SMART BIOSENSING DEVICE FOR TRACKING FISH BEHAVIOUR

E. Rosell-Moll[1], C. Piazzon[2], J. Sosa[3], M. Á. Ferrer[4], E. Cabruja[5], A. Vega[3], J. A. Calduch-Giner[1], A. Sitjà-Bobadilla[2], M. Lozano[5], J. A. Montiel-Nelson[3], J. M. Afonso[6], J. Pérez-Sánchez[1]

[1]Nutrigenomics and Fish Growth Endocrinology Group, Institute of Aquaculture Torre de la Sal (IATS-CSIC), Castellón, Spain. [2]Fish Pathology Group, IATS-CSIC, Castellón, Spain. [3]Institute for Applied Microelectronics (IUMA), University of Las Palmas de Gran Canaria (ULPGC), Las Palmas, Spain. [4]Technological Centre for Innovation in Communications (iDeTIC), ULPGC, Las Palmas, Spain. [5]Institute of Microelectronics of Barcelona (IMB-CNM, CSIC), Barcelona, Spain. [6]Aquaculture Research Group, Institute of Sustainable Aquaculture and Marine Ecosystems (IU-ECOAQUA), ULPGC, Las Palmas, Spain.

Biosensor technology for tracking individual challenged fish behaviour has the potential to revolutionize aquaculture, allowing farmers and breeders to orientate selective breeding towards more robust and efficient fish or improve culture conditions for a more sustainable and ethical production. The proposed solution within the AQUAEXCEL^{2020} EU project is a stand-alone, small and light (1 g) device (AEFishBIT), based on a tri-axial accelerometer and a microprocessor. It is externally attached to the operculum to monitor physical activity by mapping accelerations in x- and y-axes, while operculum beats (z-axis) serve as a measurement of respiratory frequency. The conducted operculum attachment protocol does not show signs of tissue damage or growth impairment in active feeding gilthead sea bream. AEFishBIT offers a wide range of new information based on individual behaviour, allowing to point out the asynchrony of movements as an indirect measure of aging and adaptability to farming environment, as well as to discriminate different coping behaviour (proactive or reactive) of gilthead sea bream challenged with low water oxygen concentrations. AEFishBIT also provides reliable information of disease outcome in fish parasitized with an intestinal myxozoan, emerging as a powerful tool for sensing the quality of the environment and improving selective breeding protocols.

Introduction

The use of new technologies for individual and non-invasive monitoring of farmed fish is becoming a demand of the aquaculture industry (1). This offers the possibility of measuring variables that are directly or indirectly related with metabolic condition, health and welfare status (2). In order to track individual fish movement responses and associate them with proper farming conditions and welfare status, a biosensor device to provide reliable and simultaneous measurements of fish physical activity and respiratory frequency was designed within the AQUAEXCEL^{2020} EU project. This smart biosensor (AEFishBIT) is composed of a tri-axial accelerometer, a microprocessor, a battery and an RFID tag for identification. The device is attached in the operculum using a fairly invasive procedure, and initial testing was conducted in gilthead sea bream and European sea bass in swimming metabolic chambers (3). The present work is aimed to further evaluate the impact of the biosensor attachment on fish welfare and growth, and to validate its functionality by analyzing biotic and abiotic factors in free-swimming fish.

Materials and Methods

AEFishBIT was used for recording and processing acceleration data from x-, y- and z-axes. Records of operculum breathing (z-axis) served as a direct measurement of respiratory frequency, whereas estimation of fish activity was derived from x- and y-axes signals. The final weight of the full packaged device is less than 1 g in air. The autonomy of the system in stand-alone mode is 6 h of continuous data recording with different programmable time schedules. AEFishBIT is protected by a registered patent (P201830305).

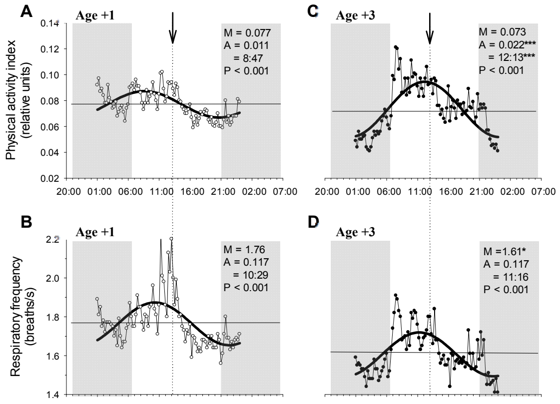
Devices were externally attached to the fish operculum using monel piercing tags with a flexible heat shrink polyethylene tube that is able to easily fit the device. The impact of this tagging procedure was evaluated in 1-year old gilthead sea bream that were fed once daily. Ten days after tagging, fish were weighed and specific growth rates (SGR) were calculated.

Functional validation was assessed in free-swimming gilthead sea bream to underline differences according to: 1) biological age (1- and 3-year old fish in 3000L tanks); 2) oxygen concentration (2-3 ppm O_2 for 2.5h in 90L tanks); 3) tank space availability (90L, 500L and 3000L tanks); and 4) disease progression in fish infected with the myxozoan parasite *Enteromyxum leei*. Devices were programmed for on-board calculation of respiratory frequency and physical activity over 2 min time windows each 15 min along two consecutive days. In all studies, rearing density was 9-14 kg/m^3, fish remained unfed over the recording time, and devices were retrieved after recording for on-board processed data download. Data analysis of physical activity and respiratory frequency was assessed through Student’s t-test and Pearson coefficients. The daily rhythmicity of the time series analysis was analyzed by a simple cosinor model.

Results and Discussion

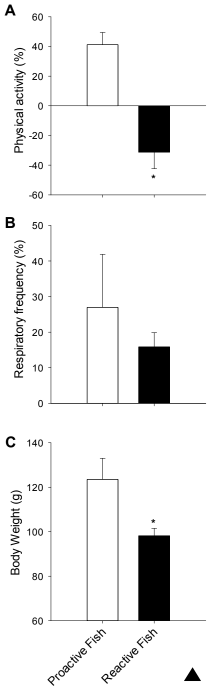
After 10 of intervention, biosensor attachment did not alter tissue integrity, feeding behavior and growth performance (SGR = 1.14 and 1.19 in tagged and non-tagged fish, respectively). Measures of respiratory frequency and jerk accelerations revealed age-related changes in basal metabolism and feeding behaviour (**Fig. 1**). According to this, the higher respiratory frequency (indirect measure of basal metabolism) in association with a more continuous physical activity are interpreted as a reliable measure of physiological age. Likewise, the occurrence of high jerk accelerations with decreasing tank size is informative of the welfare condition. In hypoxia tests, AEFishBIT also contributed to identify stress proactive fish that showed an increased size and increased physical activity for supporting escape reactions (**Fig. 2**). Finally, reduction of respiratory frequency measured by AEFishBIT was proven to provide reliable information of disease progression (parasitic enteritis) in fish parasitized with *Enteromyxum leei* (**Fig. 3**).

In summary, the present work is the proof of concept of a miniaturized device as a reliable tool for individual fish phenotyping of metabolic condition and welfare. The different behaviour patterns of free-swimming fish can be associated with a better performance or differences in stress and disease resilience. This opens new research opportunities for individual fish phenotyping of productive traits through the production cycle, but also for selective breeding in combination with other omics approaches (e.g transcriptomics, methylomics, metabolomics, microbiomics).



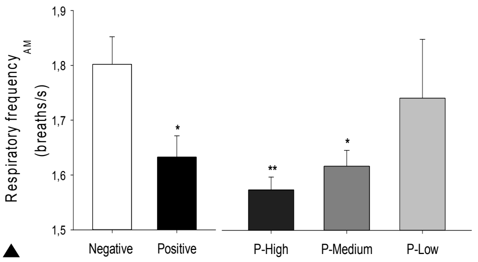
▲

Figure 1. Biological age. Jerk accelerations (**A**, **C**) and respiratory frequency (**B**, **D**). Cosinor adjust of consensus data over the course of light and dark phases. Mesor (M), amplitude (A), acrophase (ϕ), and p-value (P) for the cosinor adjust are indicated. Asterisks represent significant differences between age +1 and +3 fish (*, $P < 0.05$; ***, $P < 0.001$, t-test). Arrow and dotted vertical line mark habitual feeding time. Mesors are represented by horizontal straight lines.



▲

Figure 2. Acute hypoxia. Physical activity (A) and respiratory frequency (B) and body weight of proactive and reactive fish. Means ± SEM are represented. Changes of activity and respiratory frequency are calculated as a percentage of initial measures. Asterisks represent significant differences between proactive and reactive fish ($P < 0.05$, t-test).



▲

Figure 3. Parasite infection. The reduction of respiratory frequency (basal metabolism) correlates with the different progression stages of parasitic enteritis (P-, parasitized fish with different levels of intensity of infection). Means ± SEM are represented (n = 2-10). Asterisks represent differences with uninfected (Negative) group ($P < 0.05$, t-test).

Acknowledgments: The study has received funding from the European Union's Horizon 2020 research and innovation programme, GA no 652831 (AQUAEXCEL^{2020}).

References

1. Føre M., Frank K., Norton T., Svendsen E., Alfredsen J. A., Dempster T., et al. (2018). Precision fish farming: A new framework to improve production in aquaculture. Biosystems Engineering 173, 176-193.
2. Neethirajan S., Tuteja S. K., Huang S. T., and Kelton D. (2017). Recent advancement in biosensors technology for animal and livestock health management. Biosensors and Bioelectronics 98, 398-407.
3. Martos-Sitcha J. A., Sosa J., Ramos-Valido D., Bravo F. J., Carmona-Duarte C., Gomes H. L., et al. (2019). Ultra-low power sensor devices for monitoring physical activity and respiratory frequency in farmed fish. Frontiers in Physiology 10, 667.