Grain 5 : Reproduction and sex control

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Sex control is often needed for fish farming. Depending upon the species, farmers will need either to induce or to inhibit spawning and/or to favor the sex having the best aquaculture performances and thus sex control. Fish species exhibit an amazing diversity in their sexuality: most of them are gonochoristic, with separate sexes where individuals are either males or females during their whole life. Besides in some rare species (i.e. the molly, *Mollienesia formosa*), wild populations are exclusively composed of female individuals; they reproduce by gynogenesis with no paternal genetic contribution (see the complementary resources). Finally, other species are hermaphrodites: individuals can produce successively (successive hermaphrodites) or simultaneously (simultaneous hermaphrodite) both types of gametes (ovules and spermatozoa). In the former, individuals will either reproduce first as a male breeder, then will undergo sex reversal and finally reproduce as a female breeder (protandrous hermaphrodites), or the opposite (protogynous hermaphrodites). For both hermaphrodite categories, 2 individuals are usually needed for a reproduction. However, one species, the killifish, *Rivulus/Kryptolebias marmoratus* is able to fertilize its own ovules by its own spermatozoa (self-fertilization) when the environmental conditions threaten the population survival (see the complementary resources). In aquaculture, control of reproduction relies on 2 possible approaches. Similar to all vertebrates, fish reproduction relies on the hypothalamus-pituitary-gonadal axis (HPG). The hypothalamus controls the

pituitary through a GnRH neuro-hormone (Gonadotropin Releasing Hormone) and in the same way the pituitary controls the gonads through 2 gonadotropins (FSH and LH). Finally the pituitary gonadotropin release is regulated by gonadal steroid loops. In order to control reproduction, a first approach mimics the hormonal signal naturally controlling each organ along the HPG axis. For example, the pituitary gonadotropin release can be stimulated by GnRH injections, or the final steps of the gametogenesis through gonadotropin treatments. In the wild, environmental factors play an important role in the regulation and synchronization of the fish reproductive cycles. For aquaculture purposes, manipulation of external factors such as temperature or photoperiod can also be used to stimulate or inhibit the final steps of reproduction. The mimic of the natural floods associated with the rainy seasons under tropical conditions also constitutes another possible approach, at least for some African and Asiatic catfish species. In various aquaculture species, zootechnical performances are better in one sex than in the other. A classic example is the growth rate. Indeed growth and reproduction are often antagonistic in fish: energetic investment needed for gametogenesis (especially oogenesis) cannot be used for growth. It is therefore interesting to produce sterile individuals in which all the energy will be directed towards growth. Moreover, rearing sterile populations allows avoiding « genetic pollutions » associated with the reproduction of domestic escapees with wild breeders. In order to obtain a sterile population, before fertilization, ovules will be submitted to a thermal or a pressure shock at a very precise stage of their development. This leads to triploid sterile individuals. Lastly, in species where one sex has the best relative performances (sex linked dimorphism), fish farmers prefer using male (for instance male monosex population in tilapia) or female monosex populations (female monosex population in the case of trout, turbot, European seabass or sturgeon). Regarding tilapia, the use of male monosex

population allows to take advantage of the better growth rate of males but also to avoid precocious and continuous reproduction that would occur in a mixed population (males and females). The main approach used to produce these populations relies on hormonal sex reversal. Androgens (for masculinization) and estrogens (for feminization) are incorporated in the feed of the fry for 1-2 months depending upon the species. However, these hormone-produced populations do not benefit of a good image by the consumers and hormonal sex reversal is even prohibited or not recommended in a growing number of countries (EU, USA…). Indeed there are many concerns about the fate of the residues resulting from hormonal treatment and their possible impact on the environment, water quality, biodiversity… However, different alternatives can be used. The first one relies on a genetic control of sex determination. In the Nile tilapia (as in mammals), the males have a XY sexual genotype whereas the females have a XX one. Using estrogens, it is possible to produce functional XY females ; when mated with genetic males, they will produce YY males. Such "supermales" will be used by farmers to sire all-male progenies (YY x XX → XY). These male monosex populations will be bred and commercialized; they will never have been treated by hormones. In the rainbow trout, the male is also XY and the female XX; conversely to tilapia, females are more interesting for fish farming. Therefore, XX males (neomales) will be produced using a masculinizing treatment associated or not to gynogenesis. Such neomales will sire all-female progenies. Here again, these female monosex populations will have never been treated with hormones. Finally, in tilapia short temperature treatments (34-36°C) applied precociously (10 days post-fertilization) can strongly (or even completely) masculinize some progenies whereas others will not at all be sensitive to this treatment. Following 3 generations of breeding programs, a strong thermosensitivity can be selected allowing to produce more than 93% males through a single 10 days' treatment at high

temperature. This suggests that such an approach is feasible and should allow producing similar proportions of males than the hormonal approach but in a more sustainable way. These are the main possible technics to control reproduction or sex in fish farming.