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## ► To cite this version:

Benjamin Geffroy, Agnes Bardonnnet. The role of density in sex determination of the European eel and the identification of early sex markers. World Aquaculture 2018, Aug 2018, Montpellier, France. 848 p., 2018, Abstract Book. hal-01913017

**HAL Id: hal-01913017**

**<https://hal.science/hal-01913017>**

Submitted on 2 Jun 2020

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## THE ROLE OF DENSITY IN SEX DETERMINATION OF THE EUROPEAN EEL AND THE IDENTIFICATION OF EARLY SEX MARKERS

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Contrarily to most mammals, the majority of fish doesn't have heteromorphic sexual chromosomes (type XY/XX). In most fish, the gonad development is extremely labile and for some of them, the sex can be influenced/determined by environmental factors. This is observed in eel, where the proportion of males increases with the number of individuals at a given location or in aquaculture conditions. Two main questioning have emerged in the eel sex determination domain: **when? and how ?** does the environment affect the sex of animals.

To investigate both questions we recently conducted a 3.5-year study aiming at understanding the effect of both density and individual growth rate on sex determination (the how?). We used the von Bertalanffy growth model with mixed effect to fit our data and take into account both the reiteration of measurements on the same fish and the fact that each individual grew in different density conditions. To identify the time-window (the when?) during which the environment influence the sex of eels, we targeted genes susceptible to be differentially expressed between ovaries and testis at different stages of development in aquaculture conditions. In parallel, we conducted field studies to identify early sex markers in areas known to produce more males or more females.

Using qPCR, we detected testis-specific expressions of *pre-mir202*, *dmrt1*, *amh*, and *gsdf* and ovary-specific expressions were obtained for *aromatase*, *zar1*, *zp3* and *foxn5*. We showed that gene expressions in the gonad of intersexual eels were quite similar to those of males, supporting the idea that intersexual eels represent a transitional stage towards testicular differentiation. The combined expression of six of these genes allowed the discrimination of groups according to their potential future sex and thus, this appears to be a useful tool to estimate sex ratios of undifferentiated juvenile eel. Concerning density and growth rate, our data clearly indicate that future males reach their asymptotic size faster than future females for both length and weight, even when we set up a similar asymptotic size for both sex. Differences in growing pattern occur between 6 and 12 months of rearing in our aquaculture conditions. These differences progressively lessen as males enter in silvering phase and both slopes cross at 1225 days for length and 1515 days for weight, when males and females reach a size of 400 mm or 123 g. Finally, future females tended to present higher Coefficient Factor than future males, suggesting a central role for available energy in triggering the development of one sex.

Our findings gathered throughout both experiment (aquaculture and field context) support the hypothesis of a metagametic (environmental) sex determination and also suggests that the estimation of the quality of the environment, made by juveniles' eels, is one of the key factors influencing sex determination.

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