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Gene Conservation Laboratory

Triploid Rainbow Trout

Making All Female Sterile Rainbow Trout

The Sport Fish Division and the Gene Conservation Laboratory have implemented a breeding program to produce sterile fish for stocking lakes and streams to enhance angling opportunities. It is important to ensure that hatchery fish do not interbreed with wild fish and dilute their [genetic makeup](#) (PDF 47 kB).

Alaska sport fish hatcheries release over one million rainbow trout annually to enhance inland sport fisheries. One way to ensure these fish do not establish new populations or hybridize with wild fish is to produce sterile triploid fish that are also all females. Triploid fish have three sets of chromosomes instead of two sets.

The production of all-female triploid fish is accomplished in several steps

Step One:



Female fry, which have an XX chromosome complement, are changed into morphological males by feeding them male hormones. We refer to these sex-reversed fish as XX males. Even though these fish are genetically female they produce sperm instead of eggs. These sperm contain only X chromosomes and no Y chromosomes (the "male" chromosome"). When sperm from an XX male is used to fertilize eggs, the offspring are all female. After they are sacrificed for their sperm, these fish are discarded – they are never stocked.



Here the testes from XX males are being crushed to release sperm so they can fertilize the eggs.



Step Two:



Eggs are gently squeezed from a female rainbow trout. These eggs each contain two sets of chromosomes, at this time.

Step Three:



The eggs are fertilized with the sperm collected earlier. After a brief moment, the eggs are then gently rinsed to remove excess sperm and bacteria.

Step Four:



Exactly twenty minutes after fertilization, eggs are placed in a warm water bath maintained at 26°C (79°F) for 20 minutes. This warming prevents the extrusion of the third set of chromosomes by disrupting a process called the "spindle fiber formation". The resulting fish have three sets of chromosomes rather than two and are called "triploids". Because these fish have only X sex chromosomes, they are all females.

Step Five:



After a warm bath, the egg trays are placed in stacks for incubation. After several weeks the eggs develop into fry.

Step Six:



The groups of fish are certified for their levels of tripoidy before being released.

Step Seven:



The fry are placed into rearing tanks, and grow into fingerlings, which are released into lakes and streams. Because the fish are mature sexually they will not interbreed with wild rainbow trout.

his "heat-shock" method of producing triploids can result in a low number of fish that are diploid (two sets of chromosomes). But by creating all-female fry, we ensure that these non-triploid fish will not establish new populations in places where wild rainbow trout are not present.



The smile on this angler's face says it all. Triploid all female fish are good angling and good eating.

What is a triploid fish?

Cells that are destined to become eggs in a female rainbow trout undergo a series of cell divisions called "meiosis". These special cell divisions lead to a reduction in the number of chromosomes in preparation for fertilization which restores a normal complement of chromosomes. Meiosis is not completed, however, until the eggs are fertilized. At the time of spawning, rainbow trout eggs have two sets of chromosomes. The second set of chromosomes is usually kicked out of the nucleus to form a "barr body" (as in humans). However, gently heating the eggs prevents the barr body from being expelled by disrupting the small fibers that pull the chromosomes apart. The result is that each egg has two sets of chromosomes. Fertilization with a male sperm, which has one set of chromosomes, produces a 'zygote' with three sets of chromosomes. Triploid fish are similar to other fish, except they are unable to reproduce, because they cannot produce functional gametes.

What is a Triploid Fish?

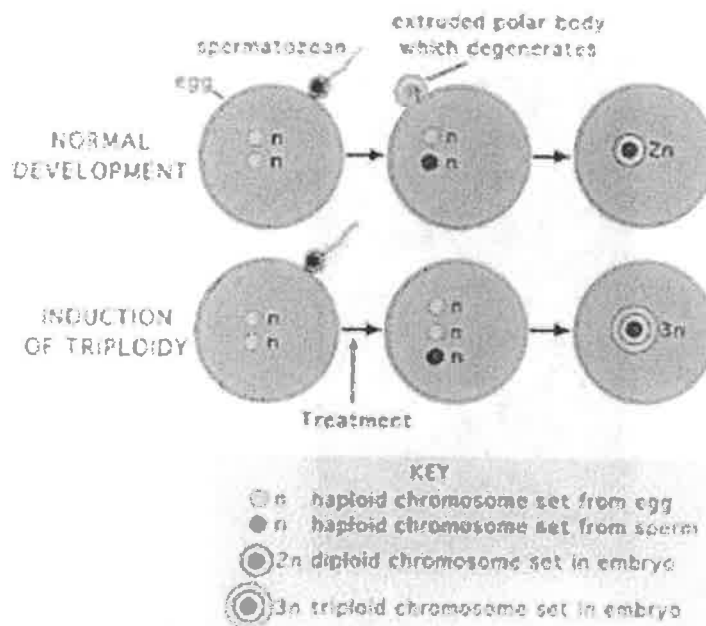
In simple terms, a triploid fish is merely a fish that is sterile. Triploid fish have three sets of chromosomes, unlike a fertile fish that have two sets of chromosomes (a diploid fish). Triploids are common in many industries; Seedless watermelons are triploids, as are bananas. Triploids are also naturally occurring.

A triploid fish is not a genetically modified organism. The genes of the fish have not been manipulated or changed in any way and do not result in the expression of any foreign or novel proteins or tissues. Triploid fish simply have 3 sets of chromosomes instead of 2 and for this reason are not able to reproduce, but are otherwise normal fish.

Recent legislation codified in the Fish and Game Code describes the rationale to use sterile fish for recreational purposes. These include the conservation of native fish genotypes (genetics) and the protection of endangered or threatened species. For these and other reasons the California Department of Fish and Wildlife is producing triploid trout eggs that hatch into fish, are raised, and ultimately result in sterilized trout released for recreational purposes.

Triploid trout are created by forcing the egg to retain a chromosome that is normally ejected during egg development. There are many ways to do this; the California Department of Fish and Wildlife uses the pressure shock treatment method.

As you can see in the graphic below, in fertilized trout eggs, normally a chromosome (N) is kicked out of the egg as a polar body at some stage of development. Using pressure treatment at a specific time in the egg development, the polar body and chromosome is retained. With 3 chromosomes the fish is sterile and cannot reproduce.



After pressurization eggs are put into vertical flow incubator stacks for hatching.



After the eggs hatch, they are raised like any other trout. Studies have shown that after 3 years of age, triploid trout tend to grow larger than a non-sterile trout due to less energy being expended for mating purposes.



For more information as to why we have begun stocking triploid fish please follow these links.

[Pre-stocking Evaluation](#)

[Senate Bill 1148](#)

[Environmental Impact Reports/Surveys](#)