# How should we monitor welfare in the ornamental fish trade?

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***Abstract:***

The global ornamental fish trade is a multibillion-dollar industry, with legal trade estimated to be worth between $15-20 billion *per annum*. Although there is existing legislation concerning the improvement of fish welfare in aquaculture and research, there is little legislation surrounding the welfare of pet fishes. The different phases of the ornamental fish trade, including curation, transportation, time spent at wholesalers/retailers and time spent in domestic/public aquaria, represent different welfare concerns. Within the animal welfare field there is increasing interest in improving welfare through the creation of operational welfare indicators (OWIs), where individual indicators are aggregated to assess animal welfare. OWIs can be morphological, behavioural, physiological, metabolic or abiotic in nature, with behaviour often considered as the foremost non-invasive method of elucidating welfare in fishes. Currently, while OWIs exist for food fish species, there are no OWIs for use within the ornamental fish trade. This review looks briefly at the stressors experienced by fishes within the ornamental trade, and then used a systematic approach (keywords behavio* AND fish* AND welfare) to identify relevant publications investigating existing behavioural measures of welfare used for ornamental fish species. Finally this review considers the potential development of OWIs for the ornamental trade.

***Keywords:*** fish; welfare; OWI; behaviour; welfare monitoring

## *Introduction*

The global ornamental fish trade is a multibillion-dollar industry, with legal trade estimated to be worth between $15-20 billion *per annum*[1,2]. It is thought that more than 125 countries participate in the trade of live fishes, highlighting the global nature of the industry[3]. The primary exporters of ornamental fishes, both directly caught, and captive bred, are Asia Pacific countries (Philippines, Japan, Burma, Indonesia, Malaysia, Singapore, Sri Lanka), the Netherlands, Germany, Spain, the United States of America (US; Hawaii), Czech Republic and Russia. The primary purchasing countries of ornamental fishes are the US, Japan and in Europe[3]. The ornamental fish trade is dominated by freshwater fish species (~2,000 spp.), accounting for 90% of the market, with the remaining 10% comprising marine species (~2,500 spp.)[1]. Of the 2 billion fishes transported *per annum*, 99% are for hobbyist aquaria, with the remaining 1% destined for public aquaria or research laboratories[3]. Due to the increasing popularity of keeping home aquaria over recent decades, a substantial increase in trade and demand for ornamental fishes has developed, with the trade growing by 14% annually since the 1970s[4,5].

Ornamental fishes are exposed to stressors and environments which may negatively affect their welfare[6] such as poor water quality, inappropriate stocking densities, diseases and injury[7], with issues comparable to those experienced in food fish aquaculture[7,8]. Fishes within the ornamental trade can experience long transport times (up to 72 h in some instances) and a large number of individual stages in their supply chain (Fig. 1). The ornamental fish supply chain comprises many different phases including curation, transportation, time spent at wholesalers/retailers and time spent in domestic/public aquaria, with each phase having very different welfare concerns (Fig. 1)[9]. A typical supply chain starts in the origin country *via* capture of wild fishes or breeding in aquaculture[1,10]. Fishes may then be held in a holding facility either with the exporter or a middleman broker prior to transportation, usually by air, to their destination country. Depending on the country, fishes may be subject to border inspection (e.g. within the European Union (EU)) and be collected either directly by a retailer or *via* a wholesaler. At the retailer, fishes will normally reside in display tanks prior to being purchased and transported to their final destination. Due to the length of the acquisition process of ornamental fishes there is a cumulative potential for decreased welfare and accountability[11]. Although there is existing European legislation concerning fish welfare in aquaculture and research (EU, 1998 and 2010, respectively), there is little legislation surrounding the welfare of pet fishes[7,12] and many countries do not have any legislation at all. Membership of organisations who aim to improve welfare within the trade e.g. Ornamental Fish International (OFI) and the Ornamental Aquatic Trade Association (OATA) is available for retailers but is voluntary, leaving the global trade with no regulatory body that monitors welfare[7,12].

One of the primary problems facing the regulation of the ornamental trade is the difficulty in monitoring ornamental fish welfare. Farmed food-fish tend to be larger in size than their ornamental counterparts making it easier to identify any welfare issues. Technological advances (see *Can we use current aquaculture technologies in an ornamental-setting***?**) within food-fish aquaculture allow monitoring of different welfare aspects including changes in condition and subtle, complex behaviours associated with poor welfare[7]. Within food-fish aquaculture, there has been an emergence of operational welfare indicators (OWIs), which are behavioural and physical indicators that provide an overall welfare assessment[13-15]. Current published OWIs are either commercial-species-specific, for example Precision Fish Farming (salmonids)[16], laboratory-species-specific, for example the Fish Behaviour Index (FBI) or generalised across species primarily used in aquaculture, such as The FishEthoBase [15]. To date, there are no OWIs published or used commercially regarding the welfare of ornamental fishes. The diversity of ornamental fish species traded, their relatively small physical size, and the international nature of the supply chain make technology used in food-fish welfare monitoring, and many of the food-fish OWIs, unsuitable for ornamental fishes. Within the ornamental trade, fishes can be more easily inspected visually (e.g. for behaviour, colouration, injuries) as they are generally housed in glass tanks making behaviour and non-invasive observations more likely candidates for ornamental fish OWIs. Therefore, following a brief overview of fish welfare and the stressors encountered by fishes in the ornamental trade, the aim of this review is to consider the potential for developing OWIs for the ornamental trade, including a review of existing behavioural measures of welfare in ornamental fishes. Identified behavioural measures are then combined with other non-invasive potential indicators that may be beneficial when developing an ornamental fish OWI monitoring tool.

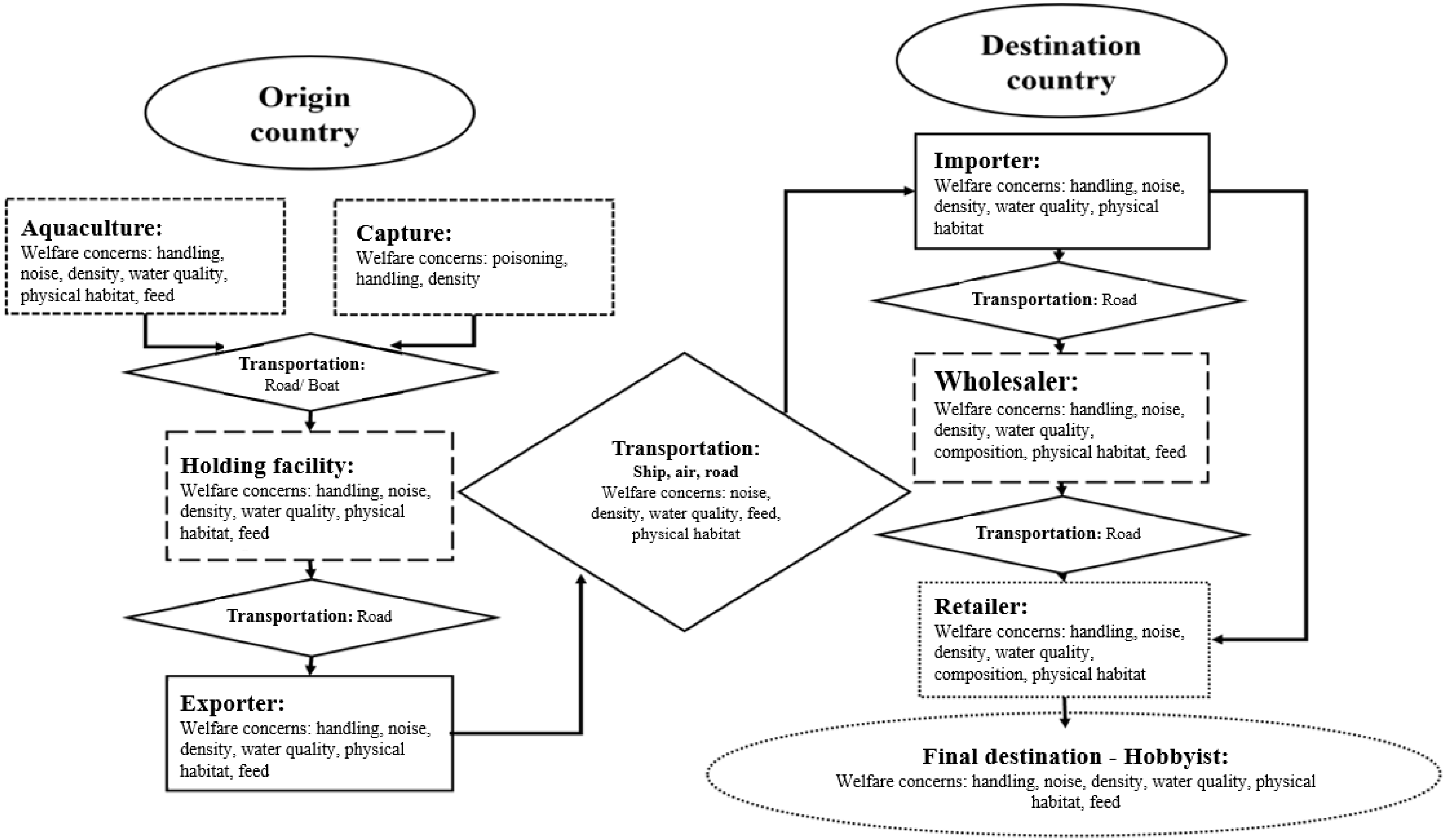


Figure 1. Transportation process for ornamental fishes with a summary of welfare concerns at each stage. Rectangular boxes, with small, dashed borders indicate when fishes are curated either from wild sources or from ornamental fish breeders. Rectangular and diamond shaped boxes with continuous borders and arrows indicate when fishes are confined in plastic bags with individuals of the same species. Rectangular boxes with large, dashed borders indicate when fishes are confined in tanks of the same species. Rectangular and oval boxes with fine dotted borders indicates when fishes are in tanks potentially with mixed species.

### *Fish welfare and the ornamental trade*

Defining and assessing fish welfare is challenging as definitions can vary considerably. For fishes, welfare has been particularly difficult to define from a scientific perspective as whether fish experience pain has been an area of controversy within the literature[6,17-20]. There is now a large body of evidence demonstrating that fish are self-aware, can become distressed, experience pain through nociceptors and to some extent experience emotions[21,22], however their sentience is not accepted by all[6,23]. Fish welfare is increasingly acknowledged as a societal issue[24-26] and over the past decade, fish welfare issues have raised scientific, political and public concern worldwide[27-29].

In terms of measurement, animal welfare is most often framed within the 'Five Freedoms'[30], and more recently the 'Five Domains'[31], both of which have been considered in the context of fishes[8,31]. The integration of behavioural, physiological and cognitive measures for determining fish welfare, particularly in relation to food-fish, has been reviewed extensively[7,15,32]. The FishEthoBase (www.fishethobase.net) suggests that 'fish welfare is guaranteed if a fish can live up to the potential of the species and develop its individuality'. When considering the welfare of fish within the ornamental trade, the stressors they encounter need to be understood[33-35], along with interaction between these multifactorial stressors[29]. The stressors experienced by fishes in the ornamental trade have been reviewed extensively[1,7,26,34-37], thus for context only a brief summary of the main stressors is given here.

*Mechanical disturbance:* In the ornamental trade, the transport of live fishes coupled with pre- and post-handling procedures can represent a significant stress for the fish[7,11,38]. Mortalities relating to mechanical disturbance within the ornamental fish trade are primarily based on estimates as opposed to empirical studies and differ drastically in number[7,37]. Ornamental fishes are usually transported in polythene bags filled with water and oxygen (air or pure oxygen) in a 1:4 ratio, respectively[25]. Air pockets within bags increase the risk of mechanical disturbance which can lead to immunosuppression [11] and an increased incidence of disease[11,39]. Through the supply chain (Figure 1), multiple stages of transport can compound the stressors experienced.

*Inappropriate stocking densities:* Appropriate stocking densities vary between species[40,41]. For some species, high stocking densities, either during transport or holding can lead to reduced water quality from increased nitrogenous waste accumulation, oxygen consumption and suspended organic solids, as well as increased aggression[42,43]. Whereas in other species, low stocking densities can lead to increased territoriality and an increase in aggressive behaviours from dominant fish repeatedly targeting subordinate individuals, leading to negative effects on physiology[44-46]. Individuals of highly territorial species should not be housed together due to the increased potential for aggression and injury[47] and species that are gregarious should be housed in groups[48]. Within all stages of the ornamental trade supply chain, fishes can be exposed to variable stocking densities, with stocking density changing, sometimes abruptly, between different stages. Physiological effects of inappropriate stocking density have been documented in ornamental fishes. For example, stocking densities have been suggested as a contributing factor in cortisol-mediated masculinisation in larval and juvenile zebrafish (*D. rerio*), with a larger proportion of males found in tanks with higher stocking densities[46,49]. Furthermore, zebrafish (*Danio rerio*) housed at a higher density (40 fish/l) had significantly higher whole-body cortisol concentrations than those housed at a lower density (0.2 fish/l)[50]. Fishes may additionally be placed in mixed-species assemblages which can affect behaviour and welfare[51] and understanding the behavioural compatibility of different ornamental fish species is essential[22,52].

*Poor water quality*: Confinement of fishes in an enclosed volume of water, particularly during transport, but also in holding conditions, can result in deterioration of water quality (in particular, elevated ammonia ($NH_3$) and carbon dioxide ($CO_2$), and reduction in dissolved ($O_2$) which can lead to increased stress and negative effects on welfare[53]. The main water quality parameters that need to be controlled are ammonia ($NH_3$), ammonium ($NH_4^+$), nitrite ($NO_2^-$), nitrate ($NO_3^-$), dissolved oxygen (DO), carbon dioxide ($CO_2$) and chlorine (Cl), and salinity if the species are marine[54]. Poor water quality can negatively impact fish growth through reduced feed consumption and/or feed conversion ratios[55,56], suggesting that water quality may affect energy partitioning and subsequent growth[57]. The capacity to transport ornamental fishes without negatively impacting their welfare requires in-depth knowledge of specific species in terms of stress tolerance, metabolism and water quality requirements[58]. Although there has been significant effort to control overall water quality within the ornamental supply chain, changes in water quality between each stage can expose fishes to significant stress and are often overlooked when assessing welfare. Prior to transport, water is often super-saturated with pure oxygen to compensate for oxygen deficiency and deterioration in combination with an increased ammonia build-up[59]. Water conditioners and additives have been created to mitigate any problems that may arise from poor water quality (see review [37]). For example, some wholesalers add zeolite, a microporous adsorbent mineral, to maintain water quality.

*Inappropriate water temperature*: Within the supply chain, ornamental fishes may be exposed to temperatures out-with their thermal tolerance range, with suboptimal temperatures affecting fish metabolism and related physiological processes such as hepatic intracellular activity and cardiac muscle contractility[60-62]. As fish are ectotherms, the temperature of their physical environment is directly correlated to physiological reactions, meaning that with increasing temperature the rate of biochemical reactions increases[34]. Consequently, it is common practice to reduce the temperature of water for transport of live fishes, for example through the addition of cool blocks in insulated boxes or transporting fishes in temperature controlled vehicles. However, optimal transport temperatures vary between species and caution must be taken so that the cooling process does not incur thermal stress in the fish, as the majority of tropical ornamental fish species are intolerant of cooler temperatures[34]. For example, low temperatures (<19.6° C) during transport compromise cardinal tetra (*Paracheirodon axelrodi*) survival[58] and changes in temperature can exacerbate declines in water quality[63,64].

*Poor feed management*: Particularly prior to transport, fishes may be food-deprived to reduce water quality deterioration through uneaten food and to reduce the amount of physical waste produced by the fish which may further compromise water quality[65,66]. During these food-deprivation periods, fishes can meet energy requirements from nutrient stores[67] but long-term under-feeding in home aquaria[68] could have detrimental effects including malnutrition and excessive competition for food. However, in home aquaria, the potential for overfeeding is more likely, with negative knock-on effects for water quality [47,69,70]. Poor management of feeding conditions can result in abnormal behavioural patterns, poor overall performance and increased disease susceptibility[71].

*Inappropriate photoperiod*: Photoperiod can affect fish welfare by influencing feeding strategy, condition factor and feed conversion efficiency[72,73]. Not only is photoperiod a main component in the synchronisation of endogenous rhythms, it also affects internal processes such as metabolic rate, growth, epithelial pigmentation, reproduction and locomotor activity[74-76]. Photoperiod is often manipulated for breeding, with significant effects on growth and reproduction[77,78]. Light intensity is also an important consideration at retail stores and for home aquaria where a trade-off exists between an intensity that optimises fish colouration to the human eye[79] but that is also conducive to good fish welfare.

*Noise and vibration*: Human-generated noise pollution affects fish behaviour[80-82] and elicits stress in fishes. For example, in zebrafish, sound exposure (112 dB re 1 μPa) altered group cohesion, swimming velocity and location of fish in the water column[81]. There is little existing research documenting the effect of noise during transport of ornamental fishes, but oscillatory movements during transport have the potential to compromise fish health[83]. A multitude of sounds and vibrations are likely to be experienced by fishes in the ornamental trade including engine noise, human speech and noise from aquarium filters as well as vibrations associated with noise. Aquarium filters mask auditory hearing of goldfish (*Carassius auratus*), specifically when filter frequency noises are at 15-19 dB (0.1-0.3kHz) and recommendations have been made for hobbyists to purchase quiet filter setups to improve welfare conditions within home aquaria[84].

*Medical treatments:* Some chemicals that are used to eliminate potential pathogenic microorganisms in ornamental fishes may be stressors in their own right. Three of the most commonly used treatments for fungal, parasitic and bacterial diseases are formalin, malachite green and acriflavine, all of which are routinely used prophylactically. Malachite green can lead to mortality if used at high concentrations[85] and also negatively affects physiological functioning (specifically carbohydrate metabolism) and behaviour in stinging catfish (*Heteropneustes fossilis*)[86,87]. Although it has a relatively low toxicity for fishes, formalin (37% formaldehyde) can have sublethal physical effects, such as gill hyperplasia in bluespotted coridora (*Corydoras melanistius*), which may have subsequent behavioural effects[88]. Acriflavine (acriflavinium chloride) is an acridine dye that is used in some non-EU fisheries and occasionally placed in transport bags with fishes coming from outside of the EU to treat external protozoan and bacterial diseases[89]. Furthermore, acriflavine can cause fish sterility as well as other species-specific differences in sensitivity[89]. Consequently, some treatments for ornamental fishes may provoke negative biological and behavioural effects.

### *Operational welfare indicators (OWIs)*

With increasing concern regarding animal production and animal use increasing over recent years, there has been a move to improve welfare and production systems through creating OWIs[29]. OWIs are individual indicators of welfare, which can be morphological, behavioural, physiological, metabolic or abiotic in nature, and can be aggregated to fully assess all aspects of animal welfare. To be classified as 'operational', OWIs need to be multi-faceted in nature[29], provide insight into an aspect of animal welfare and be easy to use without requiring laboratory analysis, otherwise they may not be used by individuals involved[90-91] Suites of OWIs and welfare measurement methods have been developed for a range of animals such as equids[92], livestock[93-96], rabbits[97], laboratory animals[98], birds[99-100], mammalian companion animals[101] and some species of food and cleaner fishes[13,92,102-104]. Existing welfare monitoring tools that use OWIs have created methods to quantify welfare, including allocating a score/grade to quantify welfare[105], a ranking system[106], computational modelling based on attribute levels[107], and needs or welfare indices ([91] and [108] respectively). Kubasiewicz *et al.* (2020) used a novel strategy of combining expert opinion on what classifies as 'most important' welfare indicators and creating specific questions to address them. These questions were then formulated to create a flow chart with scores/grades given depending on the answers, resulting in a process that was intuitive and understandable for both researchers and lay-people.

OWIs have been created for food fishes in aquaculture[15,16], which aim to incorporate aspects of their physiology, morphology and behaviour as well as information about their environment[14,15, 103,109-111]. Recently, a combination of OWIs have been used to assess lumpfish (*Cyclopterus lumpus*) welfare, including species-specific welfare indicators (external body damage, relative mass and cortisol/plasma analysis). These OWIs have been validated by a range of interested parties (e.g. farmers, scientists, wholesalers/importer/exporters)[91]. Noble *et al.* (2018)[14] suggested that for OWIs to be robust and all-encompassing they need to be (i) fit for purpose, (ii) species-specific, (iii) life stage and biologically specific, and (iv) realistic for utilisation in specific settings. For example, the FISHWELL OWIs use the 'Five Domains' model for animal welfare and apply them to salmon, employing five different categories to evaluate welfare status: resource availability, environment, health, behaviour and feelings[14]. Essentially, the evaluation of welfare depends upon an unequivocal comprehension of welfare and defined, robust indicators that unambiguously quantify the variables they are measuring. To date, there are no aggregated OWIs or welfare assessment matrices published on the welfare of ornamental fishes. However, there is the potential for a similar strategy to those used for food-fishes to be used within the ornamental fish trade due to the broad range of stakeholders, including, curators wholesalers, retailers and farmers.

### *Can we use current aquaculture technologies in an ornamental-setting?*

Technological advances over the past few decades in commercial aquaculture, have drastically improved the array of techniques that are available to monitor fishes[112] and many of these have been applied from a welfare monitoring perspective. However, these techniques have species and environment-specific limitations and do not all perform equally well. For example, the use of sentinel robotics to monitor water quality within large cage food-fish aquaculture[113] would not be suitable for use in home aquaria. In food fishes, telemetry technology advances, such as SmartTags, have been developed to measure opercular beat rate (OBR) and be used as a fish welfare indicator[114]. However, for smaller fishes, one would need to consider the size of the tag relative to the size of the fish. Telemetry can also be used to assess welfare in a fish farm setting by analysing swimming behaviour through remote sensors and cameras in tanks, cages or ponds[13]. Electromyogram (EMG) radio transmitters can be used to indicate welfare status. In European sea bass (*Dicentrarchus labrax*), muscle activity was analysed at different stocking densities through EMG and findings confirmed through haematological and serological analyses[115]. Muscle activity was significantly higher at high densities (50 kg $m^{-3}$) resulting in a higher use of anaerobic substrates and energetic reserves which, consequently, reduces the ability for the fish to manage additional stressors[115-117]. Results from telemetry technology for food fishes can therefore be used as welfare indicators in conjunction with functional, behavioural and physiological analyses to create a suite of OWIs for farmed fishes. However, these technologies are unlikely to be viable for ornamental fish culturing facilities, wholesalers or retail stores due to the small size of ornamental fishes themselves, the small size of tanks they are held in, the invasive nature of some technological approaches and relative high financial cost compared to other welfare monitoring methods. With alternative methods for monitoring ornamental fish welfare there is a lack of impetus to further advance these technologies for the ornamental fish trade.

### *Identifying appropriate OWIs for the ornamental fish trade*

Behaviour has been suggested as one of the most pertinent welfare indicators of captive animals as it provides a non-invasive indication of the biological state of an organism including the animal's physiological and mental state[13,15,118,119]. Additionally, studies have found that behaviour can be reliably assessed by observers from different occupations (e.g. veterinarians and farmers)[120], which further validates behaviour being used as a welfare indicator. Using behaviour to monitor fish welfare has the potential to improve animal health, nutrition and social interactions and has the additional benefit of being non-invasive[8,52,110,121]. The use of behaviour as a tool to monitor welfare in food-fish aquaculture has been reviewed previously[111,119], however, despite the potential use of behaviour as a monitoring tool in a broad array of settings[8,110,121], it is still considered under-utilised[119]. In an ornamental fish-keeping setting, behavioural measurements of stress are significantly easier to obtain and assess compared to physiological measures[7,122]. To identify potential behaviours that could be included as OWIs for the ornamental trade a systematic search was carried out to identify behaviours previously measured in relation to welfare of ornamental species.

A systematic search method using one search engine (Web of Science (Core Collection)) was used (August/September 2020) to identify a range of peer-reviewed studies that monitored the effects of stressors relevant to the ornamental fish trade on behaviour. Additionally, references of the identified papers were searched manually to include any other relevant papers. The search terms used to identify the papers were "behavio*", "fish*" and "welfare", with Boolean operators used. Original search terms included the term "ornamental", but this resulted in very limited search results and was therefore removed. Due to the broad range of studies analysing the welfare of food fishes, the inclusion criteria were refined to only include studies that monitored the effects of stressors associated with the ornamental fish trade in an ornamental fish species and focussed on behaviour as a measurable parameter. Only peer-reviewed primary research was included and was further limited by only including quantitative studies. Initially, titles and abstracts were assessed for relevance by the first author (MJ) and once identified, full papers were analysed for relevance against the inclusion/exclusion criteria ($n$= 23). Through manually searching references of relevant papers, additional papers were included ($n$= 13) with a total of 36 relevant papers identified. We included all relevant research papers from the search, from all publication years to date. While a systematic-type search method was used, the aim was to identify a wide range of behavioural measures that have been previously used in ornamental fishes, rather than to provide an exhaustive list of previous studies.

The results identified fish behaviours that have been widely measured in ornamental fishes and are commonly accepted as indicators of welfare (Table 1). These include feeding/foraging behaviour[123], aggression[36,124], neophobia[45,125], gasping at the water surface and locomotor activity[126,127]. Although not included within the systematic search inclusion criteria, changes in ventilation rate[128,129], and observations of physical damage (e.g. to fins, scale loss) represent non-invasive measures that could be combined with behavioural observations as suitable OWIs. Each of these potential indicators is discussed below.

Table 1. Studies that have monitored the effects of stressors relevant to the ornamental trade on ornamental fish behaviours. These studies were found using a systematic-type approach where keywords (behavio* AND fish* AND welfare) were used to identify relevant publications within the Web of Science search engine. In addition to this, the references of the identified papers were searched manually to include any relevant papers that were not found using the search terms.

| **Stressor** | **Species** | **Behavioural parameters measured** | **Outcome** | **Reference** |
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| Handling methods/ physical habitat | Three-spined sticklebacks (*Gasterosteus aculeatus*) | Assays: scototaxis (black vs. white areas of tank), diving, scototaxis and diving combined, novel object recognition (NOR) and open-field (OF) tests. Behaviours analysed in NOR and OF: exploratory behaviour, mean distance from centre of tank, mean swimming speed, latency to enter central zone. Diving and scototaxis: mean time spent in bottom of tank, mean time spent in black side of tank, mean distance from bottom corner of black tank (safest area), mean swimming speed. | Fish preferred black areas of tank than white tanks. In the open field test, handling method had no significant effect on behaviour, but some measured behaviours differed over time. In the NOR, net handled fish had a lower mean distance from the novel object than box handled fish when tested in the black tank. Fish significantly decreased mean speed after introduction of novel object, however, speed increased during the 5 minutes post-introduction. Diving and scototaxis testing: Net handled fish spent a significantly longer time in the black side of the test tank - however preference varied over the duration of the experiment. Time spent at the bottom of the tank decreased over time. | [130] |
| Handling methods | Three-spined sticklebacks (*Gasterosteus aculeatus*), Panamanian bishops (*Brachyraphis episcopi*) | Behavioural temperament assays (motivation to leave shelter) and neophobia analysis through novel object avoidance. Only *G. aculeatus* and *B. episcopi* were used for behavioural assays. | There were species-dependent and size-dependent differences in shelter emergence times depending on handling method (net/scoop). Handling method did not have a significant effect on neophobia in *G. aculeatus*. However, net-handled *B. episcopi* spent significantly longer near the novel object, with size-dependent difference in novel object proximity also found. | [131] |
| Light intensity | Acará tinga (*Geophagus proximus)*, Nile tilapia | Chasing, circling, frontal display, lateral fight, lateral threat, mouth fight, nipping and undulation. | High light intensity resulted in an increase in fighting latency in *G. proximus* and *O. niloticus* but not *P. scalare*. High light intensity decreased chasing, circling and lateral fighting | [132] |

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| | (*Oreochromis niloticus*), angelfish (*Pterophyllum scalare*) | | in *O. niloticus*; increased frontal display and mouth fight in *P. scalare* and had no effect on *G. proximus* agonistic behaviours. | |
| Light intensity | Amazonian cichlid (*Laetacara fulvipinnis*) | Specific agonistic behaviours: frontal display, mouth fight, nipping, chasing, parallel display, threat, undulation and flight. | Light intensity increases agonistic behaviours, and interferes with social hierarchy stability which may compromise welfare. High light intensity decreased fighting latency, increased threat frequency, number of attacks and flight number, resulting in a destabilised social hierarchy. | 133 |
| Noise | Zebrafish (*Danio rerio*) | Acoustic response tendency in relation to sound field conditions. | No correlation between behavioural response intensity, quality, or directionality and sound pressure or ellipticity and directivity of particle motion. However, there was a negative correlation found between freezing tendency and particle velocity level. | 134 |
| Noise | Zebrafish (*Danio rerio*) | Assays: novel tank test (NT) and light-dark test (LD). NT behaviours observed: Time at top, middle and bottom tank zones, number of bottom entries, distance travelled in each zone, absolute turn angle in each zone, total time mobile. LD behaviours observed: light zone rotations, distance travelled, mean speed and time in each zone. | Overall, fish exposed to music displayed less anxiety-like behaviours during the NT and were less active and calmer during the LD. | 135 |
| Noise | Zebrafish (*Danio rerio)* | Swimming behaviour: startle frequency, swimming speed and depth variance. Foraging behaviour: food discrimination and food handling errors. | Sound exposure affects swimming behaviour and foraging efficiency, with intermittent sound having a more profound effect than continuous sound. | 136 |
| Noise | Zebrafish (*Danio rerio*), Lake Victoria | Exploration and frequency of freezing, rapid turns and erratic swimming. | Swimming speed was species-dependent. In both species, sound exposure reduced prolonged swimming speed. Zebrafish exploratory | 137 |

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| | cichlid (*Haplochromis piceatus*) | | behaviour was not affected by sound, but cichlids changed vertical distribution and spent longer in the bottom of the tank. Overall, sound exposure can result in both similar and species-specific behavioural responses with responses not related to hearing ability. | |
| Novel environment | Zebrafish (*Danio rerio*) | Group structure, proximity, shoaling, social and spatial metrics. | Under acute stress, fish shoaled in significantly higher densities, with a lower variation in neighbour distances with increased proximity to walls compared to the same group tested 24 h later, suggesting a reduction in acute stress. | [138] |
| Nutrition | *Simochromis pleurospilus* | Cognitive performance testing. Behaviours measured: latency to leave neutral shelter, latency to enter choice area and visual cue by fish. | Fish with a change in food ration in early life significantly outperformed their constant ration counterparts during the learning test, irrespective of the direction of food ration change. | [139] |
| Physical habitat | Amazonian zebra pleco (*Hypancistrus zebra*) | Preference tests depending on time within refuges. | Fish spent longest in clay shelters followed by the natural rock shelter, outside the shelter and in the PVC shelter, with a significant difference among shelter types. | [140] |
| Physical habitat | Dwarf cichlid (*Apistogramma agassizii*) | Agonistic behaviours – chases, tail beats, bites and shelter use. | Shelter use was not influenced by enrichment conditions but was monopolised by dominant individuals. Biting frequency changed in response to habitat enrichment. | [141] |
| Physical habitat | Goldfish (*Carassius auratus*) | Motivation testing (novel) through swimming against flow and preference tests. | Fish preferred planted areas but did not discriminate between real/artificial plants. Water currents can be used as a novel method of identifying fish motivation. | [142] |
| Physical habitat | Goldfish (*Carassius auratus*) | Foraging, foraging bout number and mean foraging bout length. | Foraging was significantly affected by enrichment particle size, with particle-size dependent preferences in certain substrates. | [143] |
| Physical habitat | Three-spined sticklebacks (*Gasterosteus aculeatus*) | Learning and memory assays using three foraging compartments: latency to feed and compartment preference recorded. Temperament assay: boldness, neophobia, activity. | No effect of housing environment on learning or behaviour, but a significant effect on memory retention. | [144] |

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| Physical habitat | Zebrafish (*Danio rerio*) | Novel tank test and environmental preference choice tank, with associated observed behaviours, such as aggression. | There was no significant difference between fish in enriched and non-enriched tanks in relation to latency to enter the top half of the tank. Overall, fish from enriched tanks spent longer in the top of the tank compared to fish from barren tanks. There was no significant preference in relation to choice tank test. | [145] |
| Physical habitat | Zebrafish (*Danio rerio*) | Preference tests in relation to differing enrichment cues. | Dominant individuals in pairs significantly preferred substrate and also behaviourally excluded the subordinate individual. In groups, there were significant preferences for substrates and plants compared to barren conditions. | [146] |
| Physical habitat | Zebrafish (*Danio rerio*) | Activity level, shoaling density, aggression and time spent in bottom of tank. | Activity levels and shoaling density were not significantly affected by structure presence. In structured tanks, aggression remained high during days 1-5, and reduced by day 7. In control tanks, reduced aggression levels were observed 2 days earlier suggesting the presence of structures reduces the time it takes for dominance/subordinate establishment. | [147] |
| Physical habitat | Zebrafish (*Danio rerio*) | Feeding and chasing behaviours. | Aggression by dominants was higher in simple compared to complex habitats. Number of prey items eaten did not differ between habitats. Fish that conducted more aggression (chase rate) ate more food in both habitats. | [148] |
| Physical habitat | Zebrafish (*Danio rerio*) | Aggression, foraging (latency to feed – feed motivation), shoaling distances, feeding motivation. | Vegetation presence increases aggression whereas water flow decreased feeding latency after disturbance - however, behavioural patterns were population dependent. Environmental conditions had a significant effect on shoal cohesion, but population did not. | [149] |
| Physical habitat | Zebrafish (*Danio rerio*) | Aggression, nipping and chasing. | Fish showed reduced aggression levels in tanks with enrichment compared to bare tanks. Spatial complexity affected aggression, high aggression levels affected fecundity. | [150] |
| Physical habitat | Zebrafish (*Danio rerio)* | Preference scores from Jacobs' preference index. | A significant preference for enriched + swimming zones, with fish significantly avoiding swimming only and plain zones. | [151] |

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| Physical habitat | Zebrafish (*Danio rerio*) | Preference test. Time in each compartment, latency to exit respective compartment. | There was a significant preference for the dark chamber during the place-preference test. In the latency experiment, fish placed in the dark environment took longer to exit than fish placed in the light compartment. | [152] |
| Physical habitat | Zebrafish (*Danio rerio*), three-spined sticklebacks (*Gasterosteus aculeatus*) | Scan counts of presence in each zone which were used to calculate Jacobs' preference index (preference/ avoidance). | There were species-dependent preferences for enrichment, with *D. rerio* showing no preference and *G. aculeatus* showing preference for both shelter types. | [153] |
| Physical habitat | Zebrafish (*Danio rerio*), checker barbs (*Puntius oligolepis*) | Behavioural diversity, exploration, foraging, resting, locomotion, socio-positive behaviour, socio-negative behaviour, mating behaviour, comfort behaviour and "stereotypy". Preference tests and space use was analysed. | Fish showed similar behavioural diversity between structured and non-structured tanks. There were higher levels of "stereotypy" in barren tanks, indicating a significant preference for tanks with environmental complexity. | [154] |
| Physical habitat/ stocking density | Goldfish (*Carassius auratus*) | Foraging, position relative to cover (simple/ complex) and orientation relative to cover. | The presence of cover affected the position and orientation of fish. However, the presence of protective cover had no effect on fish positioning or orientation. Coverage had no effect on foraging behaviours. | [155] |
| Physical habitat/ stocking density | Midas cichlid (*Amphilophus citrinellus)* | Biting, chasing, charging, lateral displays, operculum flares, territoriality, foraging, cowering, escaping, resting, hovering, hiding, digging and swimming behaviour. | Dominant individuals exhibited increased aggressive bouts in relation to increased number of competitors. In subordinate fish, cowering behaviours had a strong negative correlation with increasing group size, but not habitat complexity. Aggression was not associated with varying stocking density; aggression was lower in complex tank environments. | [156] |
| Physical habitat/ stocking density | *Serrapinnus notomelas* | Attack, foraging, *per capita* aggressiveness and foraging. | Environmental enrichment had no effect on aggression, but larger group sizes reduced aggressiveness. | [157] |

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| Social composition/ physical habitat | Zebrafish (*Danio rerio)* | Behavioural assays: novel tank test (NT), light-dark (LD), and place-preference tests (PP). Behaviours measured: NT: transitions into top of tank, total time in top of tank, erratic movements. LD: transitions into white zone, total time in white zone, shuttling explorations. PP: transitions into enrichment, transitions into zone with conspecifics, erratic movements, duration and number of times spent immobile. | Fish that were housed in barren tanks on their own significantly increased anxiety-like behaviours in the NT and LD. This treatment group, as well as both group-housed treatment groups spent a significantly longer time interacting with conspecifics than the enrichment in the PP. | [158] |
| Species composition | Blunthead cichlid (*Tropheus moorii*) | Agonistic behaviours, hierarchy linearity index and aggression socio-matrices. | Aggression reduced immediately after the introduction of the novel individuals, with linearity and steepness levels decreasing alongside an increase in unknown relationships. | [159] |
| Stocking density/ light intensity/ nutrition | Zebrafish (*Danio rerio)* | Fin displays, fluttering, aggression, mouth gaping, chatter. | Stocking density and light spectra had no effect on behaviour. Food deprivation only had a significant effect on fluttering frequency, with fluttering increasing significantly in fish deprived of food for 72/ 216 h compared to 24 h. | [160] |
| Stocking density/ species composition | Neon tetras (*Paracheirodon innesi*), white cloud mountain minnows (*Tanichthys albonubes*), angelfish (*Pterophyllum scalare*), tiger barbs (*Barbus tetrazona*) | Darting, aggression, shoaling, neighbour index and interaction with tank enrichment (intra and interspecific). | Angelfish reduced aggressive behaviours in small shoals; white cloud mountain minnows and neon tetras increased natural behaviours. | [51] |

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| Stocking density/species composition | Neon tetras (*Paracheirodon innesi*), white cloud mountain minnows (*Tanichthys albonubes*), angelfish (*Pterophyllum scalare*), tiger barbs (*Barbus tetrazona*) | Darting, aggression, shoaling, latency to feed, neighbour index and time in tank enrichment. | Group size effects on behaviour are species-specific. Combinations of behavioural indicators within principal components analysis identifies group sizes which improve welfare. | [161] |
| Transportation/ physical habitat | Variatus platy (*Xiphophorus variatus*) | Biting, chasing, erratic swimming. | Lower numbers of erratic swimming and chases observed immediately post-transport in fish transported in bags with enrichment. | [162] |
| Transportation/ water quality | Variatus platy (*Xiphophorus variatus*) | Chases, biting, erratic swimming, ventilation rate. | No effect of the water conditioner Stress Coat on mortality during actual transport or body condition but did improve behavioural indicators. | [163] |
| Water quality/ Water renewal | Angelfish (*Pterophyllum scalare*) | Attack frequency and aggressive displays. | Aggressive behaviours reduced faster in tanks that had only 25% water changed compared to tanks that had 50% water changed. | [164] |

***Behavioural OWIs***

*Feeding/Foraging*

Twenty-seven percent (*n*=10) of the 36 papers found during the systematic-type search (Table 1) used changes in foraging and/or feeding behaviours as an indicator of welfare. Foraging is defined as exploration for and exploitation of resources[165], and in fishes, the behaviours associated with this can be influenced by both abiotic and biotic conditions[166-169]. Therefore, feeding and foraging behaviours could be used to detect welfare problems within the different stages of the ornamental fish trade[170,171], although this would likely not be appropriate during transport. In addition to changes to feeding motivation, feed intake is also routinely used in aquaculture as a direct indicator of poor welfare [56]. For example, after transportation when fish are placed in retail tanks, analysing the latency to begin feeding (feed motivation) and how much is consumed (feed intake) could elucidate whether there are any welfare problems that need addressing such as disease, injury or poor water quality.

*Aggression*

In both captivity and natural habitats, many fish species aggressively defend available resources, such as food, mates or territory, and may establish dominance hierarchies[172]. In natural environments, fishes may be able to escape their attacker but this is often not possible in captivity. Behaviours such as attacking (chase, bite, charge, mouth wrestling/locking) or displaying (operculum flare, lateral displaying, colouration change, vocalisation, electric organ discharges) are indicators of direct aggression in fishes[156,172]. Aggressive behaviours are frequently measured to provide insight into fish welfare; of the 36 papers identified (Table 1), 50% (*n*=18) used aggression as a behavioural parameter measurement. Aggressive behaviours can increase when a feeding technique is unsuitable for the species, for instance if fish are fed too little it can result in resource competition and guarding[173]. Aggression can also increase if social compositions and/ or densities are incompatible, for example placing two highly territorial species together or too many fish in the same tank[174], as well as when enrichment is lacking[175]. Therefore, increased pervasiveness of aggression could be an indicator that something is compromising welfare. However, according to the natural-based welfare approach, a certain level of these behaviours would be classified as natural as they would be performed in the wild[109]. For example, clownfishes (Pomacentridae family) reside in social groups where numerous agonistic interactions occur that are important to the maintenance of social hierarchy and dominance establishment[176]. Thus lack of aggressive interactions could also indicate welfare issues.

The incidence of aggressive behaviours occurs across different fish species and is diverse in its prevalence. Territorial behaviour, such as being stationed near a shelter, refuge or feeding area, can be an indicator of indirect aggression in some ornamental fish species (e.g. angelfish, *Pterophyllum* *scalare*)[177], but not all (e.g. the dwarf cichlid, *Apistogramma agassizi*)[178]. Consequently, it is imperative to create OWIs that are both species-specific and also based on the behavioural repertoire of the chosen species (e.g. whether the species is territorial or not). If the behaviour of one fish causes injury or undue stress to another, it is likely to compromise the welfare of that specific animal. Additionally, when aggression levels become excessive it can indicate that the individual exhibiting the behaviour is doing so because its welfare is compromised and it is showing stress-like behaviours. For aggression to be used as an OWI, it not only needs to take into account the welfare and behaviour of individual fish but also (if applicable) the group of fish housed together, in conjunction with a thorough understanding of the behavioural repertoire of the fish species, or group of species, whose OWI will be created.

Despite a clear link between acute short-term stress responses and coping strategies (the way in which fish cope with a stressful situation), there is limited information available regarding chronic long-term stress responses and respective coping strategies[121]. Stocking density affected coping strategies in African catfish (*Clarias gariepinus*), with higher stocking densities (considered in this study as a chronic stress) resulting in an increased feed conversion ratio and overall feeding activity, alongside lowered agonistic behaviour[179]. Evidently, coping strategies play an essential role in how individuals adapt to their housing environment and thereby their individual welfare[121]. However, as a whole, individual coping strategies should not be used as welfare indicators, but inferences from individual behaviours can potentially be made to identify a welfare problem[180,181]. Additionally, as different species have different behavioural and social traits, such as territoriality and dominance/size-based hierarchies[44,182,183], it is crucial that the person observing aggressive behaviour as a welfare indicator is familiar with the species and able to distinguish between normal and abnormal levels of aggressive behaviours.

*Swimming behaviour*

The use of swimming behaviour in the assessment of fish welfare relies on the observer's ability to distinguish normal and abnormal swimming behaviours which can be problematic. Nevertheless, it is still one of the most widely used parameters when attempting to appraise fish welfare (Table 1). The most prevalent ways in which swimming behaviour is quantified include speed, absence of swimming, rapid changes in direction, erratic swimming, tank depth variance and utilisation, startle speed, shuttling, shoaling distances and group swimming cohesion. Half of the behaviour papers identified in Table 1 (*n*=18) used swimming behaviour as a measurable parameter.

The way in which groups of fish swim together (for example in cages and ponds) is the most commonly used OWI to evaluate farmed fish welfare, either by eye for small densities or *via* the use of computer aided technology[13]. However, studies into social dynamics have found that one fish can dictate the behaviour of a whole group[184], highlighting that interpretations of group behaviours can give false indications of welfare as they do not consider the welfare of individuals within the group. This is especially true when fishes are kept at high stocking densities as they can adopt polarised schooling behaviour, purely to reduce the potential for collisions[16]. However, separating individuals from their conspecifics or social group may prevent the fish from displaying their full behavioural repertoire in an experimental setting, with social separation increasing stress intensity in very social species[138].

Swimming behaviours (such as erratic movements, chases, and latency to enter sections of a tank) are routinely used in ethograms when analysing stress in fishes. *D rerio* alter their swimming behaviour by either adjusting their swimming frequency or type of swimming patterns when exposed to negative conditions[185,186], highlighting how swimming behaviour can be an indirect indicator of poor welfare conditions. Guppies adapt their shoaling and swimming behaviour to their actual social environment and whether there is a perceived predation threat[187]. There are clear differences in natural swimming behaviours between different species, such as shoaling fishes (e.g. western rainbow fish , *Melanotaenia australis*)[188] and burrowing fishes (e.g. spined loach, *Cobitis taenia*)[189]. Thus classification of swimming behaviours as 'normal' or 'abnormal' is both species- and context-dependent.

Some ornamental fishes are bred for their elaborate ornamentation through artificial morphological selection, such as colouration, body, tail and eye morphological variants, which are deemed more appealing to consumers[190]. For example, some *D. rerio* have been genetically modified to have aesthetically pleasing exaggerated fins, which are classified as *long fin*[191]. However, *long fin D. rerio* have significant swimming deficiencies as a result of their mutant fins compared to their *short fin* (truncated fins) and wild type counterparts[191]. This is in contrast to male threadfin rainbowfish (*Iriatherina werneri*) whose lavish ornamentations do not appear to impede swimming performance[192]. The effects of morphological variations on swimming behaviour across ornamental fish species make it increasingly difficult to create generalised OWIs for ornamental fishes. While it would be unrealistic and impractical to create species-specific OWIs for individual ornamental fish species, grouping species together dependent on morphological and behavioural traits may facilitate creation of a suite of welfare monitoring tools for use within the trade.

***Non-behavioural OWIs***

*Morphological observations (colouration, fin damage, trauma)*

Although guidance exists for assessment of fish welfare by eye, there is a focus on direct signs of ill health (e.g. social isolation, lesions, bites) rather than behaviours. Stress can induce colour changes in the skin and eyes of some fishes, including ornamental species. Compared to fish reared at low density, sand gobies (*Pomatoschistus minutus*) reared at a higher density had significantly darker eyes (lower luminance values) which was positively correlated with skin darkening[193]. Furthermore, the eyes of guppies (*Poecilia reticulata*) darken when exposed to a predator and salmonids can darken their skin and eyes to indicate subordination in aggressive interactions, which further supports that colour changes can be used as an indicator of stress and negative welfare[194,195].

Physical injuries, such as dorsal fin injuries and erosion in salmonids and dislodged scales in ornamental fishes, can be suggestive of poor welfare. This is particularly true for ornamental species with long delicate fins, as there is a potential for fraying. Poor handling techniques and inappropriate transport methods can result in injuries[196], and increase susceptibility to bacteria such as the highly pathogenic *Vibrio parahaemolyticus* - a causative agent of tail rot and a common disease in ornamental fishes[197]. Consequently, using observations of physical injuries in combination with behaviour could be used to assess the welfare of ornamental fishes.

*Ventilation/respiration*

Stress results in a high oxygen demand, met by an increased ventilation rate through buccal movements and/or opercular beating. OBR is, therefore, a good indicator of stress in some situations and can be counted either automatically, for example through biosensor technology, or by eye[198]. Increased OBR may be attributable to stress and also indicative of low dissolved oxygen and gill problems[14]. However, lethargic behaviour or inactivity combined with a decreased OBR may also indicate low dissolved oxygen levels[199]. Through calculating OBR and amplitude of OBR, an index of ventilation activity can be created and from this welfare can be assessed[200].

*Infections/ disease*

Visual observations of infections and diseases and/or symptoms, such as flashing and substrate rubbing, can be used as an effective, non-invasive tool for anti-parasitic aquaculture applications in three ways: (i) being an OWI of infection status; (ii) enhancing resistance to diseases and parasites; and (iii) improving welfare through the use of encouraging behaviours via adaptive learning[119]. Due to the nature of ornamental fish infections and diseases, usually signs of infection are evident through visible indications and/or behavioural anomalies and therefore, would be easily noticeable by all observers within the supply chain. The benefits of using visual observations of infections and disease as an OWI to assess ornamental fish welfare is that fish can be observed individually (in which case, prophylactic medicinal treatment or feed deprivation interventions can be put in place) and/ or in groups (whereby quarantine or more invasive measures will need to be taken)[8].

## *Conclusion: Creating OWIs for ornamental fishes*

Ornamental fishes are classed as high value, low volume products (even though it is estimated that 2 billion individuals are sold annually)[1,3] which are traded (and transported) all over the world. It is evident that at every stage of the ornamental fish supply chain fish are potentially exposed to stressors that could to be detrimental to their welfare. Although research into food-fishes has previously dominated the field of fish welfare[201] there is increasing interest into improving welfare for ornamental fishes.

Good animal husbandry conditions and practices can produce fishes that are less stressed, less prone to disease, are in better physiological condition and consequently have better welfare. Combining the behavioural parameters identified through systematic searching of existing literature (Table 1) and the additional non-invasive measures discussed above, Table 2 presents potential OWIs for ornamental fishes. OWIs need to be fully comprehensive and incorporate multiple measurable parameters which the fishes come into contact with, to counteract any effects from avoidable stressors. Existing welfare monitoring tools have the tendency to address four welfare categories: living environment, nutrition, health/injury and behaviour. However, with increased acknowledgement that transport is a significant stressor for ornamental fishes, transport conditions should be included within the welfare categories. Aggregated welfare constructs are often developed using available scientific knowledge and input from expert panels and stakeholders[202,203] and the development of successful OWIs for the ornamental trade will depend on collaboration across a range of parties.

Due to the broad range of species being traded, a one-size-fits-all approach is not likely to be suitable. The creation of a suite of non-specialist OWIs with a focus on behaviour may aid in identifying when welfare is becoming compromised. Furthermore, with an increase in popularity in keeping ornamental fishes, creating standardised legislation and husbandry standards for every stage of the ornamental fish supply chain is essential to improve welfare. With purchasers becoming increasingly conscious of animal welfare, ensuring that fishes come from ethical sources and are not exposed to poor welfare conditions may make the ornamental fish trade even more profitable. For welfare to be improved, there needs to be a conscious effort made by all involved within the ornamental fish supply chain with a combination of stakeholders contributing towards a solution on how to rectify welfare issues as and when they arise. This starts with improving education for all stakeholders of what constitutes poor welfare and how it can be remediated, alongside in-depth research regarding the improvement of ornamental fish welfare and the development of an ornamental fish welfare monitoring tool that utilises non-invasive OWIs.

Table 2. Example of operational welfare indicators with worst- and best-case scenarios in relation to ornamental fish welfare. Worst case scenarios are likely to be indicative of very poor welfare, whereas best case scenarios are likely to be indicative of good welfare. Y/N indicates Yes/No.

| Welfare category | Operational welfare indicators | Example measures | Worst case | Best case |
|---|---|---|---|---|
| **Behaviour** | Aggression | Attack bouts<br>Aggressive displays<br>Chasing<br>Biting | Large numbers of aggressive behaviours which compromise the welfare of tank mates. | Limited numbers of aggressive behaviour bouts; welfare of conspecifics not compromised. |
| | Species compatibility | Attack bouts<br>Aggressive displays<br>Chasing<br>Biting<br>Territoriality<br>Shoaling | Inappropriate species behavioural compatibility resulting in aggressive behaviours which compromise the welfare of tank mates. | Species chosen to be housed together are compatible and able to express natural behaviours without compromising welfare of conspecifics. |
| | Swimming behaviour | Erratic movement<br>Freezing<br>Darting<br>Thigmotaxis<br>Stereotypy | High numbers of erratic movements, significant time spent immobile, significant neophobia, pacing (glass surfing), excessive thigmotaxis. | 'Normal/natural' swimming behaviour |
| | Feeding | Foraging<br>Feeding | Loss of appetite/ not eating or foraging. | Normal eating and foraging behaviours |
| **Living environment** | Physical enrichment (if appropriate for species) | Plants<br>Shelter<br>Substrate | No enrichment. | Presence of physical enrichment in tank. |
| | Social composition | Y/ N | Inappropriate social composition for species. | Appropriate social composition for species. |
| | Water quality | pH<br>Dissolved oxygen<br>Ammonia<br>Nitrite/nitrate | Outside of optimum water quality parameters. | Within optimum water quality parameters. |
| | Photoperiod | Lighting Y/ N | Inappropriate photoperiod regime for the species. | Lighting kept on a strict photoperiod regime. |
| | Noise | Quiet internal filter Y/ N<br>Physical location of tank | Loud internal filter, with the tank being placed in an area with lots of noise. | Quiet internal filter, with tank kept in a quieter area away from loud noises. |
| **Nutrition** | Appropriate nutrition profile | Y/ N | Inappropriate feed for species. | Appropriate feed for species. |

| | | | | |
|---|---|---|---|---|
| | Live feed (if applicable to species) | Y /N | No live feed supplementation. | Live feed supplementation. |
| | Feed amount | Amount suitable for species and number of fish Y/ N | Too much feed resulting in poor water quality and/or obese fish. Too little feed resulting in emaciated fish. | Appropriate amount of feed for species and number of fish. |
| **Overall health** | Evidence of injury | Lesions/ wounds<br>Missing scales<br>Fin damage | Extensive evidence of injuries. | No evidence of injury. |
| | Evidence of disease | Fin rot<br>White spot<br>Disease-related behaviours | Obvious evidence of disease (e.g. parasites, infections, disease such as white spot, fin rot, appetite loss). | No obvious evidence of disease. |
| | Evidence of malnutrition | Scoliosis<br>Obesity<br>Lordosis<br>Skin de-pigmentation<br>Rapid ventilation | Obvious evidence of malnutrition/ obesity. | No obvious evidence of malnutrition. |
| **Transportation conditions** | Water quality | pH<br>Dissolved oxygen<br>Ammonia<br>Nitrite/nitrate | Outside of optimum water quality parameters. | Within optimum water quality parameters. |
| | Noise | Packaging Y/ N | Excessive amounts of noise. | Limited amounts of noise with adequate packaging to dampen sound. |
| | Mechanical disturbance | Packaging Y/ N | No packaging to limit mechanical disturbance, potential for bag rupture. | Adequate packaging to reduce potential for excessive mechanical disturbance, fish double-bagged to prevent rupture. |

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