

Supplementary Information for

Transgenerational epigenetic sex determination: environment experienced by female zebrafish affects offspring sex ratio

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**This PDF file includes:**

Figures S1 to S5

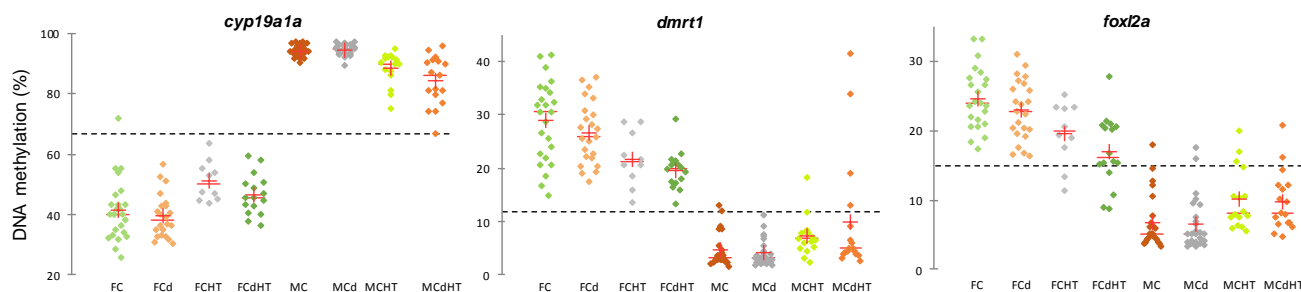
Tables S1

**Table S1:** Primers used for bisulfite sequencing and qPCR analyses (F forward; R reverse; S

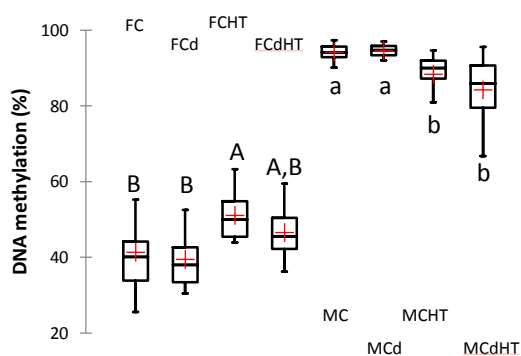
Gene	Sequencing primers	RT-qPCR primers
<i>cyp19a1a</i>	F: ATGTAGTTGTTGGGATATAAAAAGTG <b>R: CTTTAAATAAAAAAATCCACCACAACC</b> S: TGTTGGGATATAAAAAGTGT	F: AAAGGGCTCATAACGGCACT R: TGTGTGGTCGATGGTGTCTG
<i>dmrt1</i>	F: TTTGTTTTGTTTTGAATGGGGATT <b>R: TCTCCCACCAACTCTAAAATACACAAATT</b> S: TGTTTTGAATGGGGATTA	F: AAGTCCTGCAATGTCCAGCA R: GGAGGAGTATGGAGTGGGCT
<i>foxl2a</i>	F: AGGAGAGAGGGGAAAGATAG <b>R: AATTTTTCCCTCTTCAAATATAATTCAC</b> S: GAGGGGAAAGATAGG	F: CATCGCTAAGCCAAGTAGCC R: AGGGCCCAATTATTCACTCC
<i>actb1</i>		F: ATGAAGTGCGACGTGGACAT R: CTGCATACGGTCAGCAATGC
<i>eef1a1a</i>		F: ACCCTTCCCGTGGATCA R: GTGGGTTTCAGACGCACA
<i>rpl13a</i>		F: GGGAAAGGAAAAGACCCACA R: CGCTCGGCCTTCAGTT

sequencing, sequences in bold are biotinylated in 5')

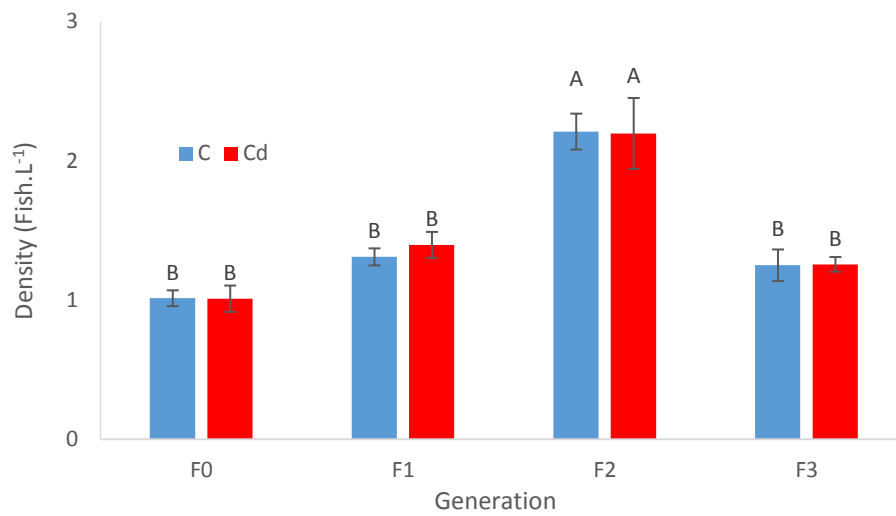
**Figure S1:** DNA methylation levels (%) of *cyp19a1a*, *dmrt1* and *foxl2a* in gonads of female (F) and male (M) zebrafish from the different experimental conditions: controls (C), exposed to cadmium (Cd) and exposed to high temperature (HT).



**Figure S2:** Box plots of the DNA methylation levels of *cyp19a1a* in gonads of females (F) and males (M) zebrafish from the different experimental conditions: controls (C), exposed to cadmium (Cd) and exposed to high temperature (HT). For each sex, means designed with different letters are significantly different ( $P < 0.05$ , Tukey's HSD test or Dunn test with Bonferroni correction). For each condition, data from all generations were combined ( $n = 10 - 24$ ).

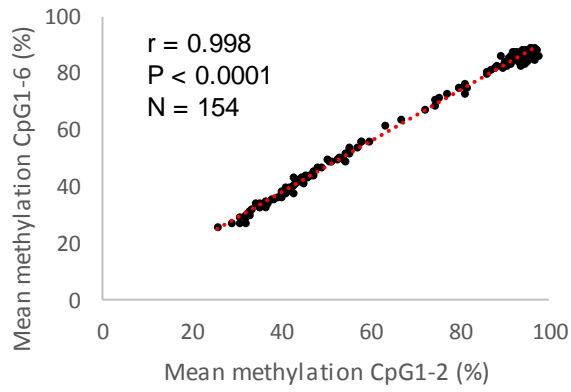


**Figure S3:** Mean ( $\pm$  SE,  $n = 4$ ) fish density determined at each generation (F0 to F3) at 170 dpf. Four aquaria were used per condition (C, control, blue; Cd, Cadmium, red). Means

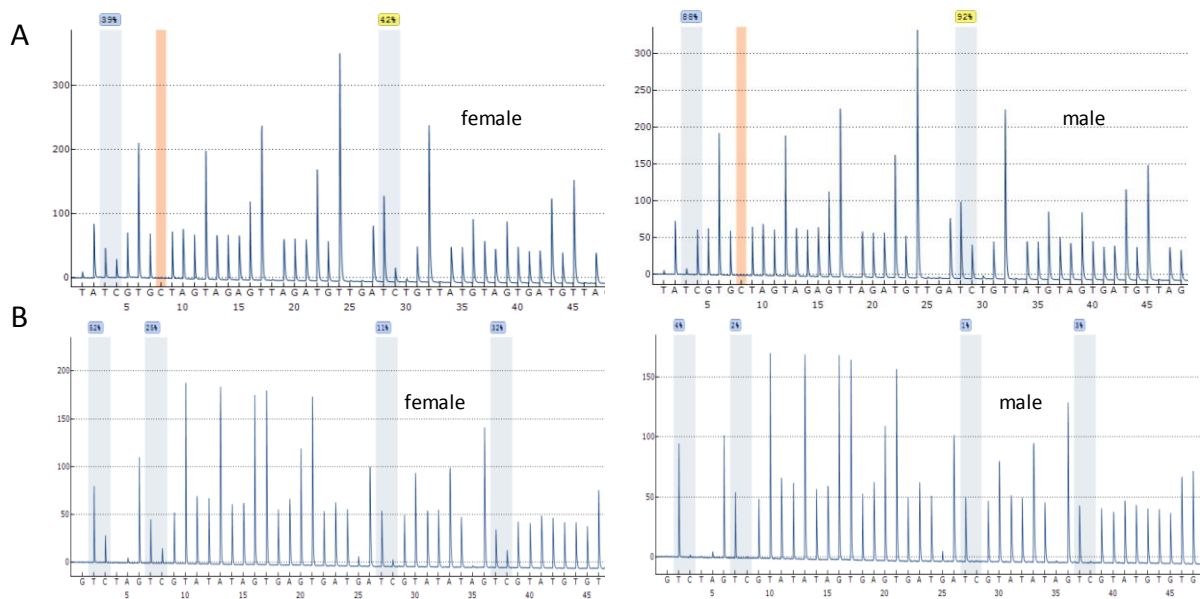


designed with different letters are significantly different ( $P < 0.05$ , Tukey's HSD test).

**Figure S4:** DNA methylation level (%) of the gonadal *cyp19a1a* promotor. The mean methylation level determined in the first two CpG sites (CpG1-2) was highly correlated with the mean methylation level determined using all the six CpG analyzed (CpG1-6).



**Figure S5:** Pyrograms obtained from gonads of male or female zebrafish for *cyp19a1a* (A), *dmrt1* (B) or *foxl2a* (C). For *foxl2a*, some polymorphisms were observed both in females and males from position 10 to 14.



C

