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# Development of tetraploid rainbow trout may yield improved triploid production

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## Several different lines can be tested for production of improved triploid offspring

Variation among tetraploid parents can be exploited by selection to yield enhanced production traits in their offspring.

Tetraploid animals have four sets of chromosomes, triploids have three sets, and diploids have two sets. In rainbow trout, this generally equates to 120, 90, and 60 chromosomes, respectively.

As part of a selection and breeding program to develop strains of rainbow trout with improved production efficiency, the authors are investigating the development of tetraploid strains that, when crossed with the appropriate diploid strains, create more productive triploid fish.

The benefits of using triploids for production purposes are twofold. First, since triploids are sterile, losses due to early reproductive maturation are largely eliminated and additional growth is achieved when growing larger fish. With rainbow trout, some of the loss due to sexual development in males can be overcome by utilizing all-female populations. Second, the problems associated with escapement and reproductive interactions with natural populations are minimized with sterile fish.

## Increased demand for triploids

Because of these benefits, the increasing demand for triploid all-female rainbow trout is being met commercially by the treatment of eggs shortly after fertilization. This produces triploid fish by retention of the second polar body.



However, while triploid treatments provide generally good yields, there is no guarantee they will be 100 percent consistent. Further, the triploid animals sold for production are subjected to a rather severe treatment whose potential impacts on performance have not been fully defined.

Crossing tetraploid parents with diploids to yield triploids circumvents both of these issues. One hundred-percent triploids result from every cross, and the fish utilized for production are not subject to any treatment. These two factors, as well as the opportunity to select and breed both parental lines for improved performance suggest the development of tetraploid strains for this purpose will be beneficial.

## Reliable tetraploid procedures

One of the first hurdles to be overcome in the authors' research was to devise treatment procedures that reliably induced tetraploids in different rainbow trout strains, as results from previous research showed major variations in strain response to standard tetraploid induction procedures. Since the genetic improvement of tetraploid lines requires the availability of genetic variation, it would be desirable to induce tetraploidy in a variety of strains and utilize crosses among them as base-line material for further genetic selection and breeding.

Initial investigations revealed differences among rainbow trout strains with respect to the timing of the first mitotic division during very early development. This stage of development, when embryos are treated to induce tetraploidy, occurs eight or nine hours after fertilization in rainbow trout incubated at

10 degrees-C. The exact time of first mitotic division depended on the population used and temperature of the incubation water. Basing the treatment time on a direct measurement of the time to first cleavage has led to more consistent production of tetraploid animals.

The induction treatment incorporates hydrostatic pressure applied with a 20-ton hydraulic press and stainless steel pressure cell. The current protocol is to treat fertilized eggs with 9,000 psi for eight minutes starting 65 percent of the time required to reach first cleavage to the two-cell stage.

Results from the protocol, which incorporates cleavage time adjustment, have revealed consistent tetraploid induction (Table 1), although improved post-treatment survival values might mandate further adjustments. The procedure has resulted in over 300 tetraploid rainbow trout from several different lines that can be tested for production of improved triploid offspring.

## Hershberger, Tetraploid induction and survival, Table

| Pressure (psi) | Treatment Duration (minutes) | Treatment Initiation Time (minutes) | Tetraploid Induction (%) | Survival (%) |
|---|---|---|---|---|
| 7,000 | 8 | 320 | 0 | 0 |
| 8,000 | 8 | 320 | 10.0 | 0.2 |
| 9,000 | 8 | 320 | 25.0 | 0.5 |
| 9,000 | 8 | 320 | 50.0 | 1.0 |
| 9,000 | 8 | 325 | 100.0 | 5.0 |
| 9,000 | 8 | 325 | 100.0 | 2.0 |
| 9,000 | 8 | 325 | 88.1 | 17.3 |

Table 1. Tetraploid induction and survival of rainbow trout embryos.

## 2005 research

Crosses among and within a limited number of tetraploid and diploid rainbow trout lines were conducted in early 2005. As expected, these crosses yielded 100 percent triploids regardless of the sex of the tetraploid parents used, and tetraploids crossed with tetraploids yielded 100 percent tetraploids. The number of abnormalities among these crosses was similar to those among the normal diploid crosses, and the mortality of the offspring was higher when a male rather than female tetraploid was used to make triploids (Table 2). This was expected to be a function of the larger sperm in tetraploids, making fertilization a little more problematic.

## Hershberger, Mortality and abnormality results, Table 2

| Cross (Female X Male) | Offspring Ploidy (%) | Crosses | Average Mortality (%) | Average Abnormality (%) |
|---|---|---|---|---|
| 2N X 2N | 2N (100) | 6 | 44.0 | 0.2 |

| | | | | |
|---|---|---|---|---|
| 2N X 4N | 3N (100) | 10 | 94.0 | 0.1 |
| 4N X 2N | 3N (100) | 4 | 73.0 | 1.0 |
| 4N X 4N | 4N (100) | 6 | 85.0 | 1.0 |

Table 2. Mortality and abnormality results in offspring from crosses of rainbow trout.

The offspring of these crosses were utilized in a small growth study to compare the performance of the triploids produced with the diploids of the same genetic stocks. Initial results indicated that triploids from some of the crosses grew faster than the diploids, whether they were grown with diploids or by themselves.

# Tetraploid production promising

To this point, experimental results suggested promising potential to create tetraploid rainbow trout strains to yield improved triploids for production. The research protocol seems to be more consistent than previous reports in its capacity to produce tetraploids. Further, variation among the tetraploid groups may be exploited by selection to yield enhanced production traits in their offspring. The authors are also looking into a procedure for yielding all-female groups of triploids when crosses with tetraploids are used.

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