Refining Severity Limits for Laboratory Zebrafish

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Aim: To identify health patterns for specific strains in order to refine phenotypic severity limits through health records

Introduction

Using our body condition scoring system, we have begun to analyse health records in order to identify patterns for specific genetic strains. This knowledge will give the fish facility users the ability to predict patterns and therefore the opportunity to lower pain and suffering, and also severity limits in different strains of fish in accordance to both the 3Rs and Animals (Scientific Procedures) Act 1986; it will also provide information about strain health to PiLs, therefore providing a more powerful tool for strain choice and colony management. This falls under the 3Rs as it is an overall refinement for health monitoring protocols, standardises the records of death and disease, and decreases the number of fish reaching protocol severity limits.

Severity Limits

Although there are guidelines to determine procedural severity limits in fish[1], little work has covered potential phenotypes that may be created unexpectedly, perhaps as a result of other procedural work, for example production of strains using genetic modification technologies. It may also be difficult to establish whether phenotypes are a result of procedural work or of husbandry practice. For example, patterns that arise in individual tanks or throughout the room may indicate a husbandry problem, whereas patterns emerging in individual strains may indicate procedural severity. Some of the patterns we have identified, suggest the development of specific abnormalities (Fig 1); this knowledge would give PiLs the opportunity to cull fish at an earlier age, therefore refining a technique and either allowing a lower severity limit to be set, or to prevent breaching a pre-existing severity limit.

ZEBRAFISH FACILITY STOCK DETAILS - ADMIN

Stock # **12888**

Line PDF ICON 1671
Tg(TCF4^{tf55} x HucGCamp5)

Date	Reason	ACTIVITY Quantity
9/6/2015	heart enlarged	1
14/5/2015	Dropsy with tumour	1
14/5/2015	dropsy with enlarged heart	3
6/5/2015	culled	?
27/4/2015	heart enlarged	1

Dof 16/5/2013

ZEBRAFISH FACILITY STOCK DETAILS - ADMIN

Stock # **12693**

Line 1932
A1 x HuC:GFP

Date	Reason	ACTIVITY Quantity
8/7/2015	Emaciated with tumour	2
25/6/2015	Tumour	1
26/5/2015	Tumour	1
6/5/2015	Emaciated with lordosis	1
23/4/2015	Tumour	4

Dof 15/3/2013

Fig. 1: Left, a genetic strain that develops a heart defect once it reaches a particlar age. Right, another genetic strain that develops tumours after a particular age. This information may be used to define an age limit for these strains.

Methods

Using the database, alongside our body condition scoring system, PCR, histology, sentinel and specific specimen screening, we have begun to analyse health records of adult fish to identify health related concerns that can be addressed by reassessing humane endpoints and severity limits. We began a preliminary analysis of our health records of three strains of zebrafish: the mutant TraNac, Wik wildtype, and a transgenic strain 1384. The TraNac strain is pigment affected, appearing transparent; we compared this to another pigment affected line, Casper. Wik is considered to be a less stable wildtype, anecdotally speaking, and was choosen on this basis; we compared this to the more stable AB x TL strain. The transgenic strain was randomly chosen, and then compared to both another trangenic strain (strain 1505) and the wildtype on which they were both created. The data collected and analysed consisted of mortality, morbidity, and relation of both to age.

The collected data was analysed using Graph Pad (v5); the data did not have a normal distribution and therefore non-parametric tests were necessary. For the wildtype data, Kruskal-Wallis and Mann-Whitney tests were used, and Krustal-Wallis and Dunn's Multiple Comparison tests were used.

Mutant Morbidity

Comparing TraNac to Casper, the specific diseases that affect each strain differs (Fig. 2). The mortality rate of Casper is higher than that of TraNac, as is the instance of both spinal curvature (scoliosis and lordosis) and under developed fish, the latter of which does not occur within the TraNac strain. TraNac, however, has a higher instance of abormal swimming and emaciation, which typically occurs concurrently. This preliminary data implies a difference in health profiles, despite the similarities of phenotype. One possibility for this is a procedural effect from the creation of the mutant; if this proves to be the case, the procedural severity potentially may need reassessment, and new humane endpoints created to prevent unauthorised pain, suffering, distress, and lasting harm.

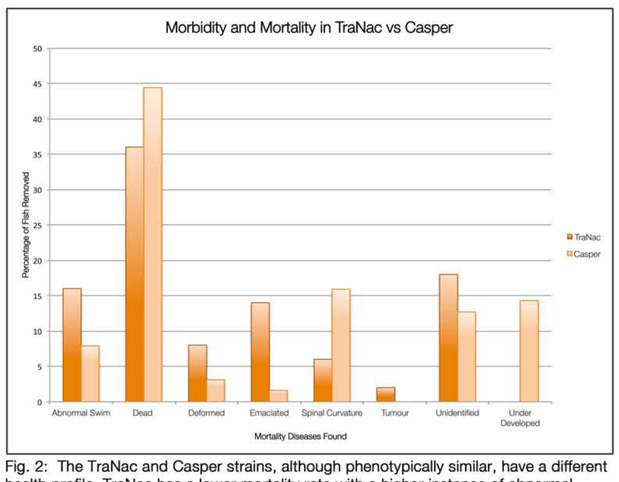


Fig. 2: The TraNac and Casper strains, although phenotypically similar, have a different health profile. TraNac has a lower mortality rate with a higher instance of abnormal swimming, emaciation; Casper has a higher mortality rate, but a higher rate of under developed fish and spinal curvature.

Fig 3: Casper and TraNac strains have the same phenotype but will have different health profiles, and potentially revised and unique humane endpoints.

Wildtype End Points

The initial comparison of AB x TL to the Wik health records indicated a similar ratio of death, disease, and spinal curvature; however, the Mann-Whitney test shows a higher mortality median for AB x TLs (Fig. 4), indicating a potential difference than initially suggested.

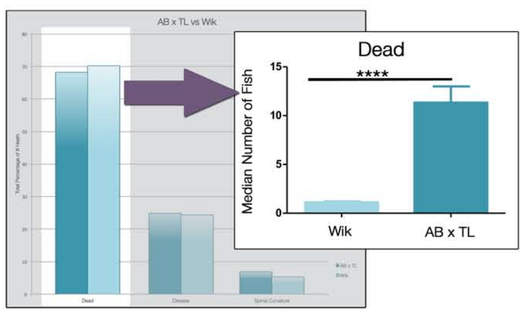


Fig. 4: (Left) An initial comparison of AB x TL and Wik wildtypes fish removed from the population due to death, deformity, or disease showed no difference in ratio. (Right) A Mann-Whitney Test shows that AB x TL have a higher median of mortality over time. (P<0.0001).

The AB x TL strain shows no obvious age-related increase in death and disease, with the frequency of both being consistant throughout the life span of the population (data not shown); the Wik, however, shows that the frequency of death and disease increases between 8 and 9 months of age (Fig 5). Whilst death and disease occurs before this point at a rate of less than 5 fish per month, both increase to over 5 per month until 14 months of age This implies that the age-related humane endpoint for Wik should be approximately 8 months to prevent pain, suffering, and distress due to disease.

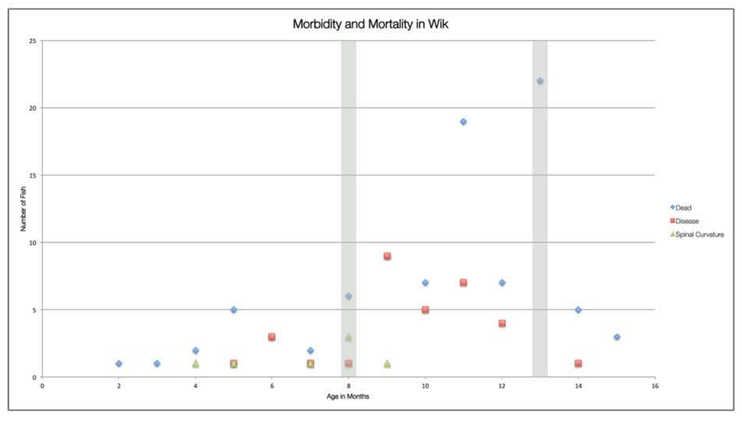
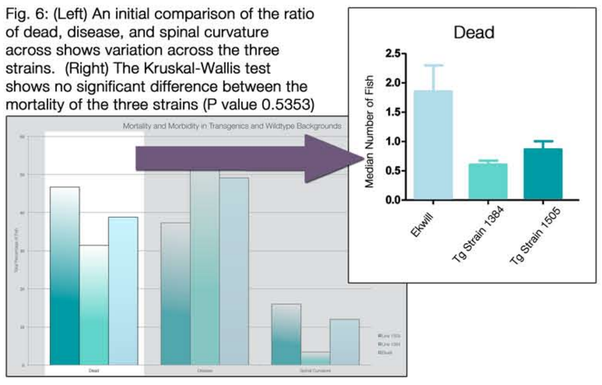


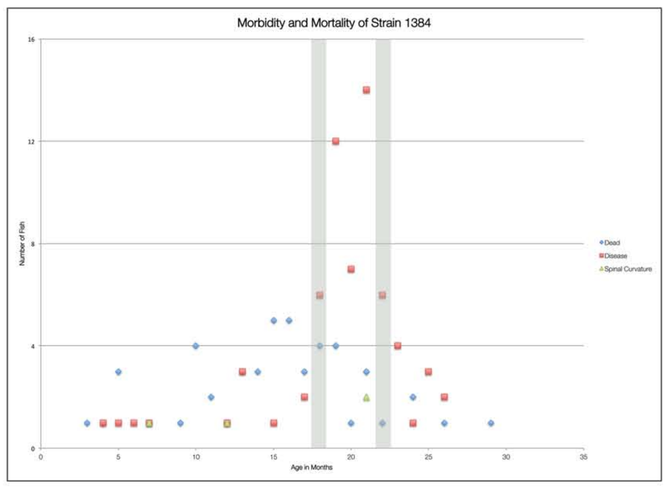
Fig. 5: Onset of ill health in the Wik strain increases at approximately 8 months of age; the age-related humane endpoint for this strain should be approximately 8 months to prevent pain, suffering, and distress.

Transgenic

The transgenic strain 1384 analysis shows that it is prone to abnormal swimming, tumours, and dropsy (data not shown). Whilst the source of these is not evident, such as procedural, this was further investigated by a comparison to both another transgenic strain (1505) and the wildtype background on which they were created (Ekwill). An initial comparison of the ratio of dead, disease, and spinal curvature shows no similarity between the three strains; furthermore, the Kruskal-Wallis test shows no significant difference between the three strains (Fig. 6).

Fig. 6: (Left) An initial comparison of the ratio of dead, disease, and spinal curvature across shows variation across the three strains. (Right) The Kruskal-Wallis test shows no significant difference between the mortality of the three strains (P value 0.5353)





The collected data for Strain 1384 shows the frequency of death and disease to increase rapidly at approximately 18 months of age (Fig. 7); whilst death and disease occurs before this point, both increase to over 5 per month until 24 months of age. As with the Wik, this implies that the age-related humane endpoint for this strain should be approximately 18 months to prevent pain, suffering, and distress due to disease.

Fig. 7 (Left): onset of ill health in the transgenic strain 1384 increases at approximately 18 months of age. In order to prevent pain, suffering, and distress, the age-related humane endpoint should fall around this age.

Conclusions and Further Work

Our current data implies that there may not be a procedural effect on fish, which would require refining procedural severity, but more standardised data is required before this can be accurately seen. The data collected thus far does allow for refining age-related humane end-points. We shall create more health profiles for strains in order to determine the most humane age-related endpoint and investigate potential procedural severity limits. We will also determine the baseline for wildtype zebrafish, particularly in terms of larval survival rates, and mortality rates of adults; we will use this to investigate if the health profiles of genetically altered strains are affected by procedures or by the parental genetic background. We require more standardised data in order accurately identify areas that require a refined severity limit. We shall continue to combine our body conditioning scoring system and conduct PCR / histological screens with database analysis to explore and understand the relationship between husbandry and procedural practice.

References:

1. Hawkins, P., et al., Guidance on the severity classification of scientific procedures involving fish: report of a Working Group appointed by the Norwegian Consensus-Platform for the Replacement, Reduction and Refinement of animal experiments (Norecopa). Laboratory Animals, 2011. 45(4): p. 219-224.

Acknowledgements:

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