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A review on induction of triploidy in fish using heat, pressure and cold shock treatments

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Abstract
Induction of triploidy in fishes is widely accepted as the most effective method for producing sterile fish for aquaculture and fisheries management. Triploidy induction refers to the production of individuals with three sets of chromosomes and can be induced in fish by inhibiting the second meiotic division followed by the extrusion of the second polar body by shocking eggs shortly after fertilization using various physical shocks and chemical treatments. Cold shock treatment and heat shock treatment for triploid induction are safe because no chemicals are used. However, these treatments require an optimal treatment temperature to be maintained in a large volume in order to treat a large quantity of eggs. Pressure treatment is also considered to be safe. However, in pressure treatment specific equipments are required and it is usually difficult to treat many eggs on a commercial scale.

Keywords: Triploidy, fertilization, heat shock, pressure shock, cold shock

Introduction
Triploidy is the most common form of polyploidy, which refers to those organisms or cells having three sets of homologous chromosomes. Triploidy is widely accepted method for producing sterile fish for aquaculture and fisheries management. Triploidy induction for aquaculture has been applied to several species of salmon [18] trout [8] and catfish [18, 23]. African catfish (*Clarias gariepinus)* is one of the most important tropical cultured fish due to high growth rate, high stocking-density capacities, high consumer acceptability and high resistance to poor water quality and oxygen depletion [1, 2].
Triploid fishes (3n) have two sets of chromosome of maternal origin (2n) and one set of chromosomes of paternal origin (1n). Triploidy induction refers to the production of individuals with three sets of chromosomes and can be induced in fish by inhibiting the second meiotic division followed by the extrusion of the second polar body by shocking eggs shortly after fertilization [11, 28]. Cold shock treatment [42, 49] and heat shock treatment [30] for triploid induction are safe because no chemicals are used. However, these treatments require an optimal treatment temperature to be maintained in a large volume in order to treat a large quantity of eggs. Pressure treatment is also considered to be safe [26]. However, in pressure treatment specific equipments are required and it is usually difficult to treat many eggs on a commercial scale. The mating of normal diploid and tetraploid fish is an alternative method for producing hybrid triploids Triploid individuals are expected to be functionally and endocrinologically sterile. Such sterility of triploid fishes (both male and female) can be of benefit to aquaculture. The blocking of complete gametogenesis particularly in female triploids during early meiotic division results in complete inhibition of oocyte development and functional sterility [20, 37, 43]. Despite gametic sterility triploid males due to meiotic inhibition of spermatogenesis, in fish species a proportion of such males are able to produce abnormal and aneuploid sperm. This ultimately leads to reproductive sterility of these males [20, 37, 43]. Production of all-female sterile triploid (3N) fishes can be important to commercial aquaculture because it mitigates negative impacts associated with gonadal maturation including immune depression, directing energy and nutrients from somatic growth, and decreased fillet quality [6, 7, 35]. Sterility of triploids can also serve to protect the intellectual property resulting from selective breeding of commercial stocks or achieve genetic containment of genetically engineered organisms.

The most common strategy for producing 3Ns in salmonids is applying a pressure or temperature shock to the egg just after fertilization to induce the retention of the second polar body during meiosis II, designated induced-triploids (3NP). An alternative strategy for producing 3N fish is to mate a tetraploid (4N) parent to a diploid (2N) parent. The resulting 3N progeny are designated intercross-triploids (3NC).

Working of triploidy

To produce triploid fish, newly fertilized eggs that have not yet shed the polar body are subjected to temperature or pressure shocks. These shocks prevent the shedding of the polar body so this extra chromosome set is retained in the fertilized egg and replicated in every cell of the developing embryo. The embryo then has three sets, one from the egg, one from the sperm, and the extra maternal set from the polar body [3].

Uses of triploidy

- 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.
  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 [42]. Sterility allows the fish to avoid the growth depression and increased mortality normally associated with maturation in fishes [38].
- 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 [42].
- 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.

Induction of triploidy using heat shock

Heat shock is an effective and widely used method for polyploid induction in fish. The heat shock is one of the common technique in triploidy of fish, but the application of heat shock to induce triploidy is not always 100% effective and can cause a detrimental side-effect and decreased viability. Other forms of ploidy and tetraploidy have been used to facilitate triploid production. Induction of triploidy in rainbow trout (*Oncorhynchus mykiss*) by heat shock has been reported by various authors. Chourrout (1980), Solar *et al.* (1984), Dillon (1988), Don and Avatalion (1986), Dogankaya and Bekcan (2014), Guner *et al.* (2016) induced triploidy in rainbow trout by heat shock. Chourrout (1980) reported that the heat shocks proved to be efficient for all tested temperatures; however, the hatching rate reached higher values at and above 26 °C and good hatching rates were obtained with the higher temperatures 30 to 50 min. after, or immediately after, fertilization. The 40 fingerlings analyzed were diploid. When the eggs were fertilized with normal sperm, a good triploidization rate was induced by means of heat shocks (27-30 °C lasting 10 min). It is supposed that the retention of the second polar body caused the gynogenetic diploids observed. Solar *et al.* (1984) reported that heat shock treatments using 26 and 28 °C for 10 min, 1 or 40 min post-fertilization yielded 83-100% triploids. The heat shocks applied at 24 °C either at 1 or 40 min, were less successful in inducing triploidy (18-30%). The high temperature shock (30 °C) produced 67% triploids when applied 1 min after fertilization. All the embryos of the groups shocked at 30 °C 40 min after fertilization died prior to hatching. The difference in the percentage of yolk sac absorption might be primarily due to differences of heat-shock application times. Dillon (1988) reported that 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 ºC beginning 20 min after fertilization for a duration of 10 min, which resulted in 60.5% survival to feeding and 100% triploidy induction. Pradeep *et al.* (2012) studied triploidy induction by heat-shock treatment in red tilapia. The best survival rate (67.0%) and triploid percentage (89.7%) was observed for the treatment at 4 minutes after fertilization. It was successfully demonstrated that 4 minutes after fertilization was the most suitable timing of heat-shock treatment for second polar body retention in newly fertilized eggs of red tilapia. Chandran *et al.* (2014) reported that heat shock induction of triploidy in the indigenous ornamental fish, (*Pseudosphromenus cupanus*) resulted in 75% triploid percentage. The eggs treated for 5 minute duration showed low survival rate. Don and Avtalion (1986) reported that triploid fish were obtained using heat-shock treatment. The optimal conditions for the heat shock (39.5±0.2 ºC for 3.5-4 min) as well as the exact zygote age (3 min) at which this heat shock was applied were studied. Results showed that this treatment gives rise to 100% of triploid fish with a satisfactory survival rate of 61% beyond the yolk sac resorption. Marx and Sukumaran (2007) induced triploidy in African catfish, (*Clarias gariepinus*) using heat shock, Maximum percentage of triploids (91.4%) was obtained in the heat shock at a temperature of 40±0.5 ºC for duration of 1 min. Uma and Chandran (2008) induced triploidy in *Gymnocorymbus ternetzi* (Boulenger). The best triploidy rate (63.6%) and yield (58.2%) were obtained with the heat shock regime of 38 °C for 4 minutes at 2.75 minutes of zygote age. Aruljothi (2015) induced triploidy in rohu, (*Labeo rohita*). Heat shock treatment at 38 °C for 1 min was found to have effectively induced triploidy upto 81.3% Venkatachalam *et al.* (2015) induced triploidy in catfish (*Clarias batrachus*) through cold shock and heat shock. Maximum Number of triploids (3n=75) 89% were obtained at temperature treatment of 38 °C for one minute. Cassani and Caton (1985) induced triploidy in Grass carp *(Ctenopharyngodon idella* Val). A heat shock of 40 ºC for 1 min, 4.75 min after activation, was the only heat treatment which produced triploidy (8%) with 81% surviving to the blastula stage. Fuhua *et al.* (2003) worked on the optimization of triploid induction by heat shock in Chinese shrimp (*Fenneropenaeus chinensis*). The highest level of triploid induction of more than 90% was obtained at 29-32 °C, starting at 18-20 min for duration of 10 min. They concluded that heat shock was found very effective way to induce triploids in this species, and could be easily used on large scale without any harmful effect on the environment as compared with chemical treatment. Parven and Gallardo (2014) reported that heat shock at 40 °C for 1 min resulted in triploidy with fry survival of 42% in hybrid catfish (*Clarias macrocephalus* × *C. gariepinus*).

Induction of triploidy using pressure shock

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. Induction of triploidy by hydrostatic pressure shock has been reported by many authors. Nigel H. McCarter (1988) reported pressure shocks of 5.6×103 kPa 4 min after fertilisation in grass carp induced triploidy in 95% of eggs. Peruzzi and Chatain (2000) induced 100% triploidy in European sea bass, (*Dicentrarchus labrax* L.) at 8500 psi for 2 min at 6 min time after fertilization for pressure shocks. Peruzzi and Chatain (2000) reported high triploid yields of (82.5-100%) in brown trout *Salmo trutta* (*Linnaeus*, 1758). Loopstra and Hansen (2008) reported that eggs pressure shocked for 5 minutes beginning at 375 or 475 CTMs post fertilization with 9,000, 9,500, or 10,000 psi of pressure in rainbow trout (*Oncorhynchus mykiss*) yield triploidy rates ranged from 93.6 to 99.1%, and average survival rates to emergence ranged from 90.6 to 100%. Dogankaya and Bekcan (2014) reported pressure shock of 48263 kpa (7,000 psi) applied to eggs for 4 min at 35, 40 and 45 minutes after fertilization, resulted in higher survival and triploidy rates at 40 min treatment. Results showed that pressure shock was more advantageous as compare to heat shock in all stages of development (embryo, eyed-stage, hatching, and first feeding) in both respects of survival and triploidy rates. Benfey, T.J. and Sutterlin, A.M., 1984 induced 100% triploidy with 70-90% survival (relative to controls) in landlocked Atlantic salmon (*Salmo salar L.*) at hydrostatic pressure shocks of 3 or 6 min at $7.0Xl0^4$ kPa (10 150 P.S.I.), when completed within 20 min of fertilization at 10 ºC. Lou, Y.D., & Purdom, C.E. (1984) induced 80-90% triploids using hydrostatic pressure applied to eggs 40 min after fertilization in rainbow trout (*Salmo gairdneri Richardson*).

Induction of triploidy using cold shock

Gheyas *et al*., (2001), Marx and Sukumaran (2007), Cassani and Caton (1985), Piferrer *et al.*, (2003) and Silva *et al.*, (2007) have successfully induced triploidy in stingray catfish, African catfish, Grass carp, Turbot and South African Catfish respectively by using cold shock. Gheyas *et al.* (2001) reported that shock duration for 10 min at 20 ºC applied 3 min. after fertilization induced triploidy up to 94 to 97% of the eggs and had the best hatching and survival percentage of the triploid larvae in stinging catfish. Marx and Sukumaran (2007) reported cold shock at 0±1 ºC for a duration of 60 minutes yielded 90% of triploids in African catfish, (*Clarias gariepinus*). While as Cassani and Caton (1985) reported that triploidy occurred most often with cold shocks at 5-7 ºC and at durations of 25-30 min starting 2.0-4.5 min after fertilization. Estimated percent triploid ranged from 50 to 100% on five occasions in Grass carp (*Ctenopharyngodon idella*). Piferrer *et al.*, (2003) [29] induced triploidy in the turbot (*Scophthalmus maximus* L.) by applying cold shocks shortly after fertilization. Shock commencement 6.5 min after fertilization, shock duration 25 min, and shock temperature between 0 and -1 ºC resulted in 100% triploidy with survival 60% of the untreated control in Turbot (*Scophthalmus maximus*). Silva *et al*. (2007) reported that the best parameter for cold shock triploidy induction in South American catfish, *Rhamdia quelen* was achieved at 4 °C, 3 min after fertilization for 20 min which resulted in 97.9±1.16% of triploid fish. This cold shock conditions resulted in a survival rate of 65.4±5.34%. Wolters *et al.* 1981 induced 100% triploidy in Channel catfish (*Ictalurus punctatus*) by cold-shocking fertilized eggs at 5 C for 1.0 hour starting 5 minutes after fertilization and hatching success of eggs cold-shocked 1.0 hour was 79%. Ueno (1982) induced triploidy in carp (*Cyprinus carpio*) using cold shock. The frequency of occurrence of triploids at 30- and 60-min of cold treatments given 5 min after fertilization was 83.3 and 91.7%, respectively. Felip *et al.* (1997) induced 100% triploidy in sea bass (*Dicentrarchus labrax* L.) at temperature shock of 0 °C, 5 min time after fertilization for duration 10 min. Ueno and Arimoto (1982) induced triploidy by cold shock in fertilized eggs of *Rhodeus ocellatus ocellatus*. The maximum percentage (95%) of triploidy was obtained from eggs treated at 5 min after fertilization. Kim *et al.* (1994) induced triploidy in mud loach (*Migurnus midepis*) by cold shocking fertilized eggs 5 min post-fertilization at 2 °C for 15 to 60 min. Best results were obtained when eggs were shocked for 60 min; 98% of fish examined in that treatment were triploids. Peruzzi and Chatain (2000) induced 100% triploidy in the European sea bass, (*Dicentrarchus labrax*) at cold shock 0-18 ºC for 15-20 min at 5 min after fertilization. Manickam (1991) induced triploidy in Asian catfish, *Clarias batrachus*. Eggs cold shocked at 5 ºC for 1 h, starting 5 min after fertilization, yielded 100% triploidy.

Significance of Triploidy and future prospectus

Triploidy induction is the only means known till date to produce sterile fish populations. In some species sterile fish reach a larger size (due to the absence of sexual maturity), which is desirable in sectors of fish farming industry. Using biotechnology to dramatically increase the growth rates of salmon and other fish species is likely to reduce production costs per unit of food produced and thereby possibly reduce costs to consumers. Triploidy can also be used to increase the viability of some hybrids, and is regarded as a potential method for the genetic containment of farmed shellfish and fish. 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.

Conclusion

Heat shock is an effective and widely used method for polyploid induction in fish and is one of the common technique in triploidy of fish, but the application of heat shock to induce triploidy is not always 100% effective and can cause a detrimental side-effect and decreased viability. Hydrostatic pressure is presently considered the most consistent method for wide-spread commercial production of triploid grass carp and rainbow trout. However, in pressure treatment specific equipments are required and it is usually difficult to treat many eggs on a commercial scale. It was concluded that induction of triploidy can serve as a basis for further work to test other shock protocols and to scale up the most practical method for a commercial production of triploid stock. Besides, attempts should be made to produce tetraploid and then to produce a hybrid triploid from tetraploid x diploid crosses. In this way, the adverse effects of shock application might be averted.

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