Behavioural performance of turbot *Scophthalmus maximus* in novel environment paradigms: non-invasive stress indicators

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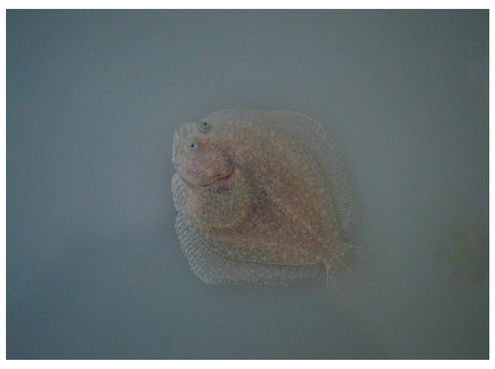
INTRODUCTION



Fish welfare concerns have gained increasing attention within the aquaculture industry, mainly to improve living conditions of farmed fish species and reduce stress. Besides these ethical aspects, better fish welfare has also been shown to positively impact disease resistance and meat quality. However, over 700 species are currently farmed worldwide and welfare standards remain still unknown for most of them. In recent years, remarkable research efforts have compromise animal health and can be continuously and easily monitored. In particular, behavioural stress indicators have been gaining relevance in fish research since they are generally effective in detecting early stress symptoms and are easyly implementable. Nevertheless, developing suitable indicators of stress requires a thorough understanding of the species' behavioural biology and stress responses. The **objective** of this study was to **evaluate the behavioural response of turbot** (***Scophthalmus maximus***), an economically important farmed species for European aquaculture, to distinct **behavioural assays** commonly used to **non-invasively** assess fish stress responses.

METHODOLOGY

Ninety-six juvenile turbot (mean ± standard deviation: 2.86 ± 0.45 cm) were randomly assigned to the stress or control group (n=48 fish/group). In the former, fish were subjected to an acute stressor prior to the behavioural testing. The acute stressor consisted of 2-minutes exposure in a 250 mL bucket (i.e., restraint event). In the control, fish were directly exposed to one of the following behavioural assays: 1) open field, 2) diving test or 3) light-dark preference test. Then, the spontaneous behaviour of each fish was recorded and measured for 10 minutes by an automated fish tracking software. Statistical differences in anxiety-like behaviours between experimental groups were determined by multiple t-tests for each test.

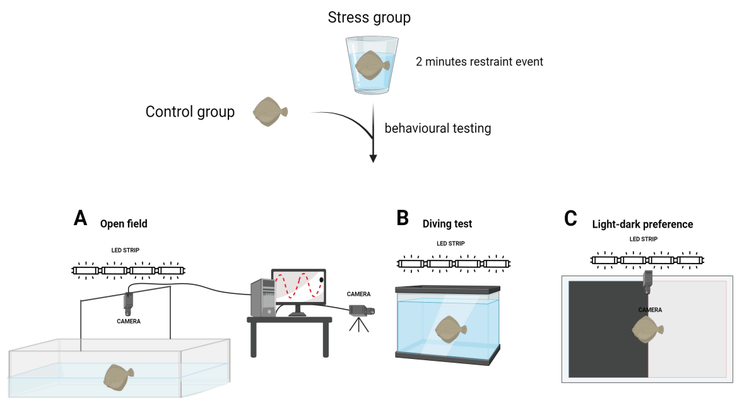


Figure 1: Scheme of the experimental design. Juvenile turbot from each experimental group (i.e., control or stress) were subjected to different behavioural assays (n=16 fish/group/test). **A.** Open field (25 x 25 x 15 cm). **B.** Diving test (15 x 6 x 20 cm). **C.** Light-dark preference (40 x 20 x 15 cm). All experimental arenas were illuminated with a white LED strip and video recordings were captured by an digitalcamera. Fish behaviour was recorded for 10 minutes and consecutively tracked by the Ethovision software.

RESULTS

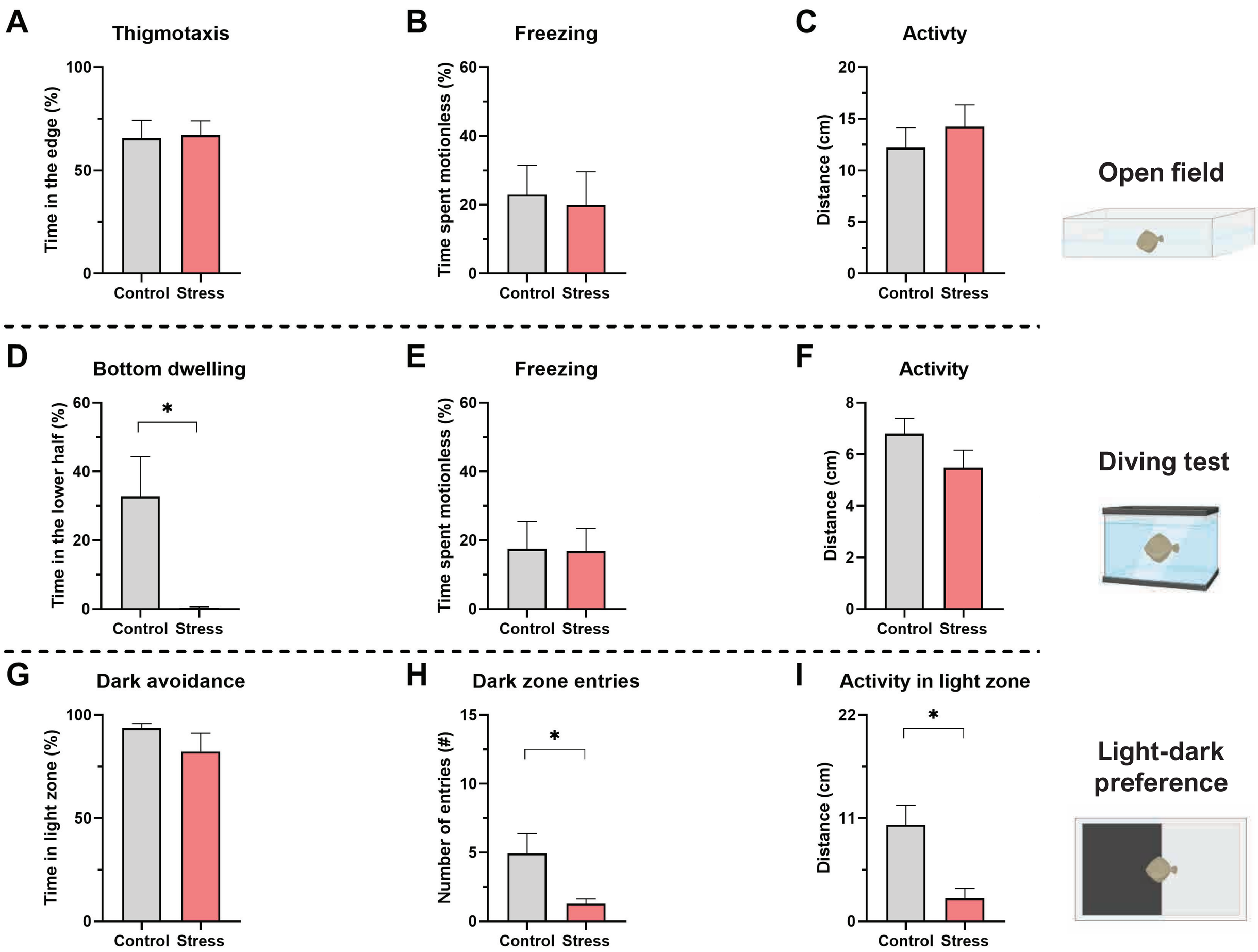


Figure 2: Behavioural performance of turbot from the control (grey) and stress (red) groups in the open field test (**A,B** and **C)**, diving test (**D,E** and **F**) and light-dark preference test (**G,H** and **I**). Data are presented as mean ± standard error and asterisks indicate statistical differences between experimental groups by means of t-tests. The *p* value was set at 0.05 for all analyses.

DISCUSSION

This study examined, for the first time, the behavioural performance of turbot in a set of behavioural tests commonly used to assess stress responses in fish. Our findings indicated that juvenile turbot exhibited **behavioural responses contrary to those previously reported in model teleost species**. Furthermore, we found that the restraint stress event used in our experiments elicited behavioural changes in two out of the three analysed behavioural tests, suggesting it can be an adequate stress challenge for the species. In the open field test, none of the analysed variables exhibited significant differences between experimental groups. This might suggest that this behavioural assay is not suitable for assessing stress in turbot, as reported in other species. On the contrary, both diving and light-dark preference tests showed clear **avoidance behaviour** in turbot. This was supported by significant spatial preferences across both experimental arenas in stressed fish. Overall, this study described for the first time **potential anxiety-like behaviours in juvenile turbot**, highlighting the importance of developing species-specific stress indicators in farmed species. Furthermore, our findings support the use of **behavioural tests as non-invasive methods to assess turbot welfare**.

CONCLUSIONS

- Juvenile turbot exhibit **avoidance behaviours** in novel environment paradigms
- **Anxiety-like behaviours** in turbot are **opposite** to those reported in **model species**
- **Light-dark preference test** and **diving test** might be useful non-invasive methods to assess turbot stress response

ACKNOWLEDGEMENTS

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