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Oysters in transition: Hermaphrodite oysters display unique DNA methylation patterns in their gills

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Oyster reefs play a major role in enhancing biodiversity and maintaining ecosystem health in the North Sea. European flat oyster (*Ostrea edulis*) restoration projects replenish wild populations with hatchery produced oysters but are limited by low reproductive output due to unknown sex ratios in breeding populations. Sex identification in flat oysters is complicated, because they are sequential hermaphrodites that alternate sex and lack clear traces of sexual dimorphism. Epigenetics, like DNA methylation, are often involved with sex reversal through influencing gene transcription. Sequential hermaphrodites represent excellent candidates for studying epigenetic mechanisms underlying sex reversal, however, most studies are limited to full males and females only. It remains unaddressed whether sex-specific DNA methylation is different in hermaphrodite individuals that simultaneously produce male and female gonad tissue. Additionally, our understanding of sex-specific epigenetics are limited to gonad tissue. Therefore, we characterized sex-specific DNA methylation by comparing whole-genome gill methylomes between 35 flat oysters of different sex phenotypes, using nanopore sequencing. Analysis resulted in numerous differentially methylated regions between male, female, and hermaphrodite oysters. Ontology enrichment analysis of differentially methylated genes emphasized the association with energy homeostasis and metabolic processes, implying a shift in energy balance between different sex phenotypes. This study is the first to compare DNA methylomes of hermaphrodite individuals with full males and females, providing novel insights into the epigenetics underlying sex reversal in a sequential hermaphrodite invertebrate. Further, we characterized sex-specific DNA methylation in gill tissue, which emphasizes the potential usage of somatic tissues as a non-lethal alternative to sex identification in bivalves.

Keywords: Sex reversal, DNA methylation, European flat oyster, Nanopore, Hermaphrodite