

Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVII

Use of flavorings to improve the palatability of the diets used in aquaculture: multidisciplinary approaches to better understand the physiological responses of teleosts in breeding

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# Introduction

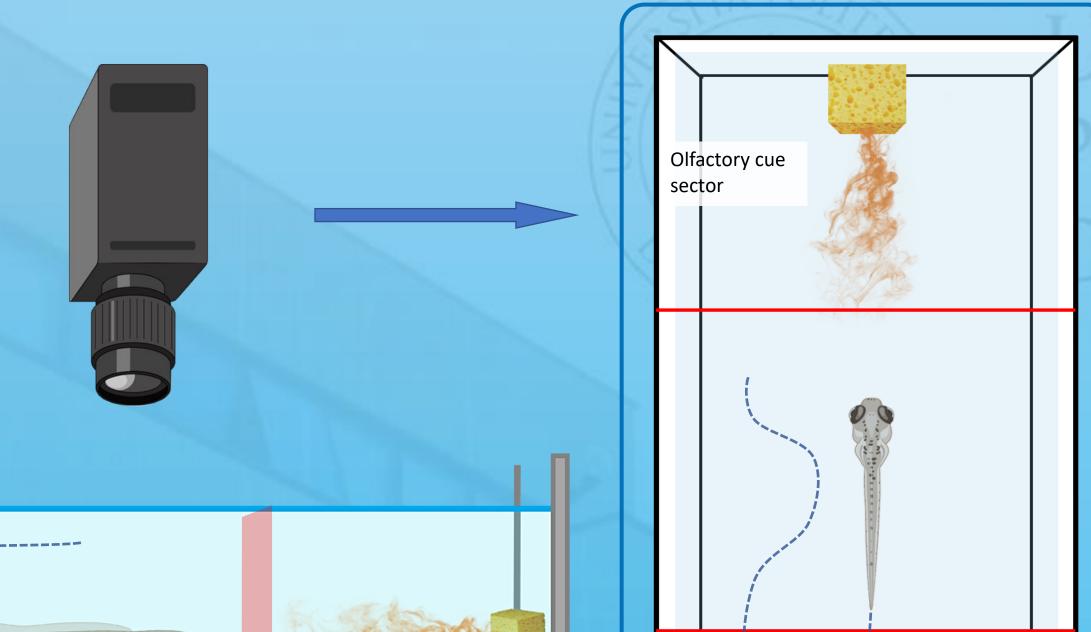
Most of the waste output in the aquaculture industry derives from feed losses, which leads to a significant increase in the concentration of nitrogen (N) and phosphorus (P) in the aquatic environment, promoting eutrophication problems. As feed represents 40–75% of production costs in this sector, reduction in waste outputs is now mandatory, and this goal can be achieved by a better management of feeding activities and by improving aquafeed palatability through specific feed additives. The present study wants to explore the application of feed additives for aquafeed formulations, possibly increasing palatability and reducing, at the same time, the above-mentioned issues. A set of different olfactory cues have been tested during a behavioural test performed on zebrafish (*Danio rerio*) larvae to identify those that positively attracted the fish.

Eight hundred zebrafish larvae aged 25 days post fertilization (dpf) were divided in forty groups of 20 individuals each and fish were singularly exposed to a different water-soluble olfactory cue (named from P01- P40).

Two cellulose sponges (side 1 cm) were inserted into the tanks: one contained the olfactory cue (1 %) while the second water and served as control for object exploration.

Video recording of explorative behaviour of

### **Materials and Methods**



Video analysis using BORIS software allowed to calculate the time spent by the subject in each sector of the tank.

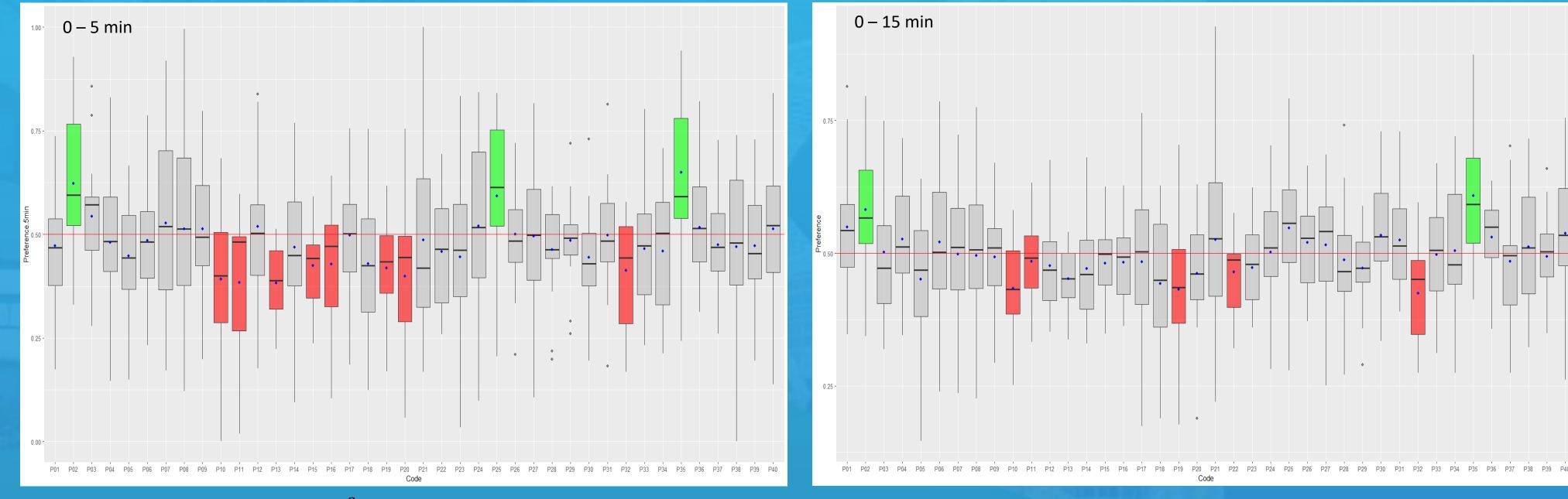
A one-way ANOVA considering the total time of analysis (min 0-15) was used to evaluate whether the larvae responded differently to several olfactory cues.

The data of the first 5 minutes (min 0-5) and the total time recorded (min 0-15) were analysed separately by computing the overall proportion of time spent close to the novel olfactory cue in these two periods of time. One-sample t-tests against the preference expected by chance (0.5) were used to test whether the preference for the novel olfactory cue was different from random choice.

zebrafish larvae; Test duration 15 min



## **Expected Results/Conclusions**



Boxplot of minutes 0-5 and 0-15 respectively, showing the average percentage of time spent by fish in the sector with the specific olfactory cue. Odours that showed a significant (positive, green; negative, red) effect are highlighted in the figure.

#### ANOVA, $F_{39,724} = 2.42$ , p = <0.0001, $\eta_g^2 = 0.12$

Analysing different time slots is essential because the effects of each olfactory cue can vary during the time in relation to its chemical nature and dilution factor. Novel or too much

concentrated odours in fact, can initially act as alarm signals evoking avoidance or defensive behaviours. To reduce feed losses in aquaculture, feed has to be ingested by fish quickly and

### thus the search of new feed additives should be focused on the olfactory cues that have a quick and positive effect on fish during the first minutes of feed administration. The best

performing odours will be added to fish feed to analyze fish behaviour, feeding habits and physiological responses.