

Refinement of a Health Monitoring System for Laboratory Zebrafish

Implications for Severity Limits

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Aim: To investigate if the use of a standardised body conditioning scoring system for zebrafish can correlate directly to the presence of disease and if it can be used to determine and refine severity limits

Introduction

Zebrafish are a rapidly expanding model in biomedical research. Currently there are no standardised methods for health monitoring of zebrafish. In this poster, we describe how we developed a non-invasive tank-side body condition scoring system (BCSS) and compared this to the results of a health screen we undertook; for the health screen, we specifically chose fish that were exhibiting visible clues of ill health. We then tracked the sampled strains through our database to identify previous patterns of ill health. We hypothesise that severity limits can be identified and refined by using the BCSS in conjunction with the health screen results and tracking patterns found in the database.

The Body Condition Scoring System: ('Traffic Light' System)

Current health monitoring systems used for laboratory zebrafish include sentinels, water quality and biofilm screening. Unlike mammals, monitoring zebrafish health and welfare using a body condition scoring system (BCSS) is rare and not well developed or utilised.

We developed and deployed a BCSS, called the 'traffic light system', (Fig. 1) (Wilson 2013) comprised of 4 stages; 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 BCS1 | 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 droopy/protruding scale | Swimming/orientation reversed Swimming on side Sitting on bottom of tank Scale loss and/or patchy loss of pigment Protruding or defective eyes | Tumors or body ulcers Decayed fins/missing caudal fin Scale loss and/or patchy loss of pigment Protruding or defective eyes | Scoliosis/hordosis |
| BCS3 | Amber I Amber II | Monitor for decline | Under conditioned Thin | Listing Gasping ¹ | Missing operculum Partial missing dorsal/pectoral fins Egg bound (not tumours) | Mild signs of scoliosis/hordosis |
| BCS4 | Green | Good Health | Over conditioned Obese | Well conditioned Steady body conformation | Swimming normal, not erratic, no signs of distress Consistent pattern/color Scales 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/color has specific descriptions to aid in health identification

Fig. 1: The four colour stages of the BCSS. Black is BCS1, and indicates dead fish; red is BCS2 and indicates immediate ill health; yellow is BCS3 and indicates a need to monitor for decline; green is BCS4 and indicates good health



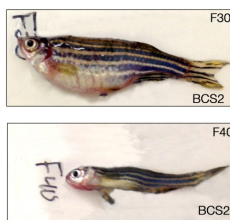
PCR and Histology Health Screen

We conducted a facility wide health screen to investigate potential causes of commonly found diseases; the fish sampled were exhibiting specific manifestations of disease, such as emaciation, scoliosis, and tumours, as well as a small sample of healthy fish. In total, 52 fish were used, numbering 0.07% of the total facility population. All of the fish were scored according to our BCSS before testing. The PCR results indicate the presence of *Mycobacteria spp* and *Pseudoloma neurophilia*; histology tests also indicated the presence of malignant tumours and granulomas.

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 (Fig. 2). All the fish exhibiting droopy, for example, which scored BCS2, all tested positive in either PCR (*Mycobacteria spp*) or histology (granulomas) (fish 30, or F30 in Fig. 2). Likewise, some fish that were emaciated, with a score of BCS2, tested positive in PCR for *Pseudoloma neurophilia* (F40 in Fig. 2), which is known to cause emaciation as well as spinal curvature. Half of the fish that exhibited no visible signs of disease, and therefore scored BCS4, tested negative in both PCR and histology tests (F49 in Fig. 2).



Fig. 2: Fish with correlating health screen results and Body Condition Scores (BCS). Left, Fish 49, or F49 was scored as BCS4, indicating no visible signs of disease; it tested negative in the health screen corresponding to the original BCS. Right, F30 and F40 were scored as BCS2 (visible disease), exhibiting droopy, and emaciation with scoliosis, respectively. F30 tested positive for *Mycobacteria spp* in the PCR results and histology revealed a granuloma; similarly, F40 tested positive for *Pseudoloma neurophilia* in the PCR and a granuloma was also seen in the histology. The BCS for these fish agree with the health screen results.



Refining the Health Monitoring System

Since the adoption of the BCSS as our standard health monitoring protocol, we have standardised the identification of diseased fish and are capable of identifying them at a much earlier stage, with improved accuracy of visual identification of the specific disease. This has led to a 54% increase of identified diseased fish, with the numbers of dead decreasing (Fig 3); this indicates that we are reducing the numbers of fish exceeding protocols' severity limits. Additionally, the number of increased using the BCSS by 52% (Fig. 3). Diseases categorised as 'Miscellaneous' was reduced by 54% under the BCSS. This has created a refinement in our health records, as they have been standardised and become more accurate than previously under our old protocol.

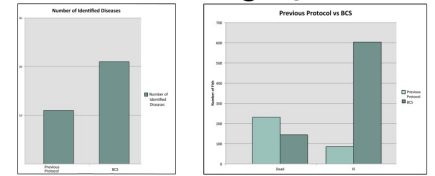


Fig. 3: Left: the number of unique identified diseases increased with the BCSS by 52%. Right: the number of diseased has increased under the BCSS, with a reflected decrease in mortality. Combined, this has refined the health monitoring within our facility

The results from our health screen indicate that our BCSS is accurate in determining when a fish has developed a disease; although the expressions may not be used for a clinical diagnosis, it succeeds in identifying diseases that may both exceed severity limits and pose a risk to healthy fish. The results also reveal that fish that would score a BCS4 can be ill with asymptomatic diseases, or be in a pre-symptomatic state; half of the fish with a BCS4 tested positive for *Mycobacteria spp* and had small granulomas. 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 consistent with the pathogens and illnesses identified (Figs. 4 and 5). 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.

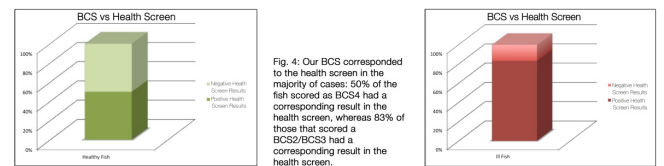


Fig. 4: Our BCS corresponded to the health screen in the majority of cases: 85% of the fish scored as BCS4 had a corresponding result in the health screen, whereas 85% of those that scored a BCS/BCS4 had a corresponding result in the health screen.

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

Fig. 5: 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

Mortality and disease are recorded in the facility database, allowing us to identify and analyse patterns that may correspond to procedural severity or husbandry / welfare. For example, patterns that arise in individual tanks or throughout rooms may indicate a husbandry related issue, whereas patterns emerging in individual strains may indicate procedural severity. Some of the patterns we have thus far identified relate to age and development of specific abnormalities (Fig. 6); this knowledge would give PILs the opportunity to cull fish at an earlier age, therefore refining techniques and either allowing a lower severity limit to be set in a project licence, or to prevent breaching a pre-existing severity limit. We have also identified that older fish seem to be more susceptible to disease; if they contract a contagious disease, they pose a greater health risk to the general fish population. This system will allow us to identify and cull fish before they reach this age. With these tools at our disposal, we can now refine severity limits, reduce numbers of animals breaching defined limits.

| ZEBRAFISH FACILITY STOCK DETAILS - ADMIN | | | | |
|--|-----------|-----------------------------------|----------|---|
| Stock # | 12888 | | | |
| Line | PDF ICON | SKU | Quantity | |
| | | Tg(TCF4 ^{+/+} × HuC:Gm5) | | |
| 9/6/2015 | | heart enlarged | 1 | 🔴 |
| 14/5/2015 | | Droopy with tumour | 1 | 🔴 |
| 14/5/2015 | | droopy with enlarged heart | 3 | 🔴 |
| 6/5/2015 | | culled | 7 | 🔴 |
| 27/4/2015 | | heart enlarged | 1 | 🔴 |
| Dof | 16/5/2013 | | | |

Fig. 6: 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.

| ZEBRAFISH FACILITY STOCK DETAILS - ADMIN | | | | |
|--|-----------|-------------------------|----------|---|
| Stock # | 12693 | | | |
| Line | PDF ICON | SKU | Quantity | |
| | | A3 × HuC:GFP | | |
| 8/7/2015 | | Emaciated with tumour | 2 | 🔴 |
| 25/6/2015 | | Tumour | 1 | 🔴 |
| 26/5/2015 | | Tumour | 1 | 🔴 |
| 6/5/2015 | | Emaciated with hordosis | 1 | 🔴 |
| 23/4/2015 | | Tumour | 4 | 🔴 |
| Dof | 15/3/2013 | | | |

References:

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

Acknowledgements:

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Further Work

With a refined health monitoring system, including the standardised BCSS and health screens, and accurate health records, we can now use our database to determine severity limits for existing strains, and identify if current severity limits should be amended in light of our findings. We will continue to conduct regular health screens to monitor the presence of the identified pathogens. With this system in use, we expect to see the number of diseased fish to decline over time, as fish will be culled before severity limits are reached, thus reducing the number of fish that may infect those that are healthy.