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## INSIGHT INTO THE WELL-BEING OF SEA BASS BY USING PHYSIOLOGICAL OPERATIONAL INDICATORS

### *L'USO DI INDICATORI FISIOLOGICI PER UN'ANALISI DELLO STATO DI BENESSERE NELLE SPIGOLE ALLEVATE*

**Abstract** - Aquaculture employs a variety of husbandry protocols that need to be evaluated in terms of health and welfare of fish. Our study demonstrated that monitoring electrical activity from red and white muscles, swimming performances and hormone levels would permit a quantitative measure of fish well-being.

**Key-words:** fish well-being, swimming performance, Ucrit, recovery test, sea bass.

**Introduction** - Assessing the well-being of free ranging fish has been fuelled by the need to evaluate anthropogenic stress from the fish perspective. Stressors are directly related to various fish handling procedures. Assessing fish handling protocols or environmental perturbations which affect fish performance, and associated energy expenditures, would permit the development of appropriate mitigative strategies. Aquaculture employs a variety of husbandry protocols that need to be evaluated in terms of health and welfare of fish. Behavioural tests can be used as indicators of short-term stress, but examples are sparse. Measurement of swimming ability, such as critical swimming speed (Ucrit) has been used as indicators of the effects of environmental challenges on the general health of fish (Randall *et al.*, 1987; Brauner *et al.*, 1994) and as welfare indicator for reared sea bass (Carbonara *et al.*, 2006). Frequently, Ucrit has been used as a physiological end point to assess the impact of environmental change (Brauner *et al.*, 1992). There have however been examples where Ucrit is not always a sensitive indicator of metabolic disturbance. Our study expands the use of simple Ucrit measures as an indicator of metabolic disturbance by examining the ability of individual fish to recover from repeated exhaustive exercise events (recovery test). Furthermore, the physiological response to the stress has been evaluated monitoring the contribution of two muscle type (red and white) to the swimming activity and the relative stress hormone levels.

**Materials and methods** – Adult sea bass were reared in 16 m<sup>3</sup> circular tanks, at a density of 10 kg (m<sup>3</sup>)<sup>-1</sup> under natural light conditions. Individuals were fed once per day to satiation. Tanks water was exchanged five times per day. Water temperature was maintained at 18 °C. The size of the fish used for monitoring the muscle electrical activity ranged between 160 and 180 g in weight, the size of those used for the swimming performance trials ranged from 160 and 700 g. Swimming performance trials were conducted using a Blažka-style swimming chamber (Thorstad *et al.*, 1997). Water velocity was calculated using a Doppler flow meter (Sigma). All the fish tested were fasted for 24 h prior to swim trials to ensure a post-absorptive state (McFarlane *et al.*, 2004). Swimming trials were conducted, in the swimming chamber, using increasing water flow velocity by steps of 0.1 msec<sup>-1</sup> every ten minutes, until individuals fatigued. Blood

samples were collected from different treatment groups and cortisol levels were measured by HPLC. Electrical activity from muscles types was monitored by implanting two pairs of wire electrodes on anaesthetized fish. One pair of electrodes were inserted subcutaneously into the lateral red musculature, a second pair of electrodes was implanted into the white musculature at a similar location but at depth of 1 cm. The duration of surgical procedure including anaesthetization, was generally 25-30 minutes. The bio-sensitive electrodes monitoring electrical activity in the two muscle types were passed through a controlled opening in the swimming chamber to the pre-amplifiers. Signals were filtered, amplified and transformed into non-differential signals. Data were subsequently recorded with a sample size of 5000 data per second.

**Results** - The averages of hard wired-red muscle electromyograms were ranging between 0.28 mV at velocity of 0.2 msec<sup>-1</sup> and 1.19 mV at the Ucrit velocity (~0.9 msec<sup>-1</sup>). The pattern was initially rather sharp and then reached a plateau at the higher velocity steps. The white muscle activity showed a different pattern. Indeed, the electromyograms (EMG) varied between 0.15 and 1.00 mV and the intensity increased exponentially between 0.2 msec<sup>-1</sup> and the Ucrit velocity (~0.9 msec<sup>-1</sup>). The Ucrit measures showed remarkable differences among the different size classes (160-700 g). Total length showed a significant positive correlation with absolute Ucrit and a significant negative correlation with relative Ucrit. The recovery tests carried out with unstressed fish showed no significant differences between Ucrit<sub>1</sub> and Ucrit<sub>2</sub>. The levels of cortisol were significantly different between stressed and unstressed fish, reaching, in the latter, values higher than 1.5 µg/ml.

**Conclusions** – Our study demonstrated that the scope for activity in sea bass is not supported solely using aerobic metabolism, though the red muscle powers the majority of the swimming ability. Monitoring EMG, swimming behaviour and performances, hormone levels would permit an estimate of the ability of fish to compensate stress directly related to various handling procedures, providing a quantitative measure of its well-being.

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