Personality traits identification based upon hypoxia test and mecano acoustic stimulation in European seabass (*Dicentrarchus labrax*)

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Introduction:

Animal personality traits can be defined as tendencies that affect behavioural and physiological responses in different contexts, vary across the individuals in a given population, and are consistent within individuals across time [1,2] .
In fish, two major personality types also named coping style, have been identified: proactive (active coping or bold or 'fight-flight') and reactive (passive coping or shy or 'non-aggressive'). Identification of personality traits in farmed fish could be useful to better understand individual adaptive capacity to environmental conditions, vulnerability to stress-related disease and to envisage selection programs to optimize production.
However, a major concern in fish personality traits identification is that the majority of tests developed to date are based on individually housed animals (feeding recovery [3], exploration and flight response [4], respirometry [5], restraining [6], and most of these tests are highly time consuming).
The European seabass, *Dicentrarchus labrax* is a major species in Mediterranean aquaculture although little is known about the effects of the early phases of domestication or selection on growth apart from classical traits of commercial interest [7,8].
Recently, tests in group situation were done with success for screening coping style in fish, including risk-taking tests [8] and hypoxia exposure [9,10].
Here we investigated for the first time how a group-based test in seabass (hypoxia test) could be predictive of individual response during a simulated attack predator (MAS : Mecano Acoustic Stimulation).

Experiment and method:

Self sorting by hypoxia in group (N=120):

Previous studies have shown that fish with divergent coping styles react differently to hypoxic conditions [11, 12]. This suggests that allowing fish to escape hypoxic conditions can be used to sort populations in respect to coping style.

Fig.1. experimental setup used to sort fish group by the hypoxia procedure.

Parameters followed:
- ✓ Escape order
- ✓O_2 level at first passage (% sat)
- ✓Emergence score (Fig. 1)
- ✓Fish escaping from hypoxia tank = Hypoxia Avoiders (**HA**)
- ✓Fish staying in hypoxic tank = Hypoxia Tolerant (**HT**)

Flight response toward a stimulus in isolated situation (N=26):

Fish were individually placed in a 400 l tank. After 2 h of acclimatization, when fish passed under the stimulation zone (zone 1, Fig. 2), the fall of a weight was triggered . Video capture started 20 min before stimulation for a total duration of 1h20. The video recordings were analyzed using the software Ethovision XT (Noldus, the Netherlands) which allowed 4 virtual zones to be defined and to track the fish swimming behaviour.

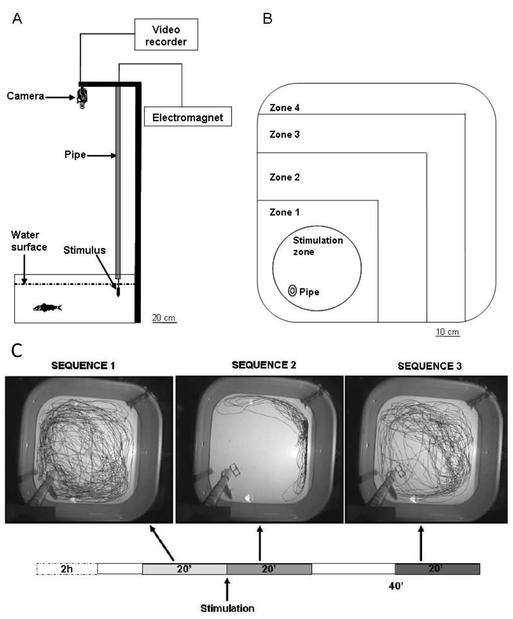

Fig.2. Scheme of the flight response experimental setup. A : tank and system to elicit the fall of the weight. B : virtual zones defined in the tank. C : example of one fish tracking during the 3 sequences [4].

Parameters followed:
- ✓Total distance travelled (in cm)
- ✓Number of entries in each zone
- ✓Angular velocity (deg/s)

Statistical analyses:

Repeated Measures ANOVA with sequence (16 five-minute periods) as the within-subjects factor and hypoxic status (Hypoxia Tolerant (HT) *vs* Hypoxia Avoiders (HA)) as between-subject factor were followed by Pearson correlations between oxygen level and angular velocity.

Results and discussion:

Total distance travelled, number of entries in Zone 3 and 4 (Fig. 3) were significantly different between **HT** and **HA** ($F_{(12, 13)}$=2.78, p=0.04, ($F_{(12,13)}$= 3.8, p=0.02) and ($F_{(12,13)}$= 3.5, p=0.03 respectively).

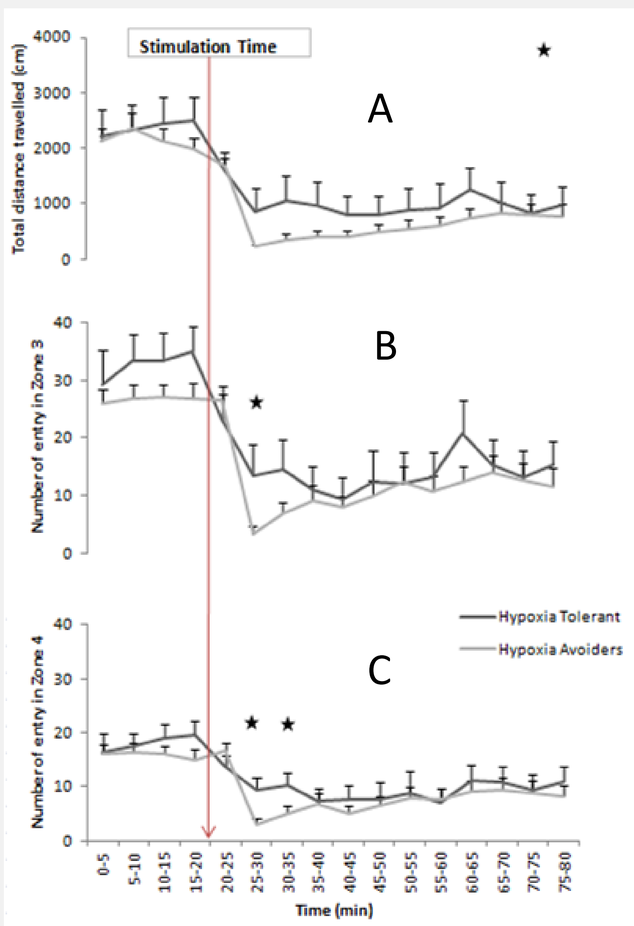

Fig.3. Behavioural variables observed before and after stimulation in the Mecano Acoustic Stimulation experiment. * Newman-keuls posthoc tests, P < 0.05.

Differences especially occurred just after stimulation : **HT** showed higher total distance travelled and higher numbers of entries in Z3 and Z4, the furthest zones from the stimulus.

In all cases, both fish categories reacted similarly to the stimulus presentation: the values of these behavioural variables decreased after stimulation which are typical indicators of fish avoidance of a dangerous area and of risk assessment [4] then increased again without reaching levels observed before stimulation showing that fish remained fearful towards the stimulus.

Lastly, there is a significant positive correlation between the O_2 level at first passage in the normoxic tank and angular velocity (Fig. 4, Rs=0.56, p=0.004).
HT and **HA** fish show different behavioural responses toward a stressful situation.

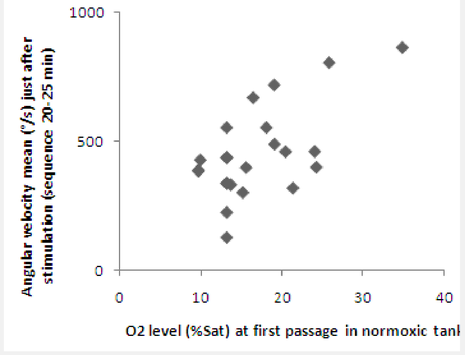

Fig. 4. Correlation between angular velocity and O_2 level at first passage in the normoxic tank.

Hypoxia test likely reflect individual metabolic needs combined with a risk taking test since we did a control experiment without hypoxia and no fish passed in the normoxic tank.
Higher metabolic needs and activity could be 2 traits of the proactive axis.

Our study highlighted that hypoxia test in group sort fish according to different behavioural responses observed in a Mecano Acoustic Stimulation experiment performed in isolated situation. These findings could be useful to develop a seabass group sorting procedure which is the first step before a potential selection program based on personality traits.

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