

Behavioural indicators of welfare in farmed fish

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Abstract Behaviour represents a reaction to the environment as fish perceive it and is therefore a key element of fish welfare. This review summarises the main findings on how behavioural changes have been used to assess welfare in farmed fish, using both functional and feeling-based approaches. Changes in foraging behaviour, ventilatory activity, aggression,

individual and group swimming behaviour, stereotypic and abnormal behaviour have been linked with acute and chronic stressors in aquaculture and can therefore be regarded as likely indicators of poor welfare. On the contrary, measurements of exploratory behaviour, feed anticipatory activity and reward-related operant behaviour are beginning to be considered as indicators of positive emotions and welfare in

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fish. Despite the lack of scientific agreement about the existence of sentience in fish, the possibility that they are capable of both positive and negative emotions may contribute to the development of new strategies (e.g. environmental enrichment) to promote good welfare. Numerous studies that use behavioural indicators of welfare show that behavioural changes can be interpreted as either good or poor welfare depending on the fish species. It is therefore essential to understand the species-specific biology before drawing any conclusions in relation to welfare. In addition, different individuals within the same species may exhibit divergent coping strategies towards stressors, and what is tolerated by some individuals may be detrimental to others. Therefore, the assessment of welfare in a few individuals may not represent the average welfare of a group and vice versa. This underlines the need to develop on-farm, operational behavioural welfare indicators that can be easily used to assess not only the individual welfare but also the welfare of the whole group (e.g. spatial distribution). With the ongoing development of video technology and image processing, the on-farm surveillance of behaviour may in the near future represent a low-cost, noninvasive tool to assess the welfare of farmed fish.

Keywords Fish behaviour · Well-being · Aquaculture · Operational welfare indicators · Mental states · Stress · Appraisal

Introduction

During the last decade, the issue of farmed fish welfare has raised increasing public and scientific concern. This has contributed to the development of policies related to fish welfare (e.g. Council Directive 98/58/EC and the recommendation on the welfare of farmed fish by the council of Europe in 2005). Stakeholders, welfare NGO's and scientists have long recognised the importance of welfare of farmed and captive animals; nevertheless, despite the increasing interest on fish welfare, there is no clear consensus on how fish welfare should be defined or assessed (Huntingford et al. 2006; Ashley 2007).

Most animal welfare definitions can be loosely categorised into 'function-based' or 'feeling-based'

definitions. 'Function-based' definitions have the fundamental assumption that welfare is correlated with biological functioning including physiological stress responses (Duncan 2005), while in the 'Feeling-based' definitions, welfare more or less equals the current emotional state of the animal (Duncan and Dawkins 1983) and in the longer term, it represents the balance between positive and negative subjective experiences (Spruijt et al. 2001). The primary basis for the concept of 'animal welfare' is the belief that animals are sentient beings with an ability to experience good or bad feelings or emotional states (Dawkins 1990).

If the fish farmer wants to improve or document fish welfare, they need methods to assess animal welfare that can be feasible on a farm. A welfare assessment system should describe the welfare of farmed fish and allow the farmer to assess the development over time and to respond appropriately. Welfare indicators that are relevant for inclusion in an operational welfare assessment system should be science-based, should measure welfare over extended time periods, should be measurable on a commercial farm within a realistic framework and should be relevant as a decision support system for the farmer. To fulfil these requirements, the welfare indicators must provide information on potential welfare problems and the causes of impaired welfare (Rousing et al. 2001). Behavioural welfare indicators have the advantage of being fast and easy to observe and therefore good candidates for use 'on-farm'. Examples of behaviour that are commonly used as an indicator of welfare are changes in food-anticipatory behaviour, feed intake, swimming activity and ventilation rate (Huntingford et al. 2006). Such qualitative assessment can be converted into quantitative measures via video recordings and software for data collection, analysis and presentation, while image analyses facilitate continuous surveillance of behaviour. In this way, behaviour can be used as a noninvasive and as an early sign of potential welfare problems.

Disadvantages of behavioural welfare indicators can be that they are often variable over time and difficult to quantify, the observers need the sufficient skills to observe the animals 'body language', and there can be large individual differences in behaviour within animal groups. It is also worth noting that some behavioural responses can be considered

normal coping activities and therefore contributing to welfare, while other responses may be considered abnormal or maladaptive, therefore hampering welfare. The differences between normal (i.e. part of an adaptive response) and abnormal behaviour are frequently unclear.

To make a robust scientific welfare assessment, other indicators are also needed, and using an integrated approach by combining behaviour, neurophysiology, pathological and molecular indicators may result in an improved welfare assessment (Huntingford et al. 2006).

Over the last few years, advanced biochemical and genomic technology has equipped researchers with robust tools for studying animals' coping systems, and the number of new welfare-related indicators has increased rapidly. Such scientific indicators vary from the measurement of cortisol excreted into water (reviewed in Scott and Ellis 2007 and Scott et al. 2008) into more complex approaches such as the up- and down-regulation of targeted genes (e.g. Terova et al. 2005).

The aim of this review is to show that behaviour can be used to assess the welfare of farmed fish and to provide both aquaculturists and the scientific community with an overview of experimental results that show how certain behaviours can be applied as species-specific welfare indicators. We review behavioural changes whose link with welfare takes a more functional-based approach (foraging behaviour, ventilatory activity, aggression, individual and group swimming behaviour and stereotypic and abnormal behaviour) and those whose link takes a more feeling-based approach (exploratory behaviour, food-anticipatory activity, preferences and reward-related operant behaviour). Whenever the information is available, the link between behavioural indicators and other commonly used welfare indicators will be provided to allow a more comprehensive understanding of welfare assessment.

The diversity of behavioural responses and coping styles

Before addressing how specific behaviours can be used as welfare indicators, it should be noted that behavioural responses under stressful conditions are dependent not only on the type of stressor but also on

the coping style that characterises an individual. Individual variation in responsiveness may explain why some conditions that are well tolerated by some individuals may be detrimental to others.

Koolhaas et al. (1999) defined coping style as a correlated set of physiological and behavioural traits consistently linked over time and across situations, which define the ability of the organism to cope with stress. There are two major types of coping styles: proactive (active coping or bold or 'fight-flight') and reactive (passive coping or shy or 'conservation-withdrawal'). Behaviourally, a proactive coping style is characterised by a high level of active avoidance, aggression, exploration, risk-taking and an active attempt to counteract the stressful stimulus as opposed to reactive coping style. Physiologically, the proactive coping style is characterised by low production of corticosteroid hormones in response to stress production, but high sympathetic reactivity, while the opposite is true for the reactive coping style (Koolhaas et al. 1999; Pottinger and Carrick 2001a; Øverli et al. 2007).

The presence of coping styles is now well recognised in fish (reviewed in Øverli et al. 2007), and its implication for aquaculture can be wide-ranging. One of the best examples of such implications comes from studies using selected lines of rainbow trout, *Oncorhynchus mykiss*, differing in stress responsiveness. Low cortisol responders (typical proactive individuals) were shown to exhibit higher growth rates as compared with high cortisol responders (Pottinger 2006; Trenzado et al. 2006). These authors attributed the growth disparity between the two lines to differences in competitiveness/aggressiveness. Low cortisol responders tend to win fights for social dominance (Pottinger and Carrick 2001b), which may provide them with a competitive advantage to access feed when fixed feeding regimes are used. In addition, low cortisol responders have lower feed waste, higher growth rates, higher feed efficiency and lower size variability as compared to high cortisol responders (Øverli et al. 2006a).

These selected lines of rainbow trout were also shown to differ in susceptibility to crowding stress. High-stress responders were shown to be more negatively affected by crowding than the low-stress responders (Trenzado et al. 2006). In addition, divergent coping styles have been shown to exhibit

different susceptibility to diseases and inflammatory challenges (Mackenzie et al. 2009).

In general, individuals' coping styles will be related to the stress response of the fish, both the physiological response and the time lag before, e.g. recovering normal feeding (Damsgård and Dill 1998) and swimming behaviour after a stress event Øverli et al. 2006b). If a disturbance causes a fish to swim away or become inactive, such 'lost opportunities' will affect other behaviours, e.g. the time to feed. Small Atlantic salmon, *Salmo salar*, reduce their feeding activities for 2 h after a brief exposure to a trout model (Metcalfe et al. 1987). Also, Dill (1983) showed that the potential risk of predation for a juvenile coho salmon affected the distance the fish was willing to swim from the holding position to take a food item, and the fish seems able to trade-off the relative risk towards the relative value of the food. An individual boldness trait may thus affect where to feed, when to feed, how much time to allocate to feeding and the foraging tactics of the animal. In addition, other authors have also made the link between coping styles and traits relevant to aquaculture such as feed efficiency (e.g. Martins et al. 2006a, 2008).

Coping styles may also affect how fast fish might adapt to new feeding systems and explore new fish farm environments. A study on Atlantic cod, *Gadus morhua*, escaping from net cages revealed consistent individual differences between the fish escaping compared with nonescaping fish, with bold individuals being more willing to swim through small holes in the net, and thus escape more frequently than shy fish (Hansen et al. 2008). These responses are generally linked with the learning ability of farmed fish. In fact, Moreira et al. (2004) showed that lines of rainbow trout selected for high and low responsiveness to stress (HR and LR-lines) also differed in memory retention in addition to cortisol production.

An individual's responsiveness to stress is influenced by its coping style or behavioural syndrome, which should be considered when interpreting the variation in behavioural responses. The extent to which an individual is bold or shy should not be used as a welfare indicator, but one may infer a welfare problem when the behaviour defined under the bold/shy continuum changes; for example, when a bold individual starts exhibiting typical shy behaviours such as decreased exploratory behaviour, one may

suspect that it is sick or chronically stressed. Still, one should interpret such changes with care, as coping styles are considerably plastic and may change with context (e.g. Ruiz-Gomez et al. 2008).

Behavioural welfare indicators

Foraging behaviour

In the context of this review, foraging is defined as the search for and exploitation of food resources (Danchin et al. 2008). Foraging behaviour has been recorded in fish in terms of daily feed intake or feeding rate, the latency or time to start of feeding, total feeding time and the activation of self-feeders. Under ideal circumstances, an animal can reach reproductive age or (in case of aquaculture) harvesting age, without having to engage in any aggressive or territorial behaviour, but cannot do so without feeding (Dugatkin 2004). In fact, reduced appetite has been considered one of the main causes for growth reduction after stress exposure (Wendelaar Bonga 1997).

Teleost fish exhibit a wide variety of foraging strategies, which can be affected by the farming systems (Turnbull and Kadri 2007), gender (Øverli et al. 2006b), the genetic strain/family group (Kolstad et al. 2004; Kittilsen et al. 2009) and other factors. Variations in foraging behaviour include where fish feed (bottom feeders, surface feeders and feeding from the water column), when they feed (e.g. nocturnal vs. diurnal as well as scheduled vs. random), how they feed (active predators, scavengers or more passive feeders) and what they feed on (animal or vegetable matter or both). This variation should be taken into consideration when interpreting foraging behaviour.

One of the most relevant aquaculture procedures that have been linked with changes in foraging behaviour and welfare is the feeding method. The techniques for delivering food should be appropriate for the particular species (Alanärä and Brännäs 1996; Alanärä et al. 1998; Andrew et al. 2002; Noble et al. 2007b). For instance, bottom-feeding flatfish have been shown to have improved welfare (measured as feed intake and feeding motivation) when fed sinking pellets compared with floating pellets (Kristiansen and Fernö 2007). In addition, Sánchez et al. (2009)

using sea bream, *Sparus aurata*, as a model showed that altering the feeding time (scheduled vs. random) affects behaviour and physiology. These authors highlight the importance of using scheduled feeding to allow fish to prepare themselves (both behavioural and physiologically) for the next, predictable, meal. Fish exhibited an increase in locomotory activity before feeding, i.e. food-anticipatory activity (see also section “Group swimming behaviour”) when a scheduled feeding regime was used as opposed to fish receiving random feeding (Sánchez et al. 2009). However, imposed fixed regimes can increase the incidence of fin damage and injury as compared to demand feeding (Noble et al. 2007b, 2008), so a farmer should bear this in mind when making decisions on utilising a fixed ration or demand feeding strategy. In addition, the use of self-feeders has been shown to improve feed conversion ratio and growth in rainbow trout (Alanärä and Brännäs 1996) and can also be beneficial for their welfare (Suzuki et al. 2008).

Aquaculture stressors can reduce both feeding motivation and feed intake. The speed with which fish resume feeding after a stressor has been used as a foraging-related welfare indicator, for example in rainbow trout (Øverli et al. 2006a). Feeding motivation, measured as the latency to start feeding, has also been used as an indicator of welfare in Nile tilapia *Oreochromis niloticus*, cultured in recirculating aquaculture systems (Martins et al. 2009). More recently, Rubio et al. (2010) used self-feeders in sea bass, *Dicentrarchus labrax*, to understand the effects on refeeding dynamics after fish are exposed to fasting periods (which may occur before harvest). These authors showed that fasting periods can increase both feeding rate and daily feeding times.

In addition to changes in feeding behaviour, changes in feed intake have been repeatedly used as an indicator of reduced welfare. A variety of stressors have been shown to reduce feed intake in different fish species. Examples include changes in water quality (e.g. in Atlantic salmon, Espmark and Bæverfjord 2009; Nile tilapia, Martins et al. 2009; sea bass, Santos et al. 2010), stocking density (reviewed in Ellis et al. 2002 for rainbow trout), anaesthetisation and vaccination (e.g. in Atlantic salmon, Sørsum and Damsgård 2004), establishment of dominance hierarchies (e.g. in Arctic char *Salvelinus alpinus*, Alanärä et al. 1998) as well as changes in the social

structure of the groups as a consequence of grading or sorting procedures (e.g. in African catfish *Clarias gariepinus*, Martins et al. 2005, 2006b). In addition, cleaning protocols (such as emptying and brushing of the tanks) have been shown to impair self-feeding in sea bass (Rubio et al. 2010).

In brief, any observed changes in foraging-related behaviours, unless coupled with a change in management practices, is highly likely to be an indicator of changes in either the motivational state, health and/or welfare status of fish. This is supported by studies showing similar central signalling systems (corticotrophin-releasing factor, CRF/urocortin activation) involved in both stress-induced anorexia and negative emotions, at least in higher vertebrates (Heinrichs and Koob 2004). Whether this is also the case in fish still needs further study. Changes in foraging behaviour and behaviours associated with foraging can therefore be one of the most important behavioural operational welfare indicators of farmed fish species and are probably the indicator most commonly used by fish farmers. However, one cannot exclude the possibility that reduced feeding is also an adaptive response to a mild stressor or in some cases even a response to positive experiences.

Ventilatory activity

Ventilatory activity is the flow of water ventilated over the gills per unit time. It is performed by bucco-opercular movements. Its aim is the renewal of water in the branchial cavity, and a healthy fish will regulate it precisely. Ventilation ensures the supply of oxygen at the exact rate required by cellular oxygen (O_2) metabolism, neither too much nor too little. Specifically, the partial pressure of O_2 in the blood leaving the gills must be regulated in a very low and narrow range, primarily to avoid oxidative stress at the cellular level. Certainly, a fundamental and central idea is that O_2 deficiency in animal tissues is the result of a mismatch between O_2 needs and O_2 supply. The balance must be finely tuned, and ventilation must be taken as the first firewall protecting the fish tissues against oxidative stress (Massabuau 2001, 2003). Accumulated scientific evidence supports the concept that oxidative damage to tissues and cellular components is either a primary or secondary cause of many human diseases. Considerably less is known about how oxidative

stress can affect animal health and well-being, but it is clearly a significant underlying factor related to dysfunctional host immune and inflammatory responses that can increase the susceptibility of any farmed livestock to a variety of health disorders (Sordillo and Aitken 2009). This must be definitively taken into account in fish farming (see for example Ritola et al. 2002; Kucukbay et al. 2006).

Counting the number of opercular movements (ventilatory frequency), and estimating their amplitude, provides an index of ventilatory activity. It is worth noting that besides laboratory tools, there are efforts to develop new field methods to record ventilatory activity by fish telemetry. SmartTags have been developed to measure ventilatory frequency and amplitude of free-swimming fish as an indicator of fish welfare (Damsgård 2008).

Well-balanced ventilatory activity is fundamental to maintaining homeostasis in terms of O₂ status, blood and tissue acid–base balance (pH) at precise set points. In an acclimated and unstressed fish, the respiratory frequency is adjusted close to a minimum possible value. Therefore, increased ventilatory activity is commonly used as a sign of stress and poor welfare in fish. Importantly, it only takes a few seconds for a fish to change its ventilatory activity in response to water oxygenation or a chemical change. This is mediated by either chemoreceptors in the buccal cavity (taste buds, Ishida et al. 1996) or O₂ chemoreceptors in the gill vascular bed (Burlinson and Milsom 2003). How fast it returns to reference levels is fundamentally variable if the health status of the fish is altered. Following a brief water O₂ change, it simply takes seconds (Eclancher and Dejours 1975).

Ventilatory activity can increase as a consequence of several aquaculture procedures or stressors. The key factors affecting ventilation activity include the levels of dissolved oxygen and carbon dioxide in the water with ventilatory activity being inversely related to water oxygenation. Frequency of ventilation increases up to a certain critical low oxygenation level after which it again decreases and the fish suffocate after a while. In contrast, the frequency of ventilation decreases when O₂ is in excess (Randall 1982; Xu et al. 2006). It is known that a slight excess of dissolved carbon dioxide levels leads to variable ventilatory responses according to the water chemistry and the fish physiological status; a large excess

can be narcotic resulting in decreased ventilatory activity (Osborne and Muntz 1906).

Several other biotic and abiotic factors have been linked with increased ventilator activity. These include artificial lighting, loud or aversive sound, arousing odours, unsuitable temperatures or substrates, restricted movement, reduced retreat space, forced proximity to humans, reduced feeding opportunities, manipulation of fish groups, transportation (e.g. Barreto and Volpato 2006; Morgan and Tromborg 2007), handling (Brydges et al. 2009), air exposure (White et al. 2008), the presence of predators (Barreto et al. 2003), presence of chemicals (Neely 1979; Israeli-Weinstein and Kimmel 1998; Pane et al. 2004; Scott and Sloman 2004), diseases (Byrne et al. 1991), nanotubes (Smith et al. 2007), cyanobacteria (Ernst et al. 2007) and poor water quality in general (ammonia (NH₄)/nitrate (NO₃) excess, etc.).

In addition, any environmental change stimulating ventilatory activity can increase the vulnerability of fish to toxic chemicals simply by increasing the gills exposure to these: ventilation increases the surface of ventilated gill lamellae, and it decreases the thickness of the unstirred boundary layers (Pierron et al. 2007; Couillard et al. 2008). The enhancement is stronger when the contaminant concentration is low because a very low concentration can then become significant in terms of exposure rate. A high concentration is always a problem because there is so much contaminant in the water that ventilatory activity is no longer a limiting factor.

In brief, during routine inspection, sustained hyperventilatory activity does not tell the observer much about the origin and/or intensity of a stressor (see Barreto and Volpato 2004) but, combined with other observations (such as location in the tanks or reactivity), it can be a sensitive indicator of fish welfare. Furthermore, correlations between increased ventilatory activity and other welfare indicators, such as blood lactate, glucose and haematocrit, are well established (e.g. White et al. 2008). Nevertheless, one should interpret an increase in ventilatory activity with caution as an increase may be linked with positive experiences and therefore not necessarily with poor welfare.

Importantly, considering the profound consequences ventilatory activity has on the homeostasis of a fish's internal environment such as control of

cellular oxygen status, oxidative stress and internal pH regulation, high ventilatory activity can be both a passive consequence of disturbances and a driver promoting various physiological dysfunctions and contaminations. The basic premise that ‘ventilatory activity should be low to moderate’ should help (1) to minimise the effects and consequences of disease and injury and (2) improve fish welfare.

Aggression

Fish in a natural environment can live alone or in groups (Brännäs et al. 2001). Group living can take the form of short-term aggregations or be more long term and highly structured (Pitcher and Parrish 1993; Grant 1997; Hoare and Krause 2003). Group living confers advantages in foraging, resource defence and predator detection (Pitcher et al. 1982; Ryer and Olla 1991, 1992; Pitcher and Parrish 1993), and shoaling is one of the most common social fish behaviours (Pitcher and Parrish 1993; Parrish et al. 2002; Hoare and Krause 2003). However, when resources or the number or territories are limited, group living becomes a disadvantage as competition for the resource increases (Pitcher and Parrish 1993). Limiting resources may lead to the emergence of competitive behaviours, which may take the form of dominance rank-based hierarchies (Jobling 1983; Jobling and Koskela 1996). This kind of hierarchy is generally established after agonistic encounters between two individuals, and the rank within the hierarchy depends on their ability to fight (Huntingford and Turner 1987). In this review, agonistic behaviours refer to ‘all the behavioural units of threat that express the dominance between at least two social partners as well as the submission in response to it’. Agonistic behaviours include attacks, bites or bite attempts, threatening displays and behaviours related to submission such as flight or immobility.

Dominance hierarchies and the associated agonistic behaviours are dependent on several biotic and abiotic factors such as an individual’s ‘boldness’ (see above), size or sex (Ejike and Schreck 1980), the spatial and temporal distribution of food (Bégout-Anras and Lagardère 2004), underfeeding (Symons 1968), feeding frequency (Noble et al. 2007a) and feed delivery rate (Hakoyama and Iguchi 1997), with the latter factors being particularly relevant for aquaculture. The effects of stocking density upon

aggression are still unclear, as some studies suggest that high density can reinforce aggressiveness (e.g. Keeley 2000), whereas others suggest that high density reduces aggression (e.g. Almazán-Rueda 2004; van de Nieuwegiessen et al. 2008, 2009).

A subordinate position within a group or social hierarchy may be a stressor (Schreck 1981). A subordinate individual can be subject to social stress resulting from attacks and repeated threats of attack from more dominant individuals for access to resources (food, sexual partners and territory). Social stress leads to marked behavioural and physiological changes in subordinates, who often show a general behavioural inhibition of food intake, aggressiveness, locomotory activity, changes in skin colorations and higher levels of plasma cortisol (Denight and Ward 1982; O’Connor et al. 1999; Winberg and Nilsson 1993a, b; Øverli et al. 1998). It is, however, notable that cortisol elevation can also occur in dominant fish within minutes of the end of aggressive encounters (Øverli et al. 1999).

Not only is the social state of an individual important for welfare but also the social stability of a group. Fox et al. (1997), for example, showed that cortisol levels are dependent on both individual dominance and social stability in the African cichlid fish *Haplochromis burtoni*.

Proxies of aggression can be used as an operational welfare indicator by counting the number of skin lesions. Almazán-Rueda et al. (2004) and Cañon Jones et al. (2010) showed a significant positive correlation between the number of scars and/or wounds and the number of aggressive acts measured by direct observation. Almazán-Rueda et al. 2004 also validated the use of skin lesion frequency to assess the welfare of African catfish in two studies (Almazán-Rueda et al. 2004; 2005) and showed that feeding methods, photoperiod and light intensity affect skin lesions frequency as well as other welfare indicators (such as swimming activity, growth, plasma cortisol and free fatty acids). Similarly, Martins et al. (2006b) used skin lesions as an indicator of aggression to assess the welfare of African catfish subjected to a simulated grading. Considering the time-consuming characteristics of direct or video-recording observations, counting skin lesions may be used as an easy and fast indicator of welfare, when it can be combined with other handling procedures. Although skin lesions serve as an

indicator of poor welfare, aggression per se is not necessarily an indicator of poor welfare, unless coupled with lesions and fin damage. A consequence of this is that adjusting the aquaculture system to reduce aggression does not simply mean that one affects the aggressors' behaviour, but depending on the species, it may allow a certain level of aggression to occur while providing the target individuals with means to protect themselves.

The measurement of the nonapeptides vasotocin (AVT) and isotocin (IT) has also been suggested as an indirect way to measure aggression. AVT and IT are synthesised in hypothalamic neurones, from where they are transported to the neurohypophysis for storage and subsequent release. In fish, arginine vasotocin and isotocin have several functions, including being related to stressful conditions (Kulczykowska et al. 2009; Kleszczyńska et al. 2006; Mancera et al. 2008). Changes in hypothalamic, pituitary and plasma AVT/IT concentrations are found in many fish species subjected to different types of stress, e.g. confinement, disturbance, high density, food deprivation or rapid osmotic challenge, which are accompanied by aggressive acts. The AVT and IT response depends on both stress stimulus type and the level of aggression. In many teleost species, AVT has been shown to affect complex social behaviours such as courtship and territoriality (Semsar et al. 2001; Salek et al. 2002; Kleszczyńska et al. 2008), and the release of both AVT and IT is associated with different levels of sociality (Thompson and Watson 2004). Neuromodulatory actions of AVT and IT probably influence basic emotional regulatory mechanisms related to approach/withdrawal behaviours and aggression in social shoaling species. In Mozambique tilapia *Oreochromis mossambicus*, neurohormonal changes are different in dominant and subordinate individuals in social groups (Almeida et al. 2009). These authors suggest that the social stress activates the AVT system in the forebrain of subordinate males in this species, yet the pituitary and olfactory bulbs in both subordinate and dominant males are the areas associated with the highest levels of both neuropeptides. The outcome of social interactions influences the expression of subsequent social behaviour by changing the internal state of the individual affecting growth rates, breeding status and potentially affecting the welfare status (social stress) of the individuals. The changes in absolute

levels of AVT and IT or the balance between them, associated with aggression, may reflect variations in internal state of the individuals. Taking into account a link between level of aggression and AVT/IT response, AVT and IT are proposed as specific neurohormonal measures of level of aggression.

Individual swimming behaviour

Swimming performance was defined by Beamish (1978) as an indicator of the progress of fish manoeuvring in water and is therefore an indicator of the ability of the fish to feed, escape predation and maintain position in a current (Beaumont et al. 1996). Some fish are strictly dependent on their swimming capacity in order to undertake migrations and achieve reproductive success (Plaut 2001; Wolter and Arlinghaus 2004).

Different swimming styles and activities have been classified by Beamish (1978) who distinguished (1) sustained swimming, where a fish is able to maintain a certain speed for long periods (over 200 min), (2) prolonged swimming that is characterised by a shorter duration (between 20 s and 200 min) and ends with fatigue and (3) burst swimming that can be maintained only for very short periods (under 20 s). Burst swimming can also incorporate (a) elevated fast-start, high-energy swimming where fish rapidly accelerate from a resting position or a baseline swimming speed and (b) swimming manoeuvres that involve increased complexity and turning angles (e.g. Domenici and Blake 1997). Every swimming activity can be related to a particular swimming behaviour. Indeed, sustained swimming is characterised by low-frequency tail beats and a slow velocity (ca. one-third of maximum speed), while during prolonged swimming, fish can almost reach their maximum velocity (Lembo et al. 2007). Several different tests have historically been used to quantitatively evaluate the swimming activity of fish (Beamish 1978). The ones most widely applied to assess the physiological condition of fish are critical swimming speed, U_{crit} (Wolter and Arlinghaus 2004), recovery tests (Farrell et al. 1998; Jain et al. 1998; Wagner et al. 2004) and muscular activity measured via electromyogram (EMG) (Cooke et al. 2000; McFarlane et al. 2004; Chandroo et al. 2005; Lembo et al. 2008) or remotely measured by physiological telemetry sensors. EMGs give a quantitative estimate of both swimming speed and metabolic costs of

activity during swimming (mostly due to oxygen consumption). Thus, EMGs are a sensitive estimator of the metabolic scope for activity (SFA) (Fry 1971) and therefore, uniquely among these indicators, can be used as an operational, on-farm, welfare indicator by quantitatively measuring swimming activity of fish within cages or tanks.

Changes in swimming behaviour could reflect how a fish is sensing and responding to its environment. However, whether such changes can be used as an indicator of poor or good welfare are species and context dependent (Herbert and Steffensen 2005).

A number of common aquacultural water quality parameters can have an effect upon swimming behaviour. For example, reduced dissolved oxygen levels (hypoxia) can reduce the swimming speeds and activity of a number of species including white sturgeon *Acipenser transmontanus* (Crocker and Cech 1997) and Atlantic cod (Schurmann and Steffensen 1994; Herbert and Steffensen 2005). This reduced activity may enable fish to survive prolonged and widespread exposure to hypoxic conditions (Crocker and Cech 1997). In contrast, Tang and Boisclair (1995) have reported that the brook charr *Salvelinus fontinalis* increase swimming speeds in response to hypoxic conditions, and this is suggested to be an escape response from an area of potentially localised hypoxia. So, any deviations from normal, be it reduced or elevated, swimming speeds can be used as a potential indicator of hypoxia, although other water quality factors can have an effect upon swimming speeds (see below).

Hyperoxic conditions can also affect the swimming behaviour of fish. Atlantic salmon exhibit reduced swimming speeds (measured by reduced tail beat frequency) when held in superoxygenated water for prolonged periods (Espmark and Baeverfjord 2009). Other water quality parameters, in addition to oxygen levels, have been shown to affect swimming behaviour. These include low water pH levels (Day and Butler 1996), increased ammonia levels (Shingles et al. 2001), carbon dioxide exposure (Conte 2004) and trace element exposure (Hopkins et al. 2003).

A number of feed management factors can have an impact upon swimming behaviour, but their effect upon welfare can be rather unclear as elevated swimming speeds can again be indicative of both reduced and improved fish welfare, depending upon

the fish species and rearing conditions. For example, elevated swimming speeds during feeding can indicate underfeeding in a number of aquacultural species such as gilthead sea bream (Andrew et al. 2004), turbot, *Scophthalmus maximus* (Huse and Skiftesvik 1985) and Atlantic cod (Björnsson 1993). However, increased swimming speeds during feeding may also indicate an increased motivation to feed and may simply be part of an individual's foraging strategy. For example, Atlantic halibut, *Hippoglossus hippoglossus*, which may be classified as a sit and wait forager, responds to feed by increasing their swimming activity (Kristiansen and Fernö 2007). This potential dichotomy of how a single behaviour can be interpreted as either poor or good welfare is a good example of how farmers and researchers should develop species-specific welfare indicators based upon the feeding ecology of the species under investigation.

Feeding regime can also affect swimming speeds and manoeuvre complexity irrespective of whether fish are fed to satiation or not. For example, cage-held Atlantic salmon parr and smolts exhibited increased swimming speeds and performed sharper turns during feeding when they received a uniform, temporally predictable ration to perceived daily satiation. However, fish fed to daily satiation using demand feeding technology did not significantly change their swimming speeds and performed moderate turns during feeding (Noble et al. 2007b, c). Fish can also exhibit increasingly complex swimming manoeuvres during feeding in response to underfeeding (Andrew et al. 2004). However, the same study also found that manoeuvre complexity increases with a high feed delivery rate irrespective of ration size, and the authors suggested that this behaviour allows the fish to remain within the feed delivery area (Andrew et al. 2004). Therefore, the same behaviour within the same species can be indicative of both poor welfare (underfeeding) or may simply be a foraging strategy to maximise an individual's feed intake. Further, a high feed delivery rate may reduce an individual's swimming speeds during feeding, as fish switch from swimming to processing and handling prey as feed encounter rate increases (e.g. Priyadarshana et al. 2006). In addition, increases in long-term EMG profiles (obtained via an EMG transmitter calibrated using a hard-wired EMG during U_{crit} tests) were also observed as consequences of the administration of

three diet formulations (organic vs. conventional) (Carbonara 2009) and in experiments evaluating the consequences of starvation (McFarlane et al. 2004).

Behaviour modifications in response to pathogens, parasites and pollutants have been well documented by the industry and in the scientific literature. These include increased activity, such as flashing on the water surface, as well as decreased activity and lethargy (Warren 1982). Altered host behaviour as a consequence of infection may be an important criterion for identifying infected fish that are experiencing reduced welfare. Disease is detrimental to swimming performance in sockeye salmon, *Oncorhynchus nerka* (Tierney and Farrell 2004), and bull trout, *Salvelinus confluentus* (Jones and Moffitt 2004). An increased parasite load can also reduce swimming performance in Atlantic salmon (Wagner et al. 2003). Mycobacterium-infected delta smelt, *Hypomesus transpacificus*, also showed a 20% reduction in swimming performance during U_{crit} tests (Swanson et al. 2002). However, a wide range of parasites only impact significantly upon the host swimming behaviour patterns when they reach a certain developmental stage or infection intensity threshold; thus, changes in individual swimming behaviour can be correlated with severe infections and could potentially indicate poor welfare (Barber 2007). In addition, injuries such as lordosis (Basaran et al. 2007) can also be detrimental to swimming performance. Therefore, reduced swimming speeds or occasions where fish cease schooling and become immobile or inactive can also be an indicator of fish being sick, a disease outbreak, increased parasite load or an indicator of injury in farmed fish.

Fish can also increase their swimming speeds in response to increased stocking density, e.g. rainbow trout (Cooke et al. 2000) and Atlantic halibut (Kristiansen et al. 2004). Similarly, EMG profiles showed that sea bass reared at higher stocking density used on average 25% more energy than at lower stocking density (Scolamacchia 2009). Other common aquacultural procedures, such as handling, chasing, air exposure and changes in light conditions, can also lead to elevated swimming speeds in Atlantic salmon and sea bass (FASTFISH 2009). Further, EMG monitoring was demonstrated to be an effective indicator of fish welfare for investigating energetic responses and stress levels related to transportation in rainbow trout (Chandroo et al.

2005). In fact, a significant posttransportation swimming activity increment was recorded. Furthermore, after a recovery period of 48 h, the oxygen consumption level continued to be significantly elevated, and the swimming performance (measured as critical speed and endurance) of the transported fish was still impaired. Poli et al. (2005) also reported increased swimming speeds during both crowding procedures and slaughter using CO₂ narcosis. Continuous lighting can increase swimming activity in African catfish (Almazán-Rueda 2004), while the type of rearing systems can have an impact upon swimming speeds. Recent research has shown that Atlantic salmon reared in submerged cages for both short- and long-term submergence periods increase their swimming speeds, which may be a strategy allowing them to cope with reduced buoyancy (Dempster et al. 2009; Korsøen et al. 2009).

In summary, farmers and researchers should have a thorough knowledge of fish swimming activity under normal rearing conditions, noting any short-term changes in swimming, such as reduced or elevated swimming speed or increased manoeuvre complexity, as an acute indicator of potentially detrimental welfare conditions. Fish farmers can monitor swimming activity using, for example, telemetry techniques associated with remote sensors (in cages or tanks) and cameras. Possible strategies to maintain normal swimming activity could be related to appropriate water qualities and stocking densities for each species, appropriate feeding regimes and satisfactory disease prevention.

Group swimming behaviour

Cultured fish are reared in large numbers at high densities, and it is impossible to assess the behaviour or welfare of all individuals. Assessments of group behaviour can be used as an operational on-farm welfare indicator and are what most fish farmers use daily to evaluate the hunger, stress level and health status of fish. Group swimming behaviour is defined as the spatial distribution and swimming activity of groups of fish held within an aquaculture production unit and covers shoal structure, polarisation, the horizontal and vertical distribution of the group and their swimming speed and direction. A shoal is defined as a group of fish that remain together

voluntary, while synchronised and polarised fish shoals are termed schools (Pitcher and Parrish 1993).

The motivational state of an individual or the tendency to perform certain behaviours will vary with its internal affective and physiological states, which again is a result of external and internal sensory and neuroendocrine signals and the subjective appraisal of these. The motivational state or welfare state of the fish will affect the ‘traffic rules’ used by the fish, which also should give observable changes in the group swimming behaviour and emergent properties of the whole group, which can be used as indicators of stress level or welfare. Model studies have shown that only one fish with different ‘traffic rules’ can affect the behaviour of the whole group (Romey 1996), indicating that this can be a sensitive indicator if we are able to correctly interpret the behaviour.

When held at high density, individual fish have to respond to the behaviour of others, and the group can adopt polarised schooling just by swimming in a way that minimises the risk of collisions (Føre et al. 2009). Polarity, group velocity and interindividual spacing are characteristics of fish shoals that strongly affect individual members (Pitcher and Parrish 1993, Parrish et al. 2002). These characteristics are group-level ‘emergent properties’: collective outcomes of behavioural interactions among members, not under direct control of any single member. Simulation models show that many of the different emergent structures can be explained by forces that act on each individual and are modulated in response to other individuals’ behaviour. Examples of forces include the following: biomechanical drag, aggregative attraction and repulsion and arrayal velocity matching (Parrish et al. 2002).

Farmed species are chosen because of their ability to thrive at high densities. In fish tanks and cages, there will usually be areas that are more favourable than the others and restricting these volumes will result in a trade-off between packing density (number of fish per cubic metre, Juell and Fosseidengen 2004) and environmental conditions. Especially in sea cages, there can be large environmental gradients, with highly variable temperature, salinity, oxygen, current speed and light conditions (Juell 1995, Juell and Fosseidengen 2004, Johansson et al. 2006, 2007, Oppedal et al. 2007). Fish are attracted to the most optimal areas and avoid the unfavourable ones as a result of both attractions to a part of the rearing

volume with preferred environmental conditions and avoidance/repulsion from areas with poor environmental conditions. Such conditions cause a trade-off between packing density and the benefits of being in the relatively best areas, and at high stocking densities, the less competitive fish will be forced to stay in suboptimal areas (Juell 1995, Johansson et al. 2006, Oppedal et al. 2007). The behaviour of the fish can also have an effect on environmental conditions, and the high local concentrations of fish can cause reduced water flow and increased oxygen consumption leading to areas with hypoxia. Frequent profiling measurements of these conditions are necessary to relate the behaviour to environmental conditions.

In most fish species, exposure to negative stimuli leads to rapid escape movements towards the bottom of the tanks or cages, and concentration of the group near the bottom can indicate relatively recent exposure to acute stressors (Stien et al. 2007, FASTFISH 2009; Bratland et al. 2010).

In situations of acute stress, the fish react initially with reflexes that transmit through the shoal by fish copying or matching the behaviours of their nearest neighbours (Føre et al. 2009). In salmon cages, it is possible to observe strange emergent structures like fast swimming in ‘tornados’ or ‘hour glass behaviour’ of streams of fish moving fast from one location to another, which is also seen in wild fish schools stressed by predators (Pitcher and Parrish 1993). This is probably caused by copying behaviour, confusion and cognitive demands on the individuals, which then surrender their control to that of the school. Emergent structures can also spread to unstressed fish since this behaviour itself signals danger and can cause stress. However, similar (but not equal) behaviour can also be a positive indicator of high-feeding motivation (in sea bass), so these indicators differ according to species, life stage and rearing method.

A common indicator of good health and coping ability is the anticipatory response of the fish to feeding and sounds or signals associated with feeding. In aquaculture cages and tanks, this can be shown as a high concentration of fish near the feeding area and increased schooling activity (Juell et al. 1994, Chen and Purser 2001). A good feed anticipatory response and feed intake can be signs of high feeding motivation and unstressed fish (Fernö et al. 1995, 2006; Folkedal 2010). Experiments with salmon subjected to classical conditioning showed

that the anticipatory response after giving a conditioned signal was affected by acute stress, and the time for recovery to normal baseline behaviours was longer than the period of elevated cortisol levels (Stien et al. 2007, Bratland et al. 2010). However, increased swimming activity prior to and during a meal can also be a sign of underfeeding or a poorly designed feeding regime (e.g. Andrew et al. 2004; Noble et al. 2007b, c). Therefore, care must be taken to avoid underfeeding as the ensuing feeding frenzy could be interpreted as a positive indicator of welfare.

Stereotypic and abnormal behaviour

Mason (1991) described stereotypic behaviour as a behavioural pattern that is repetitive, invariant and with no obvious goal or function. This definition has been revised following several studies that suggest stereotypic behaviour has an adaptive goal as it allows, for example, an increase in opioid activity that provides individuals with a reward experience (e.g. Cronin et al. 1986).

Observations of stereotypic behaviour and other abnormal behaviours have been subjected to intense study in zoo animals (Mason and Rushen 2006) and terrestrial farm animals (Von Borell 1995), and it is repeatedly used as an indicator of poor welfare. The link between the appearance of stereotypic behaviour and poor welfare seems obvious when it results in physical injury or arises during suboptimal housing and rearing conditions. However, this link is not so clear cut as these repetitive behaviours may generate positive (reward) feelings as part of a coping strategy and therefore be compatible with good welfare (Dawkins 1998). Despite the debate on stereotypic behaviour and feelings, it is generally accepted that the development of stereotypes arises as a response to frustration, discomfort or a conflict of motivation (Würbel and Stauffacher 1997).

There is very little evidence of stereotypic behaviour in farmed fish. Almazán-Rueda (2004) distinguished two stereotypic patterns in African catfish, which swim continuously and compulsively in fixed circular or triangular patterns of 10–50 s or 60–240 s. Both Almazán-Rueda (2004) and van de Nieuwegiessen et al. (2008) demonstrated that African catfish stereotypic behaviour can be used as an indicator of reduced welfare or chronic stress (related to stocking

density). Kristiansen et al. (2004) also reported stereotypic behaviour in Atlantic halibut, described as loops of vertical swimming behaviour. These authors showed an increase in stereotypic behaviour when halibut was kept at high stocking densities. Atlantic halibut were shown to develop stereotypic behaviour in shallow tanks and also when fed floating pellets instead of sinking pellets (Kristiansen et al. 2004, Kristiansen and Fernö 2007), suggesting that the rearing system and feeding method may act as chronic stressor in flatfish.

Other behaviours described as abnormal or a kind of stereotypic behaviour have been identified in Atlantic salmon (e.g. types of circular shoaling in cages Kristiansen et al. 2004) and Mozambique tilapia (e.g. vacuum pit digging when housed in the absence of substrate, Galhardo et al. 2008).

Despite the scarcity of literature, the appearance of stereotypes has been linked with aquaculture procedures and stressors and therefore can be an indicator of poor welfare in farmed fish.

Mental states and possible associated welfare indicators

The data reviewed thus far show that the majority of studies on fish welfare have been focused on identifying behavioural indicators of poor welfare (summarised in Table 1). Very little has been done both on identifying indicators of good welfare and on developing strategies to improve the welfare of farmed fish. The possibility that fish are sentient creatures and capable of affective states or feelings will contribute not only to strategies for avoiding poor welfare but also for promoting good welfare.

Feelings are evolved emergent properties arising from the central nervous system, which interfere with memory and learning processes, providing higher flexibility and better responses to dangers, thus increasing fitness (Broom 1998; Dawkins 1998). In other words, the negative and positive valence of stimuli interpreted in a conscious domain may play an important role in the mechanisms of getting what animals need and avoiding what they do not need. The emergence of consciousness in fish has been a matter of intense scientific debate (e.g. Rose 2002; Chandroo et al. 2004a, b). Some authors, e.g. Rose (2002, 2007) and Cabanac et al. (2009), argue that

Table 1 Examples of behavioural changes in responses to aquaculture procedures or stressors that could be used as species-specific operational welfare indicators in farmed fish

Welfare level	Behaviour	Aquaculture procedures/ stressors	Species	Operational welfare indicator	References
Good	Exploratory activity	Stocking density	Rainbow trout <i>Oncorhynchus mykiss</i>	Low stocking densities: ↑ use of self-feeders	Boujard et al. (2002)
	Food-anticipatory activity	Feeding method	Gilthead Sea Bream <i>Sparus aurata</i>	Scheduled feeding (vs. random): ↑ food anticipatory activity	Sánchez et al. (2009)
Poor	Aggression	Feeding method	African catfish <i>Clarias gariepinus</i>	Hand feeding (vs. self-feeding): ↑ % of fish bitten	Almazán-Rueda et al. (2004)
			Atlantic salmon <i>Salmo salar</i>	Fixed ration (vs. demand feeding): ↑ aggressive acts	Noble et al. (2007b)
	Photoperiod and light intensity	Feeding method	Atlantic salmon <i>Salmo salar</i>	Underfeeding (vs. satiation): ↑ aggressive acts	Cañon Jones et al. (2010)
			African catfish <i>Clarias gariepinus</i>	Long periods of light and high light intensity: ↑ skin lesions	Almazán-Rueda et al. (2004, 2005)
Foraging behaviour	Stocking density	Feeding method	African catfish <i>Clarias gariepinus</i>	Low stocking densities: ↑ skin lesions	Almazán-Rueda et al. (2004) and van de Nieuwegeessen et al. (2008, 2009)
			Rainbow trout <i>Oncorhynchus mykiss</i>	High stocking densities: ↑ aggressive behaviour	Ellis et al. (2002)
	Grading	Feeding method	White sea bream <i>Diplodus sargus</i>	High stocking densities: ↑ social interactions	Karakatsouli et al. (2007)
			African catfish <i>Clarias gariepinus</i>	Homogenous groups of small fish: ↑ % of fish bitten	Martins et al. (2006b)
	Water exchange rate in RAS	Feeding method	Atlantic halibut <i>Hippoglossus hippoglossus</i>	Floating pellets (vs. sinking): ↓ number of pellets eaten	Kristiansen and Fernö (2007)
			Nile tilapia <i>Oreochromis niloticus</i>	High water exchange rates: ↓ feeding latency in small size classes	Martins et al. (2009)
	Fasting periods	Feeding method	African catfish <i>Clarias gariepinus</i>	Homogenous groups of small fish: ↓ total feeding time and feeding rate	Martins et al. (2005)
			Sea bass <i>Dicentrarchus labrax</i>	↑ Feeding rate and daily feeding times after feeding is resumed	Rubio et al. (2010)
	Swimming activity	Hypoxia	Sea bass <i>Dicentrarchus labrax</i>	↓ Self-feeding	Rubio et al. (2010)
			Atlantic cod <i>Gadus morhua</i>	↓ Swimming speed	Schurmann and Steffensen (1994) and Herbert and Steffensen (2005)
	Hyperoxia	Swimming speed	White sturgeon <i>Acipenser transmontanus</i>	↓ Swimming speed	Crocker and Cech (1997)
			Brook charr <i>Salvelinus fontinalis</i>	↑ Swimming speed	Tang and Boisclair (1995)
Recovery test		Sockeye salmon <i>Oncorhynchus nerka</i>	↓ Recovery test	Farrell et al. (1998)	
		Nile tilapia <i>Oreochromis niloticus</i>	↑ and ↓ Swimming activity of schools	Xu et al. (2006)	
Swimming speed	Swimming speed	Atlantic salmon <i>Salmo salar</i>	↓ Swimming speed	Espmark and Bæverfjord (2009)	

Table 1 continued

Welfare level	Behaviour	Aquaculture procedures/stressors	Species	Operational welfare indicator	References
		Pollutants	Sockeye salmon <i>O. nerka</i>	Dehydroabietic acid : ↓ recovery test	Jain et al. (1998)
			Brown trout <i>Salmo trutta</i>	Cu: ↓ recovery test	Beaumont et al. (1996)
			Juvenile lake chubsuckers <i>Erimyzon sucetta</i>	Ash: ↓ U_{crit}	Hopkins et al. (2003)
	Infections		Delta smelt <i>Hypomesus transpacificus</i>	↓ U_{crit}	Swanson et al. (2002)
	Parasites		Atlantic salmon <i>Salmo salar</i>	↓ U_{crit}	Wagner et al. (2003)
	Temperature		Largemouth bass <i>Micropterus salmoides</i>	Low temperature: ↓ U_{crit}	Hasler et al. (2009)
	Transportation		Rainbow trout <i>O. mykiss</i>	↑ EMG	Chandroo et al. (2005)
	Underfeeding		Atlantic cod <i>Gadus morhua</i>	↑ Swimming speed	Björnsson (1993)
			Rainbow trout <i>O. mykiss</i>	↑ EMG	McFarlane et al. (2004)
			Gilthead sea bream <i>Sparus aurata</i>	↑ Swimming speed; ↑ school manoeuvre complexity	Andrew et al. (2004)
			Turbot <i>Scophthalmus maximus</i>	↑ Swimming speed	Huse and Skiftesvik (1985)
	Short- and long-term cage submergence		Atlantic salmon <i>Salmo salar</i>	↑ Swimming speed	Dempster et al. (2009) and Korsøen et al. (2009)
	Photoperiod and light intensity		African catfish <i>Clarias gariepinus</i>	Long periods of light and high light intensity: ↑ swimming activity	Almazán-Rueda et al. (2004, 2005)
	Photoperiod		African catfish <i>Clarias gariepinus</i>	Long periods of light: ↑ swimming activity	Almazán-Rueda et al. (2005)
	Stocking density		African catfish <i>Clarias gariepinus</i>	High stocking density: ↑ swimming activity	van de Nieuwegessen et al. (2008, 2009)
			European sea bass <i>Dicentrarchus labrax</i>	High stocking density: ↓ swimming speed	Santos et al. (2010)
			European sea bass <i>Dicentrarchus labrax</i>	High stocking density: ↑ EMG	Carbonara et al. (2008)
			Atlantic halibut <i>Hippoglossus hippoglossus</i>	High stocking density: ↑ swimming activity	Kristiansen et al. (2004)
	Environmental gradients		Atlantic salmon <i>Salmo salar</i>	Changes in Space use and patchiness	Juell and Fosseidengen (2004), Johansson et al. (2006), and Oppedal et al. (2007, 2011)
	Feeding method		African catfish <i>Clarias gariepinus</i>	Hand feeding (vs. self-feeding): ↑ swimming activity	Almazán-Rueda et al. (2004)
			Atlantic salmon <i>Salmo salar</i>	Scheduled (vs. on demand): ↑ swimming speed and turning angles	Noble et al. (2007b, c)

Table 1 continued

Welfare level	Behaviour	Aquaculture procedures/stressors	Species	Operational welfare indicator	References
Ventilatory activity		Stocking density	African catfish <i>Clarias gariepinus</i>	High stocking density: ↑ air breathing	van de Nieuwegesessen et al. (2008, 2009)
		Hypoxia	Nile tilapia <i>Oreochromis niloticus</i>	↑ Ventilator frequency	Xu et al. (2006)
Stereotypic behaviour and abnormal behaviours		Confinement	Nile tilapia <i>Oreochromis niloticus</i>	↑ Ventilator frequency	Barreto and Volpato (2004)
		Handling	Rainbow trout <i>Oncorhynchus mykiss</i>	↑ Ventilator frequency	Brydges et al. (2009)
		Stocking density	African catfish <i>Clarias gariepinus</i>	High stocking density: ↑ escape attempts	Van de Nieuwegesessen et al. (2008)
		Feed characteristics	Atlantic halibut <i>Hippoglossus hippoglossus</i>	High stocking density: ↑ loops of vertical swimming; ↑ surface swimming Floating pellets (vs. sinking): ↑ loops of vertical swimming	Kristiansen et al. (2004) Kristiansen and Fernö (2007)

this is not possible because their behaviour is simple and reflexive and they lack a neocortex. Yet, a growing body of evidence related to cognitive (Braithwaite 2006), neuroanatomic (Portavella and Vargas 2005; Rodriguez et al. 2006) and emotional (Sneddon 2007; Yue et al. 2008) aspects of fish behaviour provides strong support for sentience (the ability to feel) in fish. Good welfare should therefore be related to behaviours associated with attraction and positive anticipation and reward behaviour, while bad welfare should be associated with frustration, aversion, fear, pain and sickness behaviours.

The measurement of exploratory behaviour, food-related anticipatory activity (reward anticipation), preferences and reward-related operant behaviour has been suggested as indicators of positive fish emotions and welfare (Galhardo 2010; Galhardo et al. 2011a). Exploratory behaviour has been suggested to be self-rewarding, increasing the predictability of, and control over, the environment (Schulze Westerath et al. 2009). These characteristics have led exploratory behaviour to be proposed as a candidate for assessing positive welfare in terrestrial farm animals (Schulze Westerath et al. 2009). In farmed fish, very little has been done on assessing positive welfare. Indirect suggestions come from the effect of stressors on the use of self-feeders. Boujard et al. (2002), for example, showed that increased stocking densities of rainbow trout reduced the number of operant tasks (use of self-feeders) executed to obtain a fixed food reward. Other examples, although not in an aquaculture context, come from exposing rainbow trout to noxious stimuli, which has been shown to affect an individual's exploratory behaviour (Sneddon et al. 2003). In addition, Galhardo (2010) showed a reduction in neophobia when Mozambique tilapia are kept under stable social conditions, further supporting the use of exploration behaviour as a tool to assess (positive) welfare.

In certain species and under certain conditions, food-anticipatory activity can also be an indicator of good welfare (Kristiansen and Fernö 2007), as it may indicate a behavioural priority for foraging instead of being hidden or trying to escape. Increased swimming activity around feeding areas prior to feeding time is often observed in fish farms. This food-anticipatory activity may allow fish to predict the exposure to appetitive stimuli and have some awareness of control, which have been suggested to be

important appraisal modulators of the fish responses to their environment (Galhardo et al. 2011b).

Preference tests that allow animals to display their needs (Dawkins 2004) are used to assess fish welfare. Galhardo et al. (2009) showed that the lack of substrate is likely to decrease the welfare of breeding males in Mozambique tilapia. This approach considers that good welfare may not be equitable in all individuals. Indeed, recent studies suggest that stress response variability does not only depend on the individual's situation but also its cognitive evaluation of the situation, i.e. the way the stressor is appraised (Galhardo et al. 2009). Such knowledge could contribute to practical applications in animal production, of enhancing positive experiences and long-term positive emotional states, and including positive welfare indicators in on-farm monitoring systems (Boissy et al. 2007).

Reward-related operant behaviours are less used in fish welfare studies. However, Galhardo et al. (2011a) has shown how Mozambique tilapia can push a door in order to access different resources, where the degree of motivation to obtain food or social contact was clearly higher than for access to a suitable substrate.

Promoting positive welfare includes, for example, providing opportunities for exploration, which requires complex environments. Environmental enrichment aims to increase the environmental complexity in its physical, temporal and/or social dimensions. Physical complexity includes structural, visual, auditory, olfactory and gustatory stimuli, whereas temporal complexity refers to the degree to which physical stimuli changes (Carlstead and Shepherdson 2000). These levels of complexity are often missing in an aquaculture context. Indeed, when compared with the environment occupied by their wild conspecifics, intensively farmed fish are kept under highly standardised conditions, which often mean a complete lack of enrichment. Farmed fish are cultured for long periods of time in the same tanks of the same colour(s), the same shape and exposed to the same, potentially harmful, background noises. They are fed with the same diets, which have the same taste, same colours and often given at the same time of the day. In addition, appropriate social complexity is of paramount importance for some species, and often, the group size and composition are altered via grading or sorting procedures. If welfare-friendly

housing systems are to be developed for farmed fish, we first need to understand the consequences of environmental enrichment on fish behaviour, physiology and performance. This information is scarce in farmed fish. Galhardo et al. (2008) showed that the use of tank substrate affects the reproductive behaviour and physiology, including behavioural needs (see below), in Mozambique tilapia males. Höglund et al. (2005) showed that providing hiding materials affects the fear response to alarm cues from conspecifics in crucian carp, while tank design and colour have also been shown to improve farmed fish welfare (Ross et al. 1995; Karakatsouli et al. 2007). The use of feeding systems or regimes that deliver a responsive ration in relation to appetite levels can also improve welfare by reducing the chances that fish are underfed (Suzuki et al. 2008), by reducing competition (Andrew et al. 2002; Noble et al. 2007b) and by reducing the frequency of injury and damage (Noble et al. 2008; Suzuki et al. 2008). Juell and Fosseidengen (2004) suggested the use of underwater lamps to avoid crowding and improve the welfare of caged Atlantic salmon. The efficacy of these and other strategies for routine use in aquaculture needs further verification, particularly the impact of environmental enrichment on maintenance and water quality.

The assessment of frustrated behavioural needs is related to indicators of poor welfare (Ashley 2007). Galhardo et al. (2009) showed that Mozambique tilapia perform vacuum pit building in the absence of appropriate substrate, which, under normal circumstances, males use to build their spawning pits. Behavioural integrity, in which animals should be allowed to perform their natural repertoire of behaviour to guarantee their welfare, has also been assessed as an indicator of poor welfare (Würbel 2009). Despite the recognition that an aquaculture environment differs significantly from natural habitats, with a questionable biological relevance of some of the fish behavioural repertoire under natural conditions, natural fish behaviour has generally been used as an indicator of fish welfare. Foraging and swimming behaviours are examples of natural behaviours that have been taken into consideration in aquaculture procedures. As mentioned above, flatfish are bottom feeders and show improved welfare when fed sinking pellets as compared to floating pellets (Kristiansen and Fernö 2007). The swimming behaviour of certain species, such as flatfish, has led to novel tank design,

for example raceways to maximise the surface area, and allow flatfish to rest on the bottom, indeed, when denied access to a resting area flatfish develops stereotypic behaviour, while schooling salmonids, sea bream and sea bass need to swim constantly and are often cultured in circular tanks.

There is still room to introduce new techniques or strategies in order to improve the welfare of farmed fish. One of the most obvious approaches is to adapt the rearing systems not only to the physiological but also to the species-specific behavioural needs. At present, the production of the most relevant European farmed fish (i.e. Atlantic salmon, sea bream and sea bass) utilises technology that allows fairly good growth and survival rates, while the welfare level, including the mental dimension, of these species remains unclear. This is partly because there is still a lack of knowledge on the basic principles of behavioural, physiological and cognitive responses to stress under captivity. The basic question ‘what is it like to be a fish under aquaculture conditions’ remains to be answered. Thus far, the scientific community has made considerable efforts to understand *what* causes stress and *how* a stress response can be characterised in farmed fish. However, the future calls for answers to questions such as *when* is a potential stressor a real stressor, *for how long* a stressor remains a stressor, *why* is a stressor interpreted as such and *why* different individuals react differently to the same stressor?

An emergent area of fish research is the understanding of psychological mediators of stress. For example, predictability has been suggested as a tool to manipulate the way animals interpret stimuli or stressors (Wiepkema 1987; Greiveldinger et al. 2009; Veissier et al. 2009). This suggestion is supported by studies in rats that showed that the predictability of negative events (such as electric shocks) leads to a reduction in gastric ulcers as compared to rats that cannot predict the shock event (Guile 1987). Predictability of negative stimuli such as noise (Prior 2002) or introduction into an unfamiliar cage (Muir and Pfister 1986) has also been shown to result in reduced stress responses and better learning abilities in rats (Muir and Pfister 1986; Orsini et al. 2002; Prior 2002). Carlstead (1986) showed a positive effect of predictability of feeding on growth and aggressive behaviour in pigs, while the predictability of a sudden event was shown to lower subsequent emotional

responses in sheep (Greiveldinger et al. 2007). These results encourage the use of predictability to improve farm animal welfare. Very little is known about the effects of predictability on fish. Galhardo et al. (2011b) showed that Mozambique tilapia subjected to predictable confinement exhibit lower cortisol levels than fish that could not predict such stimuli. The relieving effects of predictability have been suggested to rely on a sense of both safety during periods of nonsignalisation and control by allowing self-preparation for the future events (Orsini et al. 2002; Bassett and Buchanan-Smith 2007). However, the use of highly predictable environments may lead to a hypostimulation of the HPI axis as a consequence, resulting in a situation that may be considered analogous to boredom (Wemelsfelder 1993).

The incorporation of concepts such as stability through change, i.e. allostasis (Korte et al. 2007), coping strategies (Øverli et al. 2007), appraisal and cognition (Galhardo et al. 2009) in farmed fish welfare will certainly allow a more comprehensive picture of how farmed fish welfare should be assessed, utilising both positive and negative indicators.

Conclusions

Behaviour represents a reaction to the environment as fish perceive it and therefore is a key element of fish welfare. In a developing and increasingly competitive aquaculture industry, it is of paramount importance that both farmers and researchers use species-specific behavioural signals for an early assessment of poor fish welfare. Furthermore, when considering the potential dichotomy of how behaviour can be interpreted as either good or poor (e.g. individual swimming activity) depending on the fish species, it is essential to develop an a priori understanding of the species-specific biology before drawing any conclusions in relation to welfare. Whether the same behavioural welfare indicator can be consistently used, and means the same throughout the life cycle of a fish, also needs to be addressed by future research.

It is also worth noting that fish differ in their coping strategies and perception of external events. Therefore, while one individual may interpret a situation as being highly stressful, another may interpret it as mildly stressful or even not at all stressful. Such

variation in individual responses may make an overall assessment of welfare difficult. However, with the ongoing development of underwater video technology and computer image processing, the possibility of sampling high number of individual fish at a low cost will fall increasingly within reach. Another alternative is to develop group-based welfare indicators, such as spatial distribution.

The variability in coping strategies between individuals also emphasises that not all individuals within a group will have a good welfare status. In fact, group-based indicators may hide poor welfare at an individual level. Providing the culture environment with some sort of modifications (e.g. providing escape routes, hiding places, etc.) may help synchronise the culture environment with the inherent individual variability in stress responses present in farmed fish.

The interpretation of the meaning of behavioural changes in terms of welfare will also demand the willingness to accept that these changes are influenced by other factors besides the stressor itself, including mental states. Accepting that fish can be subjected to psychological stress will contribute to the development of strategies (e.g. environmental enrichment or allowing fish to predict or anticipate changes in the environment) that contribute to good welfare practices under aquaculture conditions.

If the interpretation of welfare in commonly reared farmed fish has yet to be resolved, the welfare of new fish species for aquaculture is even more incipient. There are at present a number of new fish species being considered for fish farming, such as the bluefin tuna *Thunnus thynnus* and the sparid common dentex *Dentex dentex*. While much attention is being placed on larval/broodstock and grow-out technologies, little has been done on the needs of these species that may have an impact on their welfare. Using the same technologies and rearing systems already in use for other fish species may not be enough to ensure the welfare of these new fish for aquaculture.

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