

# GILTHEAD SEA BREAM SKIN MICROBIOTA SHAPES PROACTIVE AND REACTIVE BEHAVIOUR IN FISH UNDER HIGH STOCKING DENSITIES

S. Toxqui-Rodríguez<sup>1,2\*</sup>, F. Naya-Català<sup>1</sup>, P.G. Holhorea<sup>1</sup>, J.A. Calduch-Giner<sup>1</sup>, A. Sitjà-Bobadilla<sup>2</sup>, M.C. Piazzon<sup>2</sup>, J. Pérez-Sánchez<sup>1</sup>

<sup>1</sup>Nutrigenomics and Fish Growth Endocrinology Group, Institute of Aquaculture Torre de la Sal (IATS, CSIC), Spain. <sup>2</sup>Fish Pathology Group, Institute of Aquaculture Torre de la Sal (IATS, CSIC), Spain. E-mail: [socorro.toxqui@csic.es](mailto:socorro.toxqui@csic.es)

## Introduction

The intensification of aquaculture production must deal with inadequate stocking densities that increase the risk of health issues and welfare status due to a variety of stressful conditions such as feed competition, aggressive interactions and reduced O<sub>2</sub> availability. In gilthead sea bream facing changes in water O<sub>2</sub> concentrations, mitochondrial bioenergetics of blood cells are finely adjusted at the transcriptional level (Martos-Sitcha et al., 2017). Likewise, different O<sub>2</sub> status and rearing densities changes induce different tissue-specific expression patterns of genes related to energy metabolism and endocrine growth (liver > muscle > blood) (Martos-Sitcha et al., 2019). All this evidences the fish plasticity to cope changes in the environment, and several components of the *Gh/Igf* system have emerged as hypoxic imprinting genes during critical early life stages (Naya-Català et al., 2021a). There is now a large body of evidence linking changes in mucosal microbiota communities with fish health and welfare, affecting both local and systemic physiological functions (Naya-Català et al., 2021b). However, it remains to be unravelled how changes in mucosal microbiota composition are linked with changes in physiological traits and welfare indicators in a broad sense. Thus, this study aimed to assess how high-stocking densities in concurrence with reduced O<sub>2</sub> availability affect host physiological traits, with focus on skin microbiota and associated shifts in other behavioural and welfare indicators, using a gathered biomarker approach.

## Methods

Two-year-old gilthead seabream (450-500 g) were pit-tagged and distributed in 3,000 L tanks to achieve three different initial rearing densities (low, LD: 6 kg/m<sup>3</sup>; medium, MD: 12 kg/m<sup>3</sup>; high, HD: 22 kg/m<sup>3</sup>). Fish were fed close to satiety with a commercial diet from May to June (8 weeks) under natural photoperiod and temperature conditions. The concentration of dissolved O<sub>2</sub> varied from 6-5 ppm in LD fish to 5-4 ppm and 4-3 ppm in MD and HD fish, respectively. At the end of the trial, 10 fish per group were randomly selected for the continuous and simultaneous recording of swimming activity and respiration rates over 48h, using implanted AEFishBit devices (Calduch-Giner et al., 2023). The same fish were used for assessing external damage using a scoring system from 1 to 5. Also, from the same fish, samples of skin mucus, blood, liver and white skeletal muscle were taken for microbiota, biochemical and transcriptional analyses. For skin microbiota profiling, the 16S rRNA v1-v9 regions were sequenced with the ONT MinION device and processed with an in-house pipeline. Muscle and liver gene expression was assessed by qPCR array layouts designed for the simultaneously gene expression profiling of two tissue-specific panels of 44 genes each.

## Results

At the end of the trial, welfare scores of epidermal status varied among the three groups ( $P < 0.05$ ), showing the worst status fish held at HD and gradually improving with the decrease in density. However, correlation analysis indicated that external damage appears associated with an active feeding behaviour. Concerning skin microbiota, discriminant analysis showed that the skin microbiome of the three different groups differed in bacterial

abundance, and LEfSe analysis revealed six strong microbial markers for these experimental conditions, being the abundance of *Alteromonas* and *Massilia* largely increased in the HD group, though a closer look evidenced an opposite trend for these two discriminant taxa. At the transcriptional levels, five genes related to growth (*igf1*, *igf2*), lipid metabolism (*cyp7a1*), and oxidative metabolism (*cs*, *cox1*) were positively correlated with a higher abundance of *Alteromonas* within the HD group. Otherwise, seven differentially regulated muscle genes related to growth (*ghr1*, *ghr2*, *igf2*), antioxidant defence (*grp170*, *grp75*), and energy metabolism (*sirt1*, *hif1a*) were positively correlated with a higher abundance of *Massilia*. This integrative behavioural, metagenomic and transcriptional approach supported stressful mediated responses that serve to alert and adapt the system in different ways. Hence, a higher abundance of *Massilia* was connected to a proactive behaviour with increased skin damage and locally regulated growth, while *Alteromonas* abundance and low cortisol levels appeared related to a reactive behaviour and systemic growth regulation via the liver *Gh/Igf* system.

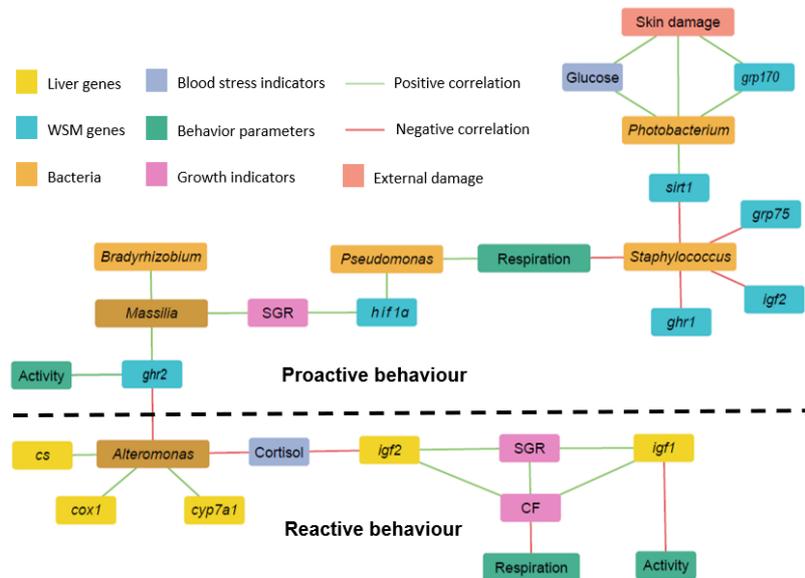


Figure 1: HD correlation network showing significant positive (green lines) and negative (red lines) correlations (Spearman,  $P < 0.05$ ) of discriminant skin microbiota (light orange, reduced abundance in HD; dark orange, increased abundance in HD) with biomarkers of external damage (red), growth (pink), behaviour (green), blood stress indicators (purple), and liver (yellow) and muscle (blue) differentially regulated genes.

### Concluding remarks

High-stocking densities had a significant impact on behavioural and physiological traits, that correlated with significant changes in the associated skin microbial population. This gathered biomarker approach served to infer and regulate new operational biomarkers for increased stress resilience in a context of global warming and intensive rearing conditions to cover the increased demand of sea food products.

**Funding:** AQUAEXCEL3.0 (H2020 #871108), EATFISH (H2020 #956697), RYC2018-024049-I&ESF.

### References

- Calduch-Giner JC, et al, 2017. *Frontiers in Marine Science*, 9:854888.  
 Martos-Sitcha JA, et al, 2017. *Frontiers in Zoology*, 14:34.  
 Martos-Sitcha JA, et al, 2019. *Frontiers in Physiology*, 10:840.  
 Naya-Català F, et al, 2021a. *Scientific Reports*, 11:416466.  
 Naya-Català F., et al, 2021b. *Frontiers in Physiology*, 12:748265.