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Induction and Verification of Triploidy in Fish

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Use of sexually sterile fish would be advantageous for several applications, such as: 1) controlling reproduction of exotic species; 2) preventing potential backcross of hybrids with either parent species resulting in intermingling of genetic material; and 3) improving growth of aquaculture species because less energy is diverted for reproduction.

A need for sexually sterile fish has prompted research in the production of triploid fish. The cells of triploid individuals have three sets of chromosomes (3n) as compared to the normal two sets (2n). Induction of triploidy has been reported in many fish species (e.g., rainbow trout, coho salmon, chinook salmon, pink salmon, Atlantic salmon, channel catfish, African catfish, plaice, grass carp, bighead carp, loach, tilapia).

Triploid sterility is thought to result from the production of chromosomally imbalanced eggs or sperm. For the processes of egg and sperm formation through embryo development and hatching to proceed successfully, the genetic code of the eggs and sperm must be perfect. The additional genetic material in the reproductive cells of triploid fish results in abnormal development that can abort the reproductive process at any stage.

Studies of triploidy in several species suggests that the development of the gonads is inhibited, and the inhibition appears to be more pronounced in females than in males. Although sperm may be produced by triploid individuals, it is highly unlikely that viable embryos will be produced if spawning occurs.

Successful induction of triploidy in many species of fish and the occurrence of natural triploid fish suggests that they are capable of survival and growth. Most studies of triploid fish have shown that they have normal or only slightly diminished survival and growth as compared to normal diploids. For some species, the growth rate of triploid fish was greater than for diploids, after the fish reached sexual maturity.

Mechanism for triploid induction

Induction of triploidy occurs after the eggs are ovulated and the sperm enters the eggs. Before ovulation, the nuclei of eggs in the ovary of fish have 4n number of chromosomes. Ovulation starts with the disappearance of the nuclear membrane in the egg and the appearance of chromosomes, leading to the first meiotic division. The first polar body is formed and pushed out of the egg, resulting in a reduction of chromosomes to 2n. The follicles, which attach the eggs within the ovary, split and partially dissolve, releasing the eggs. The eggs then flow through the genital opening of the female fish. After the sperm enters the egg through the micropyle, the genetic content of the nucleus of the egg is further reduced to 1n when the second polar body is formed (second meiotic division) and ejected from the egg. The genetic material of the sperm (1n) and the nucleus of the egg (1n) then combine to form a developing embryo.

Triploid fish have been produced by preventing the second meiotic division of the egg (after the sperm enters the egg). Therefore, two sets of chromosomes are contributed by the female and one set by the male (2n egg + 1n sperm = 3n). This procedure is usually accomplished through chemical, thermal, or mechanical methods. Chemical methods are primarily experimental and restricted to research laboratories. Thermal and mechanical methods are commonly used in production facilities. Triploid fish are commercially produced by applying thermal or pressure shocks shortly after water is added to the egg and sperm mixture. The precise timing of the second meiotic division and thus, the application of treatment to induce triploidy, varies with the species of fish and water temperature.

Triploid production

Hydrostatic pressure is presently the most consistent method for wide-spread commercial production of triploid grass carp and rainbow trout. Pressure shocks are usually administered using a stainless steel cylindrical vessel closed by a brass piston fitted with an O-ring, pressure gauge, and relief valve. An external hydraulic press is used to apply pressure to the piston (Figure 1).

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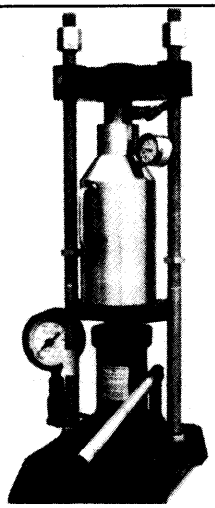

Figure 1. Hydrostatic pressure chamber and press used to treat fertilized eggs to produce triploid fish.

Optimum parameters for triploid induction of grass carp are reported to be 7,000 to 8,000 pounds per square inch (psi) for a duration of up to 90 seconds, starting 4 to 5 minutes after water is added to the eggs and sperm at 26°C (79°F). Decompression is instantaneous at the end of the treatment, and the eggs are then transferred to the incubation apparatus.

A small quantity of fertilized grass carp eggs is not pressure treated and is incubated separately as a control group. There is a strong relationship between egg viability of the control group and the ability to produce triploid grass carp, suggesting an egg quality factor. Egg quality is the general state which affects egg viability and ability to be fertilized. It has been documented that the quality of grass carp eggs is affected by the condition of the brood female, seasonal water temperature changes, and the timing of hand stripping in relationship to the time of ovulation. Egg quality, as measured by egg viability of the control group at 20 hours after fertilization, provides a good indicator of successful triploid grass carp induction by hydrostatic pressure shocks. When the egg viability of the control group is greater than 60 percent, hydrostatic pressure shocks usually result in 80 to 100 percent triploid fish with a hatch relative to controls of nearly 70 percent, at the optimum treatment parameters reported above. Few if any triploids are produced when the viability of control eggs from the same spawn is below 40 percent.

All-triploid progeny of rainbow trout have been induced by pressure shocks of 7,000 psi applied 40 minutes after water is added to the eggs and sperm, for a duration of 4 minutes, at 9.4°C (49°F).

Triploid verification in grass carp

Because hydrostatic pressure treatments seldom result in 100 percent triploid grass carp, each individual must be verified triploid before they can be stocked in the waters of many states. Triploidy in grass carp is commonly verified by taking a blood sample and analyzing the volume of red blood cells, using an electronic particle counter.

Blood samples can be taken from grass carp greater than 50 millimeters (2 inches) long. Fish may be anesthetized if necessary. The head of the fish is tilted back and the branchial artery on the isthmus is punctured with a blood lancet rinsed in EDTA between samples. Approximately 1 microliter of blood is withdrawn using either a positive displacement micropipette with disposable polypropylene tips (Figure 2) or disposable hematocrit capillary tubes.

The blood sample is immediately placed in a vial containing an electrolyte solution (e.g., Hematall Azide-Free Isotonic Diluent™, Fisher Scientific®). The cell membrane of the red blood cells are dissolved (lysed) with a lysing agent (e.g., Hematall LA-Hgb Reagent™, Fisher Scientific®), leaving the nuclei. The lysed blood cells are immediately scanned for ploidy determination with an electronic particle counter, calibrated to read both diploid and triploid red blood cell nuclei volumes. Diploid grass carp have a red blood cell nuclei volume of 10.06 cubic micrometers (μm3), while the mean volume of triploid red blood cell nuclei is 14.82 μm3.

Figure 2. Obtaining a blood sample from each grass carp to be analyzed for triploidy.

Conclusions

Sexually sterile fish are advantageous for controlling reproduction of exotic species, preventing the potential backcross of hybrids with either parent species resulting in intermingling of genetic material, and improving growth of aquaculture species. Interest in sexually sterile fish has prompted research into production of triploid fish. Triploids have been produced by preventing the second meiotic division after the sperm enters the egg, thereby resulting in two sets of chromosomes from the female and one set from the male. Triploid induction technology is used for wide-spread commercial production of grass carp and rainbow trout. Hydrostatic pressure is presently the most consistent method for the commercial production of triploid grass carp. Because these treatments seldom result in 100 percent triploid fish, each individual must be verified triploid before they can be stocked.

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