

Performance improvements by polyploidization in aquaculture

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Introduction

Polyploids can be defined as organisms with one or more additional chromosome sets with respect to the number most frequently found in nature for a given species. For several species, including both wild and farmed ones, some spontaneous polyploids are occasionally found in nature. Also, many plants used in modern agriculture are induced polyploids.

Polyploidy, particularly triploidy, can be easily induced in some invertebrates and lower vertebrates, resulting in viable animals. Regarding species cultured in Europe, polyploidy is currently induced in oysters to improve some aspects of production. According to the EU regulations (Directive 90/220/CEE of April 23 of 1990), polyploids are not considered genetically modified organisms (GMOs).

Since triploids are sterile, their use in aquaculture and restocking has been promoted by several international organizations (NASCO, FAO, ICES, etc.) in order to limit the genetic impact of escapees on wild populations. In addition, their use has been proposed as a solution to the problem of the containment of GMOs.

Summary of responses to questions

1) Effectiveness of current direct triploidization techniques

a) Are there some species that exhibit more intrinsic variations to direct triploidization treatments?

Most of cultured species can be readily triploidized, except some having an unusual reproductive strategy, such as the flat oyster and the lobster. At least in fish, species having large eggs appear to exhibit a variation of response to treatment induction with temperature shocks. Pressure treatment or crossing tetraploids with diploids seems to give more reliable results in these cases, depending on species. Cold shocks and pressure shocks are suitable for fish species with small eggs (carps, seabass, turbot, seabream, etc.).

b) What is the relevance of egg quality for successful triploidization?

For triploidization, egg quality is important to optimize production and have the best possible survival. In batch with poor quality, eggs may display different levels of promptness for the resumption of the second meiotic division with consequent decrement in the effectiveness of the triploidization treatment.

c) Importance of fine-tuned variables to maximize induced triploidy.

Depending on the objective pursued, it is more or less important to achieve a 100% triploidization rate. In order to obtain 100% triploidy, application of a precise protocol is required, including the fine-tuning of triploidization variables (timing, intensity and duration of treatment, as the most important ones). Such protocols



exist for many species farmed in Europe (e.g., seabass, gilthead sea bream, turbot, trout, oyster), but need to be optimized for others.

In the case of GMOs farmed in the open environment, 100% sterility is critical and needs to be achieved and confirmed. For non-GMOs, the higher the level of sterility achieved the better in order to limit interaction with the wild population.

d) Importance of an adequate scaling-up of the method.

An adequate scaling-up of the method is a key step if triploidization is to be applied at the large scale required for production and should be developed in collaboration with the aquaculture industry.

e) When treatment induces lower larval performances (especially survival), what level of triploidy- or treatment-induced depression can producers accept?

In some species, triploid induction implies some mortality at the early stages of development, before investment in food and resources has been made. This mortality has to be balanced with the expected ecological and economical benefits derived from the application of triploidy.

2) Performance capacity of triploids with respect to diploids

a) What is the performance of triploids in culture as compared to diploids?

Performance of triploids is species specific. Currently, triploids are farmed to maintain or improve organoleptic quality when the product is sold during or after sexual maturation of diploids (oyster, salmonids). In some cases, triploidy improves growth and survival (oysters) but, particularly in fish, it usually does not. However, it avoids growth depression and the increase of mortality associated with the sexual maturation of diploids, resulting in increased production with triploids (e.g., salmonids, turbot). Under good culture conditions, triploids perform well, but under adverse conditions (e.g., low oxygen concentration, stress, etc.), they may underperform relative to diploids. There is insufficient evidence to indicate that triploids are more prone to developmental abnormalities.

b) What information is available on the performance of triploids in the wild and do they show any sexual behaviour?

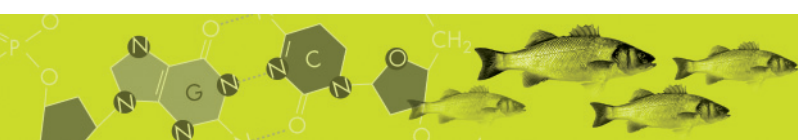
Apart from the lack of homing behaviour of sterile Pacific salmon, little is known about the performance of induced or natural triploids in the wild. Male triploid Pacific salmon display sexual behaviour in the wild and could interfere with reproduction of native individuals. There are no data on European species.

3) Degree and permanence of gonadal sterility in triploids

a) Does triploidization produce functional sterility in both sexes?

Triploidy interferes with gametogenesis in all European aquacultured species tested so far. In fish, it results in complete female sterility, but males are still able to develop testis. In some species (e.g., Atlantic salmon), these males can produce only a small amount of aneuploid sperm, capable of activating eggs, but not fertilizing them. In other species (seabass, turbot, seabream, Arctic charr), triploid males do not produce sperm. In the Pacific oyster, some triploids can develop male and female gonads. Often male oysters release sperm and, exceptionally, females can release eggs.

b) How many reproductive seasons do we have to monitor before ensuring that triploidy induces functional sterility in a given species?



To ensure that triploidy induces functional sterility in a given species, at least two full consecutive reproductive cycles should be monitored and the absence of gamete production confirmed.

c) Are current techniques for triploidy induction adequate for the biocontainment of GMOs?

For GMOs, where 100% biocontainment is required, the current technology based on the production of triploids cannot ensure it, unless a feasible method for the large scale monitoring of ploidy on an individual fish-by-fish basis is developed. Complementary technologies (physical containment, sterile hybrids, anti-sense GnRH transgenics, etc.) should be considered.

4) *Applicability of tetraploidy to generate auto- and allotriploids*

a) What are the effects on viability and reproductive performance of tetraploids?

Tetraploidization is theoretically possible, but in practice difficult to achieve. Viable tetraploids have only been produced in Pacific oyster and rainbow trout, and are fertile. In Pacific oysters, crossing tetraploids with diploids gives 100% triploid stocks. In this case, tetraploids are appropriately contained to avoid larvae or eggs escaping into the environment. In trout, production of unreduced eggs and enlarged spermatozoa in tetraploids limits their value as a tool to produce triploids.

Viable tetraploids were not possible to obtain in sea bass or carp. In other species it has not been tested and thus valid conclusions cannot be drawn.

Main research priorities for the potential use of triploidy to limit the genetic impact of escapees on wild populations

1. If sterility or biocontainment is found to be necessary for species currently produced and for which commercial triploidization protocols are not available (cod, meagre, soles, sturgeons, mussels, pectinids, abalone, etc.), these should be developed.
2. In spite of the success of tetraploidization in oysters, tetraploid fish are difficult to produce. Further research is required in this area.
3. Knowledge on the physiology and gene regulation in triploids (hormonal and immune status, functional genomics) reared in optimal or sub-optimal farming conditions should be increased.
4. The mechanisms by which triploidy affects reproduction, particularly gametogenesis, are still poorly understood and should be investigated by using a range of experimental approaches.
5. Performance of triploids under both normal and adverse conditions (stress, lower water oxygen concentration, presence of pathogens) needs to be investigated and their viability assessed in comparison with their diploid counterparts.
6. Evaluate biological and economical performance of triploids in different types of farming environments such as ponds (carp, European catfish), sea cages (seabass, seabream, cod), concrete raceways (sea bass, sea bream, turbot, halibut, freshwater salmonids, sturgeon) and recirculated systems (marine fishes). Evaluate also the organoleptic properties of triploids throughout the year.
7. Performance of induced sterile triploids in the natural environment should be investigated, taking into account possible species differences and sex, and addressing relevant traits such as survival, competition for resources and reproductive performance and behaviour.



8. Evaluate the ecological impact of the escape of small numbers of triploids from farms into the wild and the potential effects of restocking with large numbers of triploids.
9. If triploidy is going to be applied for the genetic containment of GMOs, then a reliable method is required for the low-cost, high-throughput, high-efficiency individual verification of ploidy and sterility.

Recommendations

1. Reinforce at all levels that induced polyploids are not GMOs.
2. Promote research to achieve sterility in triploids for the aquaculture industry.
3. For GMO containment purposes, 100% sterile triploid are required and must be verified on an individual animal basis.
4. For non-GMO production purposes, 100% sterility is less critical but protocols should be optimized.
5. The commercial application of triploidy should balance the advantages *vs.* the inconveniences on a species by species basis, since triploidy affects several characteristics of the animal (survival, growth, reproduction, etc.).
6. Tetraploidy is the common method for the induction of triploidy in shellfish and should be explored in other species. However, tetraploid stocks need to be contained.
7. Define and set up public information strategies (web sites, applied workshops, labelling, marketing approaches) between stakeholders in the aquaculture industry to disseminate the benefits and possible risks of triploidy.
8. Educate consumers by providing examples of how polyploidization has been used in agriculture for a long time, and how it is critical to current agriculture.

Suggested readings

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