Why Zebrafish?

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Aim: To determine if the lack of distinct facial features in fish influences researchers' choice in species model.

Introduction

Although fish are currently the second most used vertebrate model in research, it is debatable whether the same amount of care and consideration is given to them as mammalian models. Even ASPA guidance does not consider them as sentient as other species. In this preliminary work, we hypothesis, that this perception is in part due to the lack of facial muscles and structures that are common to all mammalian models, making it difficult to empathise with them and giving them a remoteness which is not seen in mammalian species. Because zebrafish cannot make facial expressions, as mammals do, it makes it extremely difficult to determine how much pain, suffering and distress they are experiencing. In this work, we will explore whether subtly altering facial expressions has an impact on people's thoughts on pain and suffering in zebrafish and if it is related to their lack of facial expressions impacting on people's level of empathy. We then go onto further examine the various reasons as to why people may or may not choose to work with zebrafish in the research.

Grimace Scales

Grimace scales have been created for various mammalian species in the research industry in order to help those working with the animals detect how much pain, suffering and distress they are in. A grimace scale is composed of various photographs of a particular species showing different facial expressions and what they mean. However, a grimace scale does not exist for zebrafish due to their inability to visually express their pain, suffering and discomfort.

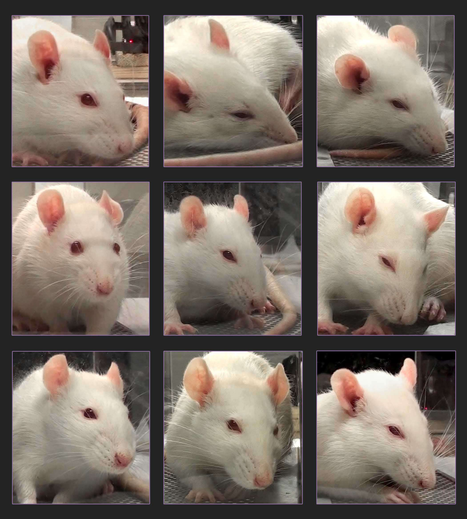
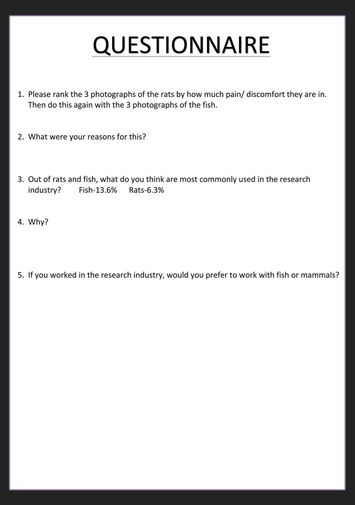


Fig. 1: A rodent grimace scale detailing the types of diagnostic features to look for when identifying pain, suffering, distress, and lasting harm. All pictures indicate a higher level of pain moving from left to right. Top row: nose and cheek flattening. Middle row: orbital (eye) tightening. Bottom row: whisker tightening. Grimace scale images reproduced with permission from the NC3Rs

Methods

In order to find out the possible cause for people choosing to work with fish rather than mammals in research, we interviewed collected 4 groups of 4 people from different areas in the industry including: researchers that work with fish, technicians that work with fish, technicians that work with mammals, and a group of people that do not work with animals at all.



QUESTIONNAIRE

1. Please rank the 3 photographs of the rats by how much pain/ discomfort they are in. Then do this again with the 3 photographs of the fish.
2. What were your reasons for this?
3. Out of rats and fish, what do you think are most commonly used in the research industry? Fish-13.6% Rats-6.3%
4. Why?
5. If you worked in the research industry, would you prefer to work with fish or mammals?

We then created a false grimace scale for zebrafish in an attempt to mimic a rodent grimace scale (fig. 2). This was done by manipulating close up photographs of zebrafish multiple times to give the fish various distinct facial expressions.

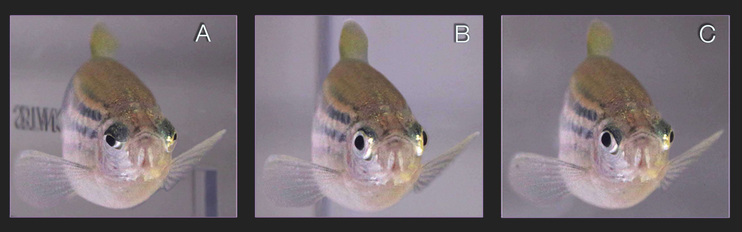


Fig. 2 (left): a copy of the questions the interviewees were asked. Above, the three edited images that they had to rank in question 1 to levels of pain being displayed. This is based on the orbital tightening poriton of a mammalian grimace scale.

Following this, we designed several questions which involved: whether zebrafish or rats had a higher percentage of use as models in research and the possible reasons behind it, and whether the interviewees would personally prefer to work with fish or mammals and why. Part of the questionnaire also involved the individuals looking at edited photos of zebrafish and ranking them from 1-3 (1 being least amount of pain and 3 being the most) and then repeating this exercise with 3 photos of fish with no difference in facial expression (fig. 3) as well as 3 photographs taken from a rodent grimace scale.

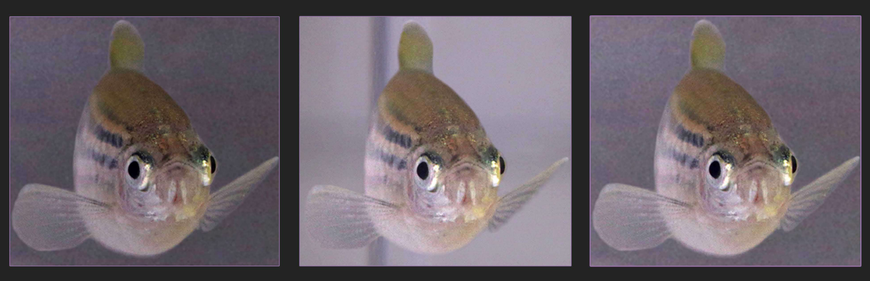


Fig. 3: the unedited images from the first question of the questionnaire. Only the background has been altered.

Finally, we compiled and analysed all of our data in order to compare what people from different backgrounds in the research industry thought and how they differed from one another.

Results

We compiled the results from each group and found that 93.7% of the interviewees guessed correctly when ranking the manipulated fish photos as conveying the most pain. 68.75% guessed correctly when ranking the mice, whilst 75% ranked the unedited fish images as identical. (Fig. 4)

When asked which had a higher research usage, 100% of aquatic and mammalian technicians believed that fish were more used over rats. However, both researchers and laymen were split at 50%. Finally, when the interviewees were asked if they would prefer to work with fish or mammals, the results were very mixed: 100% of researchers said they would prefer to work with fish, whereas for both aquatic technicians and laymen, 75% preferred to work with fish, as did 50% of the mammalian technicians. (Fig. 5).

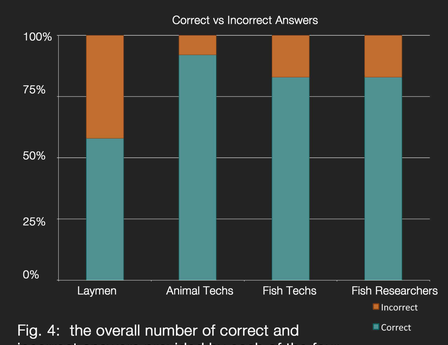
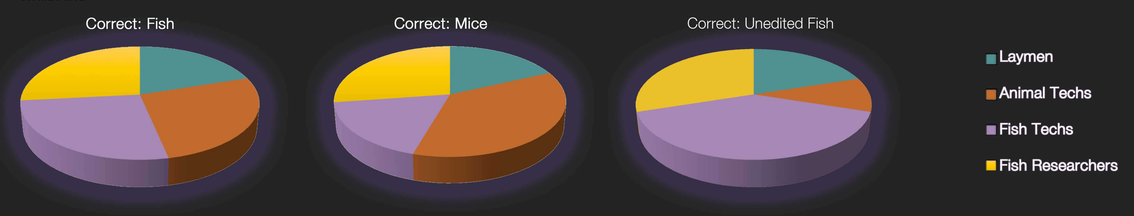


Fig. 4: the overall number of correct and incorrect answers provided by each of the four groups (laymen, animal techs, fish techs, and fish researchers). The animals techs provided the most correct answers, whilst the laymen provided the most incorrect. Fish techs and researchers were equal.

Fig. 5: the correct answers provided by each group by each category. Fish techs, more than any other group, were able to identify the unedited fish photos, whilst the



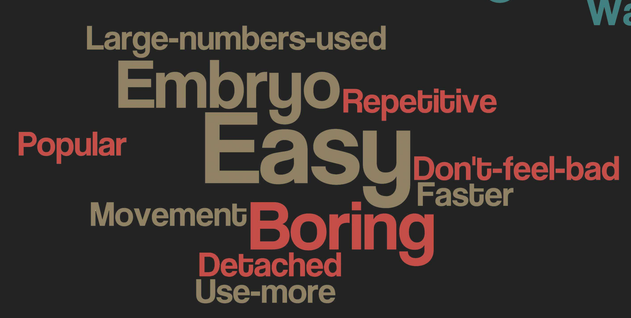
Discussion

When ranking the edited zebrafish images, the interviewees tended to give anthropomorphic responses, using emotional words like 'unhappy' to back their choices rather than looking at physical characteristics. When asked to rank levels of pain and suffering in the fish, the manipulated images induced more emotive responses, perhaps because the images were edited to mimic a mammalian grimace scale and was effective in grading the images as they were more relatable .

When ranking the mice images, we expected the animal technicians to judge correctly as they health check mammals. The majority of the other groups also guessed correctly, perhaps as a mammalian species, this is intuitive so people understand the features of pain and suffering in other mammals. This suggests people find it easier to relate to mammals due to the similarities between facial expressions.

The majority of interviewees correctly identified the unedited pictures as the same, and the altered backgrounds made no difference. We found that most people are unable to assess how much pain a fish is in purely based on expression as fish do not have the same facial muscles as mammals.

We wanted to determine if people chose to work with zebrafish in research over mammals because fish inspire less empathy and are therefore easier to work with on an emotional level as they feel less guilty. Many of the interviewees gave various scientific reasons for working with fish when asked, such as easy embryo collection and faster developmental stages. But people felt less empathy and detached from the fish and mostly provided negative words (fig. 6). Mammals inspired the words 'guilty' and 'empathy', as well as positive words.



Further Work

In order to follow on from this concept, we intend to develop a questionnaire using thematic analysis; this will entail making open ended questions designed to be more probing which will improve the depth of the answers given. Further topics to include would be the emotional toll on those who work with all vertebrates, and why people equate pain with emotion in people, but not in animals. Related to this is the idea that sentience in animals only concerns ability to feel pain, yet in humans is connected to level of intelligence. The question of an effective fish grimace scale needs to be further explored.

References:
1. T. T. Rocchetti, S. Silbert, A. Gostnell, C. Kubasek, A.C. C. Pignatari and R. Widen. 2017. Detection of Mycobacterium chelonae abscessus Group, and Mycobacterium fortuitum Complex by a Multiplex Real-Time PCR directly from clinical samples using the BD Max System. *The Journal of Molecular Diagnostics*. Vol 19, No.2

Acknowledgements:
Many thanks to the UCL Fish Facility staff: K. Dunford, H. Callaway, P. Barwood, E. Hitchcock, J. Warmsley, T. Wheeler.