Refining Severity Limits for Laboratory Zebrafish

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Aim: To investigate whether the use of a standardized tank side scoring system combined with a PCR/histology based health screen enables the refinement of phenotypic severity limits.

Introduction

Zebrafish are a rapidly expanding model in biomedical research. Currently there are no standardized methods for health monitoring, nor for identification of the potential severity of phenotypes caused by procedures. In this poster we describe the body condition scoring system we developed, and how we compared the results of a PCR/pathology health screen. We used that information and tracked patterns of illness on the facility database and refining severity limits in the general population, as well as in individual strains of zebrafish.

The Body Condition Scoring System

There is currently no widely used or published body condition scoring system (BCSS) for monitoring the health and welfare of laboratory zebrafish. In response to this, we developed and deployed a BCSS comprised of 4 stages, based on a traffic light (Table 1; Fig. 1)[1]; each grades various aspects of fish behaviour and general body condition that may be observed in a general population of zebrafish. With this, identification of disease and ill health has become more standardised and thus refined.

Body Condition Score	Traffic light colour	Meaning of traffic light colour	General appearance	General movement / swimming	Body, scale and fin	Bone formation
BCS1	Black	Immediate disposal	Dying	Little sign of life/movement	Not relevant	Not relevant
BCS2	Red	Priority to remove from system Possible signs of contagious disease Investigate	General emaciation Wasted body to head ratio General body deformities General dropsy/protruding scale	Swimming/orientation reversed Swimming on side Sitting on bottom of tank but will move in response to stimuli	Tumors or body ulcers Decayed fins/missing caudal fin Scale loss and/or patchy loss of pigment Protruding or defective eyes	Scoliosis/lordosis
BCS3	Amber I	Monitor for decline	Under conditioned Thin	Listing Gasping[1]	Missing operculum Partial missing dorsal/pectoral fins	Mild signs of scoliosis/ lordosis
	Amber II		Over conditioned Obese		Egg bound (not tumours)	
BCS4	Green	Good Health	Well conditioned Sleek body conformation	Swimming normal, not erratic, no signs of distress	Consistent pattern/colour Sexes may be physically witnessed	No signs of bone malformation

[1] Gasping in large numbers of fish is serious as it indicates a water problem and should be acted upon immediately

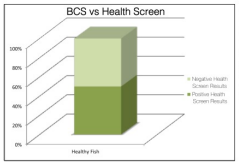
Table 1: Body Conditioning Scores and corresponding colour and action. Each score/colour has specific descriptions to aid in health identificaiton

Fig. 1: The four stages of the BCSS. BCS1/black is dead; BSC2/red is for obviously diseases fish; BCS3/amber is moniter for decline; BCS4/green is healthy

The adoption of the BCSS as our health monitoring protocol has been a refinement, as we have: improved accuracy of visual identification of disease; standardised recording; increased the number of identified diseased fish; decreased the numbers of dead, thus reducing the numbers of fish exceeding protocols' severity limits.

Health Screening and Testing

We conducted a facility wide health screen to investigate potential causes of commonly found diseases; We were able to see a similarity between the results and our BCSS; 73.6% of what we scored with our BCSS had a corresponding result from either the PCR or histology results. The results from our health screen indicate that our BCSS is accurate in determining when a fish has developed a disease. The results also reveal that fish that would score a BCS4 can be ill with asymptomatic diseases, or be in a pre-symptomatic state. This stresses the importance of constant health monitoring. Of all the fish that were tested with a visible disease, only 17% tested negative for a specific pathogen and in histopathology; the remainder showed signs consistant with the pathogens and illnesses identified. This suggests a genetic or protocol cause that is associated with the strain; if this proves true, it can be used to refine severity limits.



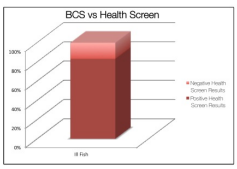


Fig. 2 (left): Our BCS corresponded to the health screen in the majority of cases: 50% of the fish scored as BCS4 had a corresponding result in the health screen, whereas 83% of those that scored a BCS2/BCS3 had a corresponding result in the health screen.

	BCS4- Visibly healthy	BCS2- Visible signs of disease
Negative results		
Positive results		

Fig. 3 (right): A small portion of the fish sampled tested negative in both PCR and histology results despite exhibiting visible signs of disease; the reverse was also true with visibly healthy fish testing positive. The former indicates a genetic or protocol cause rather than husbandry.

Severity Limits

Although there are guidelines to determine procedural severity limits in fish[2], little work has covered potential phenotypes that may be created unexpectedly, perhaps as a result of other procedural work, for example production of lines 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 4); 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.

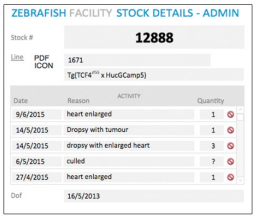
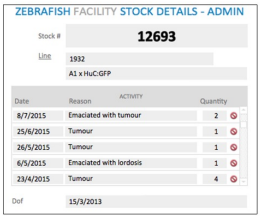


Fig. 4: Left, a genetic strain that develops a heart defect once it reaches a particular age. Right, another genetic strain that develops tumours after a particular age. This information may be used to define an age limit for these strains.



Database Pattern Analysis

Using the database, alongside our body scoring system, PCR, histology, sentinel and specific specimen screening allows us to analyse much more than patterns of potential procedural severity in individual strains. We have found a wide range of uses to which we can add refinements, both within welfare and procedural issues. For example, using pre-existing data, humane endpoints can be assessed; using patterns within the database, age-based humane endpoints may be determined to prevent suffering from disease (fig 5). As well, the effects of inbreeding can be assessed; preliminary data within our database suggests that disease can appear earlier within each successive generation (fig. 6). Additionally, baselines for wildtype zebrafish can be established, particularly in terms of larval survival rates, and mortality rates of adults (fig 7).

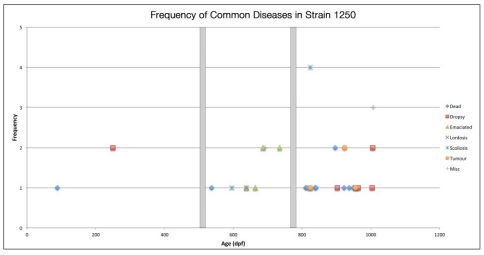


Fig. 5: Analysis of the causes of ill health in a specific strain shows few instances of any ill health in the first 500 days of life, then emaciation between the ages of 500 and 750 days before more extensive and broad ranging ill health sets in after 750 days. This might suggest a humane endpoint for this line of fish should be 500 days, before the onset of ill health.

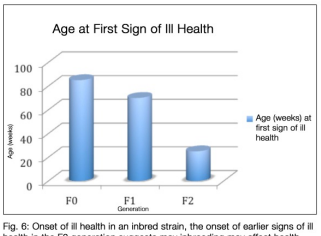
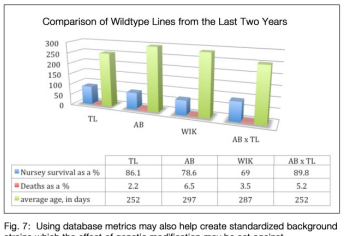


Fig. 6: Onset of ill health in an inbred strain, the onset of earlier signs of ill health in the F2 generation suggests may inbreeding may affect health and survival rates.



	TL	AB	WIK	AB x TL
Nursey survival as a %	86.1	78.6	69	89.8
Deaths as a %	2.2	6.5	3.5	5.2
average age, in days	252	297	287	252

Fig. 7: Using database metrics may also help create standardized background strains which the effect of genetic modification may be set against.

Further Work

We shall continue to combine our body conditioning scoring system and PCR / histological screens with database analysis to explore and try to understand the relationship between husbandry and procedural practice. We will be embarking on a more rigorous training regimen, to help facility staff spot and code sick fish. We intend to set up another fish room at UCL, with the intention it acts as a specific pathogen free unit, with much improved biosecurity and with the intention of comparing patterns of ill health between a regular zebrafish unit and one with a higher health status.

References:

1. Wilson, C, K. Dunford, C. Nichols, H. Callaway, J. Hakkesteeg, M. Wicks. 2013. 'Body Condition Scoring for Laboratory Zebrafish' in Animal Technology and Welfare. 12(1). pp 1-7
2. 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.

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