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Health of farmed fish: its relation to fish welfare and its utility as welfare indicator

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Abstract This brief review focuses on health and biological function as cornerstones of fish welfare. From the function-based point of view, good welfare is reflected in the ability of the animal to cope with infectious and non-infectious stressors, thereby maintaining homeostasis and good health, whereas stressful husbandry conditions and protracted suffering will lead to the loss of the coping ability and, thus, to impaired health. In the first part of the review, the physiological processes through which stressful husbandry conditions modulate health of farmed fish are examined. If fish are subjected to unfavourable husbandry conditions, the resulting disruption of internal homeostasis necessitates energy-demanding physiological adjustments (allostasis/acclimation). The ensuing energy drain leads to trade-offs with other energy-demanding processes such as the functioning of the primary epithelial barriers (gut, skin, gills) and the immune system. Understanding of the relation between husbandry conditions, allostatic responses and fish health provides the basis for the second theme developed in this review, the potential use of biological function and health parameters as operational welfare indicators (OWIs). Advantages of function- and health-related parameters are that they are relatively straightforward to recognize and to measure and are routinely monitored in most aquaculture units, thereby providing feasible tools to assess fish welfare under practical farming conditions. As the efforts to improve fish welfare and environmental sustainability lead to increasingly diverse solutions, in particular integrated production, it is imperative that we have objective OWIs to compare with other production forms, such as high-density aquaculture. However, to receive the necessary acceptance for legislation, more robust scientific backing of the health- and function-related OWIs is urgently needed.

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Introduction

Welfare of an animal is a complex issue, and a unified definition is hard to find in the literature. Nevertheless, most definitions of animal welfare take into account the three categories: feelings, nature and function. Briefly, the first is concerned with the subjective experience of the animal and implies that the animals are sentient and able to suffer, i.e. experience pain and fear (Chandroo et al. 2004). In this category, good welfare is defined as the absence of negative feelings and the presence of positive feelings (cf. the concept of the five freedoms). The second category requires that an animal is allowed to express its natural behaviour. The third category, based on functional definitions, focuses on the ability of the animal to cope and acclimate to its environment without being forced beyond its physical capacity. In this category, good welfare can be viewed upon as the ability to maintain homeostasis and normal biological functions. Ultimate reflections of good welfare from the function-based point of view are good health and absence of disease, and with respect to aquaculture, good productivity (e.g., Turnbull and Kadri 2007; Volpato et al. 2007). In this review, we focus on health and biological function as cornerstones of fish welfare. Although health and welfare are intimately connected (Moberg 2000; Ashley 2007), we are aware that a health-based approach to fish welfare is in a way reductionistic and does not take into account all components of welfare. While the physical health of an animal is fundamental for good welfare (Ashley 2007; Duncan 2005), the fact that an animal is healthy does not necessarily mean that it has a good welfare status. Thus, welfare is the broader, more overarching concept than the health concept.

In aquaculture, the term 'health' is often interpreted as 'absence of overt disease', and thus, emphasis is given to disease prevention and eradication. In this communication, however, the term 'health' is widened beyond the absence of disease to also cover pathology defined as detrimental arrangements of molecules, cells, tissues and their dysfunction (reviewed by Broom 2007). Health, from this perspective, means the ability of an animal to perform normal physiological functions and to maintain homeostasis, thereby supporting its ability to withstand infectious and non-infectious stressors. As such, good health is essential (but not yet sufficient) for good welfare (Ashley 2007; Duncan 2005). Poor health, i.e. the reduced ability of the animal to perform normal functioning, to acclimate to stressful conditions and to prevent disease, implies a bad welfare status. Importantly, it is often poor welfare itself, which is a precondition to loss of health, with the important exception that healthy animals in an optimal welfare situation may still suffer an acute infection which by definition is regarded as poor health. Stressors (e.g. handling, inappropriate husbandry conditions such as confinement or crowding leading to accentuated social interactions between conspecifics, poor water quality and pathogen occurrences) present in culture systems are potential threats to animal welfare and thereby to animal health (Huntingford et al. 2006), as they impose an allostatic load on the animal, which in the short term will impair its physiological homeostasis and in the long term its condition and health status (Broom and Corke 2002; Roger 2008). The close link between health and welfare is also evident from the 'concept of the five freedoms' that has been developed in order to secure welfare. This concept includes freedom from thirst, hunger and malnutrition, freedom from discomfort by providing adequate environmental quality parameters, freedom from distress by ensuring adequate living conditions and freedom from disease. For example, when an animal has no access to food, its welfare ('freedom of hunger') is impaired, but at the same time, starvation places the fish at risk of developing metabolic problems (Fig. 1, quadrant C) and eventually metabolic diseases and infections, which are health problems (Fig. 1, quadrant D). In this scenario, poor welfare precedes poor health. However, bad health may during certain circumstances also result in bad welfare. In Fig. 1, quadrant A, fish reared in an optimal environment, determined by the biology of the fish, experience a minimal allostatic load, show no signs of health problems, and the welfare is maximized. However, all healthy animals may be struck by an acute infection that reduces the welfare of the fish (Fig. 1,

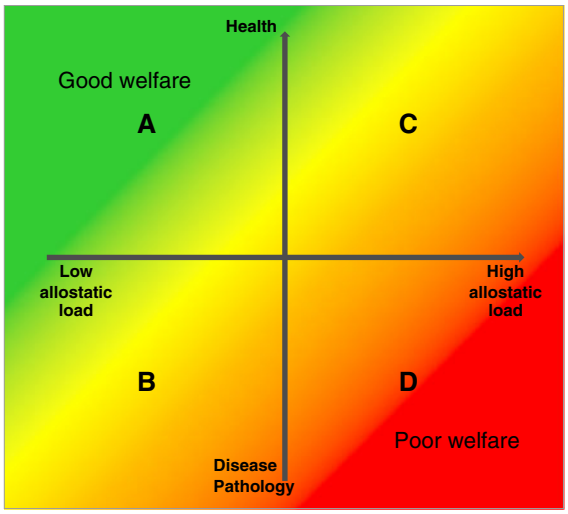

Fig. 1 The graph visualizes the distinctions and links between the concepts of health and welfare from a function-based point of view. When plotting health against allostatic load, four different conditions that fish can experience are discernible (quadrants **a**, **b**, **c** and **d**): In **a** fish are in an optimal physical environment with low level of stressors, while at the same time being healthy. Thus, the welfare is defined as good. In **b** the allostatic load is low, but pathogen-induced disease is present. As a consequence, welfare is reduced for the individual infected fish because of disease symptoms. In **c** stressor intensity is higher, what affects health and the ability of the fish to cope with its environment, but the intensity is not yet strong enough to cause disease—a situation yielding acceptable welfare. However, there is risk of increased disease susceptibility that by definition is reduced welfare. Finally, in **d** there is high allostatic load as well as disease, which lead to the worst welfare status

quadrant B), a scenario where bad health will lead to bad welfare. The inherent link between health and welfare is further emphasized by Dawkins (2006) who suggested that an animal's welfare can be characterized by asking 'does the animal get what it wants' and 'is the animal healthy?' Thus, although close and mutual links exist between welfare and health, the knowledge of how alterations in homeostasis and health status translate into welfare of the fish and vice versa is currently rather limited.

An advantage of assessing health in a broad sense, including both pathological changes and disease, is that it provides a pragmatic approach to the assessment of fish welfare in culture situations. Since health parameters are routinely monitored in most aquaculture units, they can provide information on at least some aspects of the current welfare status of the farmed fish. In addition, the health-related approach allows us to formulate testable hypotheses to develop operational welfare indicators for the practice.

This communication aims to discuss the utility of the health-related approach in fish welfare assessment. Firstly, we will examine the physiological processes through which husbandry conditions modulate health of farmed fish. This mechanistic understanding provides the basis for the second theme we develop in this communication, i.e. the potential of health parameters as operational welfare indicators. Welfare scenarios that ignore the effects on the environment are doomed to non-sustainability, an aspect that if addressed in full would spring the frame of this short review, but which we allude to at the end of Sect. 3. We will not include the question of sentience and mental health. There is an ongoing controversial discussion as to whether fish possess or lack the brain centres believed to be a prerequisite for a sentient animal; this question will be addressed in other chapters of this volume. Thus, our definition of health refers only to physical health.

Processes through which poor husbandry and welfare conditions translate into impaired fish health

Stressful farming conditions may impair welfare of the fish, and this can translate into reduced fish performance and health. The health of farmed fish is of major concern as impaired health or any disease state is neither agreeable regarding welfare considerations nor economically sustainable. Here, we discuss the processes through which poor welfare conditions in the husbandry system can affect the health status of farmed fish and favour diseases. If the fish is subjected to unfavourable husbandry conditions, this impairs diverse basal physiological functions and disrupts internal homeostasis. The adaptive responses needed to counteract this require energy, and the ensuing drain will lead to trade-offs with other energy-demanding functions of the organisms, including defence mechanisms such as immune responses and/or physiological processes such as growth and reproduction (Lupatsch et al. 2010; Anderson et al. 2011).

In order to better understand the relationships between the various processes, the following section addresses the basic necessities for life (homeostasis) and the physiological systems that maintain it

(allostasis). This discussion will also consider what the physiological limits are for coping with a sub-optimal environment, i.e. what determines the coping ability of an organism. In this context, the energetic costs of allostasis/acclimation and possible consequences on the key defence system of the fish, the immune system, will be briefly discussed.

Homeostasis, stress and allostasis

All living organisms are adapted to the environment they inhabit through the natural selection of evolution. A prerequisite for life is being able to maintain stability of essential physiological systems (homeostasis) within the ranges enabling survival of the animal, despite constant challenges from the external environment (Stott 1981; McEwen and Wingfield 2003). Stress can be considered as a state of threatened homeostasis that is re-established by a complex suite of neural, behavioural and physiological modifications through the stress response (Barton 2002). The stress responses are perhaps the most fundamental reaction preserved among all vertebrates. The primary stress response is initiated by the CNS perceiving cues from the environment and mediated by the hypothalamic–pituitary–inter-renal (HPI) axis and the autonomic nervous system (ANS), resulting in the release of stress hormones, corticosteroids and catecholamines, from the head kidney (Wendelaar Bonga 1997; Barton 2002). Once released into the blood stream, the stress hormones elicit secondary stress responses in the target tissues expressing the appropriate receptors (Mommsen et al. 1999). In general, the secretion of catecholamines is initiated almost immediately after onset of the stress response and is transiently decreased. For example, rainbow trout subjected to moderate hypoxia (<40% DO) responded with a sharp increase in adrenaline with a peak at 24 h but returned to basal values after 48 h, despite maintenance in an hypoxic environment (Thomas et al. 1991). On the other hand, the release of cortisol starts within minutes of stress onset, and elevated levels can be maintained for a longer period of time compared with adrenaline. In fish subjected to a moderate hypoxia of 50% DO, elevated plasma cortisol levels could be measured for up to 30 days after the initiation of hypoxic treatment (Sundh et al. 2010). Well-documented secondary stress responses of fish include metabolic, cellular, osmoregulatory, haematological and immunological changes, manifested, among others, as increased blood pressure and respiration, increased blood glucose and lactate levels, impaired primary barriers towards the environment, heat shock protein production, changes in ion composition, haematocrit, lysozyme activity and antibody production (Wendelaar Bonga 1997; Barton 2002). These will in turn lead to tertiary stress responses such as decreases in growth, swimming capacity, disease resistance and feeding activity and altered behaviour (Wendelaar Bonga 1997; Barton 2002).

The stress response can be described in terms of allostasis, defined as the struggle to maintain homeostasis through changes in physiological systems (McEwen and Wingfield 2003; Korte et al. 2007; Landys et al. 2006). Allostasis allows the animal to actively adjust physiological systems to meet predictable and unpredictable changes in the environment. Thus, stress is not necessarily detrimental to the individual but is an essential adaptive response to promote the best chance of survival in the face of threatening situations (Iwama et al. 1997; Ashley 2007). However, when the allostatic load turns into allostatic overload, the stress becomes distress and turns detrimental (Moberg 2000).

What then determines the scope to cope of an organism, i.e. the ability to acclimate to conditions outside the 'optimal' range? Basically, what sets the scope are the biological 'needs' shaped by millions of years of natural selection, which are therefore different for each single species. A certain degree of tolerance to the displacement from the optimal range may be present, but eventually the displacement will result in disease and/or death. The ability of a fish to acclimate to a stressor, i.e. being able to maintain homeostasis despite the stressor impact, is further affected by the magnitude and duration of the stressor as well as of the number of additional stressors. During more chronic situations, wear and tear on the allostatic systems may eventually lead to severe disturbance and death by exhaustion.

Coping with threats to homeostasis: the cost of acclimation

Energetically, there are costs associated with allostasis, meaning that if a portion of the fish's energy budget is required to cope with stress, then less energy will be available for other biological functioning components (Iwama et al. 1997; Wendelaar Bonga 1997). This is particularly true when

organisms are exposed to stressors from which they cannot escape. The most favourable response to conditions that could constitute an allostatic load on the fish is to avoid it. However, farmed fish are restricted to holding facilities and thus are usually not able to avoid unfavourable conditions. For example, a fish put under allostatic load uses energy reserves in order to acclimate to a stressful environment. Even if the fish has managed to acclimate to a stressor for weeks or months, the energy stores will eventually be depleted. This will indeed affect the ability of fish to meet another stressor or a change in the fish's endogenous development. Thus, it is when energy reserves are depleted, which is highly influenced by severity and duration of the stressor, that the fish is at overload and may no longer be able to acclimatize. When acclimation does not occur, this may give rise to pathology, disease and/or death.

Allostatic load and stress may favour diseases

Empirically, it has been repeatedly demonstrated that standard aquaculture practices can result in increased susceptibility of fish to disease (Mazur and Iwama 1993; Conte 2004; Dror et al. 2006). The questions remain, what are the underlying mechanisms behind these effects and how do allostatic mechanisms contribute? Primary and secondary stress responses are associated with substantial energetic costs, and particularly under conditions of repeated and chronic stress, this will lead to tertiary stress responses such as decreased growth, reduced swimming capacity, impaired disease resistance or lower feeding activity (Wendelaar Bonga 1997; Barton 2002). In this way, stressful husbandry conditions compromise, via the stress response, the health status of the fish and favour disease. In the following sections, the classical stress response and examples of common husbandry conditions will be used as starting points to discuss how husbandry-related stress may affect the primary defence systems of the fish: the epithelial barriers and the immune system.

Consequences of allostatic load/stress on the defence capabilities of the fish: primary barriers

The first lines of defence are the primary barriers, i.e. the mucus and epidermal surfaces in skin, gills and gut, which constitute an interface between the fish and the external world. These barriers regulate, for instance, ion, water and gas exchange with the environment, they are the initial sites of pathogen entry into the fish, and in the case of harmful substances, the barriers are the initial sites of uptake and action of these substances. The nature of the barriers ranges from the physical barrier constituted by the enterocytes and the 'fencing' tight junctions (Sundh et al. 2010), through ABC transporters that protect the fish against uptake of toxic substances into the organism (Sturm and Segner 2005) to the mucosal immune system defending against invading pathogens (Press and Evensen 1999). In this section, we focus on the barrier function of intestine and skin, while for the gills, we refer to recent reviews (e.g., Evans et al. 2005). The following discussion will provide evidence that the functioning of these primary barriers is weakened by poor welfare and stressful husbandry conditions (Fig. 2).

Main characteristics of the intestinal primary barrier The physical intestinal barrier is created by a single layer of epithelial cells, the enterocytes, which are connected to each other on the apical side of the cells through the tight junctions (TJ). TJ do not represent a rigid barrier but rather a fence, regulating the passage of ions, water and other molecules as well as immune cells, through the paracellular pathway. TJ consists of several physiologically regulated proteins forming the circumferential seals around

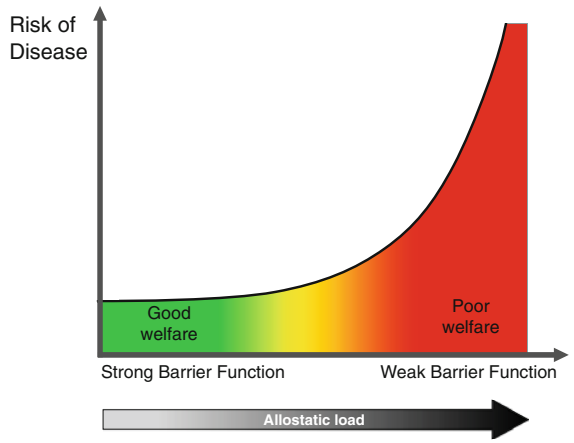

Fig. 2 The graph visualizes the connection between increasing allostatic load/stress, reduced barrier function and increased risk of disease, which from a function-based point of view means a reduced welfare

adjacent epithelial cells. These proteins are connected to the actin ring of the cytoskeleton, creating a continuous structure facing the apical side of the intestinal epithelium (Schneeberger and Lynch 2004).

The intestinal epithelial layer is protected by an extrinsic mucus layer. This is created by the apical release of mucin glycoproteins from goblet cells scattered in the intestinal epithelium (Shephard 1994). An important role of the mucus layer is to prevent uncontrolled attachment of bacteria to the intestinal epithelial cells. This is achieved by the glycoproteins forming a matrix to physically establish an effective diffusion barrier for the bacteria. The mucus layer further contains antimicrobial factors such as antibacterial peptides, lysozyme and proteases (Ellis 2001), which actively neutralize intraluminal pathogens. Moreover, the mucus is constantly replenished by the goblet cells, which results in a continuous flushing of the intestinal lumen, carrying harmful substances and pathogens away and out of the fish.

Within the gut, pathogens such as viruses and bacteria, along with bacterial toxins and other harmful substances, are mixed with nutrients and water. Although the extrinsic and physical barriers together are highly effective in preventing harmful substances from entering the internal milieu, total exclusion is impossible. Pathogens that breach the intestinal barrier will encounter the immunological barrier, *i.e.* the gut-associated lymphoid tissue (GALT).

Main characteristics of the skin primary barrier The physical skin barrier comprises an epidermis made of a stratified squamous epithelium, together with underlying dermis containing the scales and finally the hypodermis. The basic cellular element of the epidermis is the epithelial cell. Additional cell types contributing to the barrier function of the skin include the mucus-secreting goblet cells, ion-transporting cells as well as intrusive immune cells such as macrophages or various types of granulocytes.

The epidermal layer of the skin provides physical protection against pathogens and secretes mucus. The mucus is secreted by epidermal goblet cells and is composed of water and glycoproteins. Similar to the situation in the intestine, the skin mucus has dual roles: to prevent pathogen adherence by being continuously produced and sloughed off and to act as a repository of numerous innate immune factors such as lysozyme, immunoglobulins, complement proteins, lectins, C-reactive protein, proteolytic enzymes and various other antibacterial proteins and peptides (Shephard 1994). Immune cells are also present and active in the epidermal barrier of the skin, and their number greatly increases in response to skin injury or infection with pathogens.

Farming situations adding allostatic load on the primary barriers The severity of stress elicited by the different husbandry conditions tested and their impact on the functioning of the primary barriers can vary with number of stressors, their intensity and duration as well as their nature. Short-term, acute stress under fish-farming conditions is represented for instance by netting or short-term transportation. Such treatments have been shown to result in goblet cell depletion in the skin barrier, in detachment of the enterocytes from the basement membrane in the intestinal barrier (e.g. Szakolczai 1997) and in a reduction in the thickness of the protective mucus layer, giving way to increased epithelial–bacteria interaction (Szakolczai 1997; Olsen et al. 2002, 2005). Social stress resulted in flattening of the mucosal folds and loosening of the cell-to-cell contacts between enterocytes in the intestine of European eel (Peters 1982). Changes in paracellular barrier permeability due to alterations in intercellular junctions as a consequence of acute stress have been described for Atlantic salmon and Atlantic cod (Olsen et al. 2002, 2008). Similar intercellular damage has been observed in rainbow trout subjected to 15-min acute stress. Moreover, the acute stress resulted in intestinal barrier dysfunction measured as increased paracellular permeability to mannitol (Olsen et al. 2005). Cortisol is believed to be one mediator behind these changes in epithelial permeability. In fact, in rainbow trout, intraperitoneal-located slow-release cortisol implants resulted in increased paracellular permeability to mannitol concomitant with a decrease in transepithelial electrical resistance (TER) (Sundell, unpublished results). In addition, also the most initial and rapid primary stress response, adrenaline, has been shown to modulate the integrity of the intestinal barrier by increasing the permeability to Cl^- through the tight junctions (Bakker et al. 1993).

A common, often more long-term stressor in fish culture is periods of low levels of dissolved oxygen

(DO). Indeed, the DO levels have been suggested as a key limiting factor for salmon aquaculture (Ellis et al. 2002). This is supported by data from sea cage environmental studies showing that DO levels can be highly variable in both space and time and are affected by factors such as stocking density, degree of stratification, water currents, position of the sea cages and seasonal variations. Moreover, tidal cycles exhibit a major influence on the DO levels in farms situated in fjords, as these farms are sheltered from other causes of water movement like winds, waves and strong currents (Johansson et al. 2007). Cyclic drops in DO levels, reaching as low as 50% (<5 mg ml^{-1}) for several hours have been observed when the tidal current changes direction and the water current is close to zero (slack water). Not surprisingly, in late summer, the combination of slack water and high water temperatures led to the lowest and most critical DO levels within the sea cages (Johansson et al. 2007). Subjecting Atlantic salmon to such common husbandry conditions, constituting low and fluctuating DO levels in combination with high temperatures, under a series of controlled experiments led to an induction of the primary stress response reflected in elevated plasma cortisol levels (Sundh et al. 2010). Experimental analyses based solely on plasma cortisol levels would conclude that the allostatic load decreases with time, as the cortisol levels returned to basal values after 30–60 days, depending on the type of stressor. However, the impact of the stressors as assessed by measuring intestinal barrier function revealed a continued impairment during prolonged periods, even after full recovery of normal cortisol levels. Consequently, several different husbandry conditions creating constantly low and fluctuating DO levels and poor water quality result in elevated plasma cortisol levels up to 4 weeks after onset of the treatment, but not at later time points, whereas the intestinal barrier functions were disturbed throughout the experimental period. Moreover, the detrimental effects of the experimental environment on the intestinal barrier were more severe at higher temperatures, which suggest that elevated temperature per se acts as an additional stressor creating a higher allostatic load. In addition to disturbing the physical barrier, as shown by increased paracellular permeability, many of these husbandry conditions also affected the immunological properties of the intestinal barrier. An increased infiltration of neutrophils and altered gene expression patterns of important pro-inflammatory and anti-inflammatory cytokines in the intestinal mucosa was demonstrated as a result of both decreased DO levels and increased temperature (Sundh et al. 2010; Sundh et al., in preparation; Niklasson et al., submitted). An immunosuppressive effect of the stressors was suggested as IFN-γ was down-regulated by low DO, and this effect was even stronger with higher water temperature. Taken together, environmental situations commonly observed in sea cages during rearing of Atlantic salmon are apparently stressful to the fish, and this leads, in addition to transient changes in the classical stress response parameter, cortisol, to persistent alterations in the fish's defence system, in particular the immunology and permeability of the intestinal barrier.

The mucus layer in the skin has also been shown to be affected by unfavourable environmental conditions. Mucus production rates are known to increase during times of stress. For example, the mucification of acid-exposed fish during short-term exposures (within the first day of exposure) is correlated with a reduction in mucus cell numbers due to the exhausted secretion of the mucus cells. In long-term exposures to acidic water, the mucification is thought to be due to hyperplasia of mucus cells in skin and gills (Segner et al. 1988). Changes in salinity also have an influence on skin mucus production. In general, the abundance of mucus cells on fish surfaces decreases as salinity increases (Shephard 1994). A reduction in epithelium height and numbers of cell layers is also a feature of sea water-adapted fishes (Wendelaar Bonga and Meis 1981). Recently, in a study with smoltifying Atlantic salmon, it has been shown that intermixing of sea water (20‰) in combination with intensive rearing decreases mucus cell numbers and epithelium thickness, changes mucus quality and subsequently increases the susceptibility to infection with *Moritella viscosa* in Atlantic salmon after transfer to full-strength sea water (Toften et al., unpublished results). Alternatively, infestation of farmed salmon, even with low numbers of sea louse, *Lepeophtheirus salmonis*, can also reduce the number of mucous cells and results in stress-related changes to the skin epithelium (Nolan et al. 1999).

Not only low DO can be detrimental, hyperoxygenation of fresh water can also affect both intestinal and skin barrier functions. The intensification of Atlantic salmon smolt production includes

moves towards stocking at higher fish densities. As water flow and DO become limiting, oxygen is added to maintain adequate oxygen levels in the holding tanks (Wedemeyer 1996). DO levels of 200–300% saturation are commonly used in aquaculture in order to be able to reduce the specific water flow (L kg^{-1} min^{-1}) (Brun 2003). This practice can have negative impacts on water quality in terms of increased levels of metabolic wastes such as carbon dioxide and ammonia together with decreased pH (Fivelstad et al. 1999, 2004; Ellis et al. 2002). Such an environment in the FW stage of Atlantic salmon farming has been hypothesized to be a contributing factor to the increased disease susceptibility to fish pathogens observed after SW transfer. Several studies indicate that Atlantic salmon smolts have increased susceptibility to infectious pancreatic necrosis virus (IPNV) and *Moritella viscosa* after exposure to reduced specific water flow and hyper-oxygenation (Fridell et al. 2007; Sundh et al. 2009; Toften et al., unpublished results).

Subjecting Atlantic salmon parr to hyperoxic conditions for 26 days resulted in chronically elevated plasma cortisol levels (Fridell et al. 2007). An increased allostatic load was also manifested as a disturbed intestinal barrier function resulting in increased paracellular permeability and increased translocation rate of *Aeromonas salmonicida* in the distal intestine (Sundh et al.2009). These observed decreases in intestinal barrier function are thought to be responsible for an increased risk of infection. Indeed, fish subjected to a similar experimental protocol revealed increased disease susceptibility to an IPNV challenge after subsequent transfer to SW (Fridell et al. 2007).

Moreover, the IPNV challenge per se can serve as a stressor. This was shown by elevated levels of plasma cortisol after IPNV co-habitant challenge (Fridell et al. 2007). This was also reflected in the cortisol release rate into the water (Sundh 2009). Interestingly, fish subjected to stress in FW responded with a stronger primary stress response to the IPNV challenge compared with unstressed fish. This reveals that stress in one life stage may affect the outcome of additional stressors in a subsequent life stages. The IPNV challenge per se also decreased the intestinal barrier function, as shown by both an increased paracellular permeability and increased translocation of *A. salmonicida.* Thus, the disturbed intestinal barrier function may be one explanation behind the increased risk of disease from secondary bacterial infections observed during acute IPNV infections (Johansen and Sommer 2001). In summary, the commonly used husbandry practice of hyperoxygenating FW is likely to reduce the ability of Atlantic salmon post-smolts to cope with additional stressors and represents therefore a major risk to the welfare of the fish.

Another typical stressor is farming under conditions where fish densities are too high (Gytre 2004; Huntingford et al. 2006). As fish density increases, water quality parameters are affected in a complex manner yielding decreased levels of dissolved oxygen (DO) and increased levels of carbon dioxide, nitrates and ammonia as well as a decreased pH (Ellis et al. 2002). Several studies have shown that high stocking density per se can be stressful to the fish (Ellis et al. 2002; Adams et al. 2007; Schram et al. 2010). For instance, Atlantic salmon kept at a density of 70 kg m^{-3} displayed elevated plasma cortisol levels, while this was not seen in fish kept at the intermediate densities of 30 and 50 kg m^{-3} (Sundh et al., in preparation). The elevated plasma cortisol levels decreased with time, suggesting habituation. The intestinal barrier function decreased in a dose-dependent manner in response to the severity of the stress, i.e. the fish density. Again, this was observed at time points when no differences in plasma cortisol levels were detected. Increased fish density resulted in decreased TER as well as increased paracellular permeability to mannitol. The threshold density for mediating a decreased barrier function was between 30 and 50 kg m^{-3}. Additionally, local signs of inflammation were more evident in fish kept at 70 kg m^{-3} as compared to 10 kg m^{-3}, which could be an effect of increased leakage of luminal content that attracts immune cells to the mucosa. Also, the expression of cytokines in the intestinal mucosa was affected by density. IFN-γ was down-regulated in the 70 kg m^{-3} group compared to 10 kg m^{-3}, suggesting a suppression of the mucosal immune barrier. In conclusion, high stocking density most frequently also in association with poor water quality chronically stressful to the fish as eliciting a primary stress response followed by a disturbance of physical and immunological barrier properties of the intestinal epithelium. Thus, this type of environment should be regarded as a threat to the welfare and health of the fish.

What is the association between stress and disease in fish?

As described previously, husbandry-induced stress can modulate immune function and disease susceptibility of fish (Yin et al. 1995; Davis et al. 2003; Iguchi et al. 2003; Pruett 2003; Binuramesh et al. 2005; Small and Bilodeau 2005; Welker et al. 2007). Both immunosuppressive and immunoenhancing effects of stress have been described (e.g. Demers and Bayne 1997, Vazzana et al. 2002). These discrepancies are most probably related to differences in study design, stress intensity and duration, nature of stressor etc. However, despite many examples of impaired immune status and enhanced disease incidences in stressed fish, the relationship between stress and disease must not be generalized (Weyts et al. 1999; Final Report of WEALTH project no 501984, 2008). Various genetic, developmental and environmental factors modulate magnitude, duration and consequences of the stress response and may obscure the relationship between stress and immune/disease status. A brief overview of several studies performed during the last decade, as given in Table 1, may illustrate our still fragmentary knowledge on how stress modulates immune parameters and disease susceptibility. Even if significant alterations in immunological functions occur following exposure to stressors, the consequences for the overall host resistance may not always be deleterious (Pruett 2003). For example, channel catfish subjected to low water stress displayed a higher lysozyme activity than un-stressed individuals, while disease resistance to *Edwardsiella ictaluri* was significantly lower (Small and Bilodeau 2005).

Stressor effects on the immune function and disease susceptibility have mainly been attributed to elevated cortisol levels (Pottinger and Carrick 1999a; Weyts et al. 1999). Indeed, cortisol has immunomodulatory effects in fish as reviewed by Weyts et al. (1999), Yada and Nakanishi (2002) and Segner et al. (2006). Cortisol acts as a regulator inhibiting certain constituents of the immune system and enhancing others (e.g., Espelid et al. 1996; Pruett 2003; Esteban et al. 2004). Also, the regulatory effect varies with cortisol concentration, duration of stress impact (acute versus chronic stress) or species differences in stress sensitivity (Weyts et al. 1999; Ruane et al. 1999; Barton 2002; Pruett 2003; Small and Bilodeau 2005; Ruane et al. 2007). However, it is clear that cortisol is but one amongst several players in mediating the immunosuppressive effects of stressful husbandry conditions on fish. Unfortunately, the differential role of other stress-induced endocrine processes in modulating immune functions of fish is little understood to date (Pruett 2003). Also stress-induced paracrine hormone secretion in the immune system itself must not be overlooked in this context (Arnold and Rice 2000). Thus, instead of keeping a rather one-dimensional focus on cortisol, we should try to develop a more general view on how (neuro)endocrine and paracrine processes mediate the effects of stressful husbandry conditions on the fish immune system (see Verburg-van Kemenade et al. 1999, 2009).

The utility of health/disease parameters as welfare indicators

The pros and cons of using health-related parameters as welfare indicators

While the previous chapter addressed how poor welfare status, as associated, for instance, with poor husbandry conditions, translates into poor health status of fish, the focus of the present chapter is on the use of health-related parameters as indicators of fish welfare. It is a strength of the health and biological function concept that it has potential to provide such welfare indicators (WIs) for fish culture. Welfare indicators are defined as *operational welfare indicators* (OWIs) when they can be corrected instantly, whereas they are designated *strategic* when they cannot be corrected instantly. Both types of indicators are referred to as *direct* if they are related to the fish and as *indirect* if they refer to the system or rearing conditions. As discussed previously, the basis for using health/disease parameters as WI or OWI is that poor welfare conditions can result in reduced fish health and increased disease incidence. In addition, disease per se can implicate reduced well-being of the animal.

Advantages of biological function and health-related parameters such as growth, body indices or feed utilization are that they are straightforward to measure. Physical health is the one of the most universally used indicators of welfare, as is relatively

Table 1 Brief overview of 38 studies related to common stressors in aquaculture: type of stressor employed, fish species, health and function indicators, bacterial challenge tests performed

Authors	Stressor(s)	Species	Physiological indicators	Immune parameters	Bacterial challenge
Acerete et al. (2004)	Transport loading	*Perca fluviatilis*	Cortisol, glucose, osmolality, RBC, haematocrit, lactates, haemoglobin	Not evaluated	Not performed
Barcellos et al. (2004)	Tank draining, transfer, crowding	*Rhamdia quelen*	Cortisol, RBC, haemoglobin	WBC	Not performed
Barcellos et al. (2006)	Handling air emersion	*Rhamdia quelen* (fingerlings)	Cortisol	Not evaluated	Not performed
Barnett and Pankurst (1998)	Crowding, chasing, exercise	*Rhombosolea tapirina*	Cortisol, lactates	Not evaluated	Not performed
Binuramesh et al. (2005)	Confinement, sex ratio	*Oreochromis mossambicus*	Cortisol	ROS production lysozyme immunoglobulin	*Aeromonas hydrophila*
Biswas et al. (2006)	Photoperiod manipulation, handling, confinement	*Pagrus major*	Cortisol, glucose, cholesterol, total protein, Ions, SGR, haematocrit, osmolarity	Not evaluated	Not performed
Cairns et al. (2008)	Handling confinement	*Onocrhynchus mykiss*	Expression of stress-related genes	Not evaluated	Not performed
Davis et al. (2003)	Dietary cortisol	*Ictalurus punctatus*	Cortisol	Not evaluated	*Ichthyophtirius multifiliis* Channel Catfish Virus (CCV)
Demers and Bayne (1997)	Handling	*Oncorhynchus mykiss*	Cortisol, adrenaline	Lysozyme	Not performed
Dror et al. (2006)	Handling	*Carassius auratus*	Glucose, cortisol	Interleukin-10	*Aeromonas salmonicida*
Espelid et al. (1996)	Handling, cortisol injection	*Salmo salar*	Cortisol	WBC	*Aeromonas salmonicida*
Fanouraki et al. (2007)	Anaesthesia, chasing, air exposure, density, temperature	*Pagrus pagrus*	Cortisol, glucose, lactates, norepinephrine, epinephrine, electrolytes, prothrombin, ions	Not evaluated	Not performed
Gornati et al. (2004)	Density	*Dicentrarchus labrax*	HSP, metallothioneins, stress-related genes	Not evaluated	Not performed
Hosoya et al. (2007)	Handling	*Melanogrammus aeglefinus*	Cortisol, glucose, HSP70	Not evaluated	Not performed
Iguchi et al. (2003)	Density	*Plecoglossus altivelis*	Cortisol	IgM	*Flavobacterium psychophilum*
Jentoft et al. (2005)	Handling	*Perca fluviatilis*	Cortisol, glucose, growth	Not evaluated	Not performed

Table 1 continued

Authors	Stressor(s)	Species	Physiological indicators	Immune parameters	Bacterial challenge
Lankford et al. (2003)	Air immersion, temperature, daytime	*Acipencer medirostris*	Cortisol, glucose, lactates	Not evaluated	Not performed
Mazur and Iwama (1993)	Density	*Oncorynchus tshawytsha*	Cortisol, haematocrit	Not evaluated	Not performed
McCormick et al. (1998)	Handling	*Salmo salar*	Cortisol, IGF-1, growth	Not evaluated	Not performed
Ortuno et al. (2002)	Complex stressful event	*Sparus aurata*	Cortisol, glucose	ROS production complement	Not performed
Pottinger and Carrick (1999a)	Confinement	*Oncorhynchus mykiss*	Cortisol, glucose	Not evaluated	Not performed
Pottinger and Carrick (1999b)	Confinement	*Oncorhynchus mykiss*	Cortisol	Not evaluated	Not performed
Pottinger et al. (2000)	Confinement	*Leuciscus cephalus*	Cortisol, GR, glucose, lactates, steroids	Not evaluated	Not performed
Rotllant et al. (1997)	Crowding	*Pagrus pagrus*	Cortisol, glucose, haematology	Complement, Ig lysozyme, WBC, haemagglutination	Not performed
Ruane and Komen (2003)	Density	*Cyprinus carpio*	Cortisol, glucose, free fatty acids, growth Food conversion	Not evaluated	Not performed
Saeij et al. (2003)	Handling, cortisol treatment	*Cyprinus carpio*	Not evaluated	Immune gene expression	*Trypanoplasma borreli*
Small and Bilodeau (2005)	Low water stress, dietary cortisol	*Ictalurus punctatus*	Cortisol	Lysozyme	*Edwardsiella ictaluri*
Strand et al. (2007)	Shadows movements, tank cleaning	*Perca fluviatilis*	Weight, feed intake, SGR	Not evaluated	Not performed
Terova et al. (2005)	Density	*Dicentrarchus labrax*	GRmRNA	Not evaluated	Not performed
Trenzado et al. (2003)	Confinement	*Oncorhynchus mykiss*	Cortisol, glucose, lactates, amino acids, glycogen aminotransferase	Not evaluated	Not performed
Trenzado et al. (2006)	Crowding	*Oncorhynchus mykiss*	Glucose, weight, SGR, feed intake, feed efficiency, haematocrit, haemoglobin, glycogen, liver enzymes	Not evaluated	Not performed
Vazzana et al. (2002)	Density	*Dicentrarchus labrax*	Cortisol, glucose, osmolarity	ROS production cytotoxic cells activity	Not performed

Table 1 continued

Authors	Stressor(s)	Species	Physiological indicators	Immune parameters	Bacterial challenge
Vijayan et al. (1997)	Confinement, cortisol treatment	*Oreochromis mossambicus*	Cortisol, glucose, lactates, free amino acids, glycogen, PEPCK, LDH	Not evaluated	Not performed
Welker et al. (2007)	Hypoxia	*Ictalurus punctatus*	Cortisol, glucose	Lysozyme, CH50, bactericidal activity, antibody response	*Edwardsiella ictaluri*
Wilkinson et al. (2006)	Confinement, hypoosmotic stress	*Salmo salar* *Oncorhynchus mykiss*	Cortisol, GH, IGF-1, IGF-2	Not evaluated	Not performed
Yin et al. (1995)	Crowding	*Cyprinus carpio*	Cortisol, glucose, chlorides	Proteins, lysozyme, complement, phagocytic cells activity	*Aeromonas hydrophila*

Captions: RBC, red blood cells; WBC, white blood cells; ROS, reactive oxygen species; SGR, specific growth rate; HSP, heat shock protein; IgM, type M immunoglobulin; IGF-1 and -2, insulin-like growth factor 1 and 2; GH, growth hormone; GR, glucocorticoid receptor; PEPCK, phosphoenolpyruvate carboxykinase; LDH, lactate dehydrogenase; CH50, total haemolytic complement

straightforward to recognize and to measure. In comparison with primary stress parameters such as cortisol, health-related parameters are less transient but integrate the adverse consequences of low welfare conditions over time. As they are more directly related to the defence mechanisms of the fish, such as indicators of immune system and primary barrier functioning, they are reliable indicators of disturbed function and decreased welfare. Finally, as promoting health and preventing or reducing disease support the economical success of a fish farm, this is an avenue through which farm owners can be motivated to take measures for improving fish welfare.

Although health and disease parameters are objective and can often be quantified supporting their use as OWIs in practical aquaculture, they can be difficult to interpret, and the caveats in the welfare–health relationship must not be neglected (Dawkins 2006). A disadvantage is that health-related OWIs may not be very specific. For instance, parameters such as condition factor or feed conversion are not influenced by fish welfare alone but also by, e.g., feed quality or water temperature. Thus, such parameters should not be used as stand-alone OWIs but in concert with other indicators. Another critical point is the question to what extent the presence of disease in a farm system is indeed an indicator of poor welfare? As pointed out by Wolffrom (2004) and Broom and Corke (2002), presence of disease normally indicates impaired welfare. Diseases result in poor welfare through various clinical and subclinical effects, although Huntingford et al. (2006) pointed out that for specific diseases, we need further research to fully understand their welfare implications. More difficult to answer is the question whether the presence of disease in a farm is a consequence of low welfare conditions. Disease may develop as a consequence of bad husbandry and stressful conditions and indicate an underlying problem in the farming facility. As discussed earlier, stressful husbandry conditions are likely to impair defence capabilities such as the immune system or the primary barriers of fish, and this eventually leads to disease. Similarly, poor hygiene in the husbandry system can lead to an increased pathogen load in the water eventually overloading the defence capability of the fish and causing disease. However, the occurrence of disease is not necessarily caused by poor welfare conditions, but can occur also under good welfare conditions. For

instance, if the farm system is connected with a river system or in the case of cages is embedded in the natural environment, even a farmed stock kept in good welfare may show disease due to transmission of pathogens form wild stocks. Generally, with respect to welfare conditions, diseases arising from opportunistic pathogens may be more informative than diseases arising from infections with obligatory pathogens.

Alternatively, the absence of disease is not necessarily an indicator of good welfare. For instance, if a farmed fish stock is vaccinated, this may protect the fish from disease outbreaks despite poor welfare conditions. Thus, it is not so much the absence/presence alone that is meaningful as a direct welfare indicator instead, to be able to more conclusively predict the disease indicator should be embedded in a coherent scheme along with other indicators. For instance, it may be combined with a disease challenge test that informs on the immune capacity of the fish, or with measurements of stress indicators such as cortisol or barrier leakage (see WEALTH project no 501984, 2008). In addition, one should discriminate between pathogenic and non-pathogenic (i.e. nephrocalcinosis, gas bubble disease, deformations, fin damage) origins of disease, with the latter being directly indicative of poor environmental quality. Moreover, a distinction is needed between obligate and opportunistic pathogenic disease. Obligate pathogenic disease will occur even if the fish is in prime condition, while opportunistic pathogens will become a problem mainly under poor welfare conditions as the equilibrium between the fish and the opportunistic pathogen becomes imbalanced (WEALTH project no 501984, 2008).

Practical application of health-related parameters as welfare indicators in aquaculture

Scientific understanding of the processes and mechanisms relating husbandry conditions, fish health and welfare, as discussed in the previous chapter, is paramount to support the use of health parameters as welfare indicators, but practical use and applicability of these indicators is still another issue. In the following sections, we will discuss the use of OWIs for the example of rainbow trout, although the same general principles could be applied to any teleost being domesticated and introduced into artificial systems where conflicts arise between the demands of the species in question and the technology and economy of the husbandry conditions.

When addressing OWIs in fish production, it is important to focus on freshwater and marine systems separately. Furthermore, the production technologies of fry and fingerlings are so different from those for growth production of larger fish, making generalizations and conclusions difficult. Therefore, it is necessary to consider separately the different rearing systems such as flow-through, recirculation, concrete raceways, fibre-glass tanks, earth ponds and net-pen-systems both in fresh and in salt water.

In practice, a series of inspections and observations should be taken in order to determine the state of welfare on the farm (Table 2). These should refer to the fish, e.g., fish appearance (size, condition, skin, fin, eye and gill integrity and colour), fish behaviour (feed intake, location in water column, air-gasping, balance, activity), but also the system (fish density, unit size), water quality (oxygen, carbon dioxide, ammonia, BOD) (Table 3) or feed quality (essential nutrients, oxidation state of lipids, contaminants) (Table 4). Health inspections should be conducted and include virology, bacteriology and parasitology (Table 5). Serological parameters may be included if possible due to the fact that factors such as serum cortisol, glucose, lactic acid and others may reveal

Table 2 Initial observation of parameters related directly to the fish in the farms and the possibility for instant correction

Observation	Instant correction possible
Mortality	±
Condition factor	–
Epaxial musculature	–
Hypaxial musculature	–
Exophthalmia	–
Colour/pigmentation	–
Skin	–
Fins	–
Gills	–
Ulcers	–
Balance disturbances	±
Air-gasping	±
Scratching	–
Lethargy	±
Visible parasites	±

Table 3 Water quality parameters and their possible use as operational welfare indicators

Parameter	Instant correction possible
Temperature	±
Salinity	±
pH	±
Water flow	±
Turbidity	+
BOD	+
Ammonia	+
Nitrite	+
Nitrate	+
Phosphate	+

Table 4 Nutritional parameters and their possible use for OWI

Nutritional element	Instant correction possible
Proteins	
Amino acids	+
Lipids	
Fatty acids	+
Carbohydrates	+
Vitamins	+
Minerals	+
Antioxidants	+
Immunostimulants	+
Contaminants	±

both acute and chronic welfare problems. For instance, antibody titres can reflect both prior and current infections of specific pathogens. The physico-chemical conditions in the production unit can in some cases be corrected. For instance, in recirculation systems operating with limited water volumes, it is possible to replenish fish tank water (adjusting BOD, salinity), re-oxygenate water, adjust salinity by adding sodium chloride or to adjust pH by adding hydrochloric acid or sodium hydroxide. In larger enterprises such as net-pen culture in the sea, it is not always feasible to correct the problems instantly.

The issue of health conditions is a good example of a strategic OWI. If poor values of direct, i.e. fish health-related OWIs are observed, this can be difficult to correct. For instance, health inspection on the farm site may reveal that clinical signs of the fish could be caused by excessive abundance of ectoparasites/ectocommensals on the gills, fins and skin of the fish. The problems associated with this may be corrected instantly through water treatment using formaldehyde, hydrogen peroxide, sodium percarbonate, sodium chloride or other disinfectants. If the health problem of the fish is not related to superficial colonization with ectocommensals or parasites, which can easily be managed as described above, it is necessary to make a plan for correction of the adverse conditions. This may include stamping out (if the disease is not treatable), drug treatment or vaccination (if available), radical change in water source or feed source. Here, a major welfare issue in fish culture comes into play and that is the serious lack of veterinary medicines (Wall 2008) and effective vaccines (Biering et al. 2005) to treat fish diseases.

How can fish farmers, farm inspectors and authorities use health-related OWIs? Producers are required to maintain strict health management plans incorporating a number of areas such as biosecurity controls, recording of movements and mortalities. It can be envisaged that in the near future, such management plans may include a range of health-related OWIs that the individual producers are required to monitor in order to show that the highest possible standards of welfare can be maintained. The systematic observations of important farm and fish parameters may be powerful tools for a future description of the welfare systems in a particular farm environment. The inevitably robust nature of OWIs makes them useful guidelines for farmers.

If one is to assume that maintaining a good health status of farmed fish is a cornerstone of good welfare, then practical health-related OWIs allowing the producer to rapidly and reliably measure welfare is a key factor. There are currently a number of resources available to producers to allow them to maintain a good health status on the farm. These strategies range from the legislative (EU Directives, national legislation) to agreed codes of practice in various industries and down to individual management plans on each farm. From a legislative point of view, EU Council Directive 2006/88/EC consolidates and updates aquaculture health controls for all aquatic animals within the EU (Breed 2008). There have been numerous studies on the use and application of risk analysis in managing health issues in aquaculture (Murray and Peeler 2005; Peeler et al.

Table 5 Some infections with various pathogens in rainbow trout farming (freshwater/marine) and relevance as operational welfare indicators

Pathogen	Control method available	Instant correction possible	Instant correction not possible
Virus			
VHSV	+	−	+
IHNV	+	−	+
IPNV	+	−	+
Bacteria			
Flavobacterium psychrophilum	+	−	+
Yersinia ruckeri	+	−	+
Aeromonas salmonicida	+	−	+
Vibrio anguillarum	+	−	+
Parasites			
Ectocommensals			
Apiosoma	+	+	−
Ambiphrya	+	+	−
Capriniana	+	+	−
Epistylis	+	+	−
Ectoparasites			
Trichodina	+	+	−
Chilodonella	+	+	−
Skin parasites			
Ichthyobodo	+	+	−
Tetrahymena	+	−	+
Ichthyophthirius	+	−	+
Gyrodactylus	+	+	−
Argulus	+	−	+
Lepeophtheirus	+	−	+
Caligus	+	−	+
Intestinal parasites			
Spironucleus	+	−	+
Eubothrium	+	−	+
Crepidostomum	+	−	+
Eye parasites			
Diplostomum	+	−	+
Tylodelphys	+	−	+
Kidney parasites			
Tetracapsuloides bryosalmonae	+	−	+
Cartilage parasites			
Myxobolus cerebralis	+	−	+

Possibility for control indicated and if this correction can be performed instantly

2007), and this risk-based approach is reflected in the new Directive. The Directive also allows each member state to address new and emerging disease issues within their country. This may take the form of listing a particular disease, thus making it notifiable to the authorities or developing codes of practice for the management of the disease in a particular country. Codes of practice have been developed in many countries for aquaculture such as the 'Code of good practice for Scottish finfish aquaculture' and the

'Code of conduct for European aquaculture' issued by the Federation of European Aquaculture Producers. These codes provide a voluntary framework and guidelines for the industry allowing them to improve practices and health of the farmed stock and thus, by default, improving welfare of their farmed fish. In fact, the RSPCA in conjunction with Freedom Foods in the UK has produced a welfare standards document for farmed Atlantic salmon. Producers who sign up to agree with the standards are audited and if accepted are allowed to produce labelled fish, which may demand a higher premium in the market place.

One aspect that is yet to address, but which is vital to sustainability and general acceptance of aquaculture, is that health and welfare of farmed fish need to be considered in the context of the complete ecosystem to which they belong. Or, in other words, what is the cost of maintaining welfare in the box to the cost of welfare outside, including the immediate and the networked environment? The 'ecological footprint' extends well beyond the farming system itself and includes the socio-economic and ecological impacts of feed production to supply the farm, as well as 'down-stream' effects, all of which can have severe consequences on welfare for many species, including humans. This is especially dramatic for shrimp farming, which has spawned efforts towards integrated production rather than monoculture (i.e. Azad et al. 2009). Indeed, to make a real step towards improved welfare, the question arises whether rather than focusing on the consequences of technologies permitting increasing fish densities in monoculture (as we discussed previously), an adaption of integrated multi-trophic aquaculture (IMTA) may be a better way to go (Chopin et al. 2001; Neori 2008)? Of course, this would require a wide diversity of solutions, each tailored to specific ecosystem economies and climates (i.e. Pigneguy 2008). It would be interesting to investigate how the 'five freedoms' criteria for welfare in IMTA compare with conventional monoculture. As far as the authors are aware, this has not been done nor have OWIs, as outlined earlier for salmonids in monoculture, been applied to IMTA. An obvious difficulty is how to establish reliable criteria for comparison between such diverse systems. Here, the measurement of barrier function, as described previously, could be one such approach. Poor welfare conditions lead to heavy use of antibiotics and other treatment strategies to maintain health. These loads are passed onto the environment in the form of toxic wastes, high levels of organic materials and antibiotic-resistant microbial strains (Boerlin and Reid-Smith 2008). This increased risk and pressure on health and welfare outside the box is seldom assessed and does not yet figure in the OWIs for the farm, although it is essential this be done.

The seriousness of this problem and the extent of the knowledge gaps can be exemplified by several recent studies. Even to understand how the wild fish population outside the box will react to welfare measures applied on the farm, we need to better understand their welfare (Berg 2007). For example, although the diversity of potentially pathogenic bacteria was found to be very high in wild Chinook salmon fry (Evans and Neff 2009), the prevalence of individual bacterial strains tends to be low, as was also found in other juvenile salmonids (Dionne et al. 2009). The potential for overloading this natural diversity with high concentrations of pathogens released from intensive aquaculture is a very real concern. To understand the potential harmful effects of aquaculture on the welfare in the immediate environment, we therefore need more long-term studies of aquaculture localities, preferably starting even before farms are established, but in the least as monitoring programmes to follow the effects of farming strategies. An example of how valuable this can be is a 23-year monitoring programme of the emergence of columnaris disease (*Flavobacterium columnare*) in salmon fingerlings on a fish farm in northern Finland (Pulkkinen et al. 2010). The authors show how intensive farming is coupled with the evolution of more virulent pathogens, with higher transmission amongst homogeneous subsets of fish. That major antibiotics used to treat fish are also, in part, front-line reagents in treating microbial infections of other animals, including humans, demonstrates how the selection for resistant and virulent strains can have wide-ranging consequences (Boerlin and Reid-Smith 2008).

Conclusions

In this brief review, we focus on which processes and mechanisms lay the basis for maintaining biological functions and good health under the pressures imposed by the economics of successful aquaculture

production, and how this again relates to good welfare in aquaculture. This focus is chosen as well founded, and measurable health parameters are arguably the most readily adopted as operational welfare indicators for use on the farm. There is quite some effort being invested in deriving putative feeling-based and nature-based parameters and the resultant OWIs topics of other articles in this volume. One of the great challenges will be integrating these data with function- and health-based OWIs to develop comprehensive and robust welfare plans.

A definite advantage of function- and health-based OWIs is that they remain valid and applicable, regardless of whether fish can be regarded as sentient or not. For all sentient beings, protracted suffering will inevitably lead to poorer health, a measurable commodity across species boundaries. For non-sentient species, measuring biological function and health parameters or processes is probably one of the few indicators we have available as markers of welfare. This focus places a strong reliance on the reliability of parameters currently under consideration and in use and argues strongly for the development of validation based on methods measuring secondary and tertiary response indicators. For this reason, we have outlined in this review the strengths and shortcomings of the current methodologies and approaches. We have drawn attention to one of the major front lines in the fish allostatic response to environmental stressors, the primary barriers provided by skin, intestine and gills. Injury of these barriers, whether mechanically or chemically or biologically, inevitably leads to a displaced homeostasis and threatens health. Primary barrier responses, including the ensuing systemic immune responses, are areas of urgently needing further research. In addition to characterizing physiological responses, the application of system biology approaches, including functional genomics and proteomics (see for example the article by Prunet, this volume), should be at the forefront of such efforts. This is necessary for robust scientific backing of health- and function-related OWIs. Indicators based on sound physiological understanding are critical if health and, as a consequence, welfare considerations are to receive the necessary acceptance for legislation to protect a sustainable aquaculture industry where good welfare is an integral and central component.

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