CHROMOSOMAL MANIPULATION IN SENEGAL SOLE: OBTAINING OF GYNOGENETIC AND TRIPLOID OFFSPRING

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Introduction

Senegal sole (*Solea senegalensis* L.) is a flatfish (Order Pleuronectiformes) that in recent years has aroused great interest in the aquaculture mainly in southern Spain and Portugal, constituting a viable alternative to the traditional culture of sea bass and sea bream. In fact, the culture of this fish is gradually increasing due to its relatively rapid growth and a high price in the market. However, the production in captivity of Senegal sole has several problems related to aspects such as its reproduction under culture conditions, sex reversion, and absence of knowledge about the sex-determining mechanisms. There are also other problems in optimising its production. For example, in the Senegal sole, like other cultured marine fishes, females have higher growth rates than males and sexual maturation occurs in males before in females and usually prior to reaching market size. The development of the gonads during sexual maturation results in a growth reduction of length and also in this period mortality increases. Thus, all these phenomena are reflected in a heavy economic loss just before marketing.

All these aspects related to the development of aquaculture in *S. senagalensis* lead to the need for applied chromosomal manipulation techniques. Usually, it is possible to manipulate the fish reproduction to produce specimens that could contribute to improve the cultures. In fish, gynogenesis is usually achieved by fertilization of eggs with genetically inactivated sperm using radiation or chemical treatments (to obtain gynogenetic haploids), followed by heat shock (to obtain gynogenetic diploids). Concretely, in flatfishes, diploid gynogenetics have been achieved in the same species –i.e. *Scophthalmus maximus* (Piferrer et al., 2004; Cal et al., 2006)–, resulting, in general, in 100% females, indicating a sex-determining mechanism with homogametic females in the group. On the other hand, triploidy has been achieved in some flatfish –i.e. *Hippoglossus hippoglossus* (Holmefjord and Refstie, 1997). As the case of diploid gynogenetic flatfishes, triploids had been produced by means temperature shock in order to retain the second polar body normally extruded after fertilization.

Materials and methods

Trials were conducted at the facilities of the IFAPA Centro *Agua del Pino* in Huelva (Spain). Wild *Solea senegalensis* spawners were captured and stocked in batches. Eggs and sperm from single female and male in each case were obtained by stripping. Sperm was diluted (1:100) in Ringer solution and kept on ice until fertilization.

The gynogenesis procedure was basically similar to that described by Castro et al. (2003) in turbot (*Scophthalmus maximus*). It was necessary to regulate the UV intensity to ensure sperm motility of close to 25-50% after activation with seawater. UV irradiation was conducted on a ice plate with CL1000 Crosslinker UVP. To induce diploid gynogenesis, freshly ovulated eggs were fertilized at 19 ± 1ºC with UV irradiated sperm ($32 mJ \cdot cm^{-2}$) followed by a cold shock (0 to -1ºC) for 25min, starting 6.5min after fertilization. Three batches were used as controls to check eggs viability (diploids: no UV-irradiated sperm and no cold shock), inactivation of irradiated sperm (haploids: UV-

irradiated sperm but no cold shock), and retention of second polar body (triploids: no irradiated sperm and cold shock).

Gynogenetic juvenile fishes were dissected for macroscopic sex determination and sex confirmed by standard histological procedures. Haploid embryos and gynogenetic diploid and triploid progenies were checked by microsatellites genotyping and cytogenetic techniques.

Results and discussion

Three different gynogenetic diploid offspring were obtained. The gynogens rates, verified by microsatellite markers analysis, were close to 100% as it was for another three additional offspring of gynogenetic haploids. The haploid embryos showed the typical syndrome characterized by abnormal body shortening and underdevelopment of the head. All haploid larvae died within 12h after hatching.

The histological analysis in gynogenetic fishes revealed that all gynogenetic gonads were female, indicating that the male was the heterogametic sex. This sex determination is similar to that found in other flatfishes such as Atlantic halibut (*Hippoglossus hippoglossus*) by Tvedt et al. (2006). Additionally, producing gynogenetics (haploids or diploids) will be useful to the linkage map developed in this species because they allow us to calculate the distance to the centromere of the markers by half-tetrad analysis, and even to produce all-female or all-male populations.

On the other hand, triploid progenies, produced by cold-shock treatment, were also confirmed by microsatellite locus analysis with a rate of 100% but these showed very low survival. Thus, producing triploid fishes could help produce sterile specimens without problems related to sexual maturation and sometimes with a higher growth level than the diploid ones (Felip et al., 2001).

Conclusions

We have successfully applied, in Senegal sole, chromosomal manipulation techniques, previously developed in other flatfishes, in order to produce haploid embryos and 100% gynogenetic diploids and triploids progenies. Results suggest that the female is the homogametic sex in *Solea senegalensis*

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