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# Emerging indicators of fish welfare in aquaculture

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## Abstract

As aquaculture continues to grow and intensify, there is increasing public concern over the welfare of farmed fish. Stress and production-related pathologies and repressed growth are examples of the challenges facing aquaculture, and their impacts could be minimised by effective identification of the early signs of impaired welfare. Many welfare monitoring methods have been recommended, however, continuous and reliable welfare monitoring in aquaculture is not yet widespread and commonplace. The aim of this scoping review was to present an overview of the most recent developments in fish welfare assessments with a specific focus on practical translation to the aquaculture industry. A keyword-based search was undertaken to identify peer-reviewed papers published between 2014-2020 in which a novel method with the potential to be used for the assessment of fish welfare in aquaculture was introduced. The results were sorted into two categories: non-invasive and invasive methods. All methods were assessed for their advantages and disadvantages, potential applicability to aquaculture. Invasive methods were also ranked on their degree of impact. It is concluded that increased interest into fish welfare, in combination with more intelligent modern technology, has resulted in the development of newer and more refined alternatives to traditional methods of welfare assessment such as behaviour monitoring by 2D cameras and plasma cortisol evaluation. Although, in many cases, more research is needed before these methods are suitable for widespread industry use, studies that focus on increasing the precision, automation and practical applicability of these methods are a promising avenue for future research.

**Keywords**

Aquaculture; monitoring; novel; stress; welfare

## Introduction

*Global scale of aquaculture*

Over the last 50 years, global fish consumption has increased at twice the rate of population growth, reaching 156 million tonnes in 2018 with 52% of this sourced from aquaculture [1],which is defined as “the farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants" [2]. As demand has risen, the aquaculture industry has expanded and intensified [3], contributing to growing political, economic and public concern about sustainability and related issues including animal welfare [4,5]. Given this immense global scale, and the vast number of individual animals being utilised, it is critical that the welfare of fish in aquaculture is adequately assessed and addressed. China alone produces more farmed fish than the rest of Asia as a whole, as well more than the Americas, Europe, Africa and Oceania [1], although little has been published on the welfare of fish within Chinese aquaculture systems in English. Terrestrial livestock welfare has been studied extensively over the last four decades, however, fish welfare has only recently been given serious attention [6–9].

*The welfare of fish*

Animal welfare is a multi-dimensional concept, which has been defined in several ways but can generally be considered as "the physical and mental state of an animal in relation to the conditions in which it lives and dies" [10]. Animals experience poor welfare from challenges to their physical or psychological health, often separated into five overlapping and inter-connected 'domains' (nutrition, physical environment, health, behavioural interactions, and mental state) [11]. To maintain adequate welfare, animals should not experience severe or chronic challenges in any domain, and should have opportunities for sufficient positive experiences [12,13]. Historically, there has been considerable debate about the ability of fish to have conscious experiences such as distress and pain, and therefore, whether they deserve the same welfare considerations that are applied to other agricultural animals like mammals and birds [9,14]. The fish brain lacks a mammalian-like neocortex [15], which in mammals is the region predominantly responsible for complex thoughts, consciousness and pain [16]. This is important as sentience is the basis for animal welfare [17] and only sentient organisms have the potential to experience emotions, pleasure and suffering [18]. However, a lack of homology is not synonymous with a lack of function, as similar processes can occur in other parts of the fish brain, such as the forebrain [9,19,20]. Furthermore, the neocortex is not the only region of the mammalian brain involved in consciousness [21]. Research from the last two decades suggests that fish are sentient, conscious and have the capacity to suffer [9,22–24]. Consequently, government bodies, such as the European Food Safety Authority (EFSA), now acknowledge that fish are capable of suffering [25]. It is therefore imperative that fish welfare is adequately monitored, measured, and attended to within aquaculture as it is for other livestock species.

Exposure of livestock to stressful conditions is prevalent in intensive farming, and monitoring the existence of stress responses is a key part of evaluating welfare [26]. Stress is a biological response elicited when an animal is confronted with a 'stressor'- a threat, or perceived threat, to its wellbeing [27], that interferes with the dynamic equilibrium ('homeostasis') of the animal [28]. It should be noted that all stress is not inherently dire to an animal's wellbeing, and for this reason stress is often divided into two definitions: 'eustress' and 'distress'. Eustress is an everyday experience useful for survival (e.g. avoidance of predators, searching for food) and does not necessarily have a detrimental effect on the animal unless it occurs often enough [29]. Distress, on the other hand, is experienced consciously as an aversive experience [27]. This response can be recorded using behavioural, physiological, or production-based measures amongst others [30–33]. Stress in fish has three stages: primary, secondary and tertiary [34]. The primary stage involves a cascade of neuroendocrine responses such as release of catecholamines and glucocorticoids [35]. The secondary stage involves a range of physiological reactions such as an increase in heart rate and utilisation of metabolic energy stores [36]. The tertiary stress response is activated by recurrent or chronic stressors and influences whole-body performance [34,37], manifesting physically (e.g. impaired growth) [37,38], and behaviourally, (e.g. developing unusual swimming patterns) [8]. Long-term effects of stress include elevated mortality rates and increased susceptibility to disease from compromised immune function [34]. Therefore, the control of animal stress within farming systems is important from both a welfare and an economic perspective [39]. Common stressors within aquaculture include, but are not limited to, poor water quality [40], hypoxia [41], transport [42–44], overcrowding [45–47], handling [48], starvation [41], disease [30], method of slaughter [49] and predation or aggressive attacks from other fish including conspecifics [50,51]. While it is impossible to eliminate all stressors in aquaculture, welfare monitoring methods can allow for the detection and quantification of these stressors, and minimisation of their impact.

*Assessing welfare and study aims*

Scientific assessment of animal welfare is dependent on valid and standardised tools, and 'Operational Welfare Indicators' are those which are relevant, easy to use, reliable, comparable, suitable for aquaculture, and appropriate for specific systems or routines [52]. Methods that can be applied to on-farm use may also be valuable for translating to aquaculture contexts [53]. Novel methods or those in development should ideally minimise production costs, time requirements and impact on animals, while maintaining reliability and accuracy [54]. Tools or techniques that are costly, unreliable, or time consuming are unlikely to be supported by industry [54], however, they may still be useful in an experimental context. The aim of this paper was to conduct a scoping review in order to identify, summarise and appraise novel methods of welfare assessment in fish, with a focus on translation to the aquaculture industry. Methods solely applicable to wild fish welfare were outside the scope of this review.

## Methods

Literature was identified using ScienceDirect, Scopus and Google Scholar databases. This process identified research articles published in English. Search keywords included combinations of the following: farmed fish AND/OR aquaculture; stress; welfare monitoring AND/OR behaviour AND/OR method AND/OR indicators AND/OR assessment.

Inclusion criteria for research discussed in this review were the following:

1. *Welfare*:- Fish welfare assessment or monitoring was an explicit aim of the research
2. *Novel*:- the authors explicitly stated that they were proposing a new method
3. *Recent*:- All corresponding articles were published between January 2014 and January 2020
4. *Peer-reviewed*:- Article was published within a peer-reviewed scientific journal
5. *Applicability*:- Methods were developed for aquaculture or had the possibility to be adapted. Due to the wide range of fish species used in aquaculture, the methods selected were all applicable or potentially applicable for most commonly farmed teleost species
6. *Non-lethal*:- lethal methods and techniques applied during harvest were excluded

This review is discussed in two sections, separating non-invasive and invasive methods. Where details are available, methods were assessed on the following criteria: invasiveness and potential severity, equipment required, practicality, cost, time requirement, expertise and translation to other fish species found in aquaculture.

*Invasive vs non-invasive*

No fixed definitions exist for the terms ‘invasive’ and ‘non-invasive’ when applied to animal-based methods [55,56]. Non-invasive sampling may refer to methods which avoid any direct physical contact including handling, capture and restraint [57,58]. However, the same term may also refer to procedures in which the integument of an animal has not been breached [55]. According to the latter definition, methods that collect samples from the outside of the body, such as hair or skin mucous [41,59,60], would be considered non-invasive even if capture and/or handling of the animal is involved. For the purpose of this review, invasive methods include those that physically disturb the fish by handling or capture and/or affect the physical integrity of the fish, and/or had an impact on fish fitness or behaviour (Table 1). Inclusion of methods as invasive or non-invasive was based on the likelihood of impact if no empirical assessment was available. Invasive methods were also evaluated on the potential severity of their impact on fish welfare and suffering (Table 2). It is a key principle of animal-based research that protocols should minimise the impact on animal welfare [61], and therefore non-invasive or minimally-invasive methods should be used whenever possible. However, this is not only important for welfare, but also for assessing and maintaining data quality [62] as many animal-based measures including physiology, production and behaviour are affected by aversive experiences such as pain and distress [63].

**Results**

Our literature search using ScienceDirect, Scopus and Google Scholar databases yielded 17 primary research articles fitting our inclusion criteria. Of these, 10 introduced a non-invasive method and 7 introduced an invasive method.

## Part 1: Non-invasive methods

### *1.1 Introduction to behaviour monitoring of fish*

Behaviour is a commonly used, non-invasive measure of animal welfare [18,64], which can provide external signs of an animal's internal experience including negative mental states, perception of their environment, and coping responses [65–67]. For example, fish experiencing stressful conditions may alter feeding behaviour and other self-maintenance activities [66]. Behaviour monitoring can be carried out using several methods including direct human observation, manual coding of video recordings, or automated methods such as computer vision techniques [68–71]. In aquaculture, monitoring focuses primarily on group observations rather than individuals [26,72–74]). This is because production animals are generally managed at the group level, and it is challenging to track individual fish within a group non-invasively due to view obstruction and the complex swimming motion of fish shoals [75]. Additionally, farmed fish are rarely housed individually [76] and social isolation for the purposes of monitoring can itself be detrimental to welfare [77] and impractical. However, identification of individuals within groups is also advancing through techniques such as facial recognition, even for fish [78,79], which means that options may be available in the future for better individual-level monitoring. This would create important opportunities for the monitoring of welfare, because group-level behaviour does not always accurately reflect the experiences of individual animals, nor the amount or range of individual-variation existing within the group [80].

#### *1.1.1 Avoidance and anticipation testing*

Fish exhibit self-preservation behaviours, such as threat avoidance and 'anticipatory behaviour', and these can provide insight into their perceptions and affective states and therefore, welfare [76]. Two welfare tests, originally developed for terrestrial animals, have recently been adapted for teleost fish species [76]. These tests measure human avoidance [81] and food-anticipatory behaviour [82] in juvenile rainbow trout *(Oncorhynchus mykiss)* [76]. Both tests were conducted in two experimental conditions: 6 groups of fish housed in normal oxygenated conditions, and 6 groups in hypoxic conditions [76], which is known to be detrimental to health and welfare [83]. In the human avoidance test, an experimenter stood 20 cm away from a 1 m x 1 m x 0.2 m tank, housing 83–85 fish, for a five-minute period and the distribution of the group within the tank was recorded at 1-minute intervals [76]. Uniform distribution of fish indicated low-level human-avoidance, congregation of around half of the fish to the far side of the tank indicated medium-level avoidance, and complete congregation as a shoal indicated high-level avoidance [76]. Hypoxic fish demonstrated less avoidance than controls, suggesting that self-preservation behaviour, and thus welfare, was impaired. During the food-anticipatory test, fish were conditioned to associate a neutral stimulus (water flow) with feed provision in a particular area of the tank [76]. Food anticipation was assessed by group distribution towards the feeding area after exposure to the conditioned stimulus, with more clustering in the feeding area indicating higher levels of anticipation. Fish housed in hypoxic conditions displayed less anticipatory behaviour, suggesting that impaired associative learning [76].An alternative explanation is that food may have been less rewarding to hypoxic fish than to their control counterparts, as acute stress is known to reduce feeding motivation in fish [84], which also has significant welfare implications. An advantage of these behavioural tests is that they can be easily replicated, and require few tools, easily trainable skills and short amounts of time. However, an initial control group may be necessary to compare responses of unstressed and stressed fish, and this can be difficult on farms and would add to the time required for these tests. In some farming systems, such as recirculating aquaculture systems, cameras can be used to monitor feeding activity [85,86], although in commercial sea cages these methods are likely to be more difficult to apply. In the absence of cameras, scoring of the fish behaviour can be hindered by poor visibility from high stocking densities and water turbidity [75] which restricts these tests to smaller fish enclosures. Additionally, the consistency of the protocol is reliant on effective staff training, which may be difficult to ensure or control [75]. It is currently unclear whether these tests would be applicable to all species of fish in aquaculture.

*1.1.2 Fish visualisation*

In large aquaculture settings it may not be practical for each cage, tank or pond to be individually or regularly observed [87]. Computer vision technology may offer a method for welfare assessment in these conditions [54]. Computer vision uses artificial intelligence and machine learning to process digital images, and can be applied in the aquaculture context for the identification and analysis of fish behaviour [71]. As technology has advanced, computer vision has become increasingly available and affordable [88]. One limitation of direct fish observation and early computer vision techniques is that it is difficult to observe or analyse images in low light conditions [89], however, artificial lighting can disrupt the circadian rhythm of fish and cause health defects [90,91]. To overcome this limitation, sonar and optic video imaging was recently used in combination with a Deep Neural Network model to successfully generate clear images of a large group of sardines *(Sardinops melanostictus)* housed with multiple other species, under a range of natural lighting conditions [71]. Techniques such as this can facilitate regular observations of fish groups under sub-optimal lighting conditions and have the potential to be used for the detection of behavioural parameters such as swim speed, social interactions and feeding behaviour [71], although this would currently require post-image analysis involving either additional software/programming requirements or labour. There are also practical limitations as the sonar system (ARIS EXPLORER 1800 unit, Sound Metrics, Bellevue, WA, USA) used was limited to a 15 m distance [71], therefore, future studies should also consider tank size when selecting hardware to ensure image quality is maintained.

*1.1.3 Automated behaviour detection and analysis*

Quantitative assessment of animal behaviour is time consuming and requires considerable observer training [92]. Automated systems to detect fish activity or behavioural changes via computer imaging reduce the need for human labour to analyse footage [93]. Such technology has been recently used to systematically detect subtle behavioural changes indicative of stress within a group (n = 52) of Nile tilapia (*Oreochromis niloticus*) [65], as unusual behavioural patterns can be warning signs of poor mental and/or physical health [70]. A charge-coupled device camera was placed above a transparent fish tank with LED illumination from below [65]. Fish movement was detected by applying a 'motion influence map' to raw video files. Motion influence maps integrate precise characteristics of moving objects, such as acceleration, distance, direction, and visibility, at the pixel level using optical flow analysis and can monitor the activity of multiple animals [65,94]. Data is then depicted as a heat map [65]. Recognition of target tilapia behaviours was greater than 84%. Target behaviours were characterised as distinctive sudden changes in motion or direction, such as when a fish quickly moves away from an approaching conspecific or after physical contact [65]. Abrupt movements can be indicative of a stressed internal state [95], therefore this technique may be very useful for welfare monitoring of fish in groups. An advantage of this method is its efficiency after implementation: once set up no labour is required to derive behavioural information, and action can be taken when the system detects unusually high levels of fish activity. Additionally, automated analysis is not influenced by observer bias or presence, which is useful in applied contexts such as fish farms [54,96,97]. Generalisation of this method to other fish species is feasible, although this would require species-specific target behaviours to be identified and adjustments for body size and shape [65]. One disadvantage of this method is the equipment cost and expertise required for initial development and implementation, and if full automation cannot be achieved then human labour may still be required for some tasks such as image pre-selection. Another limitation is that it is requires adequate lighting, which may restrict monitoring to daylight hours, since nocturnal lighting can be problematic for welfare [90]. With further development and generalisation, this type of technology may provide valuable and feasible monitoring that is useful for both animal welfare and production.

#### *1.1.4 Quantification of feeding behaviour*

Appropriate food intake is important for good welfare as poor feeding patterns can result in high mortality and disease susceptibility [98], or indicate the presence of disease [9]. Furthermore, feeding competition is a common challenge arising in fish management that can result in agonistic behaviour between fish or restricted access to food for sub-ordinate individuals [99,100]. Therefore, monitoring of feeding behaviour is useful for welfare purposes as well as production. Intelligent feeding control methods have been summarised previously [101,102] and are in products such as and are used by commercial companies in products such as CageEye (CageEye AS, Oslo, Norway) and Akvasmart CCS Feed System (AKVA group, Stavanger, Norway) in order to assist, autonomise and optimise feeding. However, feeding behaviour is complex and cannot be used as an indicator of welfare on its own. Along with welfare state and homeostatic control, feeding behaviour is also influenced by a range of environmental factors such as temperature, reproductive season and the presence of predators [103]. Therefore, feeding behaviour should be measured along with other indicators of welfare and should not be relied on as a sole indicator.

A combined behavioural/statistical method, using a computer vision system, was developed to quantify feeding behaviour of a group (n=60) of juvenile tilapias [75]. A CCD high-definition camera was placed above an experimental tank, with an adjacent LED light source. Behavioural characteristics were determined by swimming speed, angle of motion or directional changes at the pixel level of the video using optical flow in combination with MATLAB® (The MathWorks Inc., Natick, MA, USA). Feeding activity increases with appetite, therefore this method could usefully determine hunger and satiety, identify appropriate food quantities, or to ascertain when the release of feed into a tank should stop (Ye et al., 2016), particularly if used in conjunction with automated feeding systems that are already used in aquaculture [75]. This system may improve welfare by avoiding underfeeding or overfeeding, both of which have poor health outcomes [104]. An advantage of this system is that it is insensitive to water reflections [75], which can otherwise reduce image quality [105]. It is also practical for use in high density rearing tanks, which are typical in aquaculture [101], and only requires a minimal amount of technical equipment. Application to other fish species is possible with adjustments to the mathematical models [75]. Key limitations are the system's reliance on a light source, as image processing requires light silhouettes against a dark background, and the expertise required for the initial computational complexity of the behavioural analysis.

*1.1.5 Infra-red behavioural analysis*

Many methods for automated analysis of fish behaviour rely on a light source, however, unnatural illumination may be harmful to fish [90]. Near-infrared (NIR) imaging is a low-light option that uses the electromagnetic spectrum between visible and middle infrared light (wavelengths of 780–2526 nm) [106]. Infrared imaging is also resistant to electromagnetic interference and relatively inexpensive [107]. NIR imaging, using an NIR camera and NIR 850nm light source in conjunction with MATLAB® software, has been used to quantify feeding behaviour of adult mirror carp from nocturnal images *(Cyprinus carpio var. specularis*) [108]. The key advantage of NIR imaging is that it is not reliant on natural or artificial light sources and so it is not adversely affected by dim lighting [108]. However, there is some concern that NIR wavelengths may be harmful to animals [109], although, the carp study used a wavelength of 850 nm, which is not known to have detrimental effects on fish growth or welfare [108]. While this method was developed using mirror carp, it is likely to be effective with other common aquaculture species, such as salmon or tilapia, that display a variety of feeding behaviours during their life cycle and feed 'actively', and may be able to be combined with automated feeding systems [108]. A limitation of this method is that accuracy may be reduced from mist build up on the camera lenses, as was noted in the carp study [108]. This is a result of the damp aquaculture environment, which could also affect equipment longevity.

*1.1.6 Three-dimensional fish tracking*

Automated methods of behavioural assessment often use two-dimensions captured from a single camera view [54], however, animals move within a three-dimensional (3D) space. 3D tracking monitors animal movement within all three dimensions for a more detailed analysis of motion characteristics and behavioural patterns [110]. This is particularly relevant when monitoring aquatic, flying or arboreal animals [111], because depth or height changes in their location can be determined, whereas strictly terrestrial animals may be sufficiently monitored from a single top-down view [110]. For marine animals, 3D tracking can provide information about their position, depth, trajectory and momentum [112], as well as important behaviours such as habitat use and social interactions [111,113]. This is important for welfare monitoring since stress responses in fish can be conveyed behaviourally, through swimming patterns, speed, and interactions with conspecifics [33]. Freezing behaviour, for example, is a stress response characterised by lack of movement and positioning on the floor of the environment [114]. 3D tracking can also help overcome the issue of visual obstruction, which is particularly prevalent in large sea cages where it can be impossible to monitor the entire space using 2D visualisation methods [54].

A system recently developed for fish tracking is Microsoft Kinect™ I (Microsoft Corp., Redmond, WA, USA), a structured-light sensor system [89]. Microsoft Kinect™ is "single-point" 3D tracking technology that uses triangulation between a colour RGB camera, an NIR camera and an NIR projector [54,89]. This system was tested on a small group (n=4) Nile tilapias in tank (60 x 30 with 10 cm water depth). Although this study contributes to the feasibility of new sensors for monitoring fish behaviour in 3D space, this may be challenging to scale up to real aquaculture especially since this system has only been tested with a low stocking density. The Kinect™ was placed below the tank, which was illuminated by two lamps lit at low intensities. This system was able to identify individuals and evaluate their velocity, trajectory and spacing in real time even when some visual obstruction occurred (e.g. overlapping of fish)[89]. Another advantage of this method is that it requires less calibration than stereo-vision systems and less computational power for imaging matching and analysis [89]. A disadvantage is that sensor size restrictions limit the ability to remote sense with a maximum distance from the sensor to the fish of 3 m for adequate resolution, thus decreases its applicability to commercial aquaculture settings [89].

When measuring fish depth during 3D tracking light distortion can occur from the water. To overcome this, a recent study used an infra-red camera system and modelling of the relationship between depth and light intensity from the fish, to calculate 3D trajectories from a single top-down IR camera view [107]. This model successfully tracked four banded rainbowfish *(Melanotaenia trifasciata)* in a small tank (60 x 50 x 40 cm) with a mean depth error measurement of around half a centimetre. Measurements taken in daylight were slightly more accurate than nocturnal measurements. The maximum depth at which this could be used was less than 1 m, but this may be improved using more sensitive equipment [107]. The infrared reflection system for indoor 3D tracking of fish has lower hardware costs and fewer requirements for a 3D estimation algorithm, but is not yet suitable for real-world application [115].

**1.2 Fish size monitoring**

Poor welfare may inhibit animal growth in several ways, and therefore growth rates can be useful indicators for stress and welfare [116,117]. Physiological stress responses increase energy mobilisation, which can decrease energy availability for growth [40], and inhibit the production of growth hormone [118,119]. Stress may suppress feed intake [120,121] and fish may also experience competition from other fish [121].,and hypoxia [122], which can arise from overfeeding leading to polluted water and reduced dissolved oxygen levels [123]. Body size and growth patterns differ between individuals in a population, and this has been associated with specific coping styles [124]. Therefore tracking changes in fish size over time may provide one approach to welfare monitoring in aquaculture [9], and can also benefit production and management as the growth stage informs feeding regimes, the accuracy of oxygen consumption data and administration of medicine dosages, especially when delivered via water (e.g. anaesthesia) [125]. Accurate fish size measurements also help determine the most profitable time to harvest [125]. The relationship between stress, body weight and size is not simple [47], and some species height and weight are quadratically associated [126]. Resultingly, weight is the most important variable for identifying deviations in normal fish growth. Fish weight and growth have traditionally been monitored by physical weighing of individual fish on scales, which is time-consuming and requires capture and handling so may induce stress [125]. As for feeding behaviour, a limitation of growth measurements as a welfare indicator is that growth is affected by many factors other than welfare state, and is not always impeded when welfare is poor [127–129]. Therefore, growth should be measured in conjunction with other indicators of welfare or stress.

Ultra-sonic technology has been recently used to measure the heights, and estimate the weights, of individual free-swimming gilt-head sea breams (*Sparus aurata*) without disturbance [126]. In this system, a single beam transducer is placed down-facing on the water's surface with another placed up-facing at the bottom of the tank capturing dorsal and ventral body measurements, respectively. Sound signals are sent and received by the transducers then analysed using MATLAB® to construct an echo shape for each individual fish. Measurements were captured automatically when fish swam perpendicular to the acoustic beam axis. Modelling was used to derive height measurements, and body weight was then estimated based the known relationship between height and weight for this species [126]. This method was tested in a small aquacultural environment (small tanks of 3.0 x 2.7 m with low stocking density of 0.3–0.6 fish/$m^3$). For higher stocking densities, fish on the periphery of a shoal could be used to establish average weights and heights for a group and a split beam transducer may improve performance during non-horizontal swimming, although data obtained in this way may be less accurate [126]. For use in larger tanks, adjustments to transducer size may help translate these results to other contexts [126]. These possibilities warrant further exploration.

Machine learning techniques and Kinect™ technology have been successfully used in combination with NIR hardware to automatically estimate the size and weight of individual seabass (*Dicentrarchus labrax)* [125]. Total dorsal area, total dorsal length and fish width were measured at six different points of the body [125] based on images captured by an Infrared Reflection system (IREF) when sufficient NIR light was reflected from fish. Modelling was used to estimate fish depth based on the brightness of the silhouette, and to convert image pixel size into a true pixel size for size estimation. This system is not dependent on optical lighting, and can function in dim environments as long as there is NIR illumination. Additionally, if the fish overlap each other the system can detect this using pixel intensity and can separate the data from multiple individuals [125]. Although the developed model was based on seabass, the system could be explored with other species of fish. A limitation is that due to placement of the camera above the tank, only fish swimming above the shoal during image acquisition can be assessed.

Estimation techniques of fish size measurements are reliant on the accuracy of the models used and their ability to take into account variations in conditions, movement and body shape particularly when applied on-farm. Muñoz-Benavent et al. [130] introduced three novel geometric models that could estimate the length of freely swimming adult bluefin tuna *(Thunnus thynnus)* in aquaculture farms using a stereovision system. These models were able to account for bending of the fish body during locomotion, which alters the silhouette of the fish and can affect size calculations [131]. This system recorded 3D biometric measurements from individual fish in a large group (n = 312), using two synchronised optical cameras placed in a sea cage 15 m below the water surface. Cameras were placed underwater to capture the ventral silhouettes of the fish and the footage was analysed semi-automatically, thus reducing some human intervention. Snout-fork-length measurements using this method accurately compared with data from the fish at harvest. This method is dependent on light for image capture however as size and biomass do not fluctuate during the day, this is not a limitation. This system could be adapted for other fish species by adjusting the geometric models accordingly.

Stereo-vision systems and other automated technologies have also been applied in aquaculture to manage other health and welfare challenges [102]. For example, a machine vision system was recently tested for the monitoring of sea lice burdens [132], which is a major welfare [133] and economic issue [134] in aquaculture. Within an experimental system, populations of sea lice were able to be monitored at the group level [132], although even in the research context the small louse size creates a significant challenge, particularly when they are stationary. The major challenge of these systems is in visual obstruction either from water movement, objects (e.g. food particles) in the water, and appropriate placement of cameras in order capture relevant images (e.g. feeding events) that are useful for making management decisions [102].

Table 1: Summary of non-invasive welfare monitoring methods including their suitability to intensive aquaculture, assessed by how well the method works in larger and/or more densely populated sea cages and tanks.

| **1.1 Behaviour monitoring** | | | | | |
|---|---|---|---|---|---|
| **Method** | **Test species** | **Strengths** | **Weaknesses** | **Suitability to intensive aquaculture** | **Reference** |
| Direct human observation of human avoidance and feed anticipation | Rainbow trout | Little to no costs as no equipment and minimal training is required. | Subject to worker compliance. Subject to poor visibility and thus only feasible in smaller fish enclosures. More trials are required to test suitability of these tests to other species of fish. | Low | Colson *et al.* [76] |
| Sonar and optic imaging | Sardines | Applicable at day or night-time. Translatable to other species and more densely populated fish enclosures. | Limited to distance limitation of the imaging devices used. Additional labour and possible technological costs associated with post image analyses. | Moderate | Terayama *et al.* [71] |
| Computer based monitoring of unusual behaviours | Nile tilapia | Can recognise more subtle changes which could be useful for less overt changes in behaviour and thus, more accurate evaluations of welfare. Once the system is installed and established, minimal labour is required as the system detects behaviour changes on its own. | Complex computer system, which requires large annotated datasets for training purposes. High cost of equipment. Requires adequate lighting. More research is needed to establish translational relevance to other fish species. | High | Zhao *et al.* [65] |
| Model for assessment of feeding behaviours | Tilapia | Not susceptible to water reflections. Once the system is installed and established, minimal labour is required as the system monitors behaviour on its own. | Complex computer system. Reliant on light source. Adjustments to mathematical models would be required before this method is used on other species. Some reliability impediments yet to be overcome. | High | Ye *et al.* [75] |

| | | | | | |
|---|---|---|---|---|---|
| Near-infrared imaging to quantify feeding behaviours | Mirror carp | Once the system is installed and established, minimal labour is required as the images are automatically processed and analysed. The system is functional under dim lighting. Eliminates adverse effects of splash and reflection on image quality. | Mist on lenses reduces visibility and recognition accuracy. Recognition accuracy may be subject to fish colour: high contrast colours = higher accuracy, while low contrast colours = lower accuracy. | Moderate | Zhou *et al.* [108] |
| 3D tracking | Nile tilapia Rainbowfish | Avoids severe occlusion. Functional under dim lighting. System demonstrated high (98%) tracking accuracy. | Small distance limitation (1-3m) of the imaging system. Only tested in small tanks with small group sizes. | Low | Saberioon & Cisar [89] Lin *et al.* [107] |
| **1.2 Fish size monitoring** | | | | | |
| **Method** | **Test species** | **Strengths** | **Weaknesses** | **Suitability to intensive aquaculture** | **Reference** |
| Pulse-echo waveform measurements | Gilt-head sea breams | Once the system is installed and established, minimal labour is required as the images are automatically processed. Model can be adjusted for application to other species. | Tested on a low density fish tank. More research is needed to determine efficiency in larger more densely populated tanks. | Moderate | Soliveres *et al.* [126] |
| Infrared reflection system (IREF) and geometric model | Seabass | Once the system is installed and established, the images are automatically processed. Functional under dim lighting. | Susceptible to occlusion and therefore poor functionality in densely populated fish enclosures. More research is needed to establish translational relevance to other fish species. | Low | Saberioon & Cisar [125] |
| Stereovision system and geometric model | Bluefin tuna | Once the system is installed and established, the images are semi-automatically processed. Accurate in more densely populated fish enclosures. | Requires adequate lighting. Adjustments to mathematical models would be required before this method is used on other species. | High | Muñoz-Benavent *et al.* [130] |

## Part 2: Invasive methods

Measures such as behaviour and growth are useful indicators of welfare. However, this is dependent on the nature of the specific welfare challenge and these variables may not always reveal compromised welfare states (e.g. animals selected for fast growth may have poorer health outcomes than slow growing counterparts [135]. Ideally, welfare states should be assessed using a combination of indicators including, but not limited to, behaviour, health, physiology, environment and psychology [53,136]. Not all animal welfare indicators are non-invasive, and therefore, monitoring methods should be selected based on both the expected benefits and potential impacts to the animal. The following sections outline novel methods of measuring fish welfare, where some degree of impact to the animal is expected but destruction of the fish or product quality is avoided. Potential for use within aquaculture is considered, as well as severity (the intensity of potential suffering and the domain of suffering), the duration of impact, and the potential for refinement, such as by including analgesia use (Table 2). In practice, some methods can be combined with routine husbandry events in aquaculture such as the capture/handling of fish for lice counts [30].

**2.1 Extraction of physiological data**

Physiology can provide key insights into an animal's welfare state, and many established welfare measures rely on physiological processes. For example, cortisol is commonly measured as an indicator of hypothalamo–pituitary–interrenal axis (HPI) axis activity because it is a fundamental part of the primary stress response, occurs rapidly with acute stress, and is correlated with disease susceptibility, unusual behaviour and impaired cognitive performance [30,34,137]. Blood sampling is a common technique for measuring cortisol in fish [137], however, cortisol elevations may occur from the collection process itself e.g. removal from the water, handling, and needle puncture [42,50,138–140]. Other physiological measures can have similar disadvantages, therefore, there is a need for sampling methods that deliver robust results but are practical and aligned with good welfare practices.

2.1.1 Cortisol measurements

HPI activity can also be analysed from the measurement of excreted glucocorticoid (GC) metabolites in saliva, hair, faeces, and urine, among others, and these techniques have been widely used with non-aquatic species [141]. Cortisol extraction from tank water has been previously explored [32,142–144], however, this method is generally impractical for aquaculture as sampled fish should be individually housed for the most precise measurements, and individual housing can be a stressful experience in itself [137]. While GC levels are commonly determined from blood, other body tissues can also be used. A recent study investigated whether gill tissue could be used to measure cortisol levels in rainbow trout [42]. Three to five filaments from an external gill of anaesthetised juvenile and adult fish were removed and analysed. Filament cortisol levels were positively correlated with plasma concentrations, suggesting this method could be effectively used as biomarker of stress [42]. The impact of the biopsy procedure was assessed in a second study by comparing plasma measures (catecholamines, glucose, lactate and cortisol) across three groups of fish one hour after either a gill biopsy, a blood sample, or a control (air exposure for 15 seconds). No differences were found between the groups. Gill sampling was described by the authors as relatively easy with few complications, and could be completed in less than 30 seconds by personnel with minimal expertise [42]. Unlike for plasma cortisol, sample extraction was not required prior to ELISA analysis, reducing processing time and cost, as well as the use of potentially toxic extraction chemicals [42]. However, this method is only applicable as a non-lethal procedure in larger fish, such as rainbow trout for which the required sample constitutes only a small proportion of the gill [42]. It should also be noted that both blood sampling and gill sampling require anaesthesia to reduce handling stress [42], the long-term effects of which are largely unknown [30].

The measurement of cortisol from fish scales has also been explored in a pioneer study [145] and refined in a later study [146]. Cortisol from scales was used as a biomarker of chronic stress in common carp. This method involves anaesthetising fish before ten scales are removed from the left flank in a relatively brief procedure. An advantage of this method is that these scales regenerate within days, and if done correctly permanent damage to the fish is avoided [145]. Furthermore, unlike blood sampling, the sampling procedure does not in itself cause acute stress capable of influencing the results [145]. Conversely, a disadvantage of this method is that this method is not a useful indicator of acute stress and cannot be used to identify the moment of stress.

The potential of faecal corticoid metabolites (FCMs) as a stress biomarker was recently investigated using farmed Atlantic salmon *(Salmo salar L.)* [30]. This study built on previous knowledge of using faecal cortisol to measure stress in fish [147,148] by developing a novel and more feasible method of analysis using ELISA. Previously, a preliminary step in salmon FCM determination involved drying and homogenized the sampled faeces, however, Cao *et al.* [30] reported that FCM could be measured directly from the liquid part of salmon faeces without any extraction procedure [30]. Faecal collection was carried out by "stripping", where pressure is applied to the abdomen from the pelvic fins to the anus in a smooth and systematic manner, a procedure which should be done by trained aquaculture staff. Few tools were required for sample analysis meaning that it may be practical for a fish farm context. The disadvantage of this method is the need for handling and manual stripping, both of which are known stressful experiences for fish [48,149]. Furthermore this method is only applicable to larger fish species, from which sufficient faecal volume is able to be stripped for analysis [30]. Additionally, there is a lag-time between cortisol secretion and subsequent elevation of cortisol metabolites, and this time has not yet been quantified [137], therefore, further validation is required before the impact of specific events can be determined. Because of the lag-time, increases in FCMs are generated at a slower pace than for blood cortisol, and therefore one advantage of this measure is that it is less likely to be influenced by the sampling procedure itself [30].

2.1.2 Changes to skin mucosa

The skin mucosa of fish is sensitive to external conditions, and deteriorates when fish are in poor health, have an inappropriate or insufficient diet, or experience psychological stress [60,75]. Skin is the first line of defence from harmful microorganisms or parasites [150], and if the immune system is compromised the skin becomes more susceptible to infection [151,152]. For these reasons, skin may be a useful indicator of fish welfare. Skin mucosa in juvenile fish has been measured under varying stressful contexts: simulated capture with a 3-minute air-exposure [meagre fish (*Argyrosomus regius*)], disease caused by a bacterial infection [European sea bass (*Dicentrarchus labrax*)] and fasting [gilthead sea bream (*Sparus aurata*)] [41]. Skin mucus samples were collected using sterile glass slides slid two to three times over the lateral line of the fish in a front-to-back direction. Samples were then analysed for viscosity and metabolites (glucose, lactate, protein, cortisol) using an ELISA kit. The 3-min air exposure increased glucose and cortisol metabolite levels by 1-hr post-treatment, with elevations lasting for at least a further five hours [41,153,154]. Absolute metabolite levels were also compared against protein levels, to control for variation in sample quality. The glucose:protein ratio increased after air exposure and bacterial challenge, and decreased after fasting. The cortisol:protein ratio increased after air exposure, and 7-day fasting, but not after the 14-day fasting or bacterial infection. The direct impact of this technique on fish welfare was not assessed in this study, but the primary challenge is likely to be handling of the fish out of the water, which was carried out under 'light' anaesthesia. Sample collection with a glass slide on the skin was gentle because of the necessity to prevent sample contamination from blood and other cells [41]. However, for some species a softer alternative may be needed, such as a cell scraper. This procedure may be a viable refinement to blood sampling for measures such as cortisol, and more work assessing the specific requirements of its use, including the time between challenge and response, would make a useful contribution to fish welfare science.

**2.2 Biosensors/bio loggers**

Bio-sensors and bio-loggers are devices which are attached to or implanted into animals to record components of their environment, physiology or behaviour [155]. These devices allow a large amount of systematic and detailed data to be collected. However, as attachments, they may have short- or long-term impacts on the animal [156]. Moreover, the attachment of a tag to a fish can also be a highly stressful procedure as it involves capture, handling, anaesthesia and surgery, and a subsequent recovery period [157], and tagged fish typically have higher mortality rates than untagged fish [156].

Bio-sensors/loggers have been used previously with fish, generally in an ecological context [158–160], however they may also be useful to monitor welfare states in aquaculture, particularly if they can be used to refine physiological monitoring methods. For example, blood glucose in fish typically rises in response to a stressful event and can remain elevated for more than a day [161,162]. Glucose levels have traditionally been determined by blood sampling [163,164], however, this only obtains a single data point for an individual, while on-going monitoring provides more information on responses to stress [165]. A wireless, implantable, real-time biosensor has been developed for free-swimming fish, to monitor glucose as a stress response [50]. This system, consisting of a biosensor and potentiostat, a transmitter [1.5 × 1.5 × 0.6 cm, 3 g (without battery)] and a receiver, and was recently improved by the addition of a colour switching device which allows for the visualisation of data in real-time [166]. The biosensor, which is comprised of 1.5 cm of wire and Ag/ AgCl paste (BAS, Tokyo, Japan), is implanted within the interstitial sclera fluid of the eyeball, as glucose levels at this site correlate highly with blood glucose [167], and a potentiostat (to control the voltage between working and reference electrodes) is attached to the fins using nylon thread [50]. Methods such as these which allow real time physiological assessment may be useful to monitor internal responses to stressful contexts in an environment typical of aquaculture systems, with further development, impact assessment, and refinement. However, they are currently more feasible in an experimental context due to the expertise required and the invasive nature of the method, which directly and probably substantially impact on fish welfare. Sensor use has been assessed over several contexts including: transfer between aquaria, changes in dissolved oxygen, and interactions between individuals [50]. For many of the contexts, sensor glucose levels using continuous monitoring was similar to blood glucose at sampled timepoints. It is unknown how long the system can remain functional but the longest experiment within this paper had a recovery period of 15 hours followed by 160 mins of continuous monitoring. Limitations of this technique were difficult to assess as many key details were not reported, including the implantation method and recovery from implantation. Adequate reporting of experimental interventions is critical for assessing the impact of interventions such as tagging, and as for all animal-based experiments, studies should adhere to the ARRIVE guidelines [168] in the publication process. Inadequate reporting appears to be relatively common in tagging studies in aquaculture [169] and in itself represents a barrier to the identification of welfare impacts and the ability to mitigate effects.

Heart rate and its variability are physiological measures that are responsive to stress [170], however for aquatic species, a major challenge in heart rate monitoring is attachment/implantation of a suitable recording device, and potential side effects on behaviour, physiology or welfare [171]. Of particular importance is that any device is wireless and light enough to allow normal swimming patterns. The use of implanted bio-loggers for heart rate measurement (DST milli-HRT bio-loggers, STAR-ODDI, Gardabaer, Iceland) was assessed in two consecutive studies in rainbow trout as a model species [45,165], and recently replicated in a third study using Atlantic salmon [172]. In these studies, loggers were implanted in the abdominal cavity, in close proximity to the pericardium [45,165,172]. Heart rate data were validated with electrodes in the water, which record bioelectric potentials generated from the heart [173,174], and the bio-loggers were used to record heart rate over a period of several weeks [45,172]. Logged data quality was graded using a four-point scale, and while the highest quality grading required the largest dropout of data (~65%) from the analysis it most closely aligned to the reference dataset [45,165]. In terms of impact, the loggers are relatively small (length: 39.5 mm, diameter: 13.0 mm) with a mass (11.8g) approximately 2% of the smallest trout used in the Brijs et al. [165] study. However, surgical implantation is required, and the procedure, including recovery, transportation and reintroduction, took more than 72 hrs [165]. Several practical limitations should be noted. Dataloggers do not transmit information so data cannot be downloaded until they are removed from the animal, therefore this doesn't permit real-time monitoring. This can be problematic on commercial fish farms and sea cages where it may be difficult to re-capture fish that are implanted with the dataloggers, and these devices may also fall through sea cages and be lost. For detection at the time of slaughter, metal detectors may be employed to ensure devices do not enter the food chain [175,176] although not all tags are detectable in this way. Additionally, data quality decreased when fish heart rate was low – below 25 beats/min [165]. As logger and sensor technology develop into the future, it is likely that these approaches will continue to be useful in experimental welfare science and may be successfully translated to more applied contexts, however considerations should be given for refinement options to minimise the impact of the technique on fish welfare. Additionally, careful consideration needs to be given to decisions around the number of fish tagged within a group, and how best to choose individuals ('sentinel' animals) that represent the group as a whole, particularly as tags and other attachments can cause harm and increase mortality risk [156].

### 2.3 Acoustic telemetry

Sea-based aquacultural operations commonly use large floating sea-cages [177], housing upwards of 200 000 fish [46,66]. Sea-cages accommodate a greater number of animals, provide fish with a more naturalistic environment, and water quality is much easier to maintain than in tanks [66]. However, the sea environment and distance from land creates challenges for monitoring devices normally used in fish farms on land, and it is not possible to directly view all fish within a large sea-cage due to low visibility and the volume of water. Acoustic telemetry is one option for surveying fish behaviour where visibility is not optimal, such as in sea cages [66]. Battery-powered tags are attached to a subset of fish within a group and data from these tags are directed to an acoustic receiver via hydro-acoustic signals [178]. Acoustic telemetry has been investigated for its feasibility in monitoring fish swimming activity in aquaculture settings [46,179–183]. Receivers are generally submerged near or within the fish tank/cage to capture and interpret received signals into usable data, which are then stored internally or uploaded into a wireless database [46]. Unlike radio signals, acoustic signals transmit well in salt water environments [184]. There are challenges for implementing a successful telemetry system, such as the range limitations between tag and receivers. Currently, the maximum range between acoustic tags and their receivers is approximately 1km [46].

Telemetry has an advantage over dataloggers in that the data are transmitted rather than stored, which means that they can be accessed prior to retrieval, and also that implant failures can be identified. However, one challenge is that the data is not generally available in real-time as signals are stored before downloading, post-processing and analysis [185,186]. A new wireless system using Low Power Wide Area Networks (LPWANs) technology to overcome this limitation has recently been developed for use in suspended sea cages [66]. LPWANs are commonly used in 'Internet of Things' technology [187]. In this context LPWAN nodes are added on to acoustic receivers to transmit received signals to a single gateway. This is then transmitted to a personal computer acting as a server and user interface, eliminating the need for manual access of the data from receivers and allowing real-time data access [66]. Quality of service (defined as the number of uncorrupted messages received by the server, divided by the total number of messages transmitted by the nodes) was greater than 90% [66], suggesting that this system is a feasible solution to real time monitoring of fish within complex aquacultural environments.

Table 2: A summary of invasive welfare monitoring methods including a rating of invasiveness, assessed on a 3-point scale as marginal (+), moderate (++) and severe (+++). Degree of invasiveness was determined upon consideration of the intensity of potential suffering, the duration of impact including the length of recovery time, and the number of events that could cause suffering. For example, if the fish were handled briefly and the recovery was not prolonged then this was considered marginally invasive (+). In contrast, for a period of handling with the fish being semi-permanently or permanently affected (e.g., in the instance of surgical tag attachment) then this was considered severe (+++).

| **2.1 Extraction of physiological data** | | | | | |
|---|---|---|---|---|---|
| **Method** | **Test species** | **Strengths** | **Limitations** | **Invasiveness** | **Reference** |
| Gill cortisol | Rainbow trout | Quick and easy sampling procedure. Minimal expertise required. No evidence has been found to suggest that this sampling procedure itself induces acute stress that would influence the results. | Limited to larger fish, such as rainbow trout, as this procedure is lethal to smaller fish. Cannot determine stress in real time while fish are freely swimming. | +<br>Biopsy procedure but effects are not prolonged, and the fish have been shown to have a swift recovery. | Gesto *et al.* [42] |
| Cortisol in scales | Common carp | Quick and easy sampling procedure. Not influenced by acute stress. Useful indicator of chronic stress. | Cannot be used to determine the moment of stress (not an indicator of acute stress). | +<br>Handling and the use of anaesthesia is required, although the effects are not prolonged as scales have the capacity to regenerate | Aerts *et al.* [145] |
| Faecal corticoid metabolites (FCMs) | Atlantic salmon | Minimal expertise required. No evidence has been found to suggest that this sampling procedure itself induces acute stress that would influence the results. | Cannot determine stress in real time while fish are freely swimming. Cannot be used to identify a stressor as there is an undefined lag time between stress events and faecal cortisol. Only applicable to larger fish species, like salmon, in which a | +<br>Handling and the use of anaesthesia is required, although the effects are not prolonged. | Cao *et al.* [30] |

| | | | | | |
|---|---|---|---|---|---|
| | | | sufficient amount of faeces can be stripped. | | |
| Skin mucosa | Meagre, seabass, gilthead sea bream | Provide accurate results. Fish only need to be handled once. | Effects on the welfare of the fishes are unclear and warrant further research. Cannot determine stress in real time while fish are freely swimming. | + Handling and the use of 'light' anaesthesia is required, although the effects are not prolonged. | Fernández-alacid *et al.* [41] |
| **2.2 Biosensors/bio loggers** | | | | | |
| **Method** | **Test species** | **Strengths** | **Limitations** | **Invasiveness** | **Reference** |
| Glucose monitoring biosensor | Nile tilapia | Once the biosensors are implanted, little labour is required as the system displays the fishes' glucose information in real time. Can monitor glucose in freely swimming fish. | Effects on the welfare of the fishes are unclear and warrant further research. | ++ Surgical implantation. Once implanted, the effects are prolonged. | Wu *et al.* [166] |
| Heart rate bio-loggers | Rainbow trout Atlantic salmon | Provide an accurate and continuous monitoring of heart rate in freely swimming fish. | High level of equipment required. Data cannot be remotely recorded or accessed. Results indicated that tagged fish had poorer growth compared to untagged fish. | +++ Surgical implantation of the bio-loggers and this is followed by prolonged effects. A second surgery, to remove the implants, is also required. | Brijs *et al.* [45,165] Hvas *et al.* [172] |

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**Translation capability and limitations**

This paper has focused on novel fish welfare indicators or approaches, therefore, many of these are yet to be implemented in a commercial aquaculture setting. However, on-farm translation is a critical component for achieving impact by improving fish welfare. On-farm success and uptake are likely to be influenced by the following factors: robustness (reliability and accuracy), cost-effectivity, ease of use, and appropriateness of the information given by the technology/approach (validly measures one or more aspect of welfare). In other words, they are Operational Welfare Indicators (OWIs) [188]. OWIs can be separated into three levels of use with Level 1 OWIs comprising quick, easy and observational measures such as water quality, as well as fish survival, outward appearance, and behaviour [188]. Levels 2 and 3 represent more in-depth monitoring (e.g., fish sampling and potentially laboratory analysis), which can be employed when indicated by Level 1 OWIs.

Table 3 provides a summary of the methods discussed in this paper with a focus on translational reliability to aquaculture. It is important to note that the vast majority of non-invasive methods consist of computer visualisation technology that is only currently applicable in tank systems rather than large systems such as floating sea cages. These methods have the potential to be used during specific events such as transport or fish movement. Nevertheless, as most of these methods are in early stages of development, there is the potential for future research to focus on refining these systems for implementation in a wider range of contexts or locations. This step is crucial, as many of the discussed methods have only been tested in experimental tanks with low stocking densities and it would be ill-advised to extrapolate these results to large sea cages which hold thousands of fish, without appropriate assessment of translation success. Therefore, further research should focus on commercial scale testing of these emerging methods. Aside from high stocking densities, commercial testing would allow for opportunities to test methods in other conditions typical of aquaculture such as poor visibility, large distances from sensor to fish and also assess how well complex computer models or devices fit into production systems from a logistical standpoint. A cost-benefit analysis for methods deemed appropriate for commercial systems would also assist in the uptake of these methods to industry production.

Another limitation is that each method only evaluated a maximum of two domains (Table 3). Although the domains do overlap to some degree, it should be noted that additional methods should also be used where possible to ensure that all welfare domains are considered when evaluating welfare. The more invasive methods discussed here may be useful in this goal of forming a comprehensive picture of welfare. However, it is apparent that further studies are needed to weigh the benefits of these more invasive methods against the harm to animals.

Table 3. Potential for application and translation of novel welfare indicators and methods in commercial aquaculture systems.

| **Indicator/Method** | **Reliability & precision** | **Welfare domain(s)** | **OWI[1] level (1-3)** | **Suitable aquaculture systems[2]** | **Reference** |
|---|---|---|---|---|---|
| Direct human observation of human avoidance and feed anticipation | Difficult to assess and subject to worker compliance. | Behavioural interactions | 1 | Tank | Colson *et al.* [76] |
| Sonar and optic imaging | Demonstrated reliable acquisition of images day and night. | Behavioural interactions | 1 | All | Terayama *et al.* [71] |
| Computer based monitoring of unusual behaviours | Demonstrated high accuracy in experimental settings (98.91% detection accuracy and 89.89% recognition accuracy) | Behavioural interactions | 1 | Tank | Zhao *et al.* [65] |
| Model for assessment of feeding behaviours | Demonstrated good reliability in experimental settings with low stocking rates | Behavioural interactions and Nutrition | 1 | Tank | Ye *et al.* [75] |
| Near-infrared imaging to quantify feeding behaviours | Recognition rate of 92.99% recognition rate | Behavioural interactions and Nutrition | 1 | Tank | Zhou *et al.* [108] |
| 3D tracking | 98% accuracy rate | Behavioural interactions | 1 | Tank | Saberioon & Cisar [89]<br>Lin *et al.* [107] |
| Pulse-echo waveform measurements | Demonstrated good reliability in experimental settings with low stocking rates | Health and Nutrition | 1 | Tank | Soliveres *et al.* [126] |
| Infrared reflection system (IREF) and geometric model | Demonstrated good reliability in experimental settings with minimal | Health and Nutrition | 1 | Tank | Saberioon & Cisar [125] |

| | | | | | |
|---|---|---|---|---|---|
| | occlusion | | | | |
| Stereovision system and geometric model | Up to 90% of the samples were within a 3% error margin. | Health and Nutrition | 1 | Tank | Muñoz-Benavent *et al.* [130] |
| Gill cortisol | Measurements correlated well with blood cortisol, and an increase in gill cortisol following stress was demonstrated. No evidence to suggest that results may be influenced by sampling. | Health | 2 | All | Gesto *et al.* [42] |
| Cortisol in scales | Demonstrated good marker of chronic stress. No evidence to suggest that results may be influenced by sampling. | Health | 2 | All | Aerts *et al.* [145] |
| Faecal corticoid metabolites (FCMs) | Measurements correlated well with blood cortisol. No evidence to suggest that results may be influenced by sampling. | Health | 2 | All | Cao *et al.* [30] |
| Skin mucosa | Measures skin cortisol, as well as other metabolites (glucose, lactate and protein). Measurable changes in skin mucosa following stressors were demonstrated. | Health | 2 | All | Fernández-alacid *et al.* [41] |
| Glucose monitoring biosensor | Demonstrated reliable measurements of welfare following a variety of stressors. | Health | 2 | All | Wu *et al.* [166] |
| Heart rate bio-loggers | Measurement error was <1 heartbeat per minute. | Health | 2 | All | Brijs *et al.* [45,165] Hvas *et al.* [172] |

1: OWI = Operational Welfare Indicators. Level 1: includes basic observations of fish behaviour, appearance and mortality; Level 2: includes sampling fish for a more accurate description of symptoms; Level 3: involves expert analysis of blood and tissue samples from compromised fish [188].

2: The aquaculture system that the method is currently suitable for (pond, tank, larger sea changes, or all).

## Conclusion

Much attention has been given to fish welfare over the last couple of decades and as a result there are a variety of monitoring methods available. These vary in what they measure (behaviour, growth, cortisol etc.) and their strengths and weaknesses as well as their applicability to commercial scale aquaculture in particular, which is a critical step in the translation of these methods from experimental use to typical industry practice. Candidate methods for application to industry should be reliable and accurate, and should minimise production costs, time requirements and impact on animals i.e., minimum invasiveness. Upon review of the literature, it is apparent that ideal monitoring methods are still in the early stages of development and more research is still needed before widespread industry use. This further research should focus on commercial scale testing to evaluate how well these methods would realistically fit into the environmental conditions and logistics of commercial aquaculture.

Future research should be focused on non-invasive methods, learning lessons from terrestrial precision livestock farming. With the rapid development of new technologies applied to aquaculture like remote sensing, biosensors, artificial intelligence, and machine learning we will see a significant increase of the use of operational welfare indicators (OWI) applying these technologies in the near future. The use of smart phones apps for farmers will also improve the monitoring of the different variables involved in welfare: direct, such as animal observations, and indirect, with remote data from the sensors. The increasing interest in development of offshore marine farming will also require the use of remote sensing to monitor the welfare and health of the fish because of the inaccessibility of new offshore locations. A data driven insight into the welfare of the fish will increase the power of the OWIs developed until now. It is clear from our review that Information and Communication Technologies (ICTs) hold the key to the future of aquaculture too. Not to forget that this can also lead to exclusion of small producers or specific geographical areas, not able to afford or get access to this technologies or mobile broadband, for example, and for this reason much attention needs to be taken into avoiding both for the welfare of the fish and the farmers. As a final remark, further research on positive welfare is needed to better understand the different types of environmental enrichment that can be implemented to provide the fish with a life worth living, as well as ways of monitoring and assessing it.

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