Implementation of an integrated zebrafish health management program at the UCL Fish Facility

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Aim: optimisation of fish health, welfare, and performance of research through the increase of pathogen detection and 3Rs compliance in animal health monitoring.

Introduction

Health monitoring is imperative to reduce the potential impacts of pathogens on zebrafish welfare and research studies. Hence, our current health monitoring program has been modified and refined in order to evaluate the prevalence of pathogens within the facility. This data will be useful twofold: it can identify areas that require better biosecurity, leading to improved animal welfare, and reduce variables within the research being conducted, fostering better science. The integrated zebrafish health management program includes data from the body condition score system (BCS), database analysis of recurrent phenotypes, biosecurity measures within a higher status room, the sentinel program, and the newly introduced health screening tests through quantitative polymerase chain reaction (qPCR) assays performed in-house. Further data will be provided through a diagnostic evaluation service, which will include an assessment of husbandry practices, water quality, and a clinical exam.

The Body Condition Scoring System

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 zebrafish population.

Body Condition Score	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
BSC2 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
BSC3 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)	
BSC4 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

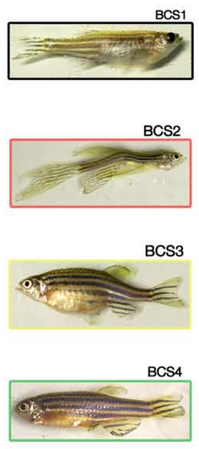


Fig. 1: The four stages of the BCSS. BCS1/black is dead; BSC2/red is for obviously diseases fish; BCS3/amber is moniter for decline; BCS4/green is healthy

The adoption of the BCSS is a refinement, as we have: improved accuracy of visual identification of disease; standardised records; increased the number of identified diseased fish; decreased the numbers of dead, thus reducing numbers of fish exceeding protocols' severity limits.

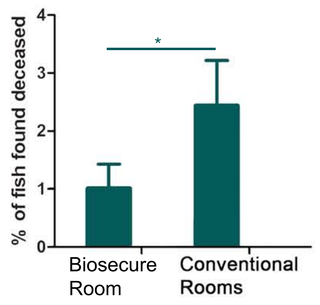
External Diagnostic Methods

Different diagnostic techniques (PCR and histology) allow us to accurately detect specific pathogens affecting our zebrafish colonies (table 2).

Pathogen	Biofilm Racks[B]	Biofilm Sentinels[B]	Quarantine Biofilm[A]	Conventional 2 Biofilm[A]	Conventional 1 Biofilm[A]	Biofilm Racks[A]	Biofilm Sentinels[A]
Mycobacterium spp	+	+	+	+	+	+	+
Mycobacterium abscessus	-	-	-	+	+	-	-
Mycobacterium chelonae	+	+	-	-	+	-	-
Mycobacterium fortuitum	-	-	+	-	+	+	-
Mycobacterium haemophilum	-	-	-	+	-	-	-

Table 2 (right): PCR report showing positive results for fish tissues, biofilms and embryos submitted to reference laboratories from different rooms. [A] =May 2017; [B] =August 2017. Biosecure embryos[A] tested negative for all tested pathogens. All samples tested negative for *Mycobacterium marinum* and *Mycobacterium peregrinum*. Fish from both the biosecure[A] and conventional rooms[A] tested negative for *pseudoloma neurophilia*, and *pseudocapillaria tomentosa*. A histopathological evaluation of fish samples from all types of rooms was negative. (Data not shown).

The introduction of a biosecurity program through the implemented of a Biosecure Room has prevented the spread of *Mycobacterium haemophilum* found in the conventional rooms.



The health monitoring program is implemented equally in both the biosecure and conventional rooms; it includes the flagging of unhealthy fish according to the BCSS and immediate removal of dead fish. All findings are recorded in the database. Fish found deceased in the biosecure room compared to the conventional rooms were significantly lower (P=0.0343) (fig. 8). This difference may be associated with the lower biosecurity measures in the conventional rooms versus the higher measures taken in the biosecure room.

Fig. 8: Fish deceased in the biosecure room vs. conventional rooms over a 10 month period. Data, represented as Means ± SEM, were compared using the Mann Whitnney test and considered to be significant at $*p < 0.05$. (p=0.0343).

Database Analysis

Our database, alongside the BCSS, allows us to analyse 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, severity and humane endpoints can be reassessed through patterns of phenotypes that arise over time (fig. 3), as can age-based endpoints, which we have used to create an age policy of 18 months (fig. 4). As well, the effects of inbreeding can be assessed; preliminary data suggests that disease can appear earlier within each successive generation (fig. 5). We conducted a facility wide health screen to identify pathogens. We saw a similarity between the results and our BCSS; 73.6% of fish scored with BCSS had a corresponding result from either the PCR or histology results. Only 17% of ill fish tested negative for a specific pathogen; the remainder showed signs consistant with the pathogens and illnesses identified (fig. 6). This suggests a genetic or protocol cause that is associated with the strain.

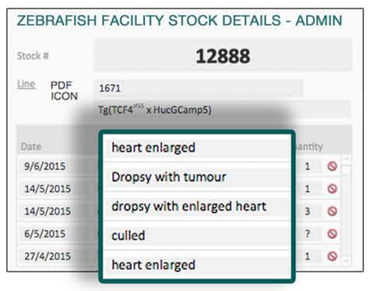


Fig. 3 (left): A genetic strain that develops a heart defect once it reaches a particlar age. Right, another genetic strain that develops tumours after a particular age. This information may be used to define an age limit for these strains.

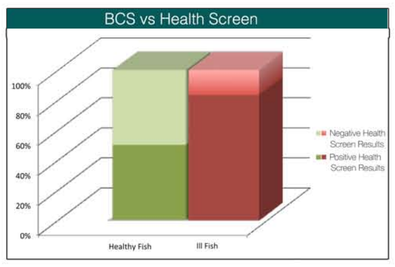


Fig. 6: Our BCS corresponded to the health screen in the majority of cases: 50% of BCS4 had a corresponding result in the health screen, whereas 83% of BCS2/BCS3 had a corresponding result in the health screen. n=100

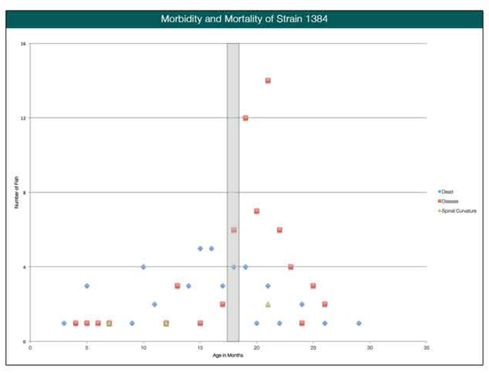
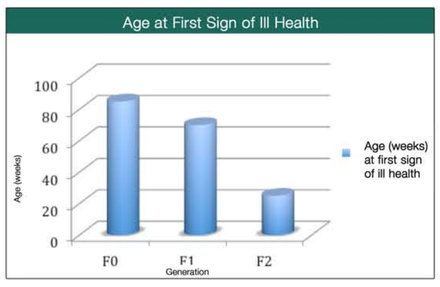


Fig. 5 (below): Ill health in an inbred strain shows earlier signs of ill health in the F2 generation suggesting inbreeding may affect health and survival rates. n = 300

Fig. 4 (left): Ill health in a transgenic strain increases at approximately 18 months. In order to prevent pain, suffering, and distress, the age-related humane endpoint should fall around this age.



Internal Diagnostic Methods

To detect *Mycobacterium spp.* and *Pseudoloma neurophilia*, DNA was extracted from liver and spleen tissues with the Dneasy Blood and Tissue kit (Qiagen) according to the manufacturer's recommendations. DNA samples were isolated from homogenated pools of 5 fish from 2 different rooms (biosecure room and conventional rooms) and also a sample of live feeds (1 L filtered rotifers culture) was screened. Here we show the results found for the qPCR assay for *Mycobacterium fortuitum.*

Sample	Lesions	qPCR
5	Lordosis, abnormal swimming and emaciation	+
8	Lack of pigmentation	+
9	Lordosis	+
10	Abnormal swimming	+
11	Severe lordosis and emaciation	+
12	Eggbound	+
13	Lordosis and cataracts	+
14	Lordosis	+
16	Abnormal swimming	+
17	-	+

Table 3: Results for Mycobacterium fortuitum. M.Fortuitum was screening using Kits Primerdesign™ Ltd. A total number of 17 samples (from sample no1 to sample no17) were tested. 10 samples out of 17 were positive for mycobacterium fortuitum. Sample 17 represents DNA from 1litre of filtered rotifer culture. Samples 8 and 9 come from sentinel fish. The rest of the samples correspond to a pool of 5 fish.

For the pathogens already identified in the fish facility we are conducting conventional PCRs using the same DNA samples from the previous test to evaluate their prevalence and the possibility of co-infections. We are conducting screens to detect the most relevant pathogens affecting our colonies (*M. fortuitum* and *M.chelonae*) and comparing the qPCR results for *M.fortuitum* with the conventional PCR tests using the primers showed below (Rocchetti *et al.* 2017).

Pathogen	Number	Oligo name	Oligo sequence	Gene
P.Neurophilia	1	PNA_03	TGA AAT GTG GTG ACC CGT TTA GG	Ribosomal RNA
P.Neurophilia	2	PNA_04	TCC TTG ACC CAT CCT TCC TGT G	Ribosomal RNA
M.haemophilum	6	MhITS1R	TGAACACGCCACCATTAC	ITS
M.chelonae	7	MC-MAG_Fw	CACGGGGTGGACAGGATTTA	ITS
M.chelonae	8	MC-MAG_Rv	TAAGGAGCACCATTTCCCAG	ITS
M.fortuitum	14	MFC_Fw2	TCACCTGATCTGCACATAATGT	rpoB
M.fortuitum	15	MFC_Rev	AGCACCTCATGCGACTT	rpoB

Table 4: Primer and probe sequences designed for the in-house PCR diagnostic assays of DNA Mycobacterium spp.

Further Work

In conclusion, this integrated health management program might help to achieve an accurate colony health status assessment or health report (specific pathogen prevalence will be estimated by conducting regular screening tests) and also secure better health information exchange of zebrafish strains between facilities across the world.

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

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