ensure sufficient genetic diversity for aquacultural purposes. Genetic diversity needs to be increased in the XX male brood stock at some future time before it can truly be said that a regional brood stock for all–female production has been established.

IMPACTS

Studies of all–female diploid rainbow trout demonstrate that:

- All–female diploid trout grow and survive as well as mixed–sex diploid trout during early life.

- Declines in flesh quality and appearance due to sexual maturation occur earlier in mixed–sex diploid than all–female diploid trout.
- All–female diploid trout grow faster than mixed–sex diploids through grow out, and survival is similar.
- All–female diploid trout show reduced non–essential activity at culture temperatures above 12.5°C (54.5°F), possibly accounting in part for their better growth as compared to mixed–sex diploid trout.

Studies of all–female triploid rainbow trout showed that:

- All–female triploid trout show somewhat reduced survival through yolk–sac absorption; production of all–female triploids via crosses of tetraploids with diploids may reduce or eliminate this problem.
- Survival beyond yolk–sac absorption in all–female triploid trout is similar to mixed–sex diploid trout under normal conditions, and it was also similar under adverse conditions in simulated transportation tests.
- All–female triploid trout showed the anticipated reduced gonadal growth.
- All–female triploid trout were clearly superior to mixed–sex diploids and all–female diploids during grow out through market size.
- Studies of muscle cell growth dynamics indicate that there is no inherent difference in the capacity for growth between diploid and triploid rainbow trout; the superior growth in all–female triploid trout appears to be primarily due to their failure to undergo sexual maturation.
- All–female triploid trout production may be the best choice for regional farmers, given their superior growth over mixed–sex diploid and all–female diploid trout.
- All–female triploid trout production appears to be an especially strong option for farmers interested in producing a larger trout, since they grew faster than mixed–sex diploid and all–female diploid trout in these studies, and all–female triploids should not show declines in flesh quality and appearance which accompany sexual maturation in mixed–sex diploid and all–female diploid trout.

RECOMMENDED FOLLOW–UP ACTIVITIES

All–female diploid and triploid rainbow trout show considerable promise for commercial aquaculture, especially in regions where breeding programs have not selected for stocks which mature at a larger size. Female rainbow trout mature at a larger size than males, so all–female diploid production reduces problems such as the declines in flesh quality and appearance prior to market size. All–female diploid trout also grew faster than mixed–sex diploid trout to market size in these studies. Producers interested in producing larger trout should give strong consideration to all–female triploid production, since the problems associated with sexual maturation appear to be forestalled indefinitely, and all–female triploid trout grew the best through grow out in these studies.

Cost–effective all–female triploid and all–female diploid production in the NCR will necessitate farmers to develop brood stocks for producing all–female diploid and all–female triploid fry. This will require production of sex–reversed gynogens for all–female production and tetraploid production for crosses with diploids to produce triploids. Field trials which compare all–female triploid, all–female diploid, and mixed–sex diploid trout would enable farmers to determine the best choice for production stocks in commercial aquaculture settings.

Hence, the following activities are suggested for follow–up:

- further production of sex–reversed gynogen brood stocks,
- production and evaluation of tetraploid brood stocks, and
- production trials for mixed–sex diploid, all–female diploid, and all–female triploid trout in commercial aquaculture settings.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED
See the Appendix for a cumulative output for all NCRAC-funded Salmonid activities.

SUPPORT

YEARS	NCRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
1990-91	$39,299	$22,669		$3,000[a]		$25,669	$64,968
1991-92	$20,500	$13,265		$5,000[b]		$18,265	$38,765
1992-93	$20,000	$14,960				$14,960	$34,960
TOTAL	$79,799	$50,894		$8,000		$58,894	$138,693

[a]Seven Pines Trout Hatchery for time, use of rearing facilities, feed, and fish
[b]Alaska Fish and Game for time, use of rearing facilities, feed, and fish.

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Publications in Print

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Manuscripts

Barry, T.P., T.B. Kayes, T.E. Kuczynski, A.F. Lapp, L.S. Procarione, and J.A. Malison. Submitted. Effects of high rearing density and low-level gas supersaturation on the growth and stress responses of rainbow and lake trout. Transactions of the American Fisheries Society.

Procarione, L.S., and J.A. Malison. In preparation. Effects of rearing density and loading on the growth and stress response of rainbow trout. Aquaculture.

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Papers Presented

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