POSTER PRESENTATIONS

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Darwin's fishes?: keeping Malawi Cichlids

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Introduction

Cichlids in Lake Malawi have evolved different phenotypes. However, at the level of the genome, they look very similar, so research is being carried out on these fish to try and understand genome evolution. African cichlids are some of the most colourful of all freshwater fish; they offer a wide variety of body shapes and behaviours. They are extremely active and can be very personable, showing behaviours such as greeting technicians and begging for food. This poster will provide information on the cichlids, including their dietary and husbandry requirements and the breeding of these animals.

Figure 1. *Rhamphochromis chilingali*.

Figure 2. *Tropheops. Sp. 'mauve'.*

Information

Cichlids have evolved rapidly into a large number of closely related but morphologically diverse species within large lakes.

- There are 850 different Malawi cichlids and all of these are more similar at the genome level than humans.
- All cichlids have some form of parental care for their eggs and fry. That parental care is either guarding the eggs or mouthbrooding.
- They have a wide range of body size from 2.5cm to 1m, however, the majority tend to be medium size and an oval shape.
- Their average life span is 4-10 years but can live to 15 years.
- Cichlids are omnivores. Many cichlids are primarily herbivores, feeding on algae and plants, others are predatory and eat very little or no plant matter, instead catching other fish, eggs or young.
- Cichlids are an aggressive species, establishing territory and hierarchy through evaluating their competitors. The most dominant males will have more vivid and bright colouration and subordinate males will assume a dull colour.

Housing requirements

- Kept at 25˚C.

- 12 hour light/dark cycle.
- Water quality is monitored weekly.
- Weekly water changes (20%).
- Nets used for catching fish are kept in a 4% bleach solution.
- Enrichment such as plants, tunnels, bricks and plant pots.
- Sand substrate for tank floor.
- Dechlorinated water.
- Air stones and water filters.
- Higher ratio of females to males to reduce aggression.
- Omnivore food – 5ml Novo Cichlid mix, 2.5ml per cross tank.
- Carnivore food – 4.5ml Novo Cichlid mix, 2.5ml black soldier fly pellets.

Figure 3. Tank set up.

Figure 4. *Calliptera "salima"* male with the plant pot enrichment.

Figure 5. Example of room set up.

Research

- The genetic and developmental basis of morphological variation in cichlid fish is currently being researched. The focus is on understanding what makes species different at the DNA level and associating specific portions of the DNA to specific pigmentation patterns.
- Studies are examining if it is possible to use *in vitro* fertilisation to create crosses between cichlid species with different colours and targeting ~15 genes to determine which underlie cichlid colour differences.

Breeding

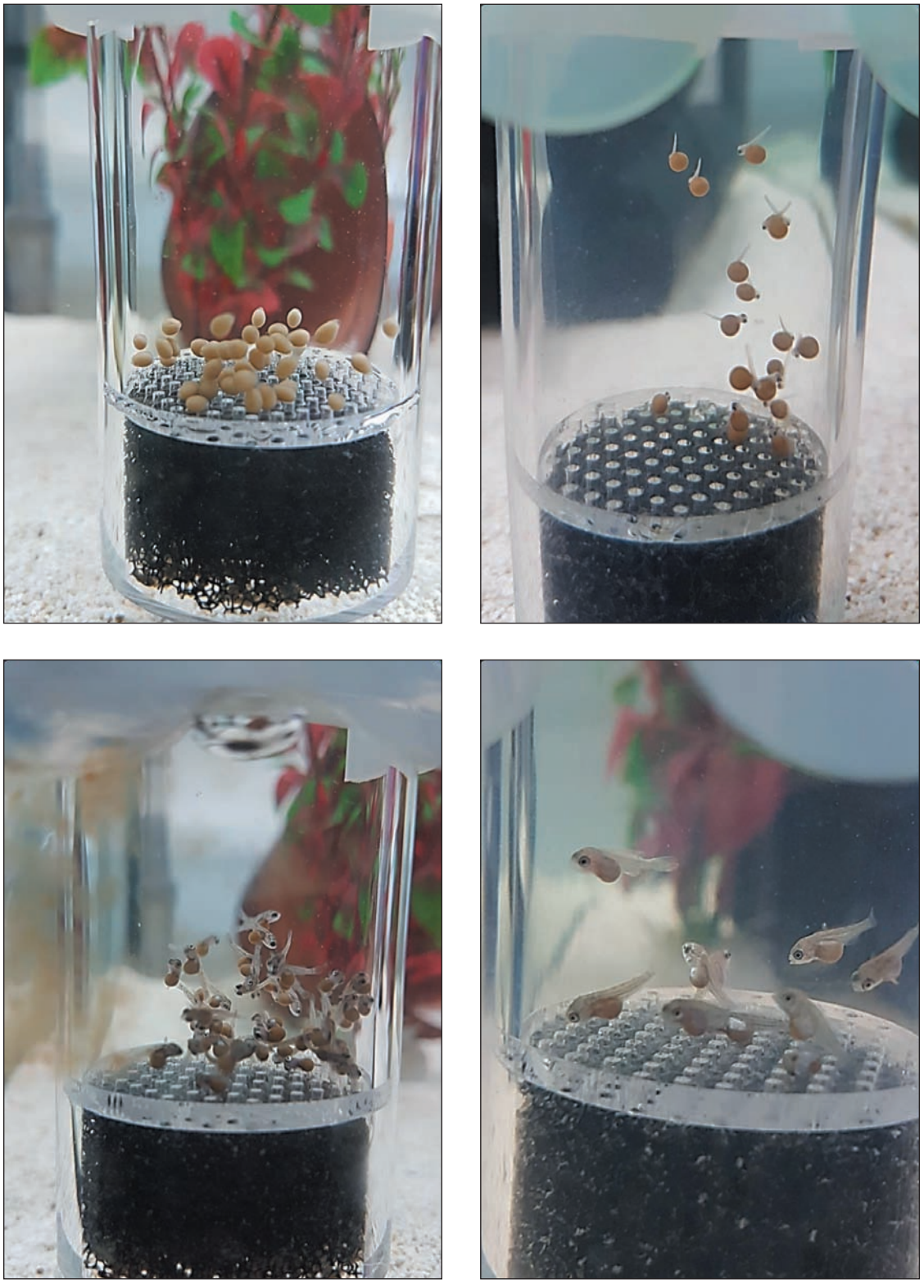
Cichlids either mate monogamously or polygamously. In the wild they will lay their eggs in caves or crevices, so providing a plant pot will help to encourage these natural behaviours. The courting process may vary slightly for different species but is similar for most of the mouthbrooding cichlids. The male will chase the females around the tank, attempting to lure one to a spawning area (either a plant pot provided or a sandy area that the male will dig out by shaking his body). The male and female will swim in circles in the spawning area. Once the female lays her eggs, she will immediately try and scoop them up into her mouth. Most adult males exhibit a unique pattern of oval-shaped colour dots on their anal fin. The male will gyrate his anal fin which leads the female to believe the spots are her eggs. She then opens her mouth to the anal fin and he discharges sperm into her mouth and fertilises the eggs.

After mating, the female will have a mouthful of

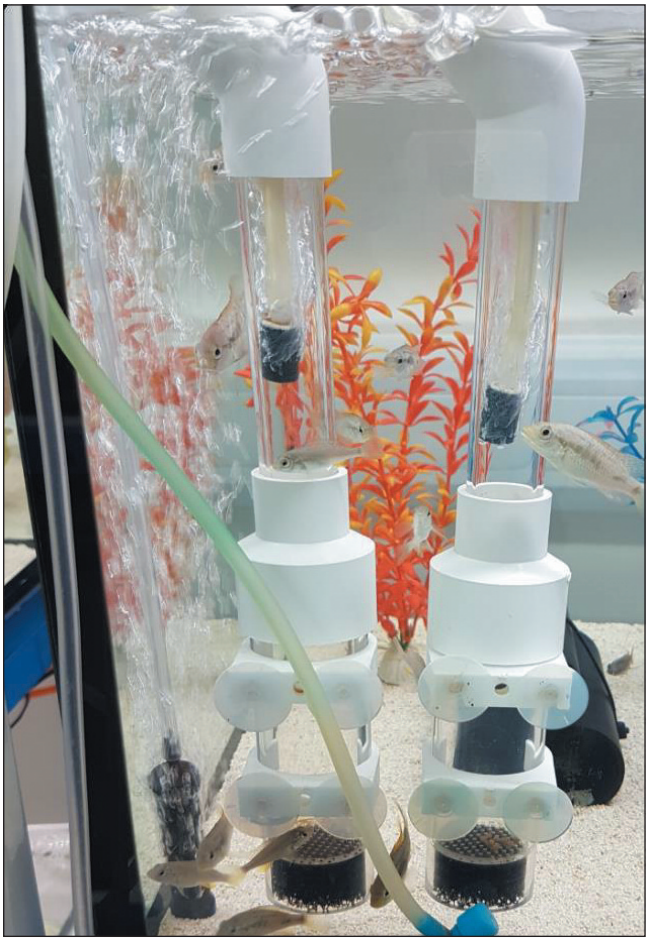
Figures 6-7. Mouthbrooders with a dropped lower jaw.

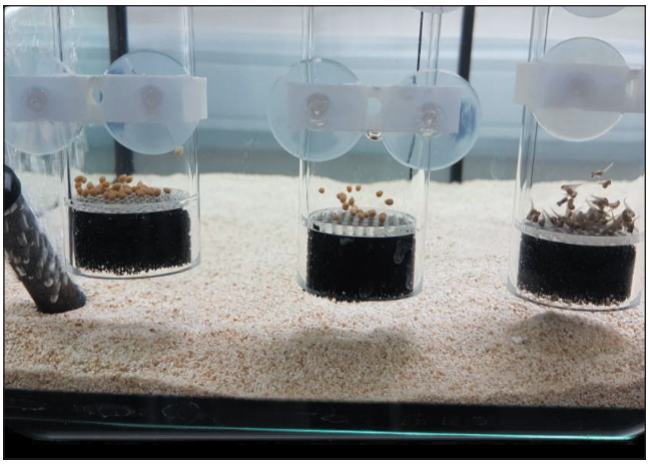
fertilised eggs. Her jaw will look very bloated and therefore mouthbrooders are easy to spot. Another sign of mouthbrooding is not eating, as many females will fast so that they do not risk endangering the eggs or fry by attempting to eat.

The eggs are removed as if they are left in the tank, when the female releases the fry, they will be eaten by the other fish. The eggs can be placed into tumblers if more fish are required, or can be fed to a carnivorous species such as the *Rhamphochromis chilingalias*, as fish are not protected under the Animals (Scientific Procedures) Act 1986 (ASPA) until they can independently feed.



Figures 8-11. Various egg tumblers with different stages of development.





Figures 12-14. Examples of egg tumblers set up in fish tanks.

Acknowledgements

The author would like to gratefully acknowledge Emilia Santos and Maggie Dinsdale for their assistance with the poster, and Sarah Manley and Sam Melvin for their work with the fish.

Stiff as a board: measuring rigor mortis in Zebrafish

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Aim

To determine the rate of rigor mortis using different anaesthetic agents.

Introduction

Schedule one killing (S1K) methods require a two-step process: a humane method of death, typically for Zebrafish an anaesthetic overdose and confirmation of death, such as confirmation of rigor mortis. There is widespread variation of anaesthetics used for S1K and there is debate about refining anaesthesia for Zebrafish, as adverse effects are becoming a concern. Anecdotal evidence suggests that different anaesthetics can inhibit or reduce the rate of the onset of rigor mortis, with some taking an hour whilst others take more than three. We conducted a trial in order to determine the rate of rigor mortis for four different agents.

Rigor mortis

Rigor mortis (RM) is a stage of death characterised by muscle stiffness. Following death, lactic acid accumulates, decreasing cellular pH.[1] This disrupts glycolysis and in turn adenosine triphosphate (ATP) levels fall, which breaks actin-myosin cross-bridges. Its depletion results in prolonged muscle stiffness (Figure 1). The rate of onset and duration of RM is dependent on many factors: size, temperature and pre-mortem exhaustion. Studies on anaesthetics used in Zebrafish are limited and the effects on RM are mostly unknown.

Figure 1. A Zebrafish that is in rigor mortis.

Four common anaesthetics are Benzocaine, lidocaine, 2-phenoxyethanol (2-PE) and MS-222 (Figure 2). Of these, lidocaine, benzocaine and MS-222 all reversibly bind to voltage-dependent sodium channels.[2] This inhibits Na+ uptake which stops the initiation and propagation of action potentials at the site of pain. The mechanism of action for 2-PE is currently unknown in fish.

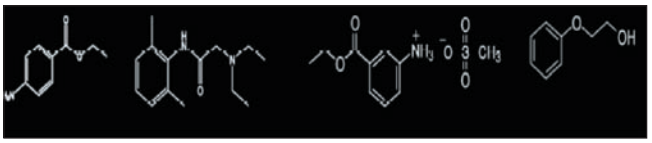


Figure 2. The chemical structure of each anaesthetic used. Left to right Benzocaine, (4-Aminobenzoic acid ethyl ester, Ethyl 4-aminobenzoate) chemically similar to MS222. Lidocaine hydrochloride, MS222, (Ethyl 3-aminobenzoate methanesulfonate). 2-Phenoxyethanol. Attributions: Benzocaine: Mykhal (Public domain). Tricaine: Edgar 181 (Public domain). 2-Phenoxyethanol. Lidocaine hydrochloride: Prisonblues at the English language Wikipedia (CC BY-SA 3.0 (http://creativecommons.org/licenses/by-sa/3.0/)).

Methods

A total of 57 hybrid/(AB:TupLF) 10 month old adult fish of the same stock were used. The fish were maintained in a recirculating 10L tank, 28.2-28.4˚C, pH (7.1-7.3, in 14:10 hour light/dark cycle and fed on a combination dry food diet. All fish used were of similar size for each

	Average Weight
Males	0.783 g
Females	1.178 g

Table 1. The average weights of each sex. The females weighed more overall.

respective sex (Table 1). The fish were scheduled for euthanasia according to S1K methods as found in the Animals (Scientific Procedures) Act 1986 (ASPA). The 2-PE dose followed UCL protocol, whilst the other three were based on published recomendations (Table 2).

Anaesthetic	Dosage	Average time of no observed opercular movement	Average time of no observed movement
Benzocaine	0.7 g/L	00:00:26	00:01:01
MS-222	0.5 g/L	00:00:38	00:01:35
2-PE	6 mL/L	00:00:12	00:00:31

Table 2. Anaesthetic doses. The time of no observed movement is the time of assured death which occurs after respiration ceases.

Lidocaine Dose	Observation
<500 mg/L	Still swimming >2 minutes
600-700 mg/L	Still swimming >2 minutes Fish exhibited erratic behaviour

Table 3. Lidocaine was too adverse; the fish did not lose consciousness quickly enough.

Figure 2. Fish immersed into overdose anaesthetic, 10 minutes after observed death transferred into glass petri dish filled with system water (remove) or transferred to dish with same anaesthetic (stay).

Lidocaine was excluded from the trial as it was deemed too adverse to use (Table 3); these fish were removed from the trial and euthanised according to UCL protocol. Each anaesthetic was tested into two different post-mortem media: 'removed' into fresh water and 'staying' in dosed water (Figure 2).

For each treatment, three fish were added to the pre-dosed water and left for ten minutes to ensure death. After a knock test to ensure death, individual fish were transferred to labelled petri dishes with the assigned post-mortem media. At 30 minute intervals, each fish was held by the caudal peduncle and measured against a protractor to measure the angle (Figure 3).

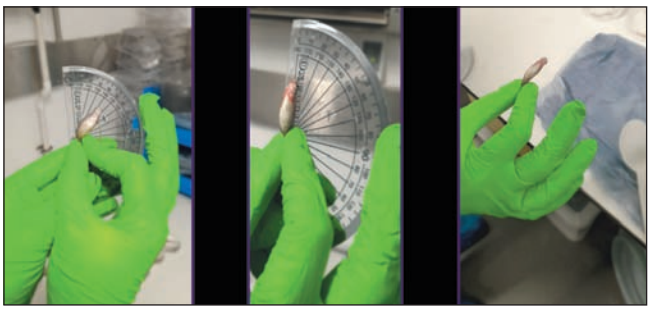


Figure 3. Every 30 minutes, the angle of each fish was measured using a protractor to determine onset/stage of rigor mortis. Most fish reached 0˚.

Results

The time points for the measured angles for each treatment were averaged and plotted to compare the rate of RM in each anaesthetic. The most notable difference was 2-PE 'stay', which reached 0˚ at a faster rate than Benzocaine and MS-222; 2-PE reached 0˚ at approximately the three-hour mark, whereas the other two took more than five hours. The maximum angle was also reached faster in the 2-PE 'remove' media but at a slower rate.

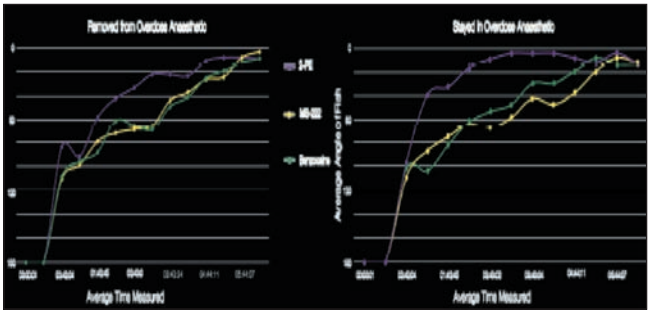
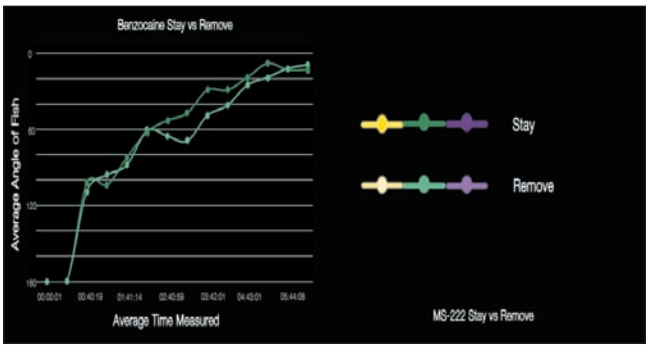


Figure 4. The different post-mortem media. The fish in 2-PE 'Stay' (right) reached the maximum angle at a faster rate than MS-222 or Benzocaine.

Each anaesthetic was individually plotted to compare the post-mortem media and its effect on the rate of RM.



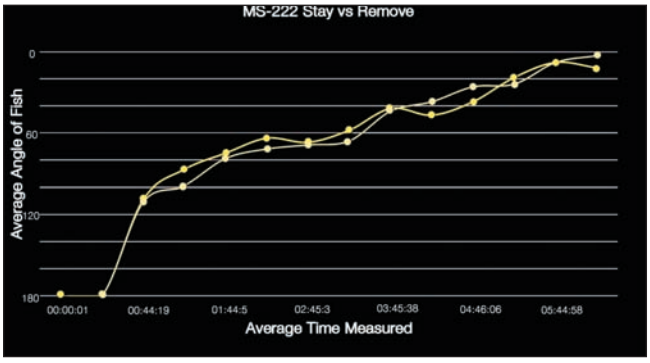


Figure 5. The three anaesthetics compared to the two post-mortem media, stay' and 'remove'. The most obvious difference was 2-PE which still reached the maximum angles in the 'stay' medium before 'removed'.

Discussion

It was noted during the trial that the fish euthanised with 2-PE exhibited signs of death sooner than the other anaesthetics by approximately 30 seconds (Table 2). Additionally, RM was induced faster with 2-PE compared to the other agents, regardless of post-mortem media (Figure 4). It is unlikely that the small acceleration of death by 2-PE caused such an increase in the onset of RM (Figure 5). Therefore, we must question the possible changes in cellular physiology by 2-PE.

When comparing the post-mortem media, it is clear that **2-PE allows for the maximum angle to be achieved first** when the fish stay in the anaesthetic. Benzocaine also appeared to work faster when the fish 'stay' rather than are removed (Figure 5). Surprisingly, MS-222 showed little, if any difference between the media; although they both work similarly at the chemical level, there is the suggestion that there is even a larger difference between them than initially assumed at the beginning of the trial.

The dosages may also play a role. Benzocaine and Lidocaine dosages are quantitatively small compared to 2-PE (Table 2).

Studies on other species suggest possible side effects of 2-PE: raised cortisol and glucose levels pre-mortem; lowered blood oxygen; increased CO_2; and reduced pH.[3] Both high levels of cortisol and decreased pH and glucose lower the rate of glycolysis, decreasing ATP, which directly increases the rate of RM.

The potential increased stress that these animals experienced during euthanasia should be of great concern. Although the data collected here is focussed on non-protected animals (i.e. dead), a potential application for this information is a **refined** use of S1K anaesthetics for Zebrafish. The exclusion of Lidocaine from the trial is a good example; it is assumed that a quick death is the ideal but for more than two minutes, the fish did not exhibit desirable anaesthetic depth, unlike the other agents which did so into in less than two minutes. Conversely, 2-PE took –30 seconds, yet there is now a question about cortisol/stress present with this agent. It is possible that we did not wait long enough in our trial to see sufficient effects from Lidocaine for S1K. As a result of this, we need to establish if **a slower, possibly less painful death** would be more ethical than aiming to achieve the quickest possible induction of death.

Further work

There are potential variables that were not explored, or explored enough, in this trial, such as strain, dosage and effect of sex/weight. This will be addressed in a repeat trial. Other factors that affect stress will be controlled further, such as handling and health. We will also attempt to establish if the increased rate of RM with 2-PE is from the agent or the increased dosage. The collected data may aid the refinement of S1K.

Acknowledgements

Many thanks to the UCL Fish Facility staff: H. Calloway, P. Barwood. E. Hitchcock, R. Davies-Green, J. Warmsley, T. Wheeler, D. Marks and V. Moiche.

References

1 **Nazir, D.** and **Magar, N.** (1962). Biochemical changes in fish muscle during rigor mortis.

2 **Collymore, C., Tolwani, A., Leiggi, C.** and **Rasmussen,2.** (2014). Efficacy and safety of 5 anaesthetics in adult Zebrafish (*Danio rerio*). *Journal of the American Association of Laboratory Animal Science* 53(2) pp198-203.

3 **Hedayati, A.A.** *et al.* (2016). Effects of 2-phenoxyethanol (2-PE) anaesthesia on some haematological and biochemical indices of Silver carp (*Hypophthalmichthys molitrix*).

Why Zebrafish?

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Aim

To determine if the lack of distinct facial features in fish influences people's thoughts 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. Even the Animals (Scientific Procedures) Act 1986 (ASPA) Guidance does not consider them as sentient as other species. In this preliminary work, we hypothesise 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 and 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 explore whether subtly altering facial expression as have an impact on people's thoughts on pain and suffering in Zebrafish and if this is related to their lack of facial expressions impacting on people's level of empathy. We then go on to further examine the various reasons as to why people may or may not choose to work with Zebrafish in research.

Grimace scales

Grimace scales have been created for various mammalian species in the research industry in order to help those working with animals detect how much pain, suffering and distress they are in (Figure 1).[1] A grimace scale is composed of various photographs of the species concerned 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.

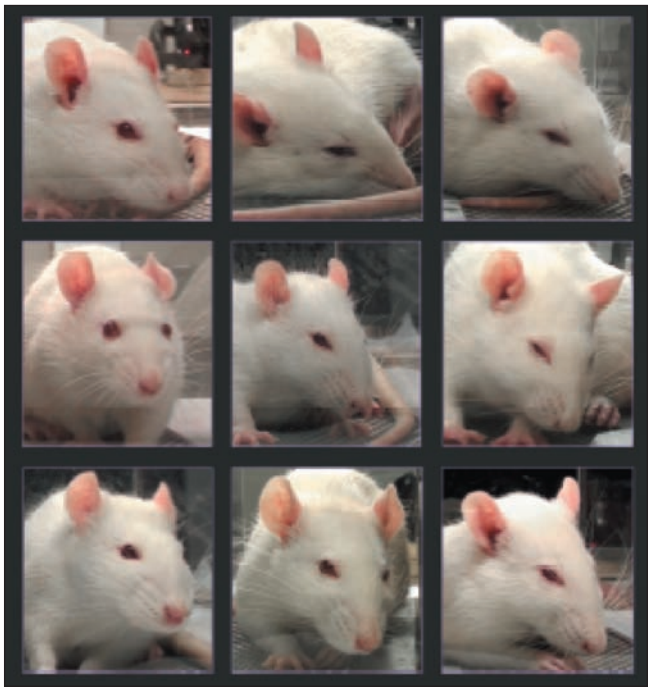

Figure 1. A rodent grimace scale detailing the types of diagnostic features to look for when identifying pain, suffering, distress and lasting harm. All pictures indicate a higher level of pain moving from left to right. Top row: nose and cheek flattening. Middle row orbital (eye) tightening. Bottom row whiskers tightening. Grimace scale images reproduced with permission from the end NC3R's.

Methods

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

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

Figure 2a. The edited Zebrafish pictures used for the interviewees to rank in order of pain. These are based on the orbital tightening portion of alien grimace scale. A: obvious or the highest pain., B: zero or no pain. C: moderate on some pain present in the animal. Zebrafish do not have eyelids and therefore this representation is entirely fictional.


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?

Figure 2b. The questionnaire used to conduct interviews for those work marking the pictures.

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, also whether the interviewee would personally prefer to work with fish or mammals and why (Figure 2b). Part of the questionnaire also involve the individuals looking at edited photographs of Zebrafish and ranking them from 1 to 3 (one being the least amount of pain and three being the most) and then repeating this exercise with three photographs of fish with no fish difference in their facial expression as we well as three photographs taken from a rodent grimace scale (Figure 3).

Figure 3. The unedited pictures of Zebrafish. The only difference between these three is the background. Interviewees were asked to rank them in order of least pain to most.

Finally, we compiled and analysed 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 guess correctly when ranking the manipulated fish photographs as conveying the most pain. 68.75% guess correctly when ranking the mice, while 75% ranked the unedited fish as identical (Figures 4-5).

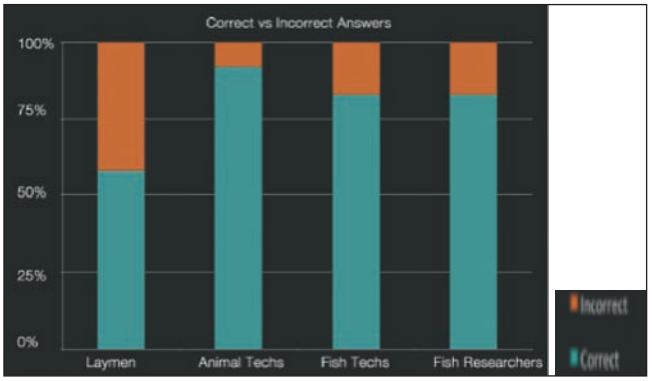

Figure 4. The overall number of correct and incorrect answers provided by each of the four groups (laymen, animal technicians, fish technicians and fish researchers). The animal technicians provided the most correct answers, whilst the laymen provided the most incorrect. Fish technicians and researchers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

Figure 5. The correct answers provided by each group by each category. All fish technicians were able to eye up identify the unedited fish photographs, whilst only some of the other groups correctly identified that the pictures were unedited.

When asked which had the higher research usage, 100% of aquatic and technicians working with mammals believe that fish were more used over rats. However, both researchers and laymen were split 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 technicians currently working with mammals.

Discussion

When ranking the edited Zebrafish images, the interviewees tended to give anthropomorphic responses, using emotional words like "unhappy quote 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 induce more emotive responses, perhaps because the images were edited to mimic mammalian grimace scale which helps 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 much as mammalian species, this is intuitive as people understand 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.

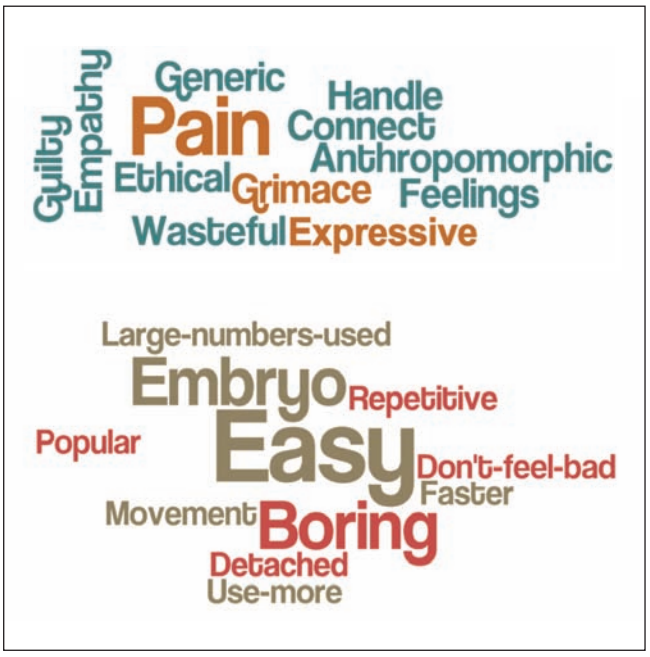

Figure 6. The two word clouds (top and bottom). The top shows the responses concerning the mammals. The orange represents the emotional responses people felt about the animals and the green represents how they perceive the animals were feeling. The bottom shows the responses to the fish with the red representing the responses that people feel about the fish and the brown showing technical terms used to discuss the fish. No group provided an emotion provided emotional terms to represent the experience of the fish.

Most interviewees correctly identified the unedited pictures as identical and the altered backgrounds had no effect. We found most people were unable to assess pain in a fish purely based on expression as they do not have the same mammalian facial muscles

We also wanted to determine if people chose zebrafish over mammals as fish inspire less empathy and therefore are easier to work with on an emotional level as they inspire less guilt.

Words clouds, based on the interviewees indicate that most had an emotive response to mammals, as well as projecting emotions onto them. Conversely fish was spoken positively in relation to ease-of-use and process. Many interviewees gave various scientific reasons for working with fish, such as an easy embryo collection and faster developmental stages. But people felt less empathy and detached from fish and mostly provided negative words (Figure 6).

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. The 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 the ability to feel pain yet in humans is connected to the levels of intelligence. The question of an effective fish grammar grimace scale needs to be further explored.

Acknowledgements

Many thanks to the UCL fish facility staff: K. Dunford, H. Calloway, P. Barwood, E. Hitchcock, J. Warmsley, T. Wheeler.

Reference

[1] **Sotocinal, S.G., Sorge, R.E., Zaloum, A., Tuttle, A.H., Martin, L.J., Wieskopf, J.S.** *et al.* (2011). The Rat Grimace Scales: a partially automated method for quantifying pain in the laboratory rat via facial expression. *Molecular Pain* 7:55

You are what you eat

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Aim

To identify any recurring differences between fish fed different diets.

Introduction

Knowing the best techniques for healthy animals is important for all animal technicians, especially when it comes to feeding. Therefore, UCL continued to explore a trial, published in *Animal Technology and Welfare*, which examined four commercially available feeds (Figure 1) and the ways they affected University College London (UCL) AB Zebrafish (*Danio rerio*).[1]

The previous trial used a feed which caused deformities, commonly in the skull and caudal vertebrae; due to this, the use of the feed was terminated. Specimens from fish fed this feed were fixed for bone staining for further study in order to understand the nature of the deformities.

Figure 1. The dry food diets used for the trial. Diet F is a combination of Diet A, B and C.

Methods

Viable AB embryos were generated from various stock, randomised and split into four trial groups: one for each of the three feeds and one for the combination feed. At five days post fertilisation (dpf) and subsequently in four or five day intervals until 56 dpf, a random sample from each group was photographed to produce length data over time (Figure 2).

At day 56 the fish were sexed and evenly distributed (Figure 3). At day 77 dpf, a random sample of both sexes from each group was weighed and photographed to correlate with length and width data.

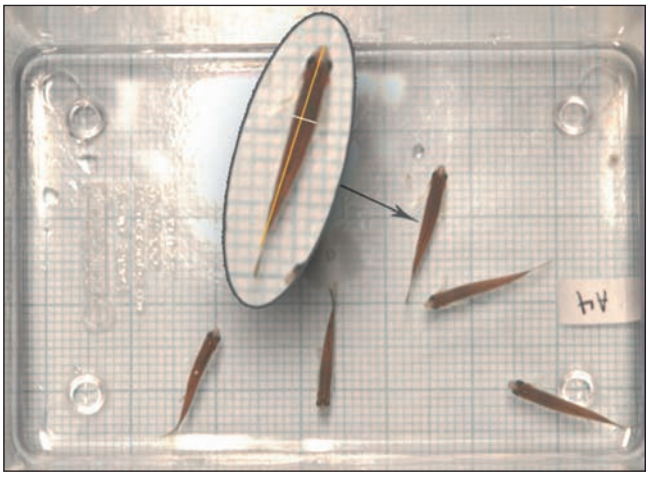

Figure 2. The setup for photographing and measuring the fish. Photographs were uploaded to Image J and distances measure using pixels.

The fish from each diet were analysed in respective sexes; due to a difference in sex ratios between the previous trial and the current, this affected the overall sample sizes when it came to comparative analysis.

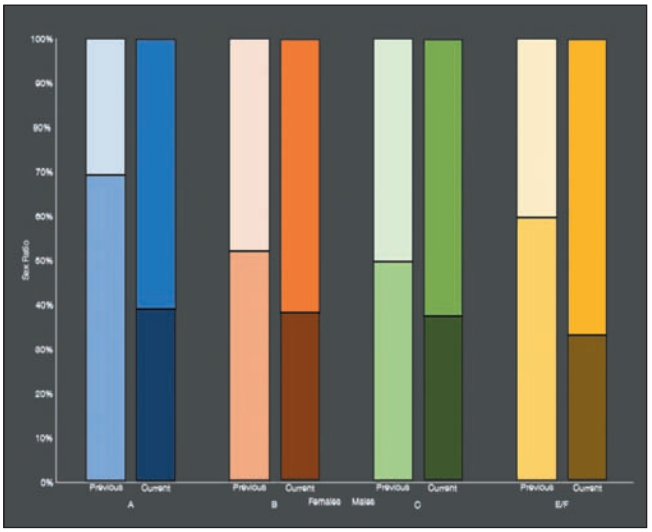

Figure 3. Comparison of sex ratios (females dark, bottom: males light, top) between the previous trial (light, left) and this trial (dark, right).

Bone staining

Deformed fish from the previous trial were culled according to Schedule 1 methods and fixed for further study via bone staining over a five day period.[2]

A preliminary test of the technique with Alizarin Red was conducted on a healthy fish (Figure 4); this was subsequently used as a reference and comparison to other samples.

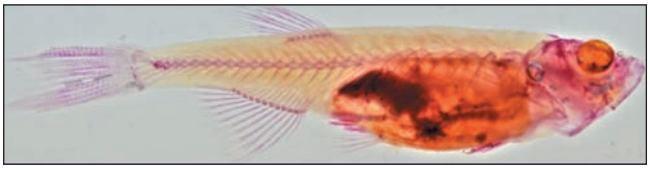

Figure 4. Healthy fish bones stained with Alizarin Red.

Another healthy fish were gutted, which caused the fish to lose it rigidity during glycerol storage, and stained with Alcian Blue for cartilage staining but was overexposed and overstained. This appears to have emphasised the bones further (Figure 5).

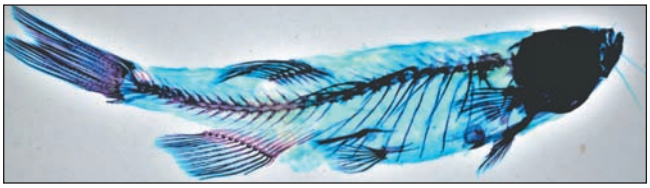

Figure 5. Healthy fish bone stained with Alcian Blue for cartilage staining.

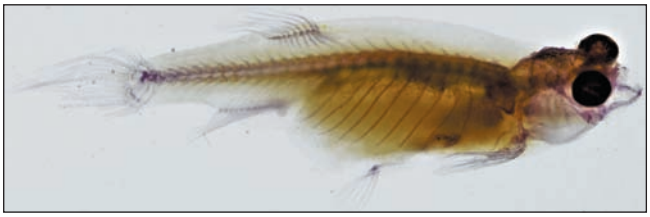

Figure 6. Diet D fish exhibited skeletal deformity and pre-caudal deformity and pre-caudal vertebrae excess tissue deposition. The skulls were truncates, leading to cranial deformities.

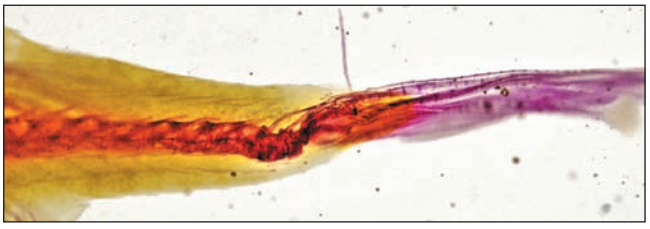

Figure 7. Diet D fish (as in Figure 6), from a dorsal view exhibiting caudal scoliosis.

Fish fed diet D developed a truncated skull (Figure 6) and caudal vertebra (Figure 7). These fish also appeared to deposit excess tissue around the pre-caudal vertebrae (Figure 6) compared to a healthy fish and fish fed other diets. Chang *et al* (2018) found that zebrafish vertebrae become brittle, thicken and calcify overtime leading to deformities such as lordosis (Figure 8).[3] The frequency of deformities observed with increasing age is likely because these physio-chemical composition progressions.

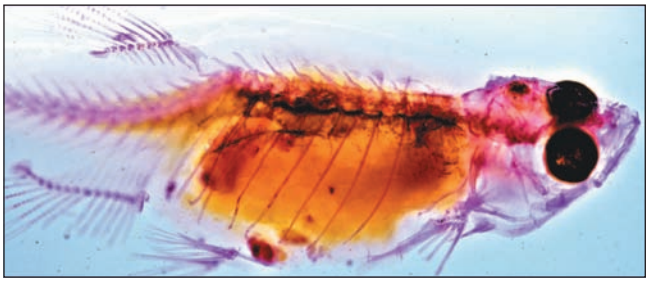

Figure 8. A particularly disfigured individual from Diet B exhibiting lordosis. The bone staining technique greatly increases the exact deformities present.

Results

Shapiro-Wilk and Kolmogorov-Smirnov tests were conducted to confirm normality: all data within groups were not significantly different ($p>0.05$). 2-way ANOVAs were conducted to identify differences between diets.

In this trial, there was a significant effect on growth between diet C and all other diets ($p<0.0133$) (Figure 10). Whereas, in the previous trial, all diets were significantly different ($p<0.0449$) with exception to growth between diets A and B. There was a significant difference between trials for diet A ($p=0.0106$) but not for B, C, or E/F (Figure 9).

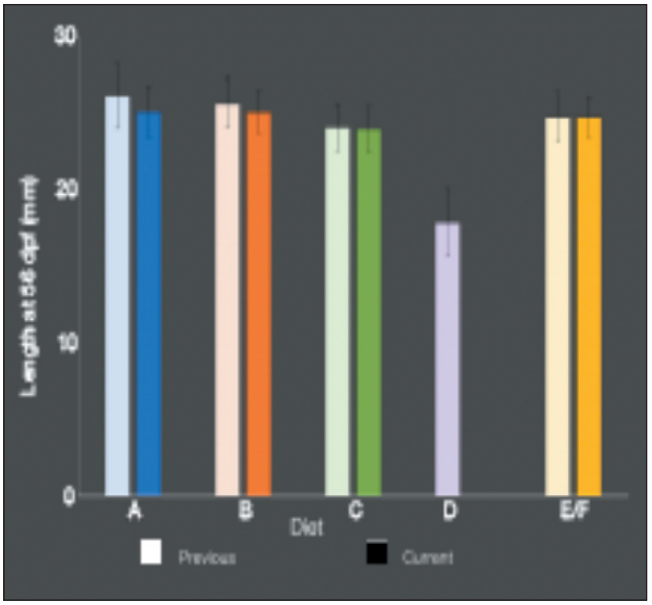

Figure 9. Comparison of growth between the previous trial (light) and the current trial (dark) at 56 dpf. Mean length (mm) ± standard deviation.

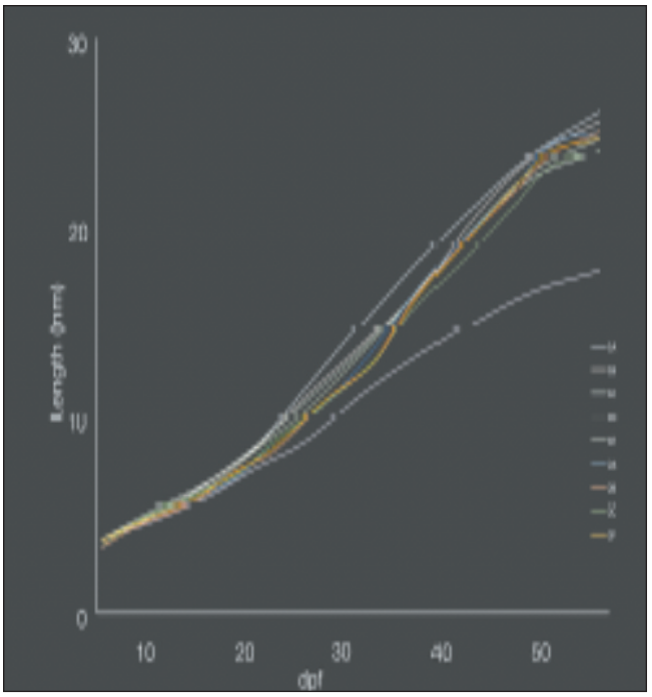

Figure 10. Comparison of growth between the previous trial (light) and this trial (dark) until 56 dpf. 1 indicates the previous trial 2 is the current trial.

As displayed in Figure 11, there was a significant effect of diet (p=0.0013) and sex (p<0.0001) on the ratio length/width. There was a significant effect on the ratio length/width of fish between Diets A and B, in males (p=0.0358) and females (p=0.0138). There was also a significant effect between diets B and C in females (p=0.0262). There was a significant effect (p<0.0001) on weight (Figure 12). There was a significant effect on female weight between Diets A and B (p=0.0069) and A and C (p=0.0128).

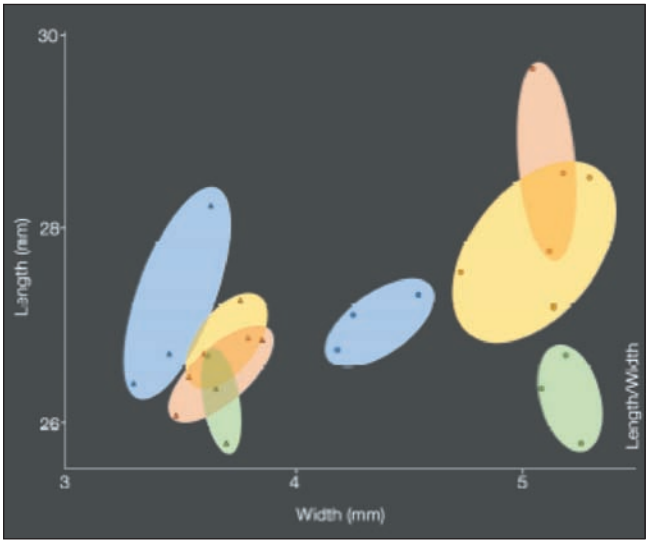

Figure 11. The mean lengths (mm) and widths (mm) of fish from each tank of the trial. Coloured ovals indicate each diet with the sexes separated. Colours indicate Diets (A,B,C,F), circles indicate females and triangles indicate males.

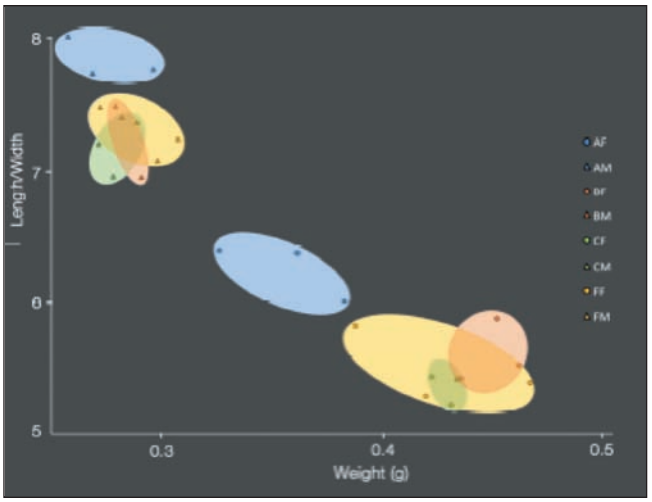

Figure 12. The mean lengths (mm) and widths (mm) ratio and mean weight (g) of fish from each tank of the trial. Coloured ovals indicate each diet with the sexes separate. Colours indicate Diets (A,B,C,F, circles indicate females and triangles males.

Discussion

Diet A consists of considerably less fibre and more ash than Diets B and C. Ash hinders protein uptake (which is potentially why Diet A fish were lighter.[3] Diet B has slightly higher lipid content which may explain why those fish had a lower length/width ration. The only diet which produced a similar survival rate between trials was C, the other diets had increased survival (-5 fish/tank) in the repeat.

Fish fed Diets A and F grew significantly longer than the fish fed Diet C. Similarly characterised by size and weight (Figures 11-12), the fish, particularly females surprisingly discrete features: Diet A fish were rather 'lanky', whereas Diet B fish were distinctly more chunky'. Diet A female fish were also significantly lighter than B and C females.

Fish fed Diets A and F grew slightly less, on average, during the trial than the previous one, due to the higher sex ratio favouring the shorter males (than longer females) and increased numbers of individuals per quantity of diets fed; however, on average Diet F fish grew marginally longer than Diet E fish (used in the original trial). This suggests that the fish fed Diet E were held back by the quarter of the diet which consisted of Diet D which was used in the earlier trial.

With the exception of sex ratios. This trial was comparable to the previous trial and shows consistent growth characteristics between diets. Bone staining is a useful tool to clarify deformities.

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[2] **Sakata-Haga, H., Uchistub, M., Shimada, H., Tsukada, T., Mitani, M., Arikawa. T.** *et al.* (2018). 'A rapid non-destructive protocol for whole-mount bone staining of small fish and Xenopus'. *Scientific Reports* 8(1) p 7453.

[3] **Chaimongkoi, A.** and **Boonyaratpalin, M.** (2001). 'Effects of Ash and inorganis Phosphorous in diets on growth and mineral composistion of seabass *Lates calcifer* (Bloch).' *Aquaculture Research* (10.1111), 32, pp 53-59.

A technologist-led approach to altering the culture of care regarding blood sampling

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Abstract

This poster will describe the implementation by Animal Technologists and Named Persons of a well-published refined bleeding technique. Establishing The Francis Crick Institute from legacy institutes required a standardised approach for many techniques, including blood sampling, which is essential for good practice. The Named Veterinary Surgeon (NVS) taught the Saphenous vein method to technologists as an alternative to bleeding from the tail vein. This poster will cover how we moved from previous methods, the reasons why and how this was then introduced as part of a technician-led culture of care.

Why not continue to use tail bleeds as the standard?

The concept of the 3Rs, and especially Refinement, give us a responsibility to constantly review existing techniques and assess if methods in use are still the most appropriate.

Using a scalpel/lancet

Tail nicks were previously being carried out using a scalpel or lancet and we found the following concerns when we were using that method:

- Repeated sampling can cause scarring to the tail especially if a scalpel is being used.
- When performing the tail nick with a scalpel, it can be difficult to get the correct amount of pressure needed for the initial cut in order to get enough blood, without too much pressure as to result in damaging the tail and causing subsequent scarring
- Animals need to be warmed in a hot box prior to the procedure in order to dilate the blood vessel, resulting in an extended period of time before the flow of blood stops after sampling.

Using a needle

We tried refining the tail bleeding technique by changing to using a needle to prick the vein instead of a scalpel but we still had the following concerns:

- In black or pigmented mice, it can be more difficult to see the tail vein through the skin. This can lead to missing the vein and having to re-puncture the tail.
- The hot box was still required, this increases the length of the procedure, resulting in extra time for the animal outside its home cage.
- Increased handling and pulling on the tail can potentially cause stress to the animal during the procedure.

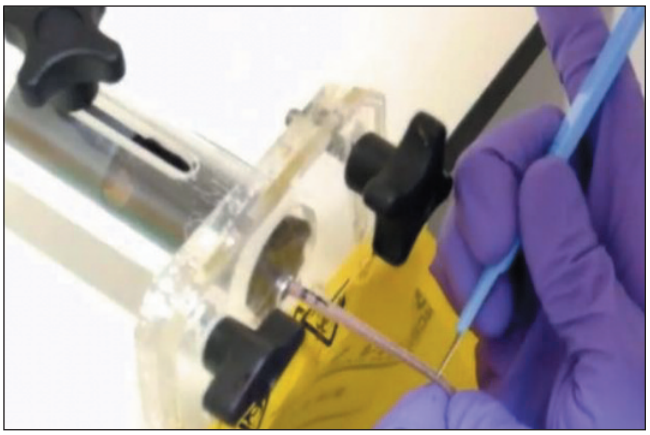

Figure 1. The tail bleed procedure.

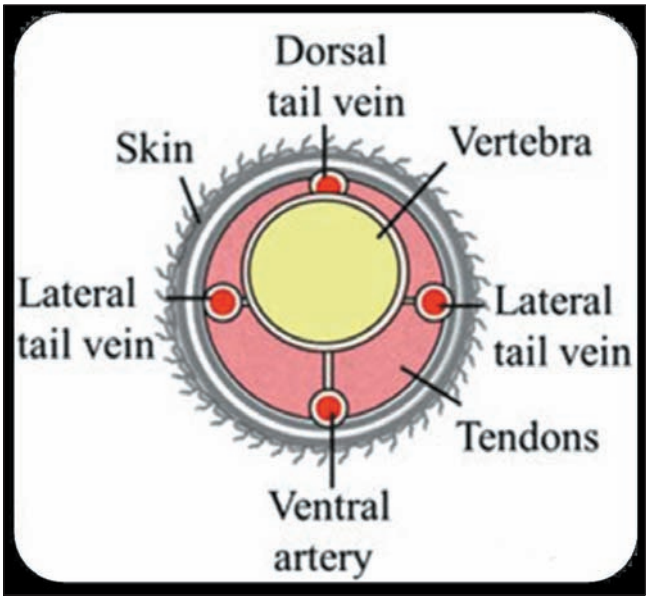

Figure 2. Cross section of a mouse tail.

What alternative methods are available?

Whilst bleeding from the tail vein is still a suitable option, there are a variety of different alternatives, although not all would be applicable as a standardised technique.

From the list of available techniques, we wanted to use one that did not require the use of anaesthetic. Our NVS and a few Animal Technologists had previously used the Saphenous vein bleed so we decided to trial it for suitability as a standardised technique in our unit.

General anaesthesia not required	General anaesthesia required	General anaesthesia required (non recovery)
• Saphenous vein	• Sublingual vein	• Cardiac puncture
• Tail vein	• Saphenous vein	• Abdominal/thoracic blood vessel
• Mandibular vein	• Retro-orbital	• Retro-orbital
• Tail snip		• Decapitation
• Blood vessel cannulation		

Figure 3. Blood collection techniques.

Advantages we found of using our chosen method of Saphenous vein bleeds

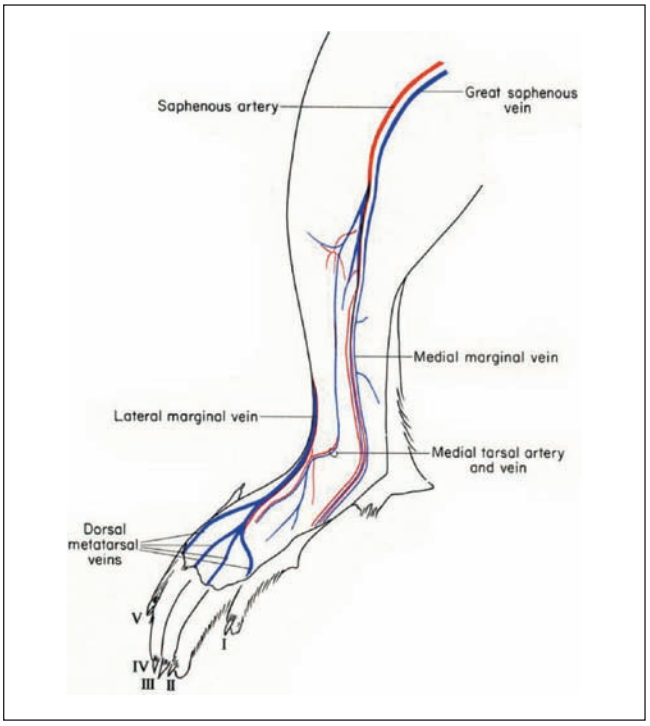

Figure 4. Mouse leg circulatory system.

From performing the Saphenous bleeding technique, we found that this method had several advantages.

- The Saphenous vein is easy to see in all coat colours. This allows the Personal Licence (PIL) holder to be more confident performing the venous puncture required for sampling.
- Either leg can be used, allowing repeat sampling alternating between legs, providing time for each leg to fully heal.
- No heating of the animal is required, eliminating the need for a heat chamber.
- The bleeding stops quickly as the vein is not dilated by heating.
- Large volumes of blood can be taken from the leg continuously with little coagulation.

Figure 5. Saphenous vein puncture.

- Reduced handling of the animal from the tail may lead to a reduction the stress to the animal during the procedure.

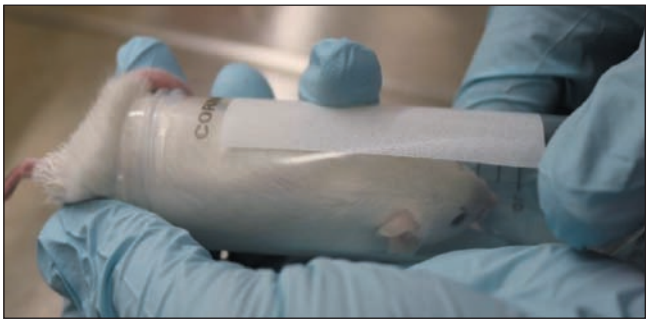

Figure 6. Mouse restrained for Saphenous bleed procedure.

- Wounds on the legs heal quickly with little to no scarring compared to what has occasionally been seen when using the tail.

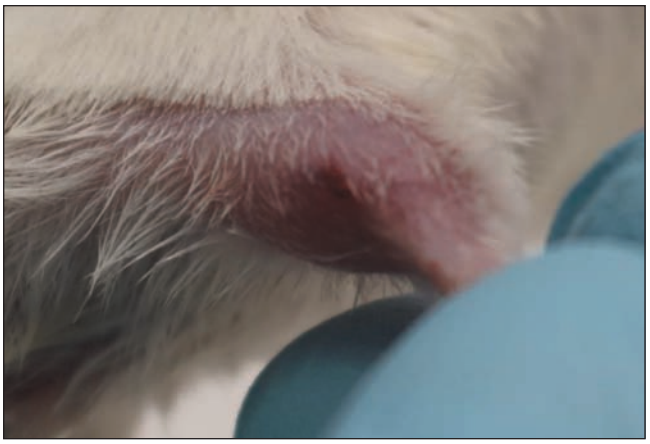

Figure 7. Wound just after procedure has finished and the bleeding has stopped.

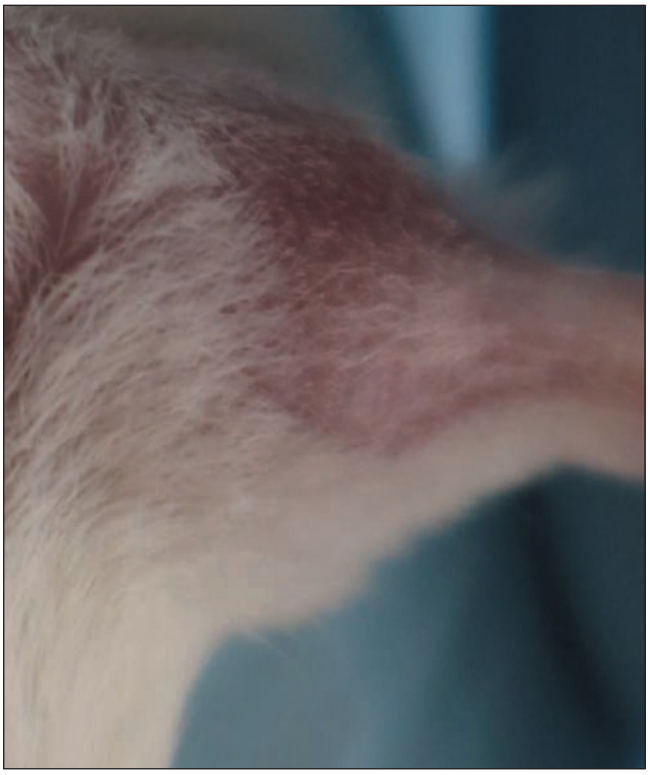

Figure 8. Healed wound 24 hours post procedure.

Refinements within the procedure

From doing this procedure we have made further refinements.

This has included using higher or smaller gauge needles depending on the volume of blood required.

We also use different sized restraining tubes depending on the size of the mouse so that it is not too tight or too loose. We found that the technician can easily and quickly restrain the animal.

Further refinements

The restrainer we currently use can be optimised further. We are looking into creating a restrainer that incorporates a thinner red plastic similar to a 50ml falcon tube and to have a variety of sizes so the most appropriate size can be used for each animal.

Figure 9. Different sized restrainters used.

Technologist driven culture of care

The differences and improvements of the Saphenous vein technique compared to previous methods led to it becoming the only method used in our unit, where appropriate.

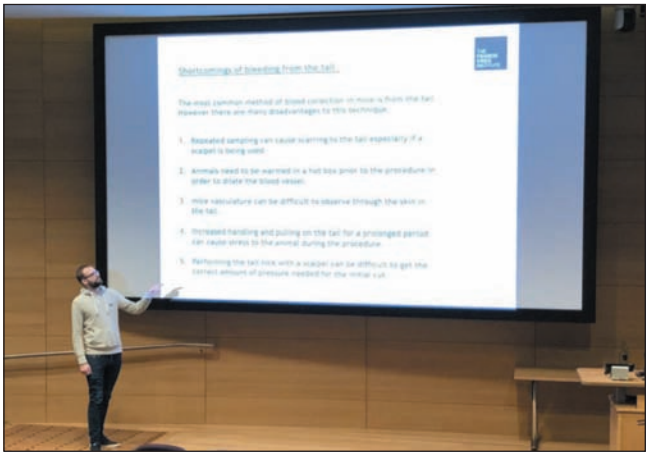

Figure 10. Blood collection presentation.

Scientific users were then encouraged to use this method over alternative techniques. The majority of Scientific users who have been shown and taught this technique now prefer it to previous methods used, this is due to the advantages of the Saphenous vein bleeding technique listed in this poster.

- cardiac puncture
- abdominal/thoracic blood vessel
- retro-orbital
- decapitation

Technologists and Scientific Users have introduced this method to visiting collaborators from partner institutes who are working on projects using blood collection; highlighting the benefits to research and animal welfare.

Technologists have given talks internally to the whole of the Biological Research Facility on blood collection via the Saphenous vein, explaining the benefits and encouraging others to take up the technique if suitable.

Future steps to take

We will to continue to highlight the benefits of using a different procedure for blood collection to the Scientific Users and Technologists who use our facilities and standardise the technique where possible. Looking forward, we would like to present at symposia and inspire others to adopt this method for use in their own institutes.

Conclusion

Although not a new technique, this method demonstrates how Animal Technologists play an essential role in implementing and demonstrating good practice whilst promoting a culture of care. Championing this technique at The Francis Crick Institute has highlighted the importance of Animal Technologists working closely with Scientific Users to promote methods of refinement and consistency of techniques, especially when different establishments and processes are involved.

Acknowledgements

Clare Brazill-Adams, Lucy Fern, Yolanda Saavedra-Torres, Helen Bailey, Luke Hitchen and Jamie Delicata.

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Figure 1. www.nc3rs.org.uk/mouse-tail-vein-non-surgical
Figure 2. www.researchgate.net/figure/Demonstration-of-mouse-restraint-location-of-blood-vessels-and-positioning-of-needle_fig1_320737499
Figure 3. www.nc3rs.org.uk/mouse-decision-tree-blood-sampling
Figure 4. www.informatics.jax.org/cookbook/figures/figure102.shtml

Variety is the spice of life; enrichment in our wildtype colony (CD1, B6CBAF1, C57BL6J)

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Introduction

Providing enrichment is a vital tool in encouraging the natural foraging behaviour of mice. We provide this by giving our mice a cardboard tunnel, tissue and a wooden chew stick.

Image 1. Shows mice interacting with paper and wooden based enrichment.

Pros: Relatively cheap, readily available, easily replaced.

Cons: Can be destroyed quickly, cannot be recycled, hard to remove mice from tunnel, mice not visible when in tunnel, absorbs water.

Due to the above pros and cons, we investigated other varieties of enrichment made from heat resistant plastic.

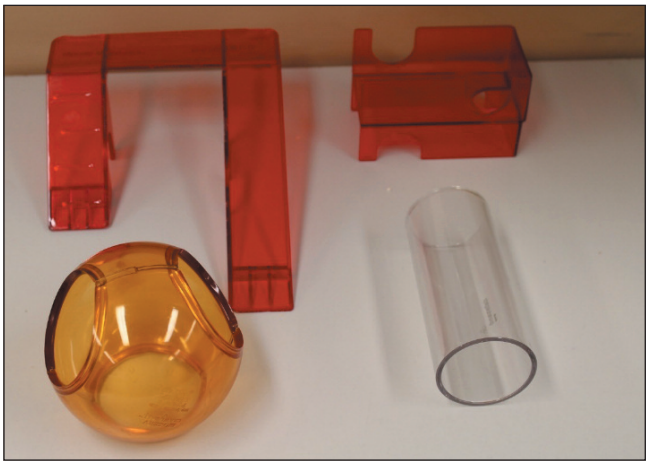

Image 2. Examples of heat-resistant plastic enrichment devices.

Images 3-6. Mice interacting with plastic based enrichment.

As with the cardboard tunnels, plastic based enrichment came with their own pros and cons.

Pros: Reusable, can be washed and sterilised, long lasting, indestructible, visibility, easy to remove mice.

Cons: Indestructible, expensive, size, storage.

Conclusion

What did we discover?:

A potential case of dominance in CD1 males (all males terminated due to fight wounds).
Wildtype animals showed no preference.
Any interaction was for a very short time. However C57BL6J males used the enrichment ball as a nest.

Potential future investigations:

Was the dominance in the CD1 males a one off?
Will the introduction of non-tail capture affect our choice of enrichment?
Could this enrichment study be done in our Transgenic colony?
Could enrichment affect the performance of stud and vasectomised males?

Acknowledgements

Linda Clark and Alastair Russell

MR and CT compatible electrical heating system for mouse imaging

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Introduction

Active heating is required to avoid hypothermia in anaesthetised animals. Small resistive MR-compatible heaters are useful where space limitations prevent the use of circulating fluids but computerised tomography (CT) imaging is severely compromised by these, due to the presence of high atomic number elements. Positron-emission tomography (PET) and Single-photon emission computerised tomography (SPECT) imaging are unaffected by these heaters unless a corrupted CT scan is used for attenuation correction. We describe a carbon-fibre sheet heater element, used with 100 kHz alternating current (AC) that is demonstrated to be MR, CT, SPECT and PET-compatible.

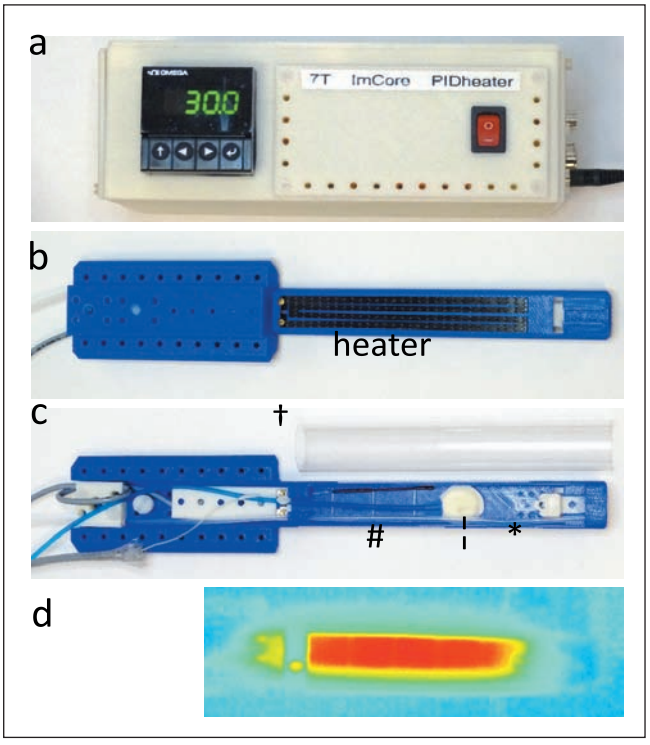

Figure 1. Carbon-fibre sheet resistive heater embedded in a 3D-printed, flat-base multimodal imaging cradle.

(a) Proportional-Integral-Derivative Controller (PID), (b) Bottom view of the cradle containing the heater element, (c) Top view of the cradle containing an adjustable mouthpiece (*) and physiological monitoring apparatus (¦, respiration; #, optical thermometer) and ( ) a cover sheet to contain anaesthetic gas, (d) thermal image of the cradle surface. The current wires and metal couplings to the carbon fibre are located beyond the imaging FOV so do not present image distortions.

The assembly is shown in Figure 1. A 250 Ω resistive heater element formed from 0.75 mm thick carbon-fibre sheet (RS, 764-8700) cut into 4 x 3 mm wide legs, each 120 mm long and spaced by 1 mm, which was glued to the underside of the cradles as shown in (Figure 1b).

A PID-controlled gain setting amplified a 100 kHz sine wave generated using a Pierce oscillator to maximum power of ca. 2 W (Figure 1a). The input to the PID was derived from a fibre optic rectal temperature system (Opsens, Accusens).

Thermal stability was validated in vivo in CBA mice.

Magnetic resonance (MR)-compatibility of the heater element was tested using MR-compatibility of the heater element was tested in a water gel phantom using single shot PRESS spectroscopy and 2D fast low angle shot (FLASH) imaging, and in vivo in the mouse using respiratory-gated 2D FLASH and cardio-respiratory-gated 3D FLASH imaging. In all cases the same acquisition was repeated for the heater turned on and off. (7T, Varian Inc, VNMR).

CT-compatibility (MILabs, Vector^{4}CT) was tested in the absence of any heater, and in the presence of either copper wire or carbon-fibre sheet heater elements.

Multimodal MR-CT-PET-SPECT imaging of kidneys was performed using ^{111}Indium Citrate (^{111}In, SPECT), ^{18}F-fluorodeoxyglucose (FDG, PET), anatomical CT, respiratory-gated bSSFP and DCE-MRI.

Results: thermal stability

The power dissipation of the carbon-fibre sheet heater resulted in homoeothermic maintenance of the animals. The PID based controller allowed automated temperature maintenance with a fluctuation of 0.1˚C at approximately 0.5˚C below the target temperature.

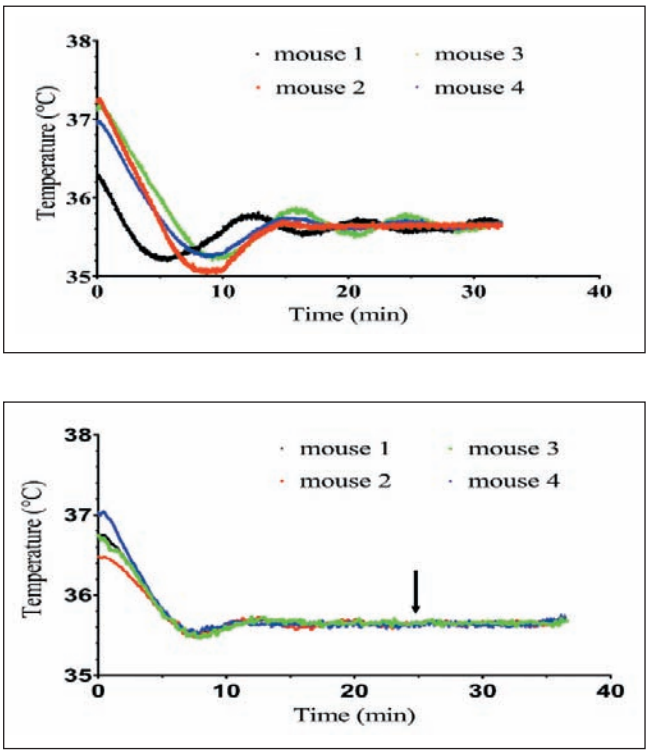

Figure 2. (A) core temperature of 4 mice placed into the SPECT/PET/CT scanner. (B) Core temperature of 4 mice placed into the MRI scanner.

The arrow indicates when respiratory-gated bssfp imaging was initiated to replicate the additional heat load of high duty cycle MRI scanning.

Conclusion

Carbon-fibre sheet resistors powered with high frequency AC under PID control:

- allow homoeothermic maintenance
- allow automated temperature control with 0.1°C fluctuation
- are compatible with multimodality imaging using MR, CT, PET and SPECT
- are small heaters
- are easy to produce
- are easy to integrate into cradles for multi-modal imaging

Results

MRI compatibility

The equivalence of spectra and images acquired in the presence and absence of AC heating demonstrates that the heater element delivers heat in a manner that does not corrupt the imaging process and the carbon fibre material does not lead to any marked image distortions.

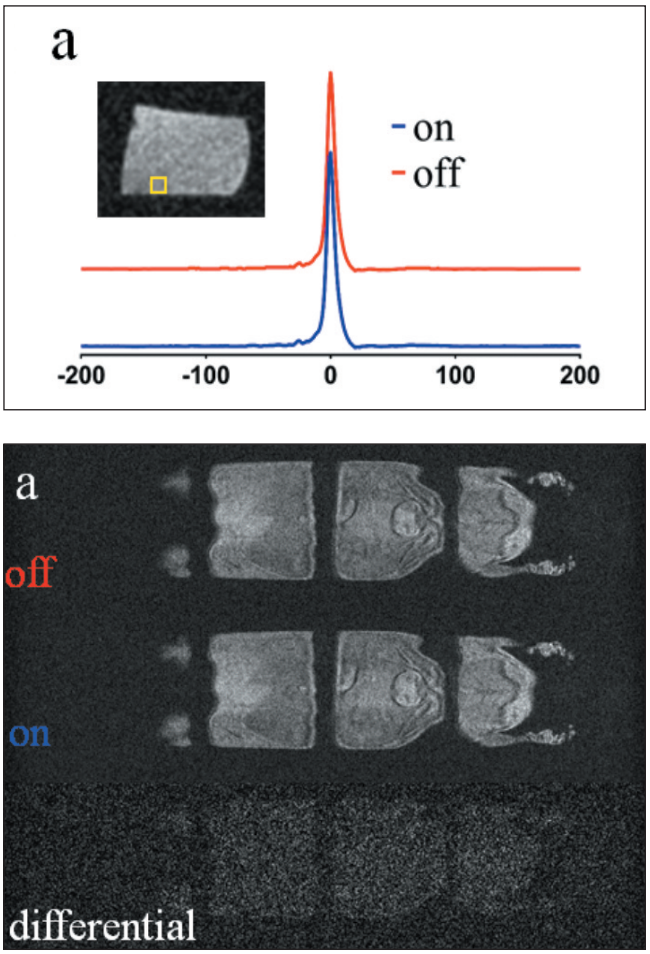

Figure 3. Impact of current flow through the carbon-fibre sheet heater element on *in vivo* MRI of the mouse. An area close to the heater was chosen to achieve this data (yellow square) as image distortions originate from current flow in the MR. (a) T1 weighted *in vivo* whole-body respiration gated 2D FLASH MRI with the heater turned off (top) and on (middle). The bottom image displays the difference between both images.

CT compatibility

The absence of attenuation-derived streak artefacts when using the carbon-fibre sheet heater provides good image quality that enables quantitative analysis of image intensities. This was not the case where the copper wire heater was used.

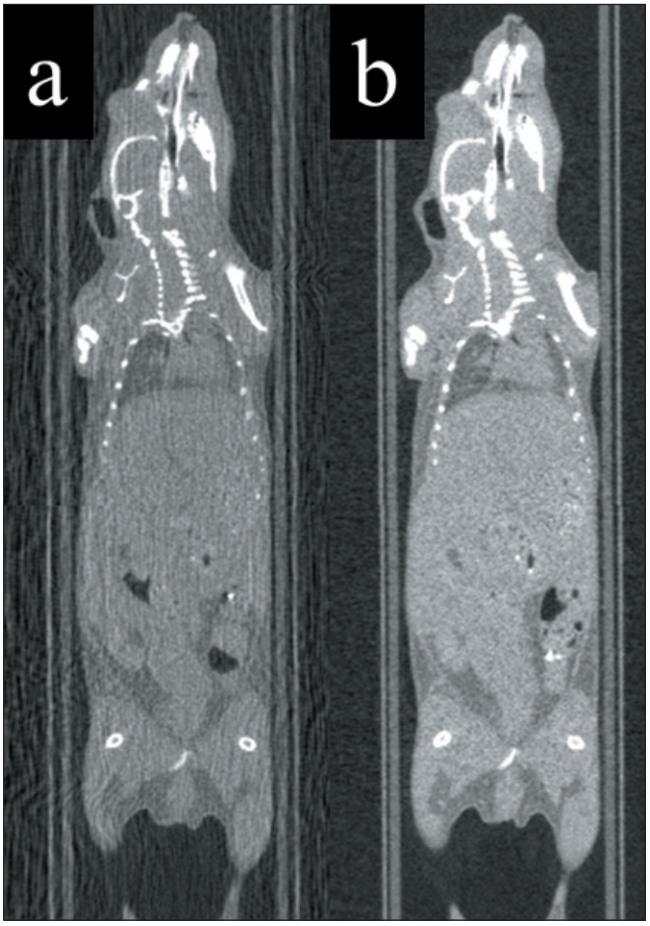

Figure 4. Impact of the heater material on CT image quality. CT imaging using (a) the 150 mm diameter copper wire heater or (b) carbon-fibre sheet heater. Streak artefacts due to the presence of the heater are absent when using the carbon fibre heater and image intensities remain intact.

Multimodal MR-CT-PET-SPECT imaging

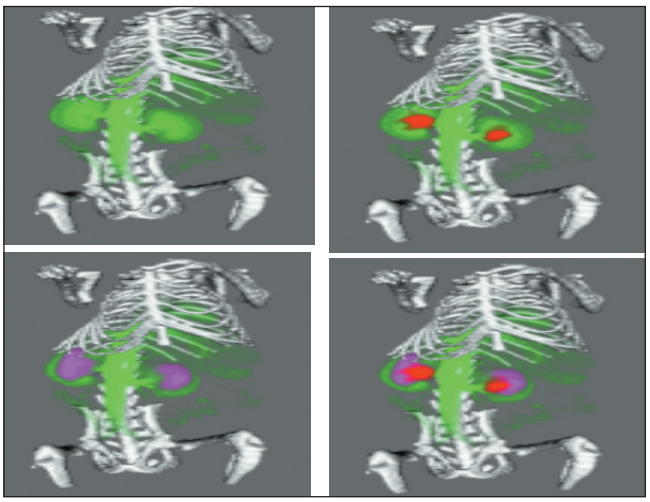

Figure 5. Multimodal imaging of a mouse using the carbon-fibre sheet resistive heater embedded in a 3D-printed, flat-base multimodal imaging cradle. The skeleton, kidneys, major vessels to the kidneys (*) are marked up. The **skeleton (white)** was imaged by CT, whilst **^{111}In-citrate (red)**, **^{18}F-fluorodeoxyglucose (purple)** and **Gadodiamide (green)** were used for SPECT, (PET)SPECT and DCE-MRI of the kidneys, respectively. Each panel shows an additional layer of the co-registered, segmented image: (a) CT + MRI, (b) CT + MRI + SPECT, (c) CT + MRI + (PET)SPECT, (d) CT + MRI + (PET)SPECT + SPECT.

Food for thought: the development of drug loaded diets improve both science and welfare

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Introduction

In our facility, we use several hormone dependent tumour models with supplementation delivered via slow release subcutaneously (s.c.) implanted pellets, implanted via trochar.

For example:

- prostate which rely on 5-α-DHT
- breast tumours which rely on 17-β-Estradiol respectively.

Administration of E2 supplementation can result in side effects such as bladder calculi and urine scald. Therefore, to improve animal welfare and to avoid steroid supply problems, we decided to develop a new way to provide hormone supplementation via the diet.

Following on from this, we then resolved to take this methodology forward into drug therapy trials.

Experiment 1

LnCap and MCF-7 respectively, either s.c. or into the mammary fat pad and were divided into the following treatment groups:

1) Control – no supplementation
2) Supplementation via slow release pellets (IRA)

Figure 1. Trochar and pellet.

– females were implanted s.c. with 17-β-Estradiol 0.1 mg 21 day release pellets.

Pellets were implanted using a 10 gauge trochar (Innovative Research of America (IRA), (Figure 1). (Males were not implanted with pellets due to unavailability.)

3) Supplementation via diet (ssniff)
i) males were fed 5-α-DHT 2.4mg/kg (Figure 2).
ii) females were fed 17-β-Estradiol 2mg/kg (Figure 3).

Figure 2. 5-α-DHT diet.

Figure 3. 17-βEstradiol diet.

Animals were weighed daily to ensure food was being consumed. Diets were colour coded to aid with identification.

Results 1

Tumours were measured weekly using

- calipers to calculate volumes
- bioluminescence using the IVIS Spectrum (Perkin Elmer) to measure both tumour size and viability (Figure 6).

The imaging results and growth curves (Figures 6 and 10) show the supplemented diet facilitated tumour growth in both cases. In the animals which received LnCap, the increase was obvious from the start, but with the MCF-7 the effects were more marked from day 14 onwards. The MCF-7 growth data showed an increase with diet but lower than with the pellets, while both the control groups showing little or nil growth at all.

LnCap SC – Tumour-day 35

Figure 4. no DHT.

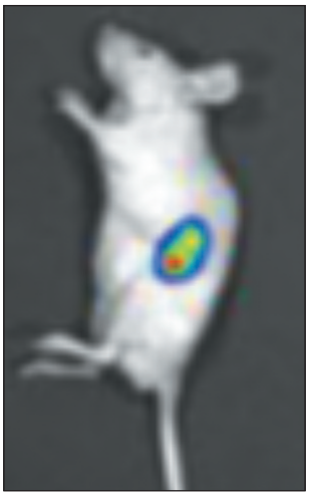

Figure 5. DHT.

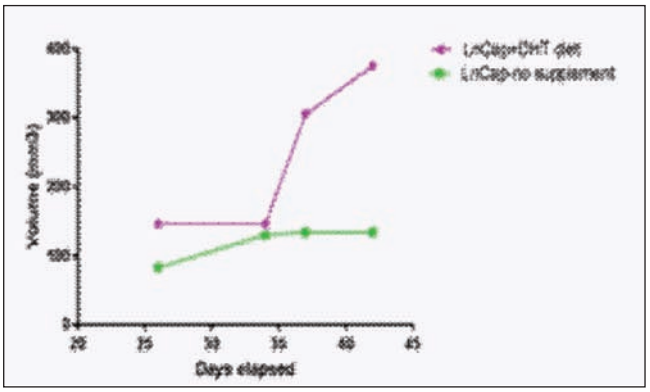

Figure 6. LnCap growth curve.

MCF-7MFP Tumour-day 14

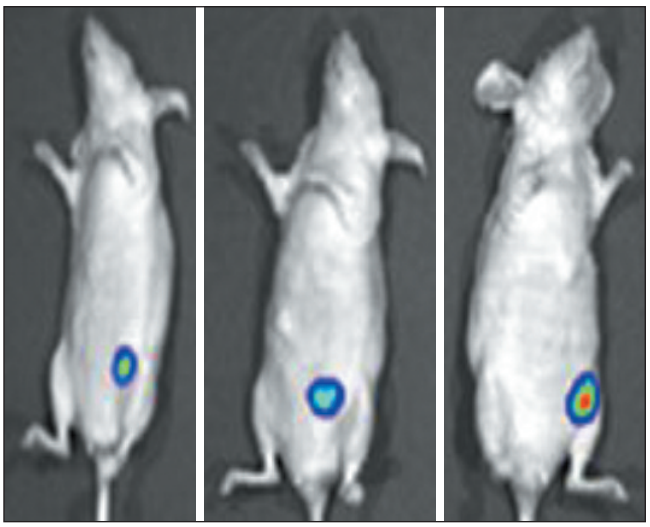

Figure 7. no E2. **Figure 8.** E2 pellet. **Figure 9.** E2 diet.

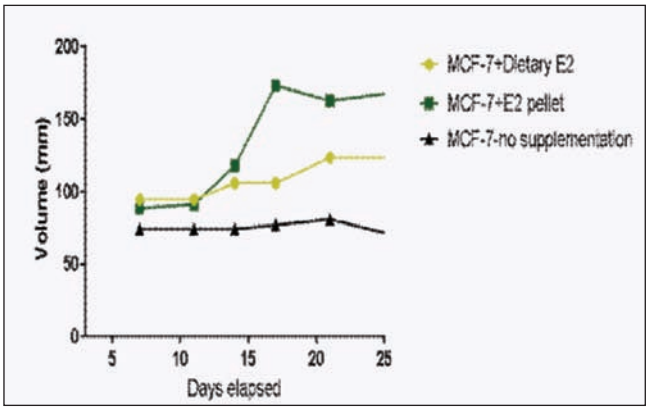

Figure 10. MCF-7 growth curve.

The slower growth rate of MCF-7 could allow a longer window of opportunity for treatment effects when carrying out therapy studies. Equally importantly, we also observed a marked reduction in side effects in mice treated with the E2 dietary supplement compared with pellets, with only some slight urinary retention which was eliminated by removing the supplemented diet for a few days, and is much easier and less invasive than removing a subcutaneous pellet.

These positive results led us to consider whether this method of delivery could also be employed in other situations. For example, there is a move towards therapeutic drugs being delivered orally, in some cases as a food supplement, so we decided to develop this methodology as an alternative to daily oral gavaging.

Experiment 2

In this experiment, a potential therapy, Substance 99, was formulated into diet and growth compared against control diet.

CD-1 NuNu mice were implanted subcutaneously with HCT-116 colorectal tumour cells before receiving Control or Substance 99 loaded diet, with daily weights to ensure consumption.

Figure 11. Substance (Sub 99) diet.

Results 2

Fuel3D scanning system to calculate tumour volumes and record thermal images.

Day 20 images

Bioluminescence Fuel3D vol. Fuel3D thermal.

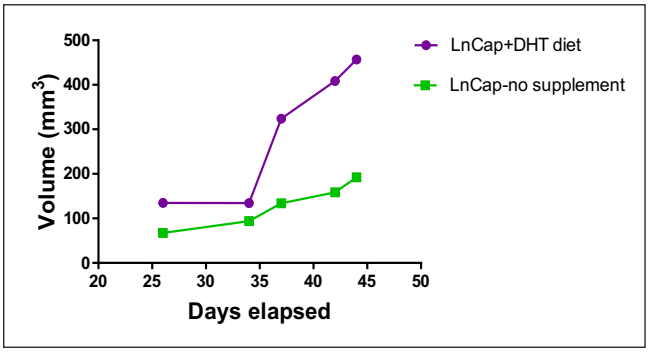

Figure 12. HCT-116 +/- Substance 99.

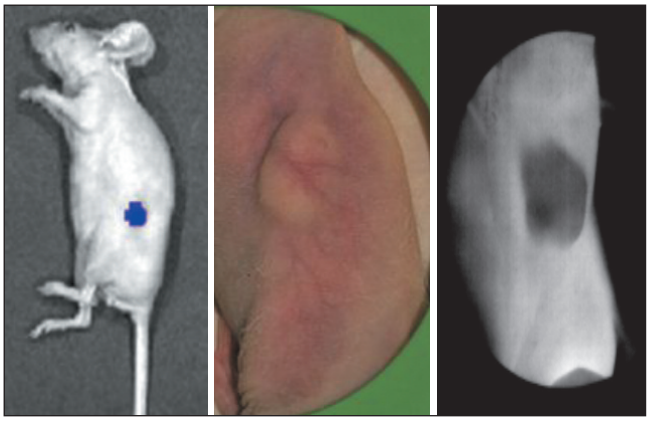

Figure 13. Sub 99. **Figure 14.** Sub 99. **Figure 15.** Sub 99.

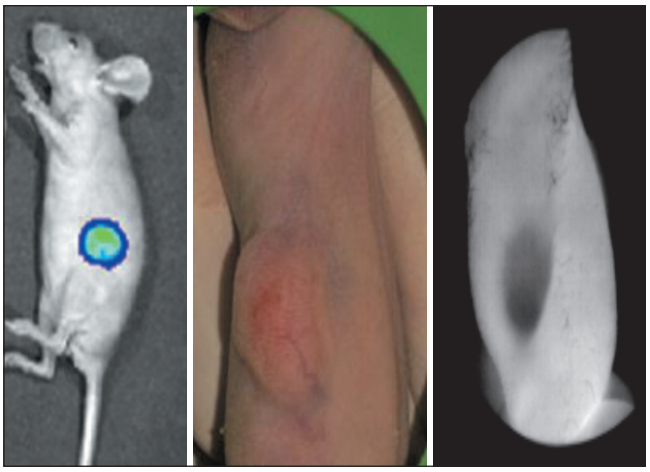

Figure 16. Carrier. **Figure 17.** Carrier. **Figure 18.** Carrier.

The results of this small pilot study show a slight difference between the groups with some growth delay shown in the Substance 99 treatment group. We believe this demonstrates that therapies can be delivered via diet and this study gives us the confidence to pursue this method of delivery in a full scale experiment.

Conclusions

Delivery of hormones via the diet is a major refinement in welfare terms by:

- Reducing the need for an invasive implant and possible removal procedure.
- Reducing deleterious side effects.
- Promoting tumour growth.
- Ease of delivery for the Animal Technologist.

Similarly, our pilot therapy experiment demonstrates that delivery of drug therapies via diet rather than daily oral gavage is both:

- a welfare improvement and
- potentially a translationally more accurate model

Therefore, we believe the potential to deliver compounds via diet, rather than by more invasive techniques, should always be considered wherever experimentally appropriate.

Acknowledgements

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