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

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Abstract

Fish are increasingly used as experimental animals in a wide range of research fields. As of 2022, a quarter of all experimental animals used in Europe are fish, with an upward trend. Less than 20% of these are zebrafish. At the same time, 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 assessment guidelines and easy-to-use tools is a challenge for researchers (particularly, for newcomers to the field of fish), for ethics committees, and prevents the rigorous implementation of the 3Rs (particularly, refinement).

Here, we present an adaptable, user-friendly scoring tool for fish. The parameters contained in the tool are based on a literature review, have been validated by expert interviews, and weighted by a fish pathologist. The tool allows to score individuals as well as groups and provides a visualization of trends. We provide the associated literature and give detailed examples and instructions on the adaptation and use of the scoring tool.

We hope that this tool to score fish welfare will empower researchers to include welfare assessment in their routines, foster discussions on fish welfare parameters among scientists, facilitate interactions with ethics commites, and most importantly, enable continous 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, 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

Supplemental File 3: Collection of fish welfare parameters

Supplemental File 4: Scoring tool

Introduction

The phylogenetic diversity of the fish family (~34'000 species (1)) is mirrored by a wide range of research areas that use fish as models. Experiments on fish range from toxicology to developmental biology, and from modelling complex human diseases (2) to behavioural experiments (3). External fertilization and the transparency of many fish eggs facilitate developmental research approaches (3, 2), and fish are often used to probe the response of aquatic organisms to the impact of global change, ocean warming and ocean acidification (4). Teleost fish share key genetic, anatomical and physiological properties with humans (5, 2) and therefore are valuable organisms to investigate basic evolutionary concepts (6–10), stem cells and regeneration (11), the impact of chemicals and pollutants on organismic health (12), or pharmacology (3).

In 2020, a quarter (27.4%) of all animals used for research in the EU were fish, and their relevance has been increasing (2015: 13.1%; (13)). The relevance of zebrafish (*Danio rerio*) as the most widely known experimental fish model has however been declining in recent years. Currently, they constitute no more than 13% of all experimental fish (13). Numerous other fish species are used in research (2, 14), such as medaka (*Oryzias latipes)* (15), fat head minnow (*Pimephales promelas*) (16), trout (*Salmonidae*) (17), cichlids (*Cichlidae*) (18), sticklebacks (*Gasterosteidae*) (19), guppies (*Poecilia reticulata*) (20), mollies (*Poecilia latipinna*) (21), or gobies (*Gobidae*) (22), to just name a few. Evolutionarily, some of these species are more distantly related than humans and armadillos (23).

In accordance with widespread legal requirements to implement the 3Rs (Replace, Reduce, Refine (24)) in animal experiments, the awareness and demand to objectively assess welfare in animal experiments continues to increase. 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. The 3R concept serves a foundation for laws and inspires recommendations (25). For example, each member state of the EU has ratified legislation regarding the use of animals in research, and the EU legislation itself features several directives governing authorisations, monitoring, husbandry, and practices in animal experimentation.

The implementation of measures towards the third R, refinement, is very much dependent on the monitoring and documentation of animal welfare during husbandry and experimentation. Accordingly, monitorings of animal welfare are legally required in many countries. Legal definitions of animal welfare are often derived from the concept of the five freedoms (Freedom from hunger and thirst, from discomfort, from pain, injury and disease, from fear and distress, and freedom to express normal behaviour; (26). Swiss legislation, for example, defines animal welfare through dignity and well-being. Animal dignity is addressed by refraining from inflicting non-justifiable pathocentric (i.e. those perceived as strains by the animals such as pain, suffering or harm) and non-pathocentric strains (i.e. those perceived as ethically unacceptable, such as humiliation, major interference with the animals' appearance or abilities, and excessive instrumentalization), while well-being is understood as absence of disturbances in bodily functions and behaviours, clinical health, and the avoidance of pain and distress (27). Whatever definition is used, 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. A challenge in the use and implementation of score sheets is the lack of a universal agreement on the nature of animal welfare (28). EU regulations, for example, contain no notion of welfare (29). The

lack of definitions is particularly striking for fish, which are treated as sentient by law (29) while other sources express a need for debate on the matter (30, 25, 31). Despite key initiatives (32–36, 28, 31), a dearth of high-quality approaches towards fish welfare (28), and an according lack of directives regarding experimental fish in national legislations, pose a challenge for refinement in fish research. Knowledge on fish's emotions and behaviour changes due to stress is scarce (5, 37, 38, 28), and their subjective pain perception is sometimes discussed controversially (39, 31). In summary, hardly any validated clinical and behavioural indicators of wellbeing are currently available for fish.

The establishment of scoresheets for fish is challenging because of the previously mentioned diversity of fish species, with their distinct behaviours, morphology, physiology, and environmental requirements (5). Also, characteristics well established for rodents are absent, such as vocalisations, body language, and spontaneous as well as induced behaviours such as grooming (40, 41, 34). Fish do not produce human-perceptible sounds, and do not display overt and familiar social bonding behaviours like licking that can be used for e.g. pigs (41). While decipherable behaviours have been identified in some fishes, many remain understudied or uninterpretable (38). Also, fish are often housed in considerably large groups, which complicates individual welfare monitoring, can result in welfare problems (e.g., hierarchy or territory issues (28)), but also expands the set of observable and measurable behavioural indicators.

Opportunities for knowledge exchange and training on fish welfare scoring are presently limited. Fish research publications typically do not include score sheets (28, 5), which limits open conversation about useful and implementable quantitative or qualitative parameters for welfare evaluations. Also, good welfare practices depend on skilled staff (5, 38), but training opportunities for fish researchers, or for authorities and ethics committees deciding on fish experimental permits,

are often scarce or lumped with other “non-rodent species”. A potential ally in fish welfare is the field of aquaculture, where fish welfare expressed by disease incidence, growth rates and meat quality translates to business revenue (e.g., MyFishCheck (42)), but interactions between the fields are usually limited since they focus on distinct species. Together, the lack of expertise and frameworks with regard to welfare scoring poses a serious challenge for researchers in the implementation of refinements, particularly when they are new to fish work.

This publication aims to facilitate refinement for fish researchers through welfare scoring. We aim to provide a versatile, multispecies, modular, literature-based, and user-friendly scoring tool and thereby empower resarchers to assess the welfare of their animals, implement refinement strategies, and fulfil legal welfare monitoring requirements.

To develop this tool, we first systematically listed and weighted 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 interviews with researchers and veterinarians working on different fish species and experimental systems. We then defined possible, easy-to-identify deviations from the desirable state for each parameter, and weighted mild versus severe deviations from the ideal state. Next, we developed a modular scoring table that a) accommodates individuals as well as groups of fish and b) can be adapted easily for the situation. Systematisation, non-intrusiveness, and modularity were main criteria during development.

We are convinced that this scoring tool will simplify welfare monitoring for fish researchers worldwide, facilitate 3R implementation as well as licence application procedures, and serve as useful starting point for discussions within and between labs with regard to fish welfare.

Methods

Scoping review

To gather the published state of the art regarding fish welfare assessments, we conducted a scoping review (43) 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 relevant documents containing welfare parameters that were retained (Supplemental File 2), a list of welfare parameters was compiled. In total, the literature search yielded 63 potential parameters (not shown).

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 scoresheet 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. The responses are not shared to maintain confidentiality for the participants.

Parameters

The 68 parameters identified in the literature were narrowed down to a meaningful, feasible set of 2based on the expert interviews and an assessment by a diagnostic and research veterinarian and fish pathologist with 20+ years of experience in fish health and fish welfare (co-author HS). 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 (44) and buoyancy (45) 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. The literature pertaining to each parameter and the species for which the parameter was mentioned were also documented (Supplemental File 3).

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. Opinions on welfare were often based on years of experience working with fish, rather than on explicitly scorable, model specific, and objective criteria. While experience is not a bad guideline per se, the criteria derived from experience are inaccessible to a newcomer and entirely subjective. Some facilities stated that certain types of physical/morphological aberrations or behavioral signs were induced by standard lab conditions and were therefore currently not considered in their score sheets as welfare issues.

With regard to a scoring tool, 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 physical 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.

Score sheet

The score sheet developed based on the review and the interviews contains 21 potentially relevant parameters for individuals, and 10 parameters for groups (Supplemental File 4). These 21 parameters are based on literature on 17 distinct fish species from 11 taxonomic families

(Supplemental File 3, second tab). They are classified into the categories morphology, skin, fins, eyes, gills/opercula, infection, swimming behavior, feeding behavior for individuals, and 2 additional categories group behavior and mortality for groups.

For individuals, the status with regard to 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 groups, 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.

In the score sheet for individuals, skipping the assessment of a parameter in the individual score sheet results in a score of 1. We propose to implement this as measure 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 to adapt the scoresheet and delete the parameter (see section on adaptation on how to do that). In the score sheet for groups, an orange color provides a warning if the percentages entered do not sum up to 100.

The tool provides a total, median, mean and maximum score for each assessment. Both these integrative and the individual scores are color coded 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 sheet 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 with heavy borders from the scoresheet tab to the trends tab. 31 data points (e.g., for one month) can be accommodated within the current dimensions of the trend tab.

Use examples

Below, we demonstrate three hypothetical examples of common research scenarios, and how the score sheet could be used to monitor welfare or refine experimental conditions. These include 1) a pre-trial to identify useful welfare scoring parameters for a genetically modified 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 example 1 (Figure 2), a hypothetical strain of genetically modified zebrafish with developmental defects is to be included in a chemical exposure experiment. The researchers are confronted with the task to identify welfare parameters that are suited, in this particular impaired strain, to detect any additional impairment caused by the experiment. They score a control tank of the strain over a month. Scores relating to anatomy (morphology and eye scores) are constitutively high due to the developmental impairment. Swimming behavior is also impacted by the impairment, 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. After the pretrial, the researchers come to the following conclusions: They pay particular attention to skin and feeding parameters, since these parameters display variation already without treatment. Mortality rates are taken into account during sample size and power calculations. Together, the data collected during the pretrial is used to design an experiment-specific score sheet and to justify animal numbers for the final animal experimentation permit application.

In example 2 (Figure 3), a hypothetical wild species is brought to the lab for a behaviour experiment. The researchers are confronted with the task to determine a suited acclimation period before the experiment. They catch a several fish for a pre-trial and assess their welfare for a month. During this time, no signs of external damage, lesion or infection are observed. However, behavioral abnormalities (stereotypical swimming and startle responses to movement and sounds) are observed during the first two weeks. These reactions decline in intensity and disappear in week 3. After the pretrial, the researchers conclude (and have data to show) that a three week acclimation period is required and sufficient to avoid confounding influences on the planned behavioral experiments.

In example 3 (Figure 4), researchers want to assess the immune status of two different zebrafish strains, A and B. The researchers are confronted with the task to determine a suited experimental duration and termination criteria. A small number of fish from each strain is therefore exposed to a 4-week pretrial. In strain A, first skin defects are observed two weeks after infection. Skin lesions continuously increase in severity. Three weeks after infection, swimming issues (loss of buoyancy) are starting to be observed and increase in prevalence until the end of the pretrial. Strain B (not shown) displays minor skin defects throughout the experiment. After the pretrial, the researchers conclude that the large-scale experiment can be terminated after week 3. At this timepoint, meaningful differences can be observed between the strains, while animal suffering can be minimized.

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.

Finally, we would like to point out that the operationalization of termination criteria (endpoints where an experiment has to 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). We hope that this sheet will facilitate the definition of termination criteria. We also hope that the repeated use of (this or similar) score sheets will facilitate conversations on termination criteria among fish researchers.

Discussion & Conclusion

Applicability

The diversity of fish species kept for research purposes, and their housing both as single fish and large swarms 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 phenotypes, 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.

Implications

We hope that this score sheet benefits fish 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 objective scoring options. Secondly, the tool potentially fosters within-group interactions and cross-facility communication regarding welfare and termination criteria. This may improve “schoal-knowledge” 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 needs 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 behaviors. 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 and personnel resources of research groups and can conflict with time requirements of experiments. We strongly recommend prioritizing the monitoring of few meaningful parameters at longer time intervals over intense monitoring plans that 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 must be sufficient to consider aspects of pain, health, and welfare in fish. However, scoring of pain remains challenging in fish. An alternative perspective is to focus on positive welfare concepts (46) for fish. Positive welfare exceeds current efforts to avoid negatives (pain, fear, stress) and additionally focuses on positive emotions, engagement, and quality of life. In fish, which feature highly diverse lifestyles and needs, a first step towards positive welfare is to acknowledge species-specific needs both in practice and regulations.

Recommendations

In summary, this manuscript provides a well-founded, adaptable welfare scoring tool to the wider fish research community. We are convinced that with this or similar tools, monitoring fish welfare in a semi-objective manner is indeed feasible. Our recommendation is to engage in cross-species and cross-field efforts to build on and improve the presented monitoring tool. For example, the design and implementation of an adaptable welfare-monitoring smart-phone app would be extremely valuable for the community. Also, visual tracking applications (47) and sensors have great potential to facilitate fish welfare monitoring in the future.

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Fig. 1

Score Sheet for individuals

Animal Exp. Permit
ID of Tank / Individual

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

External signs			18		13		13		13		13		13		13
Morphology	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)	ound, egg-bound for longer	3	na	1	na	1	na	1	na	1	na	1	na	1
	other deformities	no other deformation	0	na	1	na	1	na	1	na	1	na	1	na	1
Skin	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	ormal beat rate for the specie	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	ormal activity level for specie	0	na	1	na	1	na	1	na	1	na	1	na	
	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	rary loss of balance and orien	2	na	1	na	1	na	1	na	1	na	1	na	1
	position in tank	upies tank as typical for spec	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
	stereotypies	observation of stereotypies	2	na	1	na	1	na	1	na	1	na	1	na	1
Feeding	feeding	reacts normally to food	0	na	1	na	1	na	1	na	1	na	1	na	1
Comments															

Score sheet for groups

Permit
Tank or individual

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	###
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	###
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	###
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	###
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	###
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	###
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	###
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	###
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	###
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	###
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	###
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	###
Mortality	% survivals (0), % deaths (3)	100			0	0.0	0			0	###	0			0	###	0			0	###	0			0	###	0			0	###	0			0	###
Comments																																				

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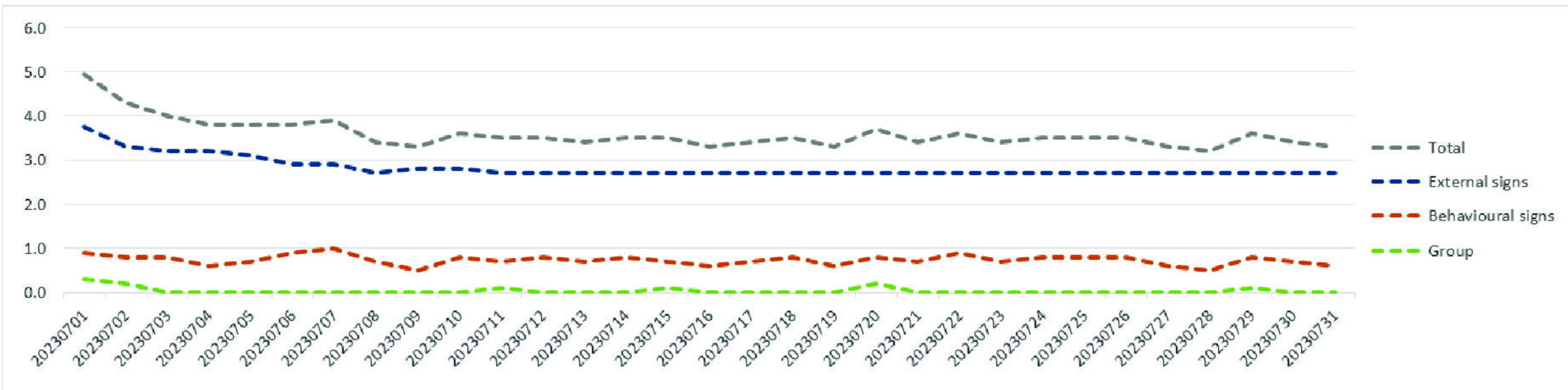
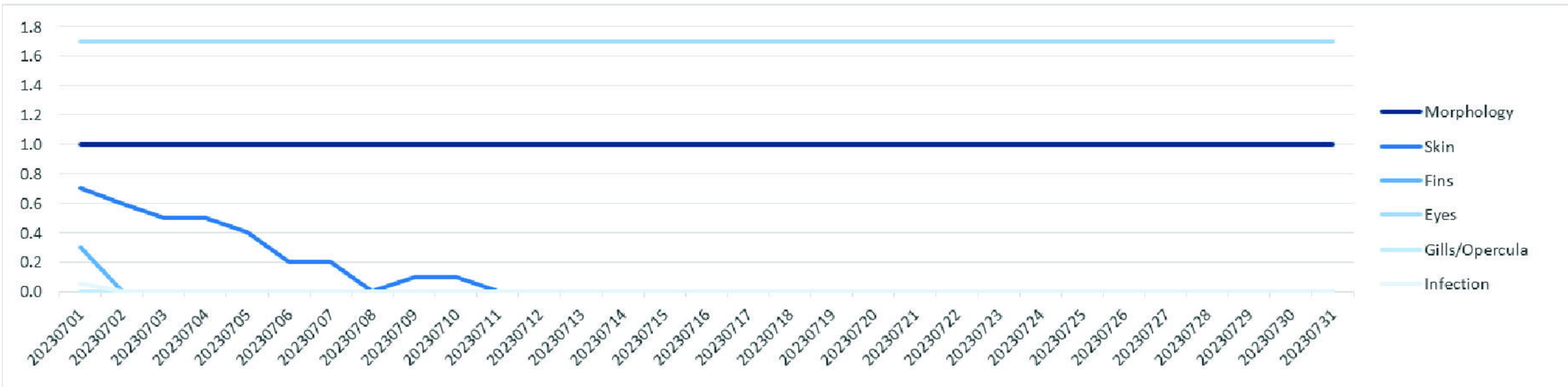
Fig. 2

	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	0.7	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





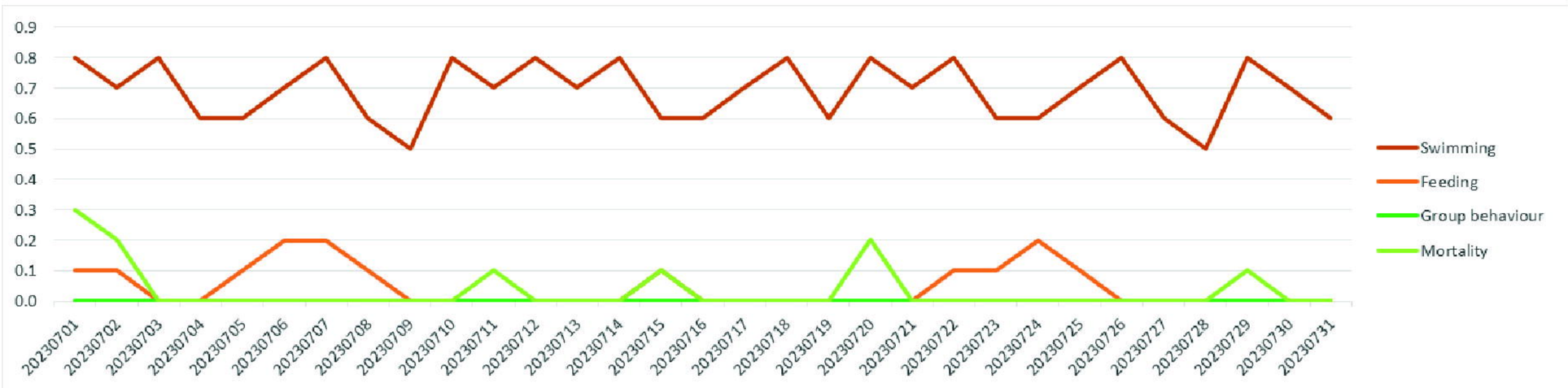


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Fig. 3

	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

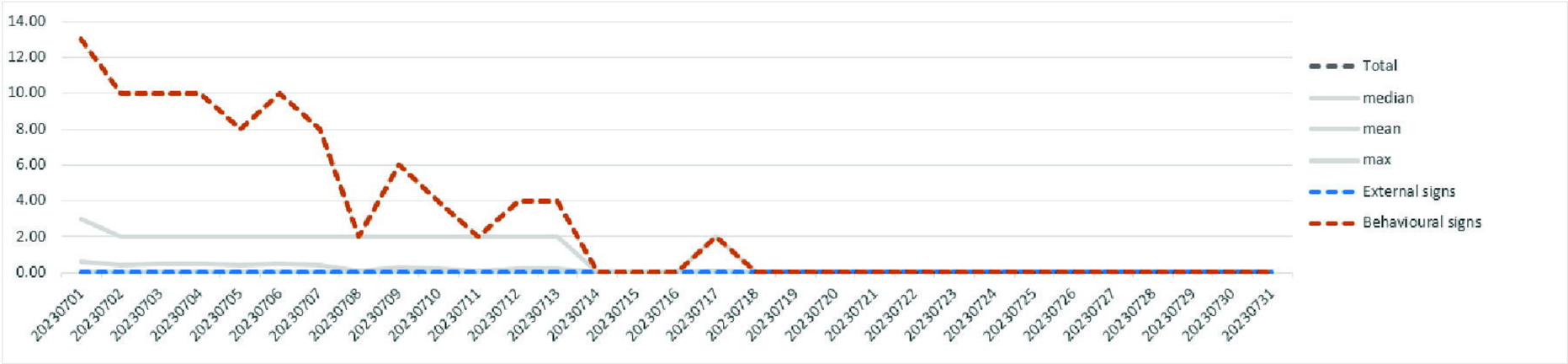

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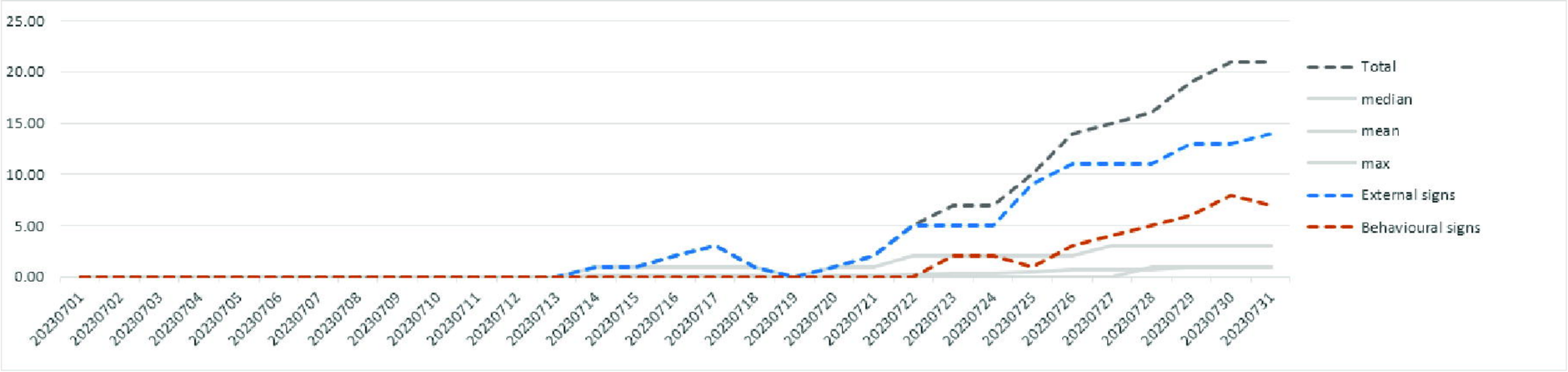
Figure example 3

	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