An adaptable, user-friendly score sheet to monitor welfare in experimental fish

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Abstract

Fish are increasingly used as experimental animals across research fields. Currently, a quarter of all experimental animals used in Europe are fish. Less than 20% of these are standard model species. Welfare assessments for experimental fish are in their infancy compared to rodents. This can be attributed to the diversity of species used, the relative recency of fish as go-to model for research, and challenges to assess welfare and pain in non-vocal underwater species. The lack of guidelines and tools presents a challenge for researchers (particularly, for newcomers), for ethics committees, and for implementing refinement measures.

Here, we present an adaptable, user-friendly score sheet for fish. The parameters contained in the excel tool are based on a literature review, have been validated by expert interviews, and evaluated by a fish pathologist. The tool allows to score individuals as well as groups, calculates summary scores and visualizes trends. We provide the underlying literature, give use examples and provide instructions on the adaptation and use of the score sheet.

We hope that this tool will empower researchers to include welfare assessment in their routines, foster discussions on fish welfare parameters among scientists, facilitate interactions with ethics committees, and most importantly, enable the refinement of fish experiments.

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Author contributions

IAK conceived the project, HS and IAK designed the analysis with input from MFG, MFG collected the data and performed the analyses with support from AB and HS, HS and IAK supervised and guided analyses, MFG wrote the manuscript draft, HS, IAK, AB and MFG edited the manuscript.

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Declaration of conflicting interests

The authors declare that there is no conflict of interest.

Research ethics

No animals were used for this research.

Data availability

All data used are available in the supplementary material.

Supplemental File 1: Literature search parameters

Supplemental File 2: List of retained and analysed literature, initial parameter list and refinements, expert questions

Supplemental File 3: Collection of fish welfare parameters

Supplemental File 4: Score sheet

Introduction

A wide range of research areas use fish as experimental models. Topics include toxicological tests [1], pharmacology [2], developmental biology [3,4], complex human diseases [5] and behaviour [6]. Fish are also often used to probe the response of aquatic organisms to the impact of global change, ocean warming and ocean acidification [7]. Teleost fish share key genetic, anatomical and physiological properties with humans [5,8] and therefore are valuable organisms to investigate basic evolutionary concepts [9–12], stem cells and regeneration [13], the impact of chemicals and pollutants on organismic health [14](12), or pharmacology [15].

In 2020, a quarter (27.4%) of all animals used for research in the EU were fish. Zebrafish (*Danio rerio*) as the most widely known experimental fish model constituted 13% of all experimental fish [15], but numerous other fish species beyond zebrafish are used in research [5,16], such as medaka (*Oryzias latipes*) [17], fat head minnow (*Pimephales promelas*) [18], trout (*Salmonidae*) [19], cichlids (*Cichlidae*) [20], sticklebacks (*Gasterosteidae*) [21], guppies (*Poecilia reticulata*) [22], mollies (*Poecilia latipinna*) [23], or gobies (*Gobidae*) [24], to just name a few. Evolutionarily, some of these species are more distantly related than humans to armadillos [25].

In accordance with widespread legal requirements to implement the 3Rs (Replace, Reduce, Refine [26]) in animal experiments, the awareness and demand to objectively assess welfare in animal experiments continues to increase, also in research using fish. The 3Rs postulate to replace, if possible, animal models with non-living models, to reduce the number of animals necessary to fulfil an experiment, and to refine the experiments to avoid or reduce negative impacts, and they serve as foundation for laws and recommendations on animal welfare in research[27]. Measures supporting the third R, refinement, are very much dependent on the monitoring and documentation of animal welfare during husbandry and experimentation, which

is legally mandatory in many countries. The tool commonly used to measure welfare are score sheets.

The aim of score sheets is to document the state of welfare and to detect alterations in welfare between groups, individuals, or time-points in a semi-quantitative, semi-objective manner. However, one can only measure what one can define and quantify, and animal welfare remains a tricky concept particularly for fish [28]. EU regulations, for example, contain no notion of welfare [29]. National definitions of welfare vary (ranging from the five freedoms [30] to dignity- or well-being-focused approaches [31]). While fish are treated as sentient by law [29], and despite key initiatives [28,32–37]), some sources still express a need for debate on the matter [27,32,38]. Knowledge on fishes´ emotions and behaviour changes is available on some species [8,28,39,40], but their subjective pain perception is sometimes discussed controversially [32,41]. Consequently, while the literature does discuss potential signs of distress in fish, evaluation tools for validated clinical and behavioural indicators of wellbeing are hard to come by for fish other than zebrafish [42–44].

In addition to these conceptual challenges, fish score sheets need to account for the previously mentioned diversity of species with distinct behaviours, morphology, physiology, and environmental requirements [8], and the absence of characteristics well established for rodents, such as vocalisations, body language, and spontaneous as well as induced behaviours such as grooming [35,45,46], or for agricultural species [46]. While decipherable behaviours have been identified in some fishes, many remain understudied or uninterpretable [40]. Fish are often housed in considerably large groups, which on the one hand complicates individual welfare monitoring and can result in specific welfare problems (e.g., hierarchy or territory issues (28)), but on the other hand expands the set of observable and measurable behavioural indicators beyond individual characteristics. If sourced from the wild, they come with a range of common background conditions [47].

Finally, in addition to these system-inherent challenges, training opportunities taking fish welfare into account are presently severely limited. Good welfare practices depend on skilled staff [8,40], but courses for fish researchers, authorities and ethics committees are usually part of a very broad education on "non-rodent species". Fish research publications typically do not include score sheets [8,28], which limits open conversation about useful and implementable quantitative or qualitative parameters for welfare evaluations. A potential ally in experimental fish welfare is the field of aquaculture, where fish welfare translates to disease incidence, growth rates and meat quality and thus to business revenue (e.g., MyFishCheck [48–51], but limited mutual interactions impede the transfer of welfare related tools. Together, these challenges result in a lack of resources, expertise and frameworks with regard to fish welfare scoring in experimental research, which poses a serious challenge for researchers in the implementation of the third R for fish, particularly when researchers are new to the organism.

This publication aims to provide fish researchers with a resource facilitating the task of refinement. We provide a versatile, multispecies, modular, literature-based, and user-friendly score sheet and thereby empower researchers to assess the welfare of their animals, implement refinement strategies, and fulfil legal welfare monitoring requirements in an evidence-based manner. To develop this tool, we first systematically listed and evaluated fish welfare parameters. This entailed a scoping literature review and an exhaustive compilation of clinical and behavioural parameters that could be used as indicators of the welfare state of fish. We also conducted qualitative guided conversations with researchers and veterinarians working on different fish species and experimental systems, and reduced the list of potential indicators to a handful of individual and group parameters. Possibility of systematisation, non-intrusiveness, and modularity of parameters (e.g. module "behaviour") were choice criteria. For each parameter, we then defined possible, easy-to-identify deviations from the desirable state, and defined mild versus severe deviations from the ideal state. We provide these parameters in the

context of a modular MS office-based scoring table that a) accommodates individuals as well as groups of fish and b) can be adapted easily for the species, the experimental context or the housing situation.

We are convinced that this score sheet will simplify welfare monitoring for fish researchers worldwide, promote the use of pre-studies to optimize animal numbers and experimental conditions, facilitate 3R implementation as well as licence application procedures, and serve as useful starting point for discussions within and between labs regarding fish welfare.

Methods

Scoping review

To gather the published state of the art regarding fish welfare assessments, we conducted a scoping review [52] on welfare indicators for juvenile and adult fish. The main research question was “Which welfare indicators and score sheets are currently used for fish?”. PubMed and Web of Science were queried with 40 combinations of the terms "fish", "rating", "welfare", "indicator", "score board", "score card", "monitoring", "assessment", "humane endpoint", "perch", "trout", and "zebrafish" (Supplemental File 1). When a search combination received too many hits to screen and select, the search term was refined. The relevance of papers was then assessed by screening title and the abstract. Papers were retained if these suggested the paper could contain information about fish welfare. Retained papers were then screened for welfare indicators and retained if they mentioned such parameters. Reference sections of retained publications were additionally screened for publications that did not come up in the original search and were subjected to the same screening process. 84 documents were directly retrieved from the databases in round 1. An additional 15 relevant documents were identified from snowballing the references of the round 1 documents. From the 99 documents containing welfare parameters that were retained (Supplemental File 2), a list of welfare parameters was compiled. In total, the literature search yielded 68 potential parameters (Supplemental File 2).

Expert interviews

To understand practical considerations and the diversity of needs and implementation situations, ten fish facilities in Switzerland housing zebrafish, perch, trout, stickleback, and cichlids for research on RNA, epigenetics, genetics and genomics, development, health and infection biology, and behaviour were visited. The persons in charge of running the facility were qualitatively interviewed in semi-structured interviews with an interview guide. Questions included fish species, husbandry parameters, types of experiments done, current approaches to

measure welfare (tool, criteria, frequency, group or individual level, time, responsible person, treatment of transgenic lines, reasons to not use a score sheet if none was used, source of the tool), wishes and thoughts towards score sheets (what would make it easy to use, digital or analog), and considerations concerning the collected parameters (relevance of parameters, additional suggestions, species-specifics, how to group parameters, potential endpoints, relevance of a total integrating score). Answers were recorded with pen and paper and later transcribed into an excel sheet. Also, 4 additional conversations were conducted with experts on animal welfare to understand what they would expect from or look for in a scoresheet. Questions are included in supplemental file 2. The responses, except for a summary and individual quotes, are not shared to maintain participant confidentiality.

Parameters

The 68 parameters identified in the literature were narrowed down to a meaningful, feasible set of 29 parameters in a first round based by co-author HS, a diagnostic and research veterinarian and fish pathologist with 20+ years of experience in fish health and fish welfare, and further refined to 21 parameters for individuals and 10 parameters for groups based on the expert interviews and on discussions within the team (who feature experience working with zebrafish, trout, perch, and gobies, and rodents). Specifically, the following steps were taken: a) non-invasively observable parameters were selected (for example, blood cortisol levels as stress indicators require invasive measurements), b) related parameters were grouped (for example, swimming equilibrium [53] and buoyancy [54] were summarised to navigation problems), c) a verbose explanation for possible states was developed (for example, “normal for the species”, “mild weight loss/gain”, and “severe weight loss/gain” for the parameter “nutrition”), d) considerations that could affect scoring were collected (e.g., recent feeding may cause non-problematic abdominal swelling), and e) observable states were linked to numeric scores with 0 = ideal state, 1 = not ideal, 2 = observe, act if possible, and 3 = act immediately, potentially terminate (Table 1, see Supplemental File 3 for additional literature and species information).

Results

Expert Interviews

Expert interviews confirmed the idea that fish welfare assessments and their use in refinement are an area of development with unsatisfactory options for fish researchers. A frequent practice was to copy other researcher's score sheets and to satisfy administrative requests ("Once the canton of … requested more parameters, so we just added one of them"). Opinions on welfare were often based on years of experience working with fish, rather than on explicitly scorable, model specific, and objective criteria. Only 3 of the 10 facilities indicate using a score sheet as welfare tool. Two facilities stated not using a score sheet because it was not compulsory in their region. Usually, more than 1 person was in charge of assessing fish health and state at the same time. Some facilities stated that certain types of physical/morphological aberrations or behavioral signs were considered as induced by standard lab conditions and were therefore currently not considered in their score sheets as welfare issues.

With regard to a score sheet, isolated parameters were generally deemed insufficient to conclude on the welfare state of individuals and groups. Also, behavioral signs of impairment were considered more relevant than physical signs, which may be linked to the current lack of understanding regarding the relevance of e.g., injury and related pain for fish wellbeing. The relevance of an integrative total score was generally supported as reasonable given the mutual impacts of parameters (e.g., damaged fins might lead to altered swimming behavior). A request was to include potential measures to alleviate the identified issues in a score sheet, since euthanasia of impacted fish would often create issues for experimental design. A digital version was preferred, and a tablet-compatible app was suggested by several participants.

Score sheet

The score sheet developed based on the review and the interviews is available as Supplemental File 4. It contains 21 potentially relevant parameters for individuals, and 10 parameters for groups. These 21 parameters are based on literature from 17 distinct fish species from 11 taxonomic families. They are classified into the categories body condition, skin, fins, eyes, gills/opercula, infection, swimming behavior, feeding behavior for individuals, and 2 additional categories (group behavior and mortality) for groups.

For individually housed or marked fish, for example in cichlid behaviour experiments [6], the status for a certain parameter is assessed verbosely by choosing from a drop-down menu (Figure 1). For example, for fin integrity, researchers can choose between "fin in healthy condition", "slightly eroded / slightly missing", "moderate fin loss" or "severe fin loss / missing fin". The sheet then automatically calculates a numerical score. For fish housed in groups, which is for example usually the case for zebrafish, the estimated percentage of fish in the tank with certain issues is recorded (Figure 1). For example, 80% of fish may be scored, for a particular parameter, to display "no abnormalities / lesions", 10% with "slight abnormalities / lesions", 5% with "moderate abnormalities / lesions" and 5% with "severe abnormalities / lesions". A weighted average is then calculated for the tank based on formulas already implemented in the score sheet. If percentages do not sum up to 100 due to entry errors, the respective cells in the excel sheet turn orange.

In the score sheet for individually housed or marked fish, skipping the assessment of a parameter in the individual score sheet results in a default score of 1 to avoid the false assumption of good welfare based on non-scoring. If parameters cannot be scored because it is either not feasible or not relevant regarding the circumstances, we recommend adapting the score sheet and deleting the parameter (s) (Supplemental File 4 features instructions on how to do that).

The score sheet tool provides a total score, a median, mean and maximum score for each assessment. Both these integrative and the individual scores are color coded from green to red on a relative scale for easy visual overview. 7 assessments (e.g., for one week) are designed to fit on an A4 printout for easy collection and recording of past assessments. In addition, a trend tab allows the tracking and visualization of integrative and individual scores across time. To visualize trends, users need to copy-paste values (without formulas) from areas outlined with heavy borders from the score sheet tab to the trends tab. Up to 31 data points (e.g., for one month) can be accommodated within the current dimensions of the trend tab.

Use scenarios

Below, we use three hypothetical common research scenarios, to demonstrate how the score sheet can be used to monitor welfare or refine experimental conditions. These scenarios are 1) a pre-trial to identify useful welfare scoring parameters for a genetically modified zebrafish strain with morphological impairments, 2) a pretrial to define a suitable acclimatization period of wild-caught individuals before an experiment, and 3) the monitoring of fish welfare during an infection trial to identify the appropriate termination point.

In scenario 1 (Figure 2), a strain of genetically modified zebrafish with developmental morphological defects in spine and eyes, and slightly elevated mortality, is to be included in a chemical exposure experiment (similar to e.g. [55]. The researchers are confronted with the task to identify welfare parameters that are suited, in this impaired strain, to detect any additional impairment caused by the experiment. To start with, they score a control tank over a month using the tool for fish that are housed in groups. Scores relating to anatomy (morphology and eye scores) are constitutively high due to the developmental impairment. Swimming behavior is also impacted by the strains' defects, resulting in overall high behavioral scores. Skin parameters and feeding behavior fluctuate slightly during the observation time. Mortality scores

indicate that strain mortality is 10% higher than usually observed in the wildtype. Based on these documented and quantified observations, the researchers come to the following conclusions for their main exposure experiment: they will pay particular attention to skin and feeding parameters, since these parameters display variation already without treatment, and may therefore be sensitive to stressors. Also, they take the 10% mortality rate into account during sample size and power calculations and start the experiment with 10% more animals than required to achieve the desired power. They design an experiment-specific score sheet where they record anatomy and swimming behavior but – given that these parameters are high to begin with, reducing their sensitivity – flag them as potentially unhelpful. They also use the data to justify animal numbers in their animal experimentation permit application.

In scenario 2 (Figure 3), individuals from a wild fish species are caught and brought to the lab for a behavior experiment (choice between two compartments, e.g. [56]) before being released again. The researchers are confronted with the task to determine a suitable acclimation period before the experiment: not too long to avoid unnecessary long captivity, not too short to avoid confounding impacts of the stress during capture on the experiment. They catch several fish for a pre-trial and assess their welfare individually for a month. Figure 3 shows data from one representative individual. During the entire observation period, no signs of external damage, lesion or infection are observed. However, a diverse range behavioral abnormalities (e.g., stereotypical swimming and startle responses to movement and sounds) are observed between d1 and d13. These reactions decline in intensity and disappear in week 3. From d19 onwards, no behavioral abnormalities are observed. Based on the pretrial, the researchers therefore conclude (and have data to show) that an 18 day acclimation period is both required and sufficient for the planned behavioral experiments, and choose their setup accordingly to ensure the reliability and validity of their data.

In scenario 3 (Figure 4), researchers want to perform a drug screen on zebrafish infected with a certain pathogen (similar to e.g. [57]) to find compounds that are active against said pathogen. The researchers are confronted with the task of determining an experimental duration that is long enough to show differences between control and treatment, and short enough to avoid unnecessary suffering. They also need to determine a termination score (i.e. a cutoff where a sick animal is euthanized and removed from the experiment to avoid excessive suffering). A group of 10 fish is therefore used for a 4-week pretrial under control conditions (pathogen infection without drug) and scored with the group tool. The score sheet reveals that first skin defects are observed 14 days after pathogen exposure, and continuously increase in severity. On day 23 after pathogen exposure, swimming issues (loss of buoyancy) are starting to be observed and increase in prevalence until the end of the pretrial. Based on this pretrial, the researchers determine to run the drug screen for 25 days, since meaningful differences between control and treatment should be observable by this timepoint without causing unnecessary suffering of experimental animals. They also decide that they will terminate any tank x compound combination where the total score exceeds 11 two days in a row, since this score should not be reached in the control within 25 days and would indicate that the compound causes harm.

Use and adaptation of the score sheet

Scoring 21 parameters on multiple fish every day is clearly not a feasible task. We want to emphasize that a key step in the use of this (and any other) score sheet is the adaptation phase. This phase includes a) test scoring of the parameters, followed by b) a choice of parameters that are truly useful to assess welfare in the given species, situation, and experiment, and c) creating an experiment-specific score sheet by deleting non-informative rows from the score sheet, adding parameters, or adapting the weighting.

We strongly encourage a rigorous and information-driven reduction of the score sheet to achieve maximum information and maximum compliance with minimal effort. The score sheet

file contains instructions to help with the adaptation and implementation phase (Supplemental File 4, tab "instructions"). The adaptation phase encompasses 7 steps: “Adapt to setting”, "Get familiar", "Generate baseline", “Adapt criteria and weights", "Termination criteria". We hope to discourage uncritical use of the score sheet with these instructions, since this would most likely lead to frustration and non-compliance. In addition, the score sheet file contains instructions for use after adaptation. The use phase is based on three steps: "Data entry", "Documentation", "Statistics". The aim is to encourage sustainable long-term preservation strategies and support good data management practices, e.g. for reporting to ethics committees.

Discussion & Conclusion

This publication aims to provide a flexible framework to develop suited score sheets across a number of species, experimental scenarios, and housing conditions. This is by no means the first attempt to develop score sheets for experimental fish (see, for example, a zebrafish-specific pain and distress score sheet in [58] Table 3). However, to our knowledge, it is the first attempt to create an adaptable tool that is explicitly not supposed to be used as is, but rather to be molded by the researcher to really fit their needs regarding welfare monitoring.

Applicability

The diversity of fish species kept for research purposes, and their housing both as single fish and large groups is a challenge for welfare assessment. The presented work tackles this in two ways. First, the developed score sheet remains adaptable. Parameters can be removed (which is actually recommended), changed, or added (e.g., for highly specific behaviours, such as mouth brooding or nest building). Second, the score sheet emphasizes the importance of relative assessments. It does not provide absolute numerical cutoffs a priori but empowers users to develop their own meaningful termination criteria based on the respective species, strain, facility, and housing conditions.

The operationalization of termination criteria (endpoints where an experiment must be terminated and the animals are euthanized to prevent further suffering) is one of the most important aspects and purposes of score sheets. How to arrive at a combined measure of maximally tolerable burden is one of the most important, but also most difficult and controversial parts of welfare assessments. Importantly, termination criteria depend heavily on the weighing of interests and the purpose of the experiment (which extent of suffering is justifiable for the expected gain of knowledge). This score sheet should facilitate the definition of termination criteria and facilitate conversations on termination criteria among fish researchers.

Implications

This score sheet should benefitfish and fish researchers in five distinct ways. Firstly, the tool aims to empower the individual researcher. Our interviews revealed a prevalence of subjective assessments which may or may not reflect animal welfare criteria, a certain acceptance of stereotypies as given, and a dependence on few knowledgeable care takers. All these aspects could benefit from more objective scoring options. Secondly, the tool potentially fosters within-group interactions and cross-facility communication regarding welfare and termination criteria. This may improve collective welfare knowledge in the fish research community and inspire initiatives and research towards better welfare assessment in fish. For example, our interviews revealed that standard laboratory conditions are generally assumed to guarantee good welfare. One way to assess this would be to conduct a project using the tool across facilities and housing conditions. Thirdly, the tool may facilitate regulatory processes surrounding animal experimentation. In Switzerland, score sheets are required to obtain animal experimentation permits. This tool may thus support researchers in defining and submitting evidence-based monitoring and stop-criteria. Fourthly, ethics committees are usually not familiar with welfare of fish. This tool should facilitate the work of ethics committees to support well-planned and well-monitored experimentation with fish, and the communication between researchers and

committees. A fifth aspect relates to teaching. Score sheets are valuable tools to discuss fish needs, health, and welfare, and can be used to train junior researchers in accurate and attentive evaluation of signs and behaviours. For example, a course could entail the application of a score sheet to strains with known physical and behavioral impairments, or to a range of species.

Limitations

From the practical perspective, welfare monitoring can pose a challenge regarding the time requirements and personnel resources of research groups . We strongly recommend prioritizing the monitoring of few meaningful parameters at longer time intervals over more complex monitoring plans which fail in the long-term. A key factor in welfare monitoring is compliance and constancy, and these need to be given priority over perfection due to overburdening the researchers or animal care takers. From the conceptual perspective, discussions on animal welfare are often impacted by debates on fish nociception. We support the point of view that the presence of protective responses and avoidance learning may be sufficient indicators of pain, health, and welfare in fish, but agree that scoring of pain remains a challenge.

Recommendations

In summary, this manuscript provides an objective, adaptable welfare score sheet tool to the wider fish research communityallowing monitoring of fish welfare in a semi-objective manner Prospectively, it would be valuable to engage in cross-species and cross-field efforts to build on the presented monitoring tool. Also the development of an adaptable welfare-monitoring smart-phone app would be extremely practical for the daily monitoring. Also, visual tracking applications [59] and sensors have great potential to facilitate fish welfare monitoring in the future.

Figures

Table 1. Parameters. Welfare parameters, explanations of these parameters, and signs to look for. Includes scores in numbers and in verbose expressions. This table is translated into an interactive scoring tool in Supplemental File 4.

Figure 1. Score sheets. A – Score sheet for individual animals. **B –** Score sheet for groups of animals. These are screenshots taken from the interactive scoring tool provided as Supplemental File 4.

Figure 2. Application Scenario 1. Example for a group scoring of an impaired mutant line of zebrafish. View of the trends tab. The trends tab translates the scores into visual patterns, on the one hand through score-dependent coloring of the cells, on the other hand through trend lines. Each value in the top table corresponds to a datapoint in the trend lines (some pointed out by circles and arrows). The summary scores, external signs, and group/behavioural signs are each collated in a separate trend line figure.

Figure 3. Application Scenario 2. Example for an individual scoring of a wild-caught fish during the acclimatization period. View of the trends tab. Both the score-dependent coloring on the top and the trend lines at the bottom suggest that the fish is stressed until day 13 of the acclimatization. No behavioural signs of stress are observed after day 19.

Figure 4. Application Scenario 3. Example for an individual scoring of an experimental fish exposed to a compound. External signs (i.e. skin defects) are observed before the appearance of behavioural signs and get progressively worse over the course of several weeks. Based on these data, the end point of the planned experiment is determined after onset of signs, but before welfare is too compromised.

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A - Score sheet for individual animals

Animal Exp. Permit

ID of Tank / Individual

Termination criteria:														
	Date	20230701		yyyymmdd		yyyymmdd		yyyymmdd		yyyymmdd		yyyymmdd		yyyymmdd
	Sored by	Jenni D.												
	Total score		24.0		21.0		21.0		21.0		21.0		21.0	
	Total median		1.0		1.0		1.0		1.0		1.0		1.0	
	Total mean		1.1		1.0		1.0		1.0		1.0		1.0	
	Total max		3.0		1.0		1.0		1.0		1.0		1.0	

External signs			18		13		13		13		13		13		13
Body condition	nutrition state	severe weight loss	3	na	1	na	1	na	1	na	1	na	1	na	1
	swelling, proliferative lesion	normal rounded abdomen	0	na	1	na	1	na	1	na	1	na	1	na	1
	spine	slight scoliosis	1	na	1	na	1	na	1	na	1	na	1	na	1
	egg-bound (female)	-bound, egg-bound for longer per	3	na	1	na	1	na	1	na	1	na	1	na	1
Skin	other deformities	no other deformation	0	na	1	na	1	na	1	na	1	na	1	na	1
	scales	protruding scales	3	na	1	na	1	na	1	na	1	na	1	na	1
	skin lesions	small superficial lesions	1	na	1	na	1	na	1	na	1	na	1	na	1
	skin color	color normal for the strain	0	na	1	na	1	na	1	na	1	na	1	na	1
Fins	fin state	moderate tissue necrosis	2	na	1	na	1	na	1	na	1	na	1	na	1
Eyes	eye state	two sided damage	3	na	1	na	1	na	1	na	1	na	1	na	1
Gills/Opercula	gill state	normal operculum and gills	0	na	1	na	1	na	1	na	1	na	1	na	1
	ventilation	normal beat rate for the species	0	na	1	na	1	na	1	na	1	na	1	na	1
Infection	skin health / infection signs	few small infected areas	2	na	1	na	1	na	1	na	1	na	1	na	1

Comments

Behavioural signs			6		8		8		8		8		8		8
Swimming	activity level	normal activity level for species	0	na	1	na	1	na	1	na	1	na	1	na	1
	breathing	normal breathing	0	na	1	na	1	na	1	na	1	na	1	na	1
	fin posture	fins are clamped	2	na	1	na	1	na	1	na	1	na	1	na	1
	navigation	poorer loss of balance and orientat	2	na	1	na	1	na	1	na	1	na	1	na	1
	position in tank	ccupies tank as typical for specie	0	na	1	na	1	na	1	na	1	na	1	na	1
	scratching	no scratching, no jumping	0	na	1	na	1	na	1	na	1	na	1	na	1
Feeding	stereotypies	observation of stereotypies	2	na	1	na	1	na	1	na	1	na	1	na	1
	feeding	reacts normally to food	0	na	1	na	1	na	1	na	1	na	1	na	1

Comments

B - Score sheet for groups

Permit

Tank or group ID

Termination criteria:									
	date	20230701	yyyymmdd	yyyymmdd	yyyymmdd	yyyymmdd	yyyymmdd	yyyymmdd	yyyymmdd
	initials	Jenni D.							
	Total score	5.8	###	###	###	###	###	###	###
	Total median	0.2	###	###	###	###	###	###	###
	Total mean	0.7	###	###	###	###	###	###	###
	Maximum score	3.0	###	###	###	###	###	###	###

0 no abnormalities/lesions
1 slight abnormalities/lesions
2 moderate abnormalities/lesions
3 severe abnormalities/lesions

External signs		0	1	2	3	5.5	0	1	2	3	####	0	1	2	3	####	0	1	2	3	####	0	1	2	3	####	0	1	2	3	####	0	1	2	3	####	0	1	2	3	####
Morphology	overall body morphology	50	0	0	50	1.5	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###
Skin	scales, skin integrity	0	0	0	100	3.0	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###
Fins	fin lesions	50	0	50	0	1.0	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###
Eyes	eye lesions	100	0	0	0	0.0	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###
Gills/Opercula	gill appearance	100	0	0	0	0.0	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###
Infection	infection or parasites	100	0	0	0	0.0	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###

Comments

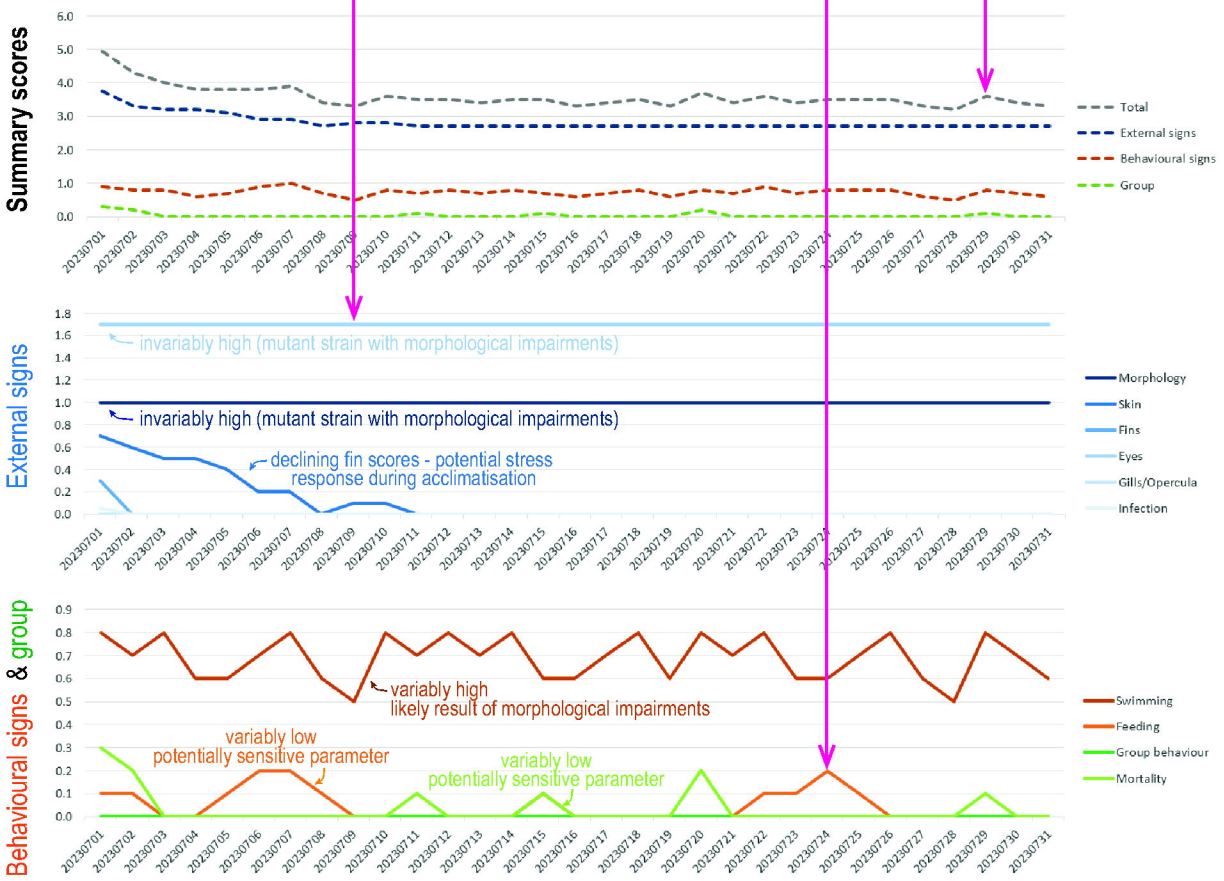
Behavioural signs		0	1	2	3	0.3	0	1	2	3	####	0	1	2	3	####	0	1	2	3	####	0	1	2	3	####	0	1	2	3	####	0	1	2	3	####	0	1	2	3	####
Swimming	swimming	90	0	0	10	0.3	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###
Feeding	position in tank	100	0	0	0	0.0	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###

Comments

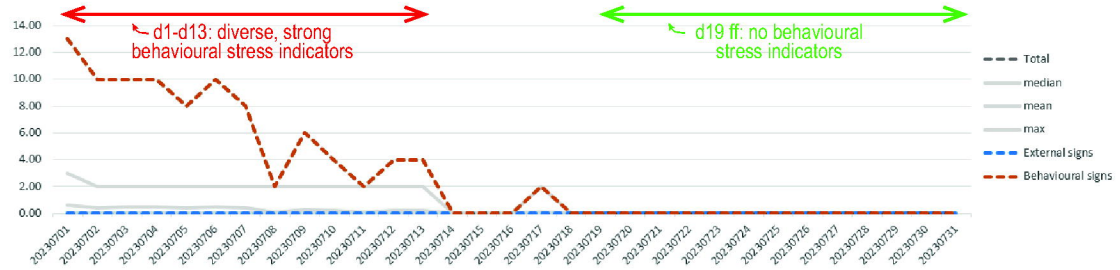
Group		0	1	2	3	0.1	0	1	2	3	####	0	1	2	3	####	0	1	2	3	####	0	1	2	3	####	0	1	2	3	####	0	1	2	3	####	0	1	2	3	####
Group behaviour	interaction patterns (aggression, ...)	90	10	0	0	0.1	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###	0	0	0	0	###
Mortality	% survivals (0), % deaths (3)	100			0	0.0	0			0	###	0			0	###	0			0	###	0			0	###	0			0	###	0			0	###	0			0	###

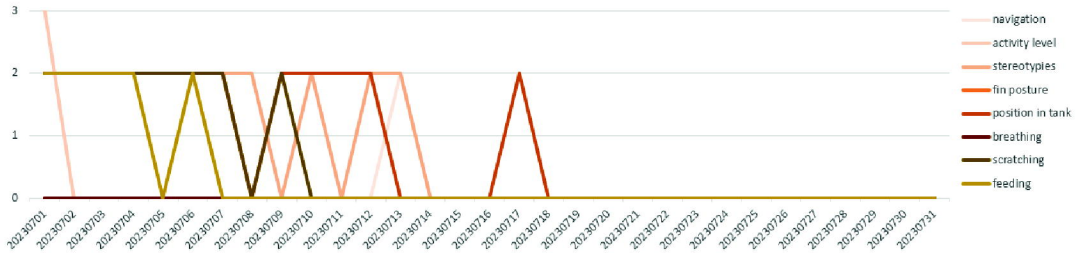
Comments

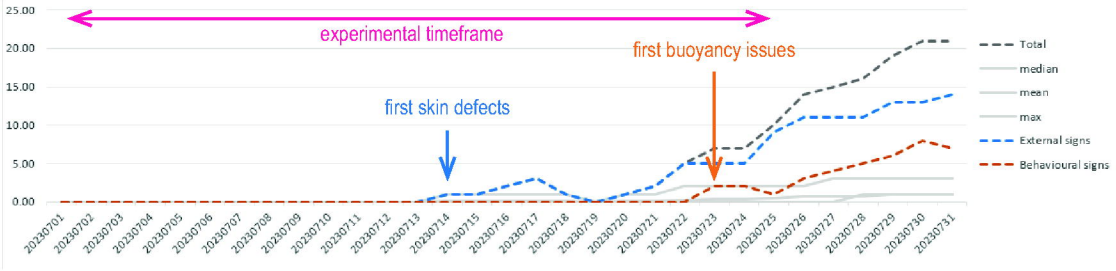
	20230701	20230702	20230703	20230704	20230705	20230706	20230707	20230708	20230709	20230710	20230711	20230712	20230713	20230714	20230715	20230716	20230717	20230718	20230719	20230720	20230721	20230722	20230723	20230724	20230725	20230726	20230727	20230728	20230729	20230730	20230731
Total	5.0	4.3	4.0	3.8	3.8	3.8	3.9	3.4	3.3	3.6	3.5	3.5	3.4	3.5	3.5	3.3	3.4	3.5	3.3	3.7	3.4	3.6	3.4	3.5	3.5	3.5	3.3	3.2	3.6	3.4	3.3
median	0.1	0.2	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mean	0.2	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.4	0.4	0.4	0.3	0.4	0.4	0.3	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.4	0.4	0.4	0.3	0.3	0.4	0.3	0.3
max	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
External signs	3.8	3.3	3.2	3.2	3.1	2.9	2.9	2.7	2.8	2.8	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
Morphology	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Skin	0.7	0.6	0.5	0.5	0.4	0.2	0.2	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fins	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Eyes	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Gills/Opercula	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Infection	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Behavioural signs	0.9	0.8	0.8	0.6	0.7	0.9	1.0	0.7	0.5	0.8	0.7	0.8	0.7	0.8	0.7	0.6	0.7	0.8	0.6	0.8	0.7	0.9	0.7	0.8	0.8	0.8	0.6	0.5	0.8	0.7	0.6
Swimming	0.8	0.7	0.8	0.6	0.6	0.7	0.8	0.6	0.5	0.8	0.7	0.8	0.7	0.8	0.6	0.6	0.7	0.8	0.6	0.8	0.7	0.8	0.6	0.6	0.7	0.8	0.6	0.5	0.8	0.7	0.6
Feeding	0.1	0.1	0.0	0.0	0.1	0.2	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Group	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Group behaviour	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mortality	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0



	20230701	20230702	20230703	20230704	20230705	20230706	20230707	20230708	20230709	20230710	20230711	20230712	20230713	20230714	20230715	20230716	20230717	20230718	20230719	20230720	20230721	20230722	20230723	20230724	20230725	20230726	20230727	20230728	20230729	20230730	20230731
Total	13.00	10.00	10.00	10.00	8.00	10.00	8.00	2.00	6.00	4.00	2.00	4.00	4.00	0.00	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
median	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
mean	0.62	0.38	0.48	0.48	0.38	0.48	0.38	0.10	0.29	0.19	0.10	0.19	0.19	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
max	3.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	0.00	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
External signs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
nutrition state	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
spine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
swelling, proliferative lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
egg-bound (female)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
other deformities	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
skin lesions	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
skin color	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
scales	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fin state	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
eye state	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
gill state	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ventilation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
skin health / infection signs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Behavioural signs	13	10	10	10	8	10	8	2	6	4	2	4	4	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
navigation	2	2	2	2	2	2	2	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
activity level	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
stereotypies	2	2	2	2	2	2	2	2	0	2	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fin posture	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
position in tank	2	2	2	2	2	2	2	0	2	2	2	2	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
breathing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
scratching	2	2	2	2	2	2	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
feeding	2	2	2	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0





termination criterion

	20230701	20230702	20230703	20230704	20230705	20230706	20230707	20230708	20230709	20230710	20230711	20230712	20230713	20230714	20230715	20230716	20230717	20230718	20230719	20230720	20230721	20230722	20230723	20230724	20230725	20230726	20230727	20230728	20230729	20230730	20230731
Total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	2.00	3.00	1.00	0.00	1.00	2.00	5.00	7.00	7.00	10.00	14.00	15.00	16.00	19.00	21.00	21.00
median	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00
mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.05	0.10	0.14	0.05	0.00	0.05	0.10	0.24	0.33	0.33	0.48	0.67	0.71	0.76	0.90	1.00	1.00
max	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	2.00	2.00	2.00	2.00	2.00	3.00	3.00	3.00	3.00	3.00
External signs	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	3	1	0	1	2	5	5	5	9	11	11	11	13	13	14
nutrition state	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
spine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
swelling, proliferative lesion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
egg-bound (female)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
other deformities	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
skin lesions	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	1	1	2	2	2	2	2	3	3	3	3	3
skin color	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
scales	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	0	1	2	1	1	2	2	2
fin state	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2
eye state	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
gill state	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ventilation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	2	2	2	3
skin health / infection signs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	2	2	2	2	2	3	3	3
Behavioural signs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	1	3	4	5	6	8	7
navigation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	1	1
activity level	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
stereotypies	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fin posture	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	2	2	1
position in tank	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
breathing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	2	2
scratching	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
feeding	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1