

Body condition scoring for Laboratory Zebrafish

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Summary

Zebrafish are a rapidly expanding model in biomedical research. The numbers reported in Home Office returns do not always show the true numbers of Zebrafish actually held in facilities, as much work is conducted on embryonic forms that do not need to be included. The true husbandry requirements of Zebrafish remain elusive; much work is still required to identify their actual needs. This lack of information is reflected in many unstandardised husbandry methods, the true impact upon some scientific disciplines is now beginning to be understood. Currently there are no standardised methods for health monitoring of Zebrafish.

In this paper we describe how we have developed and described a tank side body condition scoring system, which may also be called the traffic light system. We created a pilot study to trial this system, using 45 volunteer participants from a variety of backgrounds. We asked them to score fish in various different conditions without any training and then again after receiving limited training to try to identify whether the participants' responses could be standardised. From our trial we determined that using Body Condition Scoring is a way of standardising health monitoring and how such a system could be implemented.

Although the idea of Body Conditioning Scoring for a Zebrafish facility does require further work, we believe that it has many advantages over current methods being used. It can standardise basic health monitoring within a facility and complement other health monitoring processes such as sentinel, water quality and biofilm screening.

Introduction

Zebrafish (*Danio rerio*) are fast becoming the animal model of choice for a range of scientific disciplines, beyond the more traditional developmental biology^{1,2} and large scale mutagenic screens^{3,4}. The disciplines now using Zebrafish embryos include drug and small molecule screening^{5,6} and adults for tissue

regeneration studies^{7,8} and cancer research^{9,10}. Although Zebrafish have become an increasingly popular model, Home Office statistics show fish in general represented 15% of all returns in 2011¹¹, this does not tell the whole story – much work is done on embryonic forms, so colony size can be much larger than is suggested by Home Office returns. The speed and growth rate of scientific interest in Zebrafish may have also have outpaced the speed at which husbandry methodology has advanced.

There is a lack of general information about the husbandry requirements of Zebrafish, although studies have been made of them in the wild^{12,13}, information about their true husbandry requirements remains elusive¹⁴. This has led to a variety of various husbandry protocols being developed (personal observation) and few, if any, truly standardised methods used in the husbandry of Zebrafish. One way of introducing elements of standardisation across the facility could be to introduce a body condition scoring system. This paper describes a pilot study of a body condition scoring system devised and trialled by UCL fish facility staff.

Scoring systems are broadly used in many areas and allow for monitoring of various aspects of animals' health and behaviour. In agriculture, body condition scoring systems are common for assessing farm animal health, primarily cattle and sheep – using body condition as an indicator. For example, in dairy herds they can be used as an indicator of body fat and potential milk yield¹⁵, as well as general health¹⁶, in sheep, as a measure of body fat¹⁷ and in horses using estimated weight as an indicator of body condition^{18,19}. In laboratory mammalian species, scoring systems have been developed for a variety of different purposes – from those used to assess welfare and health under a variety of circumstances to those which aid scientific research more directly such as assessment of chimeric mice²⁰. Body conditioning scoring systems have been developed as non-invasive methods for scoring in both rats²¹ and mice²² and are used to assess health and general welfare, especially when body weight may not

be applicable – such as in cancer tumour models. Scoring systems have been developed to assess and monitor depth of anaesthesia in laboratory species, usually determined by respiration rate, heart rate, blink reflexes and various pinch reflexes. More recently, systems have been developed to determine pain by facial expression – grimace scales in both mice²³ and rabbits²⁴.

Although there has been use of scales to both classify and monitor aspects of fish husbandry and welfare both within the scientific community – for example guidance on the severity classification of procedures of fish has been published²⁵ and in fisheries ecology²⁶ and more general aquaculture the use of body condition indices is relatively common^{27,28}. In monitoring any aspect of Zebrafish health and welfare the use of body condition scoring is rare and not well developed or utilised. Although condition factor indices measuring growth and mass have been used in assessing Zebrafish condition²⁹, this type of body condition scoring is inappropriate for large-scale use as weighing and measuring the fish would be a substantial stressor to the fish. Additionally the large numbers of fish housed in many facilities would also make this extremely labour intensive and practically impossible. Other types of body condition scoring used for mammalian species may also be difficult to translate directly to fish, as they may involve handling animals¹⁵⁻¹⁷. In developing our body conditioning scoring system we have tried to address some of the problems presented by other systems for body condition scoring and recognise some of the difficulties presented by body conditioning scoring of Zebrafish. We have developed a body conditioning scoring system that is non-stressful to the fish, can be performed at the same time as the daily checks required by The Animal (Scientific Procedures) Act, 1986, is easy to learn and introduces a standardised method.

Method

This trial comprised of two parts – the development of a traffic light system where various stages of body condition were graded and a second part when we assessed the suitability of the method, where different groups of volunteers were asked to use the system to score the body condition of different fish.

All work was carried out in accordance to the Animals (Scientific Procedures) Act, 1986 – no work was carried out under a specific project licence as this study involves a variation of standard husbandry procedures. The fish used for this trial were from the UCL fish facility general adult population and healthy adults were representative of a mixed population of sex, ages and genetic status from a total of approximately 50,000 Zebrafish (*Danio rerio*). The microbiological status of these fish was untested.

All Zebrafish were held in either 3 or 10 litre tanks on recirculating systems at no more than 5 fish per litre and each tank has 6 water changes per hour. The fish water is conditioned from reverse osmosis water, with marine salts added back. The water is changed 6 times per/hour. The water parameters are temperature 28°C +/- 1°C, PH 7 +/- 0.5, conductivity 450 – 600 S, ammonia 0mg/litre, nitrites 0-0.3mg/litre and nitrates 0-25mg per litre. Dissolved gases and water hardness are not routinely measured.

The fish are fed combinations of a dry microencapsulated diet and Instar I *artemia*, two – three times daily.

It should be noted that it is rare to find such extreme examples of ill health within any Zebrafish population and we have calculated that the examples we have shown represent less than 0.01% of our total fish population at any given time that could be classified with a body condition score of BSC2 / amber or 3 / red.

The “traffic light” Body Conditioning Scoring System for Zebrafish

The traffic light / BCS (body condition scoring system) comprised of 4 stages (Table 1 and Figure 1). These 4 stages grade various aspects of both fish behaviour and general body condition. The four stages indicate various stages of health / decline of health that maybe observed in a general population of Zebrafish, especially if the population is large³⁰.

BCS1/Black. Fish presenting these symptoms have little swimming movement, little gill movement. If these are isolated cases these fish should be removed from the systems, if there are high numbers of fish presenting these signs further immediate action is necessary as this may indicate a water quality issue.

Body Conditioning Score 1

Remove immediately

Dead-immobile, white/grey colour, missing body parts

Dying-laying on bottom of tank, not moving when provoked



Figure 1.a: Body Conditioning Score 1 / Black

BSC2/Red. Fish presenting these symptoms should be removed from the system, as many of these conditions can represent underlying infectious disease such as mycobacteria³¹ and *Pseudoloma neurophilia*³². Other explanations may be genetic, water quality issues and age related³³.

Body Conditioning Score 2 Remove immediately



Club tail-tail deformity



Scoliosis and lordosis-curvature along spine



Emaciated-wasted, low body to head ratio



Lesion



Scale and pigmentation loss



Cataracts



Popeye-protruding eye



Tumour



Severely eggbound

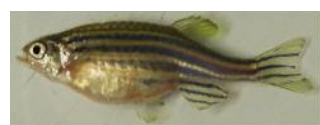


Drosy-scales puff outwards

Also includes:
Reversed orientation-swimming
at an extreme angle
Decayed fins

Figure 1.b: Body Conditioning Score 2 / Red

Body Conditioning Score 3 Monitor Also includes Gasping Missing dorsal or pectoral fin



Obese/Eggbound (start of)



Missing operculum



Thin/Small for age



Listing-swimming at a slight angle, or slowly correcting angle

Figure 1.c: Body Conditioning Score 3 / Amber

BCS3/ Amber I & Amber II. Representing both over and under conditioned fish. Fish presenting these symptoms require monitoring, as none of the conditions are necessarily serious in themselves and maybe the result of genetics. If listing and gasping are not in isolated fish this is a serious problem – as it is an indication of gas saturation³⁴ within the water and must be acted on immediately.

BSC4/Green. The vast majority of any fish Zebrafish population should fall into this category. Healthy Zebrafish should have good, symmetric body shape, complete fin complement, not showing any signs of distress when swimming or breathing and it should be easy to determine the sexes through body shape. Male body shape is more bullet-like; female is more rounded, especially in the abdomen.

Body Conditioning Score 4 Looks like: Well conditioned Sleek body Consistent pattern and colour Sexes visible

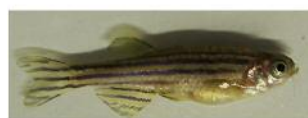


Figure 1.d: Body Conditioning Score 4/ Green

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
BSC2	Red	Priority to remove from system Possible signs of contagious disease or system failure Investigate	General emaciation Wasted body to head ratio General body deformities General drosy/protruding scales	Swimming/orientation reversed Swimming on side Sitting on the bottom of tank but will move in response to stimuli	Tumours or body ulcers Decayed fins/missing caudal fin Scale loss and/or patchy loss of pigment Protruding / defective eyes	Scoliosis/lordosis
BCS3	Amber I	Monitor for decline	Under conditioned Thin	Listing Gasping ⁴	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

⁴Gasping in large numbers of fish is serious as it indicates a water problem and should be acted upon immediately

Table 1. The different stages of the Body Condition Scoring system / traffic light system

The Trial of the “traffic light” Body Conditioning Scoring System

Six fish, representing at least one fish representing each of the stages were selected from the main facility. After the trial all fish were culled in accordance with the Animals (Scientific Procedures) Act, 1986, Schedule 1. All fish staged at either black or red would have been culled and all other fish were surplus to scientific requirements and were due to be culled by a Schedule 1 method.

All volunteer participants were ranked according to previous knowledge of fish and animal husbandry. We determined four different categories within the volunteer participants –

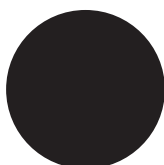
Category 1 – Fish technicians, responsible for the daily care of fish and researchers whose primary work is done with Zebrafish and have responsibilities under Animals (Scientific Procedures) Act, 1986.

Body Conditioning Score 1

Looks like:

Dead – immobile, white/grey colour, missing body parts

Dying – laying on bottom of tank, not moving when provoked



Body Conditioning Score 2

Looks like:

Emaciated – wasted body to head ratio

Tumour

Reversed orientation – swimming at an extreme angle

Decayed fins

Scale and pigmentation loss

Scoliosis/lordosis – curved back

Popeye – protruding eye

Dropsy – scales puff out



Body Conditioning Score 3

Looks like:

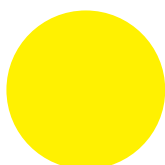
Thin

Gasping

Listing

Missing operculum

Missing dorsal or pectoral fin



Body Conditioning Score 4

Looks like:

Well conditioned

Sleek body

Consistent pattern and colour

Sexes visible

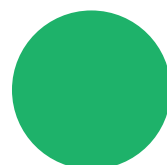


Figure 2. Body Condition Scoring sheet used to train participants

Category 2 – Students who see the fish regularly but neither conduct research nor perform husbandry duties.

Category 3 – Experienced animal technologists who work with mammalian species but do not work with fish.

Category 4 – Participants who had neither knowledge of Zebrafish as a research model nor of the husbandry of any animal used in research.

Five individual tanks housing the fish were arranged, one was coded BCS1/black (dead), one was coded BCS4/green (healthy) and the remainder were variously coded BCS2/red and BCS3/amber and had various different conditions as stated and shown in Table 1/ Figure 2. The individual tanks were transparent and housed individual fish, in order for the participant to view the fish completely before assigning it a BCS/traffic light colour. A 10-litre tank was also set up containing either one or two fish from categories BCS1/black – BCS3/amber (various stages of ill health) and eight to ten BCS4/green (normal, healthy) fish, for the participants to view.

Each participant was asked to give a BCS/traffic light colour score. The fish in the individual tanks were given a BSC/traffic light score. The scoring for the 10-litre tank had to be assigned according to the BSC/traffic light score of the least healthy fish, not an average score or a score based on the majority of BCS4/green fish but a score that could inform someone else what the worst scenario was within the tank. Each participant was asked to score all the tanks initially, according to their own experience and knowledge of fish; no help was given at this stage. Once complete, the participant was given a short amount of training (5-10 minutes) – shown a BCS/traffic light scoring sheet (Figure 2) with the various levels/stages explained, including examples of diseases/ill health with accompanying pictures. Then, with the *aide memoire* of the BCS/traffic light scoring system, they were asked to rescore all tanks.

Results

In total 45 volunteers participated in the trial, over the course of nine different events. The number of participants from each of the 4 different categories was as follows –

Category 1 – 21

Category 2 – 5

Category 3 – 7

Category 4 – 12

We then decided to combine categories 2-4 to analyse some of the results – to give us two groups:

Group 1 – Participants familiar and working with

Zebrafish in a research setting.

Group 2 – Participants not familiar with Zebrafish in a research setting.

Using the two groups (Figure 3), we found that at 64% of group 1 (fish technicians and research workers using Zebrafish) were able to correctly score the fish before training and 74% were then able to correctly score fish after training. The combined categories of 2 – 4, as group 2, were able to correctly score fish in 53% of all cases prior to training and increased to 63% after training.

Interestingly, a breakdown of the categories within group 2 (Figure 4) showed a significant rise in the category 2 participants (students familiar with Zebrafish – but not conducting either husbandry or research) ability to correctly score the Zebrafish rose from 56% prior to training to 84% after training. Conversely, category 4 participants' ability to score the fish after training dropped slightly 57% to 53%, although this is not significant (data not shown).

A one way ANOVA was performed on the data. A P value of 0.039 for the data generated before training indicates that there is a significant difference between the groups. A P-value of 0.042 for the data generated after training indicates that there is still a significant difference between the groups.

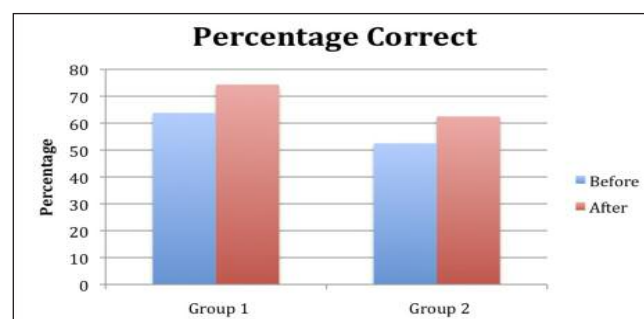


Figure 3. The difference in results between group 1 and group 2

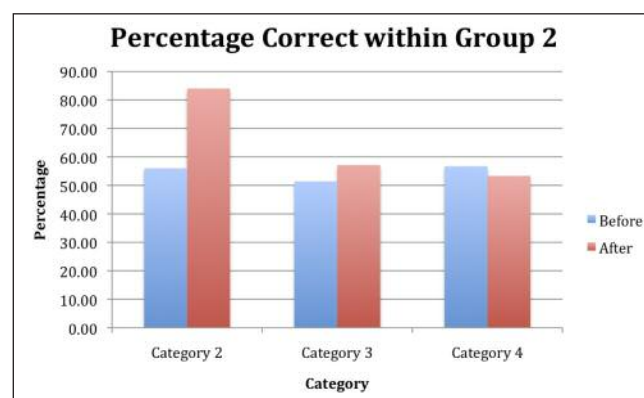


Figure 4. The differences in results between the 3 categories in group 2

Discussion

Although this is a very preliminary pilot trial, the results show that even with a very limited amount of training, some degree of standardisation from a visual inspection can be achieved. It is unsurprising those participants with the most experience working with Zebrafish could identify and score the most accurately. What is more surprising is that students who were used to looking at Zebrafish but had no research or husbandry experience, appeared to be able to score the different stages of health the most accurately after training, suggesting that what may be required prior to any training is for people to spend some time generally familiarising themselves to the species. The failure of participants in category 4 to improve scoring after training also suggests this, as well as a need to improve and lengthen the time spent training.

We recognise that more work needs to be done to fine tune the different stages in the traffic light system, which we believe can be achieved by greater range of images in and longer, more extensive periods of training. We recognised that participants from all categories had problems identifying single fish with a status other than BSC4/green in a tank of otherwise healthy fish. Fish displaying distended abdomens (egg bound or tumours) or displaying dropsy were also not obvious to all participants. This will be a problem and require further work to overcome, as all participants, were looking at 5 tanks in total, however, in a real life situation the number of tanks examined could be in the thousands.

This is not a perfect system – some things would need additional training and/or explanation – for example gasping at top on tank by many fish may indicate gas bubble disease (gas super saturation) and a problem with the systems pushing in gas under pressure to the tanks – this is an emergency and not to be treated as amber (BCS3), which would only be seen in individual fish. Since large number of individuals housed together may make it difficult or impossible to pinpoint individual conditions, this may be a better method for identifying conditions that affect larger numbers of fish, or at least for conditions that have a profound phenotype. It would be less successful for those conditions which have subtle expressions. As living fish are always in water it may also make visualisation of health status more difficult and this could also be impeded by non-handling, something more likely to happen with a mammalian species.

However a BCS/ traffic light system does have several advantages in monitoring fish health and welfare. Not only can it allow standardisation of monitoring fish, it can also enable tracking and observations of specific conditions – for example it may help to identify outbreaks of infectious disease in fish colonies – especially mycobacterium³¹ and *Pseudoloma*

*neurophilia*³². It can also be used as an indicator of age, through the effects of both fecundity and fertility and used as a marker of possible ill-health that have a genetic link, for example it may be possible to track health problems in individual lines/strains using a BCS/ traffic light system³⁵. A BCS/traffic light system can be used to monitor fish housed singularly or those housed in groups, therefore it would make monitoring of individual tanks and groups of fish possible. It is important to remember that the health and welfare of each individual fish is important, irrespective of how many fish a facility may hold overall.

A BCS/traffic light system would be beneficial to animal technologists, who may not be familiar with fish health and welfare and for new staff, as well as a useful tool in explaining decline or lack of condition to other interested parties.

A BCS/traffic light system has advantages over some other condition scoring methods e.g. using K factors²⁹ as length and weight may vary from facility to facility, biased by the lack of standardised conditions, such as diets and water quality. Furthermore BCS is a more rapid and consistent system, causing no stresses to the fish, and is a better indicator of health status.

As the trend for using Zebrafish in research increases and the size and holding capacity of Zebrafish facilities correspondingly increases – to the point where facilities hold thousands, perhaps hundreds of thousands of Zebrafish, a health monitoring and scoring system such as we suggest here is likely to become an additional part of any Zebrafish health screening and monitoring process.

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