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Fish welfare: Current issues in aquaculture

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Abstract

With the continued growth of the aquaculture industry and increasing scientific discussion over the potential for negative events to give rise to suffering, research into the welfare of cultured fish is vital. How we define and measure welfare is cause for debate, particularly in fish. However, research into the effects of aquaculture procedures on welfare is crucial to produce data and recommendations for best practice and future legislation. Both behavioural and physiological measures of welfare are necessary for correct interpretation and while there is a wealth of knowledge on the physiological consequences of many aquaculture practices it is now equally important for us to understand the behavioural responses to these practices and to relate them to fish welfare. Here I review the scientific data that allows us to interpret the effects of disease, handling, transport, food deprivation, and slaughter technique on fish welfare. The effects of stocking density, also an area of welfare concern, are complex and appear to comprise of numerous interacting and case specific factors. Investigation into the relative importance of these factors, particularly through behavioural studies, will serve to improve welfare. Stocking density, diet, feeding technique, and management procedures all have strong effects on stress responses, subsequent stress tolerance, health, and the occurrence of aggressive behaviour. Strategies to reduce disease susceptibility, minimise stress responses, and avoid aggression are, therefore, vital. However, caution should be taken when interpreting ‘‘abnormal’’ fish behaviour and further research is required to allow us to establish the importance of the expression of ‘‘natural’’ behaviours. Collectively this growing area of research serves to improve our knowledge of the impacts of aquaculture and intensive farming procedures on fish welfare and is the first step in improving welfare wherever possible.

Keywords: Behaviour; Stress; Stocking density; Slaughter; Management practices; Handling; Stereotypies

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1. Introduction

1.1. Aquaculture: a growing industry

It is estimated that global production from capture fisheries and aquaculture supplied about 132.2 million tonnes of fish in 2003. Of this total, 32.9 million tonnes were from aquaculture production (FAO, 2004). Aquaculture has seen a worldwide expansion over the past 20 years and it seems that growth is set to continue (Naylor et al., 2000). World total demand for fish and fishery products is projected to expand by almost 50 million tonnes to 183 million tonnes by 2015, and it is expected that out of this increase, 73% will come from aquaculture, accounting for 39% of global fish production (FAO, 2004). Alongside, and perhaps partly due to this rapid expansion, the welfare of farmed fish has received increasing attention. Fish welfare is an important issue for the industry, not just for public perception, marketing and product acceptance, but also often in terms of production efficiency, quality and quantity (Broom, 1998; Southgate and Wall, 2001; FSBI, 2002). However, in a number of areas, conflicts between welfare and production exist, where procedures are seemingly associated with diminished welfare at the level of the individual fish. Such practices have been the subject of much commentary and discussion in recent years (Bernoth, 1991; Lymbery, 2002; Hastein, 2004). However, although organisations such as the Farm Animal Welfare Council (FAWC), the Scottish Executive, and the Humane Slaughter Association (HSA) have published recommendations regarding the welfare of farmed fish (FAWC, 1996; HSA, 2005; Scottish Executive, 2002), this is a growing area where empirical research is lacking and these reports identify the need for an improvement in scientific knowledge on which to base future guidelines and potential legislation where necessary. It is, therefore, essential that there is good communication between the latest published scientific research, veterinary advice and the practical experience of the aquaculture industry (FAWC, 1996; Scottish Executive, 2002).

1.2. Concepts of welfare and measurement

Animal welfare is by no means a straightforward concept. The two major issues here are the meaning or definition of animal welfare and how best to objectively measure it (Broom, 1991a,b; Dawkins, 1998; Mendl and Paul, 2004). The production of accurate and objective guidelines for the levels of acceptability in all aspects of fish welfare will only be possible with the resolution of these issues. FAWC have, therefore, based their guidelines on the ‘‘Five Freedoms’’ framework, which defines ideal states rather than specific levels of acceptable welfare (FAWC, 1996). Freedom from hunger and thirst, discomfort, pain, injury, disease, fear and distress, as well as the freedom to express normal behaviour, provides us with a logical framework with which to assess welfare issues. Physical health is the most universally accepted measure of welfare and is undoubtedly a necessary requirement for good welfare. It is important to remember that poor health can be both a cause and a result of poor welfare. However, for many, good animal welfare goes beyond just physical health and also involves a lack of mental suffering. This aspect of welfare, therefore seeks to understand subjective experiences of non-human animals and proposes the conscious experience of suffering in these animals (Broom, 1991b). This is a controversial issue, particularly when it comes to fish. Concepts of animal welfare have traditionally been applied to those which are considered to have the ability to experience pain, fear and suffering and as such have been associated with species with a higher level of cognition when compared to fish. However, there is scientific debate regarding the ability of fish to experience pain and fear. While some reviewers have argued that fish lack essential brain regions or any functional equivalent, making it untenable that they can experience pain and fear (Rose, 2002), others suggest that there is anatomical, physiological, and behavioural evidence that make it conceivable that nociception in fish is experienced and that they have the potential to experience suffering in the form of pain and fear (for review see Braithwaite and Huntingford, 2004; Chandroo et al., 2004a,b; Ashley and Sneddon, in press; empirical studies include Portavella et al., 2003, 2004a,b; Sneddon, 2003; Sneddon et al., 2003a,b; Dunlop and Laming, 2005). It is unlikely that animals with a different brain structure to humans would experience anything like the emotions that humans feel when experiencing pain and fear and it is impossible to know exactly what a fish experiences. However, if an animal experiences suffering or discomfort, the nature of the pain or fear they perceive is no less important (Ashley and Sneddon, in press). If we are to give fish the benefit of the doubt here, it follows that while health is essential for good fish welfare, good health does not necessarily mean good welfare (Broom, 1991a). However, our ability to scientifically and objectively measure welfare beyond the levels of disease, injury and ill health is a further complication (Broom, 1991b; Dawkins, 2003; Mendl and Paul, 2004).

There is no single measure of welfare and although a wide range of physiological, biochemical and behavioural measures are used to assess welfare, none of these are considered reliable in isolation and multiple measures need to be taken (Broom, 1997). This is partly due to the need for careful interpretation of the functional context of many of the measures used (Dawkins, 1998). Physiological stress responses such as cortisol release, for example, are autonomic responses that indicate activity or arousal rather than being specific to poor welfare. These measures may, therefore, be affected by a number of different parameters (Dawkins, 1998; FSBI, 2002). Interpretation of behavioural measures in isolation is also subject to doubt (Dantzer, 1986, 1991; Barnett and Hemsworth, 1990; Broom, 1991b; Mason, 1991b; Dawkins, 1998). Behavioural and physiological measures are intrinsically linked and are dependent on one another for correct interpretation with regard to welfare (Dawkins, 1998). Appropriate combinations of welfare measures will, therefore, be specific to a given situation, but the greater our understanding of both the behaviour and physiology associated with a given situation the better equipped we are to answer questions of welfare.

To this end the aim of the current review is to summarise the science based knowledge associated with selected key issues relating to the welfare of fish in aquaculture. Collectively this work serves to improve our interpretation of the effect of aquaculture practices on fish welfare, and provides the foundation on which government and public bodies may base future guidelines and legislation. Perhaps due to the nature of the aquaculture setting and the importance of product quality, a large body of research has focussed on the physiological aspects of welfare in fish. However, as the debate on the ability of fish to suffer develops, behavioural aspects will become increasingly important for balanced welfare assessment. Therefore, where possible, this review aims to highlight the value of behavioural research in improving welfare.

As fish welfare is a growing area of research this is by no means a comprehensive review covering all aquaculture fish species or indeed all situations where welfare may be a concern. One of the major themes of this subject is that different species have differing biological and environmental requirements and show differing responses to aquaculture conditions. Therefore, much work remains to be done, particularly considering the growth in the aquaculture of species such as cod (*Gadus morhua*), halibut (*Hippoglossus hippoglossus* L.) and tuna (*Thunnus* sp.) (FAO, 2004).

2. Stress: a possible indicator of poor welfare

There is an extensive literature on the biology of stress in fish and the physiological and behavioural responses of fish to a wide variety of physical, chemical, and biological stressors, including those seen in aquaculture (Wendelaar-Bonga, 1997; Iwama et al., 1997; Barton, 2000a, 2002; FSBI, 2002; Conte, 2004). Measures of physiological stress response naturally feature prominently in studies of welfare. However, stress response is an adaptive function in the face of a perceived threat to homeostasis and, as suggested above, stress physiology does not necessarily equate to suffering and diminished welfare. In the short term, stress responses serve a very important function to preserve the individual. Welfare measures in aquaculture are, therefore, largely associated with tertiary effects of stress response that are generally indicative of prolonged, repeated or unavoidable stress (Barton, 2002; FSBI, 2002; Conte, 2004). These include both the direct and indirect maladaptive effects of chronic, long term stress, such as reductions in growth (Barton et al., 1987; Pickering et al., 1991; Pankhurst and Van der Kraak, 1997), suppressed reproductive function (Contreras-Sanchez et al., 1998; McCormick, 1998, 1999; Schreck et al., 2001), diminished immune function (Einarsdottir et al., 2000) and disease resistance (Pickering, 1992; Balm, 1997). So while stress responses will not tell us all we need to know about the welfare of fish, concurrent deleterious effects in several of the above areas may provide a strong indication that welfare is poor (Broom, 1988; FSBI, 2002).

The primary stress response in fish involves the release of catecholamines and activation of the hypothalamic–pituitary–interenal (HPI) axis. Corticotropin releasing factor from the hypothalamus acts on the pituitary to synthesise and release corticotropic hormone, which in turn stimulates the synthesis and mobilisation of glucocorticoid hormones (cortisol in teleosts) from the interrenal cells (Schreck, 1981; Wendelaar-Bonga, 1997). Both catecholamines and cortisol initiate secondary and tertiary stress responses and many of the adverse changes described above are associated with the long-term effects of high cortisol levels (Schreck et al., 2001). HPI activation results in energy source mobilisation, depletion of glycogen stores, and an increase in plasma levels of glucose, along with high muscle activity, anaerobic glycolysis and an increase in plasma lactate. Therefore, the levels of both glucose and lactate in the plasma are often used alongside cortisol to assess stress levels (e.g. Arends et al., 1999; Acerete et al., 2004). Measures of the expression of stress related genes might provide useful and sensitive biomarkers to diagnose stress and improve welfare in the future (Gornati et al., 2004; Ribas et al., 2004). Chronic stress exerts a strong effect on haematology (Montero et al., 2001), metabolism (Mommsen et al., 1999; Dibattista et al., 2005a), neuroendocrine function (DiBattista et al., 2005b), and hydromineral balance and osmoregulation (Wendelaar-Bonga, 1997). As will become apparent, these factors have subsequent relevance to welfare following a stressful event.

Avoidance of the maladaptive consequences of prolonged, repeated and long-term stress is clearly a central welfare goal in aquaculture. As such, the assessment of potential methods to reduce stress responses in aquaculture species is an active area of research. An example is the investigation into the feasibility of selectively breeding fish to minimise their responsiveness to stressors (Pottinger, 2003). Although the process of fish domestication is at a relatively early stage, some cultured species (i.e. salmonids) are inherently less easily disturbed in comparison to wild strains (Huntingford, 2004; Overli et al., 2005). It appears that cortisol response to stressors varies between individuals and that this characteristic has a moderate to high heritability (Pottinger et al., 1992, 1994; Fevolden et al., 1999; Overli et al., 2005). High responding (HR) and low responding (LR) lines of rainbow trout (*Oncorhynchus mykiss*) have been generated by selection for consistently high or low cortisol response to a standard confinement stressor (Pottinger and Carrick, 1999; Pottinger, 2003). While lines of HR and LR selected fish have shown divergent cortisol response to confinement stress (Pottinger et al., 1992; Pottinger and Carrick, 1999), they have also shown other clear behavioural and physiological differences. For example, LR fish show both a lower cortisol response to stressors and also a quicker resumption of feeding when placed in a novel environment (Overli et al., 2002b, 2004). However, while LR and HR fish displayed similar growth rates when maintained in monoculture, fish from the LR line grew larger than did HR fish when maintained in mixed groups (Pottinger and Carrick, 2001). LR fish were found to be more aggressive and competitive than HR fish, LR fish showing a strong tendency to become dominant over HR individuals in size matched staged fights for social dominance (Pottinger and Carrick, 2001). Non-aggressive locomotor response to a smaller conspecific intruder was also higher in HR fish (Overli et al., 2002b). The research on these fish points to these behavioural differences being closely linked or associated with not only cortisol response but also central neuroendocrine signalling systems (Overli et al., 2004, 2005). Behavioural and physiological responses to stress have common control mechanisms in the brain and the monoamine neurotransmitters serotonin (5-hydroxtryptamine, 5-HT), dopamine (DA) and norepiniphrine (NE) play a vital role here, as well as being involved in aggressive behaviour (Winberg and Nilsson, 1993). It would seem that selection for stress responsiveness in rainbow trout is also associated with changes in the function of these brain monoaminergic systems (Overli et al., 2001) as HR trout reacted to stress by an increase in concentrations of 5-HT and DA in the brain stem and NE in the optic tectum and telencephalon, whereas LR did not (Overli et al., 2005). Therefore, cortisol response to stress may be part of a complex trait that incorporates several behavioural and physiological responses. Such traits may represent different strategies or styles of coping with stress (Overli et al., 2004). It will be important to understand whether it is possible to select for stress responsiveness alone or only as part of this complex trait. Further work is required to elucidate the mechanisms of integration between behavioural profile (stress response, aggression, etc.), cortisol response and monoamine influence (Overli et al., 2002a; DiBattista et al., 2005b), as well as the effect of the co-occurring traits on welfare in the aquaculture environment. While these investigations are likely to provide welfare benefits in their own right, the feasibility of manipulating stress responsiveness by selective breeding remains unclear.

Beyond inherited stress responsiveness, a positive conditioning procedure, pairing a stressor of lowering water levels with feeding, has been shown to moderate the severity of response of juvenile chinook salmon (*Oncorhynchus tshawytscha*) to subsequent transportation as well as enhancing survival compared to controls (Schreck et al., 1995). Although repeated inescapable stressors would be detrimental to welfare, if this method was employed using short term, acute mild stressors to condition fish, an improvement in welfare may be possible.

Diet may also play an important role in stress sensitivity. African catfish (*Claria gariepinus*) receiving a diet with high supplementation of ascorbic acid (Vitamin C) during early development showed lower stress sensitivity (Merchie et al., 1997), although common carp (*Cyprinus carpio*) fed large doses of Vitamin C showed a more pronounced cortisol increase in response to stress when compared to fish fed recommended levels of the vitamin (Dabrowska et al., 1991). Juvenile gilthead sea bream (*Sparus aurata* L.) fed a Vitamin E deficient diet showed faster elevation of plasma cortisol levels in response to stress and a lower survival rate than control fish (Montero et al., 2001). Feeding rainbow trout (*O. mykiss*) glucan in low doses, several weeks prior to a stressor, such as transport, shows potential for reducing the immunosuppressive effects of stress (Jeney et al., 1997; Volpatti et al., 1998).

3. Health: fundamental to welfare

Health is a fundamental measure of welfare. However, poor health may also represent an indicator of further welfare concerns as chronic stress can suppress immune function. For example, Iguchi et al. (2003) investigated the influence of rearing density on disease susceptibility in ayu (*Plecoglossus altivelis*). Fish in high experimental rearing densities exhibited more elevated serum cortisol concentrations and more suppressed immunoglobulin M concentrations when compared to fish in lower densities. A higher mortality, possibly caused by bacterial coldwater disease, was seen in the more stressed, high-density experimental group. Poor health itself can also lead to further diminished health and welfare through a number of different mechanisms, including impaired acute (adaptive) responses to further stressors, stress related reductions in immunocompetence, reduced feeding and negative social interactions (e.g. see Tort et al., 1998a,b; Damsgard et al., 2004). When investigating the effects of infestation with an ectoparasite in rainbow trout (*O. mykiss*), Ruane et al. (1999) found that the parasitized fish showed an impaired response to a subsequent acute confinement stress when compared to non-parasitized controls. The stress associated with ectoparasite infestation became apparent following confinement, with the parasitized fish showing immunosuppression.

Stress is, therefore, a major factor in the health of farmed fish (Wedemeyer, 1997). While illness is not always connected to poor environmental conditions (FSBI, 2002) the aim of effective aquaculture is to minimise exposure and, importantly, susceptibility to pathogens and parasites, and minimise sources of physical damage. Aquaculture practices present many situations where stress and physical injury can strongly increase susceptibility to naturally occurring pathogens. Prevention is the ideal and this can only be achieved through good management protocols, and optimal system design (Rottmann et al., 1992; Conte, 2004; also see subsequent sections regarding handling, transport, and stocking density). Infectious diseases in cultured fish, treatment, and the role of stress in fish disease are reviewed by Wedemeyer (1997), Post (1987), Toranzo et al. (2005), and Nougayrede (1995). A full description of each of the many different parasitic, bacterial, viral, or fungal diseases seen in aquaculture is beyond the scope if this review. However, there are several areas that provide a clear illustration of the reciprocal links between health and welfare and how scientific research can lead to improved welfare.

For example, diseases associated with low temperatures over winter periods have been described in a number of different species (Salte et al., 1994; Macdonald, 1995; Southgate and Jones, 1995; Tort et al., 1998b) and although some of these winter diseases are clearly associated with specific bacterial pathogens (e.g. *Vibrio salmonicida*, the causative agent of coldwater vibriosis in salmonids), research on gilt head sea bream (*S. aurata*) indicates that severe immunosuppression in winter months may play an important role (Tort et al., 1998a,b). Beyond immunisation with vaccines (Toranzo et al., 1997), addressing this immunosuppression may be the key to improved welfare and survival. Tort et al. (2004) have shown that an adapted diet providing a supplementary dosage of vitamins and trace minerals to assist the immune system, with a high palatability and nutrient density to maximise feeding and nutrient intake during the winter period when feeding rate is low, provides some degree of immune protection and may help to counteract and reduce some of the effects of winter syndrome. Different feeding regimes after an outbreak of coldwater vibriosis in Atlantic salmon (*Salmo salar* L.) have been shown to have a strong impact on the development of the disease (Damsgard et al., 2004) and the level of dietary iron and omega-3 fatty acids also affect survival of this disease and furunculosis. This may be related to the levels of nutrients that are available to the pathogen (Blazer and Wolke, 1984). As iron is an essential growth factor for most bacteria, deprivation through low levels in the diet

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(Rorvik et al., 2003) or through food deprivation (Damsgard et al., 2004) may increase fish survival rates.

Fin rot describes a variety of lesions including erosion, splitting of fin rays, nodular thickening and extensive loss of tissue and is a common problem in farmed Atlantic salmon (*S. salar* L.) and rainbow trout (*O. mykiss*) (Turnbull et al., 1998; Ellis et al., 2002). It can be caused by abrasion with the environment (e.g. nets and cages; Ellis, 2002) but often occurs as a result of aggressive interactions, which may also increase susceptibility to infections through the effects of chronic stress (Turnbull et al., 1996). Secondary infection with a range of bacteria can occur, such as *Aeromonas salmonicida*, the causative agent of furunculosis (Turnbull et al., 1996). The use of injectable vaccines for diseases such as furunculosis has superseded less effective antibiotic treatment (FAWC, 1996; Lillehaug, 1996). However, immunisation is likely to be stressful to the fish as it involves handling, anaesthesia, and injection. Some vaccines require an adjuvant for adequate efficacy, but these are associated with inflammation, granuloma and pigmentation at the site of injection, impaired growth and reduced carcass quality (Midtlyng, 1997; Koppang et al., 2004, 2005). Adhesions in the peritoneal cavity and between organs have also been described (Hastein, 2004), although these may have more determinants than the type of vaccine and vaccination procedure (Vagsholm and Djupvik, 1999). While the vaccines do improve overall welfare, the development of vaccines with equal efficacy and reduced side effects or alternative oral vaccines may provide further improvements (Bogwald et al., 1994; Midtlyng, 1997).

Sea lice are a serious welfare concern. A number of different species of parasitic copepods, often collectively referred to as sea lice, erode the fish skin causing tissue damage and may also act as a vector of other diseases (Johnson et al., 2004). Indirect and direct losses due to sea lice in salmonid aquaculture globally are estimated to be greater than US$100 million annually (Johnson et al., 2004). These are mostly from the costs of treatments and management strategies, reduced growth rates, and the costs of carcass downgrading at harvest (Johnson et al., 2004). Medical intervention such as dichlorvos or hydrogen peroxide has traditionally been used. However, some sea lice have developed resistance to these chemicals (Jones et al., 1992; Treasurer et al., 2000; Tully and McFadden, 2000) and alternative treatments for sea lice are currently being sought (Mordue and Pike, 2002; Revie et al., 2002; Stone et al., 2002; Treasurer et al., 2002). A vaccine against fish lice may be plausible (Woo, 1997) and as susceptibility to lice has been shown to have a genetic component in salmon (*S. salar* L.) and brown trout (*Salmo trutta* L.), selective breeding towards louse resistance may provide welfare improvements (Jones et al., 2002; Glover et al., 2004). However, selective breeding takes time and although theoretically possible, financial and practical constraints may limit this approach (Glover et al., 2005).

Biological control of lice is an alternative and cleaner wrasse (*Labridae* sp.) have been stocked commercially with farmed salmon (*S. salar* L.) to reduce parasite levels (Tully et al., 1996; Treasurer, 2002; Wall, 2005). However, this situation appears to provide poor welfare for the wrasse as they may be attacked and eaten by the larger salmon. Chronic unavoidable predator stress is, therefore, also a concern. Adequate refuges should be provided for the wrasse along with food supplements when natural sea lice levels are low (Treasurer, 2002). FAWC recommend removal of wrasse prior to grading and during times of food deprivation (FAWC, 1996).

Viral diseases are now one of the main threats to fish health in aquaculture. Viral diseases of particular importance in salmonids include infectious pancreatic necrosis (Park and Reno, 2005; Roberts and Pearson, 2005), infectious haematopietic necrosis (Bergmann et al., 2002), viral haemorrhagic septicaemia (Fichtner et al., 1998), infectious salmon anaemia (Hovland et al.,

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1994), sleeping disease (Graham et al., 2003), and heart and skeletal muscle inflammation (Kongtorp et al., 2004). Traditional vaccines developed over the past 20 years have shown only moderate success and there are relatively few commercial vaccines and specific therapeutics with adequate efficacy (Ifremer, 2002). Therefore, increased knowledge of these diseases along with the development of alternative anti-viral treatments (Lorenzen and Olesen, 1997; Lorenzen and LaPatra, 2005) and the potential for selection for disease resistance (Midtlyng et al., 2002) are crucial to future improvements of welfare.

Non-infectious production related problems are recurrent in aquaculture and are of welfare concern. These include deformities of the heart (Poppe and Taksdal, 2000; Brocklebank and Raverty, 2002; Johansen and Poppe, 2002; Poppe et al., 2002), swim bladder (Poppe et al., 1997), and spine (Vagsholm and Djupvik, 1998; Silverstone and Hammell, 2002). These deformities undoubtedly affect welfare both directly, through poor health, and indirectly, through reduced ability to compete for food. Many of these deformations are thought to be caused by hereditary and/or environmental factors, as they are not associated with specific infectious agents. For example, high temperatures during incubation of eggs may be a causal factor associated with heart deformities in Atlantic salmon (*S. salar* L.) (Poppe and Taksdal, 2000). Affected fish tolerate stress very poorly and show a high mortality rate during stress, such as transportation, due to impaired cardiovascular function, cardiac failure, or heart rupture (Brocklebank and Raverty, 2002; Poppe et al., 2002). Spinal deformities in farmed Atlantic salmon (*S. salar* L.) are also common (Vagsholm and Djupvik, 1998) and both genetic and environmental factors appear to make up a complex aetiology. Vitamin C deficiency (Halver et al., 1969), an excess of dietary leucine (Choo et al., 1991), the use of oxytetracycline (Toften and Jobling, 1996), and environmental pollutants (Bengtsson, 1975) have been associated with spinal deformity. Vagsholm and Djupvik (1998) found a number of risk factors throughout the salmon rearing process and recommended that risks of spinal deformities could be reduced by increasing the smolt weight to around 0.1 kg at seawater introduction, vaccinating the smolt just before sea water introduction and raising at sites where salinity and temperature vary as little as possible. Genetic factors also play a substantial role in the spinal deformities seen in salmon and it is recommended not to select breeders from families showing high incidences of deformed fish, to prevent the increase in the genetic susceptibility in the population (Gjerde et al., 2005).

4. Aquaculture procedures that compromise welfare

4.1. Overview

Stressors in aquaculture are unavoidable and reducing stress and its harmful effects is a fundamental goal for successful growth and production as well as welfare. The effects of a wide range of aquaculture practices on the stress physiology of fish are well documented (for review see Pickering, 1992; Wedemeyer, 1997; Conte, 2004). Different species display a wide variation in physiological responses to stressors associated with aquaculture. Elevations in plasma cortisol levels can differ by as much as two orders of magnitude among different species of fish following identical stressors (Barton, 2000a,b, 2002; Congleton et al., 2000; Conte, 2004). There is, therefore, economic and welfare related pressure to ensure that both the frequency and the effects of a number of key management practices are reduced to a minimum. In particular, research into optimum handling and transportation methods and species-specific responses can help to achieve this aim.

4.2. Handling

Handling and transport are inherently stressful events (Barton et al., 1980; Davis and Schreck, 1997; Sharpe et al., 1998). Removal from the water elicits a maximal emergency physiological response and should only be carried out when absolutely necessary. Air exposure for 3 min resulted in a 50-fold increase in plasma cortisol levels within 30 min in gilthead sea bream (*S. aurata*) (Arends et al., 1999). Care must be taken at all stages to avoid abrasions and removal of scales and the fish's protective mucous coat, which serves as a physical and chemical barrier to infection as well as being important in osmoregulation and locomotion. Where applicable this should involve the use of wet hands and species appropriate nets, keeping the fish moist during handling. Excessive weight loading on fish at the bottom of nets and brailles should also be avoided (Conte, 2004; HSA, 2005). The use of a braille lining allows some water to be retained in the net and, therefore, provides some protection from abrasion. Moving water along with the fish should cause fewer injuries and appears to be the least stressful technique (FAWC, 1996). As such, the use of fish pumps and transfer pipes appears to be preferable for welfare. However, effective management should ensure that the design of the system provides appropriate speed and delivery rate, minimises potential for abrasion, and minimises the time spent in the pipe. Prolonged periods in pipes, particularly in warm conditions, should be avoided and pipes should be flushed through to ensure no fish are left inside after use (HSA, 2005).

Excessive crowding of fish prior to management procedures can be stressful, with potential decreases in oxygen levels and water quality, increased chances of abrasion, and rapid changes in light intensity (HSA, 2005). While some species adapt well to high loading densities (Barton et al., 2000; Ruane and Komen, 2003) others show prolonged elevated cortisol levels following confinement procedures (Barnett and Pankhurst, 1998; Barton et al., 2003). Barton et al. (2005) have also shown that a fish's capacity to respond to acute stressors such as crowding may be altered by the effects of long term holding conditions. Gilthead sea bream (*S. aurata*) held at high density over 14 days showed a suppressed cortisol response to acute handling, likely the result of negative feedback from chronically elevated cortisol (Barton et al., 2005). Stress during crowding may also affect response to further stressors such as net capture (Schreck et al., 1989; Ruane et al., 2002). Recovery periods are therefore often beneficial, particularly during transport (Iversen et al., 1998; Jonssonn et al., 1999). A cumulative effect on cortisol levels was seen when turbot (*Scophthalmus maximus* L.) were confined a second time, 4 h after an initial net capture (Waring et al., 1997). A 24 h recovery period between multiple handling procedures avoided this cumulative effect. Stressors associated with crowding can be kept to a minimum by appropriate management techniques. HSA guidelines (2005) suggest good crowding management includes a slow and gentle technique, assessment of water quality, the addition of oxygen to the water if levels fall below a critical 6 mg/l, and, perhaps most importantly, close monitoring of the behaviour and activity of the fish. A simple scoring system is given to help train staff to recognise acceptable levels of activity and stress (HSA, 2005).

Where handling is prolonged such as during stripping and milking, sedation should be used to reduce stress response (FAWC, 1996; Scottish Executive, 2002). Although the grading of fish can be stressful through capture and handling, limited grading may be advantageous to welfare by reducing size differences, thus preventing aggression, reducing feeding competition, and removing maturing fish from a population. As the following sections will describe, stocking density and feeding methods have a strong influence on the levels of social interactions, dominance hierarchies and, subsequently, growth in captive fish. The use of species appropriate feeding techniques and stocking densities can, therefore, limit heterogeneous growth within a

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group of fish and thus the need for frequent grading (Volpato and Fernandes, 1994; Alanara and Brannas, 1996; Alanara et al., 1998; Brannas et al., 2003).

4.3. Transport

A wide variety of transportation techniques are used within aquaculture, but all should aim to minimise stress, optimise water quality and oxygen levels, and minimise the build up of metabolic wastes and ammonia (see food deprivation below). Transportation involves capture, loading, transport, unloading and stocking and so can induce large stress responses that can affect fish over a prolonged recovery period (Specker and Schreck, 1980; Davis and Parker, 1986; Schreck et al., 1989; Iversen et al., 1998). In Atlantic salmon (*S. salar* L.) smolts, many of the disease outbreaks take place during the first months of transfer to the sea following transport in well boats (Iversen et al., 2005). This study found that the loading process was a more severe stressor than the transport itself, with plasma cortisol returning to resting levels during the time in the well boats in four out of five transports. Only minor plasma cortisol increases were observed during unloading. It may be that the well boats provide an important recovery function (Iversen et al., 2005). Specker and Schreck (1980) also found that the greatest stress response occurs during loading and the first few hours of transport in Coho salmon (*Oncorhynchus kisutch*) smolts. A recent study, using electromyogram telemetry, indicates that rainbow trout (*O. mykiss*) show vigorous swimming activity and elevated oxygen consumption during transport (Chandroo et al., 2005). While activity levels returned to baseline within 48 h, beyond this period, swimming performance, measured as critical speed and endurance, was still affected. The provision of a recovery period following transport is clearly important for welfare and subsequent survival (Erikson et al., 1997; Iversen et al., 1998; Tipping, 1998; Jonssonn et al., 1999; Finstad et al., 2003; Iversen et al., 2003). The net loss of sodium and chloride ions in freshwater fish, associated with the stress of transport means that certain species may benefit from dilute salt solutions or a current during, before or after transportation (Carmichael, 1984; Pickering, 1992; Wedemeyer, 1997). Anaesthetic agents may also be used to sedate fish prior to transport and/or slaughter (HSA, 2005). At low doses these agents may reduce activity and stress. Iso-eugenol based anaesthetics show good potential in reducing stress (Tort et al., 2002; Iversen et al., 2003) and may be used to sedate fish in New Zealand, Australia, Chile and Korea. However, this method has yet to be licensed in Europe and North America.

Careful acclimation, to new environmental temperatures, water chemistry and light levels (see Mork and Gulbrandsen, 1994), is also important for wellbeing. As with all aquaculture procedures, stockmanship and effective husbandry skills are of paramount importance during transport. Adequate training, knowledge of the signs of stress and appropriate working conditions, are vital to fish welfare.

4.4. Food withdrawal

Fish are often deprived of food before certain management procedures are carried out and this is designed to reduce physiological stress during the procedure. Temporary starvation prior to transport, treatment of disease, and transfer of smolts from fresh water to seawater, serves to evacuate the fish's gut and to reduce metabolism, oxygen demand and waste production. As well as being beneficial to welfare, a reduction in metabolism prior to slaughter may also alter the qualities of the flesh (Johansson and Kiessling, 1991; Einen et al., 1998; Gines et al., 2002). Prior to slaughter, Atlantic salmon (*S. salar* L.) are often deprived of food for some days or weeks.

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However, the FAWC and HSA recommend that salmon should not be totally deprived of food except during a period of up to 72 h before slaughter or a handling procedure. For trout these periods are recommended to be up to 48 h only. FAWC guidelines also suggest that fish should not be deprived of food for any other reason such as conditioning and adjustment of body composition (FAWC, 1996).

As fish are ectothermic, periods of food deprivation may be of less detriment than in endotherms (FSBI, 2002). However, fish do have motivational mechanisms for feeding when nutritional reserves are low (e.g. Metcalfe and Thorpe, 1992). It is not uncommon for Atlantic salmon (*S. salar* L.) and rainbow trout (*O. mykiss*) in the wild to survive long periods of food deprivation (e.g. over winter). However, individual salmon have been shown to vary in their ability to reduce metabolic costs when food availability is low (O'Connor et al., 2000). During the return migration in freshwater prior to breeding, adult salmon may cease feeding altogether for extended periods (Kadri et al., 1995, 1996). Such long periods of anorexia are not seen during other life stages, including those seen in aquaculture. However, salmon do show seasonal variation in food intake in aquaculture (Smith et al., 1993; Simpson et al., 1996; Blyth et al., 1999). As detailed above, diet, particularly levels of vitamins and trace minerals, has an important influence on immuno-competence, the development of diseases after outbreak and the response of fish to stressful aquaculture events. There is potential for these factors to interact with brief food deprivation to affect welfare in either a positive or negative way.

There have been relatively few studies on the effects of starvation on stress physiology or behaviour (Barton et al., 1988) and the majority of work in this area concerns the effect of prolonged starvation on growth, muscle protein and fat composition (Sumpter et al., 1991; Chung and De, 1998; Einen et al., 1998; Rios et al., 2002; Pirhonen et al., 2003; Tripathi and Verma, 2003; Lemieux et al., 2004). The impact upon welfare of food depriving a fish that has previously been fed regularly is not known. Therefore, food depriving farmed fish for short periods under appropriate conditions (e.g. temperature and season) may not cause welfare problems (FSBI, 2002). However, it is important to consider other effects of starvation and reduced nutrition, such as changes in metabolic activity (Martinez et al., 2003) and changes in behaviour related to competition, and the potential for increased aggression. For example, reduced food abundance can cause changes in territorial behaviour strategies and activity patterns in brown trout (*S. trutta* L.) (Alanara et al., 2001; Brannas et al., 2003).

5. Slaughter techniques

Humane methods for animal slaughter are based on the principle that the animal is killed quickly with minimum fear and pain or suffering (FAWC, 1996). However, many aquaculture slaughter methods have been developed not to minimise stress but to achieve product quality control, efficiency and processor safety (Conte, 2004). There is an increasing awareness in the aquaculture industry of the need to ensure that slaughter takes place under humane conditions and that fish should be stunned prior to slaughter by a method that causes immediate loss of consciousness that lasts until death (FAWC, 1996). The development of new commercial technologies to produce humane slaughter techniques for aquaculture is an active area of research.

From a welfare point of view, the most important factors in slaughter technique are the methods of handling fish during transfer to the killing facility up to the point of stun and the immediacy of loss of consciousness caused by the stunning method (Southgate and Wall, 2001; HSA, 2005). The technique used to kill the fish should do so quickly following successful

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stunning, to avoid regain of consciousness. Minimising pre-slaughter stress and the use of humane slaughter methods also improves product quality in a number of areas (Robb et al., 2000a; Skjervold et al., 2001; Southgate and Wall, 2001; Robb and Kestin, 2002; Poli et al., 2005). As with transport, sedation with anaesthetics prior to slaughter may reduce the stress associated with the event. So called ‘‘rested harvesting’’ also improves flesh quality (HSA, 2005).

Aquaculture slaughter techniques are very diverse and fish species vary in their response to different methods (e.g. sensitivity to oxygen deprivation; Morzel et al., 2003). However, the most prevalent methods have been investigated in recent years in order to assess both the associated welfare and product quality. When investigating suitable slaughter methods, consciousness can be assessed by measuring visually evoked responses (VERs) and somatosensory evoked responses SERs (e.g. responses to pain stimuli) in brain activity using electroencephalography (EEG) (Robb et al., 2000b; Kestin et al., 2002; Robb and Roth, 2003; Van de Vis et al., 2003). In certain species, behavioural indicators have been shown to correlate with brain activity (Robb et al., 2002a) but the use of such indicators alone may not be sufficient (Van de Vis et al., 2003). Adequate training of operators in the identification of behavioural indicators of consciousness is vital (Kestin et al., 2002; HSA, 2005; Poli et al., 2005).

Many of the current commercial techniques are unacceptable in terms of welfare (FAWC, 1996). Removal from water followed by asphyxiation in ice slurry is often used to slaughter rainbow trout (*O. mykiss*). This is likely to cause a prolonged period of distress before death, as the time to death when fish are removed from water has been shown to be as long as 14 min at a temperature of 2 °C (Kestin et al., 1991; Robb et al., 2000b; Lines et al., 2003). Immersion in ice slurry or asphyxia is used to slaughter gilt-head bream (*S. aurata* L.) and in both cases loss of brain function is not immediate (loss of VERs occur at 5–5.5 min; Van de Vis et al., 2003).

Salmon and trout have previously been killed by immersion in CO_2 saturated water, which causes narcosis and loss of brain function (e.g. Erikson et al., 1997). However, this only occurs over several minutes, during which the fish appear severely distressed (Robb et al., 2000a,b). Following a minimum of 4 min immersion, the fish are removed and their gills cut. The CO_2 stun method causes a reduction in flesh quality (Roth et al., 2002) and concern over welfare has led to a reduction in its use. While some Scandinavian countries still use this method, it is banned in the UK for all but emergency kills. Gill cutting without prior stunning is also used and is unacceptable as VERs are not lost immediately (Robb et al., 2000b).

Current commercial slaughter methods for eels consist of a ‘‘de-sliming’’ phase where salt or liquid ammonia are added to eels in a dry tank. This denatures the mucus that protects the skin and the process may take as long as 20 min (Robb et al., 2002b; Van de Vis et al., 2003). The eels are then washed and subsequently eviscerated. Although the whole process takes about 1 h, the point of death may occur after the procedure has been completed (Robb et al., 2002b; Van de Vis et al., 2003). Eel slaughter using live chilling followed by freezing in cold brine at −18 °C does not meet the criteria for humane slaughter (Lambooij et al., 2002). However, Robb et al. (2002b) have shown that the use of electric stunning tongues applied to either side of the eels head can instantly and effectively stun the eel and that increasing the duration of the application can cause death. Electrically stunning batches of eels in freshwater combined with nitrogen flushing can also achieve immediate unconsciousness (Van de Vis et al., 2003), therefore, humane slaughter of eels is possible.

When applied correctly, percussive or electrical stunning methods appear to achieve humane slaughter in Atlantic salmon (*S. salar* L.), gilt-head sea bream (*S. aurata* L.), turbot (*S. maximus* L.), and rainbow trout (*O. mykiss*) (Robb et al., 2002a; Morzel et al., 2003; Van de Vis et al., 2003). The UK trout industry appears to be moving towards electric stunning as its preferred slaughter method (Lines et al., 2003).

When carried out in water, electrical stunning can be efficient and humane as it can cause very rapid transition to insensibility (Robb and Roth, 2003). Fish need not be singled out, restrained, handled, or removed from the water before they are insensible (Robb et al., 2002a; Lines et al., 2003). Death may then occur as a result of bleeding, asphyxiation or as a direct result of the stun. However it is important to use the appropriate electric field strength; too weak and the stun may only cause paralysis rather that insensibility, too high and carcass damage such as haemorrhages, blood spotting, and broken bones may result. (Robb et al., 2002a; Lines et al., 2003; Morzel et al., 2003). However, carcass damage is influenced by the frequency of the alternating current and damage free humane stunning is possible (Robb et al., 2002a).

It is important to point out that in order to maintain humane slaughter techniques, the efficiency of percussive stun or electric stun methods relies on adequate training and working conditions for those undertaking the operation (FAWC, 1996; Scottish Executive, 2002). For example, it is very difficult to maintain accuracy when percussive stunning manually over a prolonged period on a commercial level. The advent of automated percussive stunning equipment, therefore, represents a welfare improvement. More recent models avoid the need for the operator to handle the fish as the design encourages the fish to swim into the entry channels. These machines, therefore, have potential to further improve welfare as they reduce handling to a minimum and keep fish in water until seconds before being stunned (HSA, 2005). Automated systems provide more accurate and consistent stunning, provided they are used and maintained correctly with an appropriate delivery system. Ineffective stunning may occur where fish are of incorrect size or deformed. Therefore, it is important that all fish are monitored and a manual stun applied if the initial stun is not effective (HSA, 2005).

6. Stocking density: a complex issue

Stocking density is a pivotal factor affecting fish welfare in the aquaculture industry, especially where high densities in confined environments are aimed at high productivity. Although rarely defined, stocking density is the term normally used to refer to the weight of fish per unit volume or per unit volume in unit time of water flow through the holding environment (Ellis, 2001). The stocking density at any point in time will increase as fish grow or decrease following grading. Stocking density is, therefore, hard to measure in the field. The concept of minimum space for a fish is more complex than for terrestrial species as fish utilise a three-dimensional medium (Ellis, 2001; FSBI, 2002; Conte, 2004). Because fish are dependent on this medium for both physiological and behavioural needs the welfare concerns associated with stocking density should address both the carrying capacity of the holding environment and the spatial and behavioural needs of the species. Carrying capacity refers to the maximum number of fish that an environment can support through oxygen supply and removal of metabolic waste and will be determined by, amongst other things, the oxygen consumption rate of the fish and their response to metabolic waste products such as CO_2 and ammonia (Ellis, 2001). Beyond providing for the physiological needs, the FAWC recommends that fish ‘‘need sufficient space to show most normal behaviour with minimal pain, stress and fear’’ (FAWC, 1996). Stocking density is, therefore, an area that illustrates both the significance of species differences and the existence of a complex web of interacting factors that effect fish welfare.

For species that live naturally in large shoals, low rather than high densities may be harmful, whereas for territorial species the opposite may be the case. Reductions in stocking density may

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reduce the physical spread of disease, but stocking density will also have an effect on many other aspects of welfare such as the water quality, particulate matter, fish to fish interaction, and fish to housing interaction (Ellis et al., 2002). In different systems and with different species the way these factors combine to affect welfare will differ, making it difficult to provide blanket guidelines, much less legislation on maximum densities (Ellis et al., 2002). Within species there may even be age dependent differences in stocking density effects (Jorgensen et al., 1993; Greaves and Tuene, 2001).

As the above might imply, studies in both laboratory and aquaculture settings show that the effect of stocking density on measures of welfare varies between species. For example, sea bass (*Dicentrarchus labrax* L.) showed higher stress levels at high densities, as indicated by cortisol, innate immune response, and expression of stress related genes (Vazzana et al., 2002; Gornati et al., 2004). High stocking densities in juvenile gilthead sea bream (*S. aurata* L.) also produce a chronic stress situation, reflected by high cortisol levels, immunosupression, altered metabolism (Montero et al., 1999). In contrast, Arctic charr (*Salvelinus alpinus*) feed and grow well when stocked at high densities but show depressed food intake and growth rates at low densities (Jorgensen et al., 1993). These differences did not seem to be due to the formation of dominance hierarchies in the low-density group. However, beyond certain high densities, stress related behavioural responses do re-occur (Ellis et al., 2002). In halibut (*H. hippoglossus* L.), a non-social flatfish that spends most of its time resting on the sea floor, tolerance for high stocking density appears to be stage dependent. Greaves and Tuene (2001) showed that small juvenile halibut had better growth and fewer injuries from aggressive encounters at the highest densities whereas Kristiansen et al. (2004) observed abnormal activity and surface swimming, reduced food consumption, and reduced growth rates with increased stocking density in adult halibut.

There is considerable evidence for decreasing welfare associated with high stocking densities in rainbow trout (*O. mykiss*). The effects of high density include decreased growth, reductions in food intake, food conversion efficiency (Refstie, 1977; Holm et al., 1990; Boujard et al., 2002) and nutritional status, fin erosion, gill damage, a reduced immune capacity (Ellis, 2002; Ellis et al., 2002), and alterations in swimming behaviour (Anras and Lagardere, 2004). When reviewing some 43 studies examining the effects of density on such parameters Ellis et al. (2002) noted that the magnitude of the effects tended to be dependent on study specific conditions and increased disease incidence did not seem to be a generic effect of density. High densities are associated both with increased competition, aggression, and physical injury (due to increased contact between fish and between fish and the housing/net), and with degradation in water quality. However, it seems that excessive aggressive behaviour and a poor feeding response may also occur when trout are held at very low densities (Ellis, 2002; Ellis et al., 2002). Although the relative contributions of aggression and degradation of water quality vary with conditions, experimental evidence indicates that water quality is the key factor in relation to density affecting welfare in rainbow trout (Ellis et al., 2002).

Recently, Turnbull et al. (2005) carried out a study on the effect of stocking density on Atlantic salmon (*S. salar* L.) in marine cages under commercial production conditions. A multivariate analysis was used to combine four commonly used measures of fish welfare, including condition of the body and fins and plasma concentrations of glucose and cortisol, into a single welfare score. This score was consistent with the evaluation of welfare by experienced farmers, and, therefore, appears to be a good measure of welfare in real environments. High stocking densities were associated with a reduction in welfare but only above a threshold of 22 kg m^{-3}. This suggests that below this threshold increasing density did not reduce welfare in these situations. When the effects of stocking density and sampling period were taken into consideration, the reduction in welfare at high stocking density did not seem to be dependent on reduced water quality or associated with increased rates of social interaction. Also the time since the last stressful husbandry event was not significantly associated with welfare. However, the sampling period and the location of the cage was a significant predictor of welfare even when adjusted for stocking density (Turnbull et al., 2005). These results show us that a range of other factors combine with stocking density to affect welfare and these factors will vary from site to site and perhaps with time. A density threshold for one set of conditions may, therefore, not to be relevant to another. This study also serves to illustrate the value of qualitative assessment of welfare by experienced animal keepers. Integration of observations of behaviour, feeding response and gross appearance can, at the very least, aid interpretation of other quantitative measures of welfare. The simple scoring system to monitor crowding behaviour in salmonids given by HSA is a good example of how such observations can improve welfare (HSA, 2005). It has been proposed that such qualitative assessment of behaviour in pigs has validity and important potential for future welfare measurement (Wemelsfelder et al., 2001; Wemelsfelder and Lawrence, 2001). As concepts of welfare in fish develop, the value of a similar approach in aquaculture should not be underestimated.

It is clearly very hard to propose within species maximum stocking densities for all situations. However expressed, stocking density alone will not directly predict welfare. A complex matrix of factors appears to influence the effect of stocking density on a range of welfare measures. It has been suggested that a more productive approach to ensure welfare is to study the specific and dynamic components of the overall effect such as behaviour, water quality, health, stress physiology, and gene expression (Ellis, 2002; Ellis et al., 2002; Turnbull et al., 2005).

7. Behavioural approaches to improve stocking density effects

Species relevant behavioural observations in particular may provide further understanding of density effects and perhaps produce practical steps to improve overall welfare as well as health. For example, behavioural observations of Atlantic halibut (*H. hippoglossus* L.) in experimental tanks suggest causal mechanisms behind the reduced growth and feeding with increasing densities in adults. Although the natural activity patterns of this largely solitary fish species are not well documented it is thought that they spend extended passive periods on the sea floor. Kristiansen et al. (2004) observed that with increasing density the tendency of the fish to leave the bottom increased and that there was a negative correlation between the subsequent vertical surface swimming and growth. Negative growth rates were seen in many individuals with high activity levels, indicative of poor husbandry and poor welfare. The origins of the surface swimming behaviour are unclear, though it may represent an effort to move away from an area or unfavourable situation. If this is the case and the behaviour repeatedly fails to achieve this aim, as in a high density restricted aquaculture situation, then this may represent a further welfare concern. The increased tendency to leave the bottom when fish are crowded also means that fish landing after swimming are more likely to come into contact with resting fish and initiate further activity. Considering these observations the authors suggest measures that may reduce the effects of density such as the use of multiple overlapping floors, alternative substrates, and an altered social environment with regards to fish size and species.

Acoustic telemetry has been used to monitor the swimming activity and positional data for rainbow trout (*O. mykiss*) within three different rearing densities on an experimental fish farm using circular tanks (Anras and Lagardere, 2004). At the lowest density the fish showed a preference for the outer circles of the tank (where the feeder and water inlet were positioned) and spent long periods in a stationary position. However in the highest stocking density there was a greater residence in the inner circle of the tank (where the water drained) and swimming patterns were chaotic, probably due to crowding and contact between fish, as suggested by observed fin damage in this group. Under natural conditions and low density rearing, rainbow trout show low intensity swimming activity during the day and a drop in nighttime swimming activity. However, in the high density experimental group high levels of activity were recorded and the day–night rhythm was not seen. Remote measurement of behaviour in this way will enable better interpretation of parallel changes in health and stress physiology.

Atlantic salmon (*S. salar* L.) in sea cages do not occupy all the available space and seldom distribute themselves randomly (Juell et al., 2003). Swimming depth and behaviour is strongly influenced by environmental factors such as temperature and light (Oppedal et al., 2001). Artificial photoperiods are used in cage rearing of Atlantic salmon as this may increase growth rates and postpone sexual maturity (Oppedal et al., 1997). The swimming depth of Atlantic salmon (*S. salar* L.) is strongly influenced by light levels to enable the maintenance of schooling behaviour, which relies on visual contact (Ferno et al., 1995). Recent studies, using echo-integration to observe swimming depth and fish density, have shown that both natural light and the photoperiod and positioning of artificial lighting exert a powerful influence on the vertical positions of the fish throughout the day and, as a result, affect the ‘‘real’’ or observed fish density (e.g. density within the shoal rather than the cage as a whole) (Juell et al., 2003; Juell and Fosseidengen, 2004). These studies have shown that the swimming depth and density of caged salmon can be manipulated by the use of underwater lamps and that appropriate positioning of these lamps can be used to avoid crowding, particularly in the upper water layers, which may be considered sub optimal with regards to welfare (Juell and Fosseidengen, 2004).

8. Aggressive behaviour

Stocking density naturally has a large effect on social interactions between fish. In species where social hierarchies are formed, such as salmonids, these may lead to chronic social stress (Ejike and Schreck, 1980; Schreck et al., 1997; Wedemeyer, 1997; Alanara et al., 1998), reduced feeding, changes in metabolism, down regulation of digestive function and suppression of growth in low ranking fish and higher growth rates in dominant individuals (Abbott and Dill, 1989; Holm et al., 1990; Jorgensen et al., 1993; Adams et al., 1998; Dibattista et al., 2005a). Also, the high levels of activity associated with agonistic behaviour and chronic social stress (e.g. Abbott and Dill, 1985; Andrew et al., 2002) may result in greater energy expenditure, elevated metabolic rates (Sloman et al., 2000), decreased food utilisation efficiency (Li and Brocksen, 1977; Jorgensen et al., 1993) and impaired immune function (Pottinger and Pickering, 1992). Beyond social stress, conspecific attacks are often the primary cause of fin damage, particularly in those fish that are already immune suppressed, and so secondary infection may follow (Abbott and Dill, 1985; Turnbull et al., 1996, 1998). Food availability is therefore an important factor when it comes to stocking density (Holm et al., 1990) and how captive fish are fed has a strong influence on the levels of social interactions (Alanara and Brannas, 1996; Adams et al., 1998; Andrew et al., 2002, 2004). For example, aggressive interactions are a major cause of injuries to the eyes, tails and pectoral fins, causing secondary infections and mortality in farmed Atlantic halibut (*H. hippoglossus* L.). The majority of damaging contact between fish appears to occur during feeding. Greaves and Tuene (2001) observed that in experimental tanks some interactions were misdirected feeding attempts. However, the majority of attacks were directed at fish that had won food pellets from aggressors. The majority of aggression occurred early in hand feeding sessions and so, to avoid aggression, the authors recommend hand feeding where food is spatially dispersed but concentrated in time. The use of automatic feeders throughout the day to prevent the build up of appetite and allow all fish the opportunity to feed to satiation may achieve this aim (Greaves and Tuene, 2001).

Andrew et al. (2002) have shown that varying feeding method can alter aggressive interactions in Atlantic salmon (*S. salar* L.), gilthead sea bream (*S. aurata* L.) and European sea bass (*D. labrax* L.) in sea cages. The behaviour of control fish that were fed to apparent satiation at set times was compared to those that were fed on demand using interactive feedback systems. Similar effects on swimming speeds and feeding intensity were seen in all three species and suggested stronger and significantly higher levels of aggressive scramble competition in the control fish.

It is clear that stocking density, the nature and level of food availability, and social behaviour act together to affect welfare. The effect of hierarchy and stocking density on the use of demand feeders has been investigated in rainbow trout (*O. mykiss*) and Arctic charr (*S. alpinus*, Alanara and Brannas, 1996). At low densities, in both species, top ranked individuals were able to dominate the feeders, performing the majority of trigger activations. However, this ability was reduced at higher densities, where a small group of fish dominated the activation of the feeder trigger (Alanara and Brannas, 1996). As discussed, such domination of a food source is likely to lead to heterogeneous growth rates with low ranking individuals gaining little access to food (Alanara et al., 1998). However, it seems that in both species, some low ranking individuals may apply an alternative strategy to attain adequate growth by feeding at night when dominant individuals are less aggressive (Alanara and Brannas, 1997). Thus, changes seen in activity and behaviour with increasing densities, described in the previous section, are likely to be intrinsically linked to the availability of food experienced by the individual and the levels of aggression and social hierarchy within a group.

The chronic social stress and high cortisol levels observed in subordinate fish are associated with a suppression of aggressive behaviour, locomotory activity and feeding levels (Overli et al., 2002a). These changes in behaviour are likely to be related to cortisol-induced changes in brain monoamine levels, particularly 5-HT and DA (DiBattista et al., 2005b). Increased brain 5-HT activity is associated with inhibition of aggression and subordinate behaviour in fish, while increased DA activity is associated with aggressive, competitive behaviour and dominance (Winberg and Nilsson, 1993). Increased dietary levels of the 5-HT amino acid precursor, L-tryptothan, has been shown to suppress aggressive activity in juvenile rainbow trout (*O. mykiss*) without affecting food intake or plasma cortisol levels (Winberg et al., 2001). Similar dietary supplements have been found to decrease aggressive behaviour in cod (*G. morhua*) (Hoglund et al., 2005) and size heterogeneity and cannibalism in juvenile grouper (*Epinephelus coioides*, Hseu et al., 2003). It has been suggested that this may be a promising management strategy, since the effects of the dietary L-tryptothan will be most pronounced in the most aggressive, dominant individuals that eat the most food and thus strong dominance hierarchies and heterogeneous growth rates may be avoided (Winberg et al., 2001; Hoglund et al., 2005). Such a strategy maybe particularly useful during periods of reduced feeding where competition is high.

An alternative approach to reducing aggression may be possible through the presence of larger conspecifics. The presence of a large dominant fish may suppress aggression among its smaller companions. This was illustrated by a small-scale experiment on Atlantic salmon (*S. salar* L.) parr where 0+ fish that had been housed with a 1+ fish showed significantly reduced aggression amongst themselves and significantly increased growth rates when compared to controls (Adams et al., 2000). Although the larger fish attacked the smaller ones, the attack rate was an order of magnitude lower than that seen between smaller fish in the control group. The observations suggest a more stable social hierarchy with less overt aggression. Whether this situation represents improved welfare and a less stressful social environment for the small fish requires further investigation. Here again, caution should be taken in interpreting stress physiology or arousal in relation to welfare and suffering. While stress measures may tell us little about potential suffering here, behavioural observation may provide further insight (Dawkins, 2003).

Background or substrate colour may be another factor that affects levels of aggression (e.g. in salmonids). Dark body colouration has been suggested to signal social subordination in Atlantic salmon (*S. salar*) and Arctic charr (*S. alpinus*, O'Connor et al., 1999; Hoglund et al., 2000, 2002). A study investigating the levels of aggression seen in the latter species showed that in pairs of fish interacting on a white background, both fish showed initial pale colouration and high levels of aggressive behaviour (Hoglund et al., 2002). Fish interacting on a black background showed initial dark colouration and a lower frequency of aggressive interactions. While one of the fish on the white background became subordinate and took on a darker body colouration, the subordinate fish on the black background did not show any additional darkening. This would suggest that the use of dark substrate has potential for reducing aggressive behaviour and social stress in Arctic charr (*S. alpinus*). Background colour also affects agonistic behaviour in Nile tilapia (*Oreochromis niloticus*, Merighe et al., 2004). This work serves to illustrate that knowledge of socio-biology of different aquaculture species may help to improve welfare.

9. Abnormal behaviour: a welfare concern?

Changes in behaviour can be useful welfare measures where they represent clinical symptoms of poor health or provide early warnings when other symptoms are sub clinical, such as altered swimming behaviour associated with parasitic infections (Post, 1987; Furevik et al., 1993). Beyond this, a wide number of stressors have been shown to cause changes in fish behaviours such as activity, avoidance, predator–prey interactions, feeding, shelter seeking, aggression, and learning (Schreck et al., 1997). These changes may represent an adaptive response mechanism. However, chronic or unavoidable stressors may deny this function and so behavioural changes may become maladaptive. The expression of abnormal behaviour in fish kept in high-density aquaculture is, therefore, of potential welfare concern. However, caution should be taken in interpreting the expression of abnormal behaviour alone in terms of welfare and suffering as, other than those where physical injury occurs, abnormal behaviours do not necessarily indicate poor welfare and might even be compatible with good welfare (Dawkins, 1998).

It has been suggested that the vertical swimming behaviour in Atlantic halibut (*H. hippoglossus* L.) described above (Kristiansen et al., 2004) and the circular shoaling behaviour seen in Atlantic salmon (*S. salar* L.) in sea cages (e.g. Oppedal et al., 2001; Juell et al., 2003) may represent abnormal behaviour or a kind of stereotypy comparable to the pacing seen in some zoo animals (Lymbery, 2002; Kristiansen et al., 2004). Stereotypies are fixed sequences of behaviour performed repetitively in the same way with no obvious function and have been used as indicators of reduced welfare (Mason, 1991a,b). While some stereotypies cause injuries and are clearly of welfare concern, the consequences of others are not so clear and there is no one to one correspondence between stereotypy and welfare (Barnett and Hemsworth, 1990; Dantzer, 1991; Mason, 1991a,b). The causes of stereotypies and what they tell us about welfare are a matter of debate (Dawkins, 1998). There are suggestions that some stereotypies are mechanisms that help animals to cope with environmental change, as reduced behavioural and physiological signs of stress have been observed with such behaviours (Dantzer, 1991; Mason, 1991a,b; Dawkins, 1998). The origin of a stereotypy may be previously adverse conditions where a behaviour pattern was repeatedly elicited, and so they do not necessarily show current suffering. Levels of stereotypy cannot be used to indicate levels of well being (Mason, 1991b). However the repeated expression of a behaviour that represents failed attempts by the animal to alter its environment is thought to indicate poor welfare at some stage. Irrespective of whether they represent a stereotypy, the loops of vertical swimming behaviour observed in Atlantic halibut (*H. hippoglossus* L.) at high experimental densities are, possibly, a welfare concern. Understanding the origin of these apparently functionless behaviours is the key to interpreting how they relate to welfare in fish. For example, it has been suggested that the surface breaking behaviour of Rays (*Raja* sp.) kept in public aquaria represents stereotypic behaviour (Casamitjana, 2004). However, there appears to be a temporal link between this behaviour and scheduled feeding and it has been suggested that the behaviour is a method of foraging adapted for the captive situation (Scott et al., 1999a). Changing the method of food delivery away from the surface has been found to significantly reduce the level of the behaviour (Scott et al., 1999b). The proposals to reduce vertical swimming patterns in Atlantic halibut (*H. hippoglossus* L.) described above may have analogous results.

Although wild salmon do mass in shoals when preparing to migrate back to their home river to spawn, the circular shoaling seen in salmon in sea cages has been described as abnormal when compared to fish in the wild at a similar life stage (Lymbery, 2002). However, without further investigation, it is unknown whether both the activity level and shoaling behaviour are indeed functionless in this situation or whether they form an adaptive functional response to the captive environment with no detriment to welfare. The above examples illustrate the importance of investigation into the origins and reinforcers of such behaviour as well the behavioural and physiological consequences of denying this behaviour. It is interesting to note that swimming depth of Atlantic salmon (*S. salar* L.) in sea cages may represent a compromise between predator avoidance, hunger, bright light avoidance, and the ability to maintain this shoaling behaviour (Fraser et al., 1994; Ferno et al., 1995; Oppedal et al., 2001; Juell et al., 2003; Juell and Fosseidengen, 2004). Investigations into the consequences of the break down of a shoal in low light levels may be useful.

10. The freedom to express normal behaviour

Freedom to express normal behaviour by providing sufficient space, proper facilities and company of an animal's own kind is one of the five freedoms defined by the FAWC (FAWC, 1996). In this sense the stocking densities and confinement seen in the aquaculture industry may be a concern and the inability to express natural behaviours, such as migration, may cause suffering. However, the ''naturalness'' of an animal's behaviour does not necessarily have a direct positive or negative correlation with welfare. This becomes clear when we consider that many natural behaviours are shown in response to adverse conditions such as predator avoidance (Dawkins, 1998, 2003, 2004; FSBI, 2002). There appears to be no way of predicting the importance of a particular behavioural pattern to a given species and the effect its deprivation has on the welfare of an animal. The only way to assess whether the behaviour is necessary or not for welfare is through empirical tests to discover the mechanism of control and the behavioural and physiological consequences of denial of expression (Dawkins, 1998; Cutts et al., 2002). The example of Atlantic salmon (*S. salar* L.) migration has been used to good effect here (FSBI, 2002). If the control mechanism for migratory behaviour is based simply on continuous swimming and the search for improved feeding grounds, then supplying these things in the captive environment may reduce the motivation to migrate and avoid related suffering. However, if based on an intrinsic drive to move to new areas, confinement might well cause suffering (FSBI, 2002).

Even if we are to accept the subjective experiences of fear, pain, and suffering in fish, further research is required to enable us to distinguish between stimuli that are aversive, as opposed to those that are stimulating, as well as those that are important reinforcers. To this end choice tests that allow fish to express their natural preferences (e.g. see Olsen, 1986; Atland and Barlaup, 1996; Atland, 1998; Mirza and Chivers, 2003), particularly in the context of the aquaculture environment (e.g. depth choice seen in Juell et al., 2003), should be used in combination with the physiological measures described above (Broom, 1991a,b; Dawkins, 1998; Dawkins, 2003; FSBI, 2002). With appropriate interpretation, such experiments may tell us how certain practices cause detriment to welfare and which behaviours are important for welfare.

Aimed at reducing stereotypies and deleterious behaviour, and increasing natural behaviours, environmental and feeding enrichment strategies have been used in both zoos (Carlstead et al., 1991; Robinson, 1998; McPhee, 2002; Renner and Lussier, 2002; Bashaw et al., 2003) and terrestrial farms (Mench et al., 1998; Sato, 2001). However, it should be clear that any enrichment does provide an improvement in the biological functioning and welfare of the animal. Therefore, given the argument above, the assessment of potential enrichment programs for fish in aquaculture is very difficult. For example, the use of a dark substrate has the potential to reduce levels of aggression in Arctic char (*S. alpinus*, Hoglund et al., 2002). Also, when red porgy (*Pagrus pagrus*) were exposed to a crowding stressor in different coloured background tanks, fish on a light background colour showed a reduced stress response when compared to those on a black tanks and background effects may be enhanced by coloured illumination (Rotllant et al., 2003; Van der Salm et al., 2004). Thus, species relevant changes to rearing environments may have clear welfare benefits. Feeding and environmental enrichment during rearing appears to affect behaviour and may improve survival rates of hatchery reared Atlantic salmon (*S. salar* L., Brown et al., 2003) and Chinook salmon (*O. tshawytscha*, Maynard et al., 1996) when released to augment wild populations. Following release into the wild, steelhead trout (*O. mykiss*) reared in enriched environments containing submerged structures, overhead cover, gravel substratum, and underwater food delivery, showed altered competitive ability and aggression when compared to conventionally reared fish (Berejikian et al., 2001). In terms of social dominance and aggressive behaviour, fry raised in enriched environments were more similar to naturally raised fish than were fish raised in a baron hatchery environment (Berejikian et al., 2000, 2001). Conventionally reared fish showed unnaturally high levels of intra-group aggression that did not translate to successful acquisition and defence of territories when released, whereas fry reared in a natural stream environment appeared to tolerate the presence of conspecifics. Fish reared in an enriched environment showed an intermediate behavioural profile in this respect.

Whether such environmental manipulation can be used to alter the behavioural profile to improve welfare (e.g. reduce aggression) within fish farms remains to be seen and will surely be dependent on the social behaviours of individual species.

11. Conclusions

Current understanding of the welfare issues discussed in this review are summarised in Tables 1–3. Continued research will only further improve our ability to identify and assess areas of welfare concern within aquaculture and allow us improve welfare wherever possible. Such improvements will benefit aquaculture productivity as well as fish welfare. It is clear that the

Table 1
A summary of the fish welfare issues relating to fish health in aquaculture

Area of welfare concern: health	Welfare issues involved	Sources of improved welfare
Winter diseases		
Several diseases associated with low temperatures	Although many are clearly associated with specific bacterial pathogens, immunosuppression during winter may play a large role (Tort et al., 1998a,b)	Immunisation (Toranzo et al., 1997). Adapted diet providing a supplementary dosage of vitamins and trace minerals to assist the immune system (Tort et al., 2004) and altered feeding regime controlling level of nutrients available to the pathogen (Damsgard et al., 2004; Rorvik et al., 2003; Damsgard et al., 2004)
Fin rot		
Abrasion with the environment and/or aggressive interactions cause fin damage and secondary infection may follow (Turnbull et al., 1998; Ellis, 2002)	Injectable vaccines have superseded antibiotics although vaccines and adjuvants are associated with inflammation and granuloma, as well as the stress of handling anaesthesia and injection (Midtlyng, 1997; Koppang et al., 2004, 2005)	Vaccines with improved efficacy and reduced side effects as well as oral application may improve welfare (Bogwald et al., 1994; Midtlyng, 1997)
Sea lice		
Parasitic copepods may cause severe tissue damage (Johnson et al., 2004)	Lice have developed resistance to traditional chemical treatments (Jones et al., 1992; Treasurer et al., 2000; Tully and McFadden, 2000)	Potential alternative controls include vaccination and selective breeding towards louse resistance (Woo, 1997; Jones et al., 2002; Mordue and Pike, 2002; Revie et al., 2002; Stone et al., 2002; Treasurer et al., 2002; Glover et al., 2004). Biological control with cleaner wrasse should consider wrasse welfare (Treasurer, 2002; Wall, 2005)
Viral diseases		
Examples: infectious pancreatic necrosis (Park and Reno, 2005; Roberts and Pearson, 2005), infectious haematopietic necrosis (Bergmann et al., 2002), viral haemorrhagic septicaemia (Fichtner et al., 1998), infectious salmon anaemia (Hovland et al., 1994), sleeping disease (Graham et al., 2003)	Traditional vaccines developed over the past 20 years have shown only moderate success and there are relatively few commercial vaccines and specific therapeutics with adequate efficacy	The development of alternative anti-viral treatments such as DNA vaccines (Lorenzen and Olesen, 1997; Lorenzen and LaPatra, 2005) and selection for disease resistance (Midtlyng et al., 2002) may improve welfare

Table 1 (*Continued*)

Area of welfare concern: health	Welfare issues involved	Sources of improved welfare
Non-infectious production related deformities		
Deformities of the heart (Poppe and Taksdal, 2000; Poppe et al., 2002), swim bladder (Poppe et al., 1997), and spine (Vagsholm and Djupvik, 1998; Silverstone and Hammell, 2002)	Fish with heart deformities show a high mortality rate during stress due to impaired cardiovascular function, cardiac failure, or heart rupture (Brocklebank and Raverty, 2002; Poppe et al., 2002). Both genetic and environmental factors may contribute to spinal deformities. (Vagsholm and Djupvik, 1998; Halver et al., 1969; Choo et al., 1991; Toften and Jobling, 1996; Bengtsson, 1975)	High temperatures during incubation of salmon should be avoided (Poppe and Taksdal, 2000). Spinal deformities may be reduced by increasing smolt weight at seawater introduction, vaccinating, and reducing salinity and temperature variations (Vagsholm and Djupvik, 1998). Fish from families showing a high incidence of deformities should not be used for breeding (Gjerde et al., 2005)

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Table 2
A summary of the fish welfare issues relating to aquaculture management procedures

Area of welfare concern: aquaculture procedures	Welfare issues involved	Sources of improved welfare
Grading, handling, crowding		
Inherently stressful (Barton et al., 1980; Davis and Schreck, 1997; Sharpe et al., 1998; Conte, 2004)	Many procedures, such as grading, are aimed at improving welfare. There is a large variation between species in stress response to procedures (Barnett and Pankhurst, 1998; Barton et al., 2000, 2003; Ruane and Komen, 2003) and handling stressors can affect subsequent stress response (Schreck et al., 1989; Ruane et al., 2002; Barton et al., 2005)	Appropriate supplementation of dietary Vitamins C + E, and glucan may protect against the adverse effects of chronic stress (Merchie et al., 1997; Dabrowska et al., 1991; Montero et al., 2001; Jeney et al., 1997; Volpatti et al., 1998). The appropriate use of good crowd management, suitable nets, careful handling, recovery periods and movement using fish pumps and transfer pipes is preferable (FAWC, 1996; Scottish Executive, 2002; Conte, 2004; HSA, 2005). Appropriate feeding technique and stocking densities may avoid frequent grading
Transportation		
Inherently stressful as may involve capture, loading, transport, unloading and stocking (Specker and Schreck, 1980)	Transport stressors can affect fish over a prolonged period (Davis and Parker, 1986; Schreck et al., 1989; Iversen et al., 1998, 2005)	Adverse effects may be reduced by suitable acclimation and recovery periods (Conte, 2004; Iversen et al., 2005) as well as species appropriate use of anaesthesia (Erikson et al., 1997; Sandodden et al., 2001; Finstad et al., 2003; HSA, 2005) and dilute salt solutions (Carmichael, 1984; Pickering, 1992; Wedemeyer, 1997)
Food withdrawal		
Starvation prior to slaughter, transportation, and other management practices	May benefit welfare by reducing metabolism, oxygen demand and waste production. Although Atlantic salmon and rainbow trout show long anorexic periods in the wild (Kadri et al., 1995, 1996), the welfare effect of food deprivation in aquaculture is not known. Deprivation for short periods under appropriate conditions may not diminish welfare	Starvation for up to 72 h for Atlantic salmon and 48 h for rainbow trout should only occur where beneficial to welfare (FAWC, 1996) and empirical studies on the effects of starvation on stress physiology or behaviour are required

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Table 2 (*Continued*)

Area of welfare concern: aquaculture procedures	Welfare issues involved	Sources of improved welfare
Slaughter		
Slaughter should be as humane as possible—fish should be stunned prior to slaughter, causing an immediate loss of consciousness that lasts until death (FAWC, 1996)	De-watering followed by asphyxiation in ice slurry of rainbow trout and gilt head sea bream (Kestin et al., 1991; Robb et al., 2000b; Lines et al., 2003; Van de Vis et al., 2003); immersion in CO_2 saturated water followed by gill cut or gill cutting alone for Atlantic salmon and rainbow trout (Robb et al., 2000b; Robb et al., 2002a); and de-sliming followed by evisceration of eels (Robb et al., 2002b; Van de Vis et al., 2003) do not meet the criteria for humane slaughter	Percussive or electrical stunning methods appear to achieve humane slaughter in Atlantic salmon, gilt-head sea bream, turbot, and rainbow trout (Robb et al., 2002a; Morzel et al., 2003; Van de Vis et al., 2003; HSA, 2005). The use of electric stunning tongues or electrically stunning batches of eels in freshwater combined with nitrogen flushing can cause immediate unconsciousness (Robb et al., 2002b; Van de Vis et al., 2003)

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Table 3
A summary of the fish welfare issues relating to stocking density, aggression and altered behaviour in aquaculture

Area of welfare concern	Welfare issues involved	Sources of improved welfare
Stocking density		
Pivotal factor affecting welfare in a number of different ways (e.g. through aggression, water quality, and activity/feeding patterns)	The effect of stocking density comprises of numerous interacting and case specific factors. Sea bass show high stress levels at high densities (Vazzana et al., 2002; Gornati et al., 2004). Arctic charr show low growth and food intake at low and very high densities (Jorgensen et al., 1993; Ellis et al., 2002). Halibut tolerance for high stocking density appears to be stage dependent (Greaves and Tuene, 2001; Kristiansen et al., 2004). Rainbow trout show a decrease in welfare at high densities, water quality being a key factor (Ellis, 2002; Ellis et al., 2002). High stocking densities, above a given threshold, are associated with reduced welfare in Atlantic salmon in sea cages. Site-specific factors also have an effect on welfare (Turnbull et al., 2005)	Feeding pattern and floor space may be altered to improve the effect of density on welfare in halibut, also see aggression below (Kristiansen et al., 2004). Salmon swimming depth and shoal density can be manipulated by artificial light levels (Juell et al., 2003; Juell and Fosseidengen, 2004), and feeding patterns can alter aggressive interactions in several species including Atlantic salmon (Andrew et al., 2002)
Aggression		
Formation of social hierarchies may lead to injuries (Greaves and Tuene, 2001), chronic social stress (Ejike and Schreck, 1980; Schreck et al., 1997; Wedemeyer, 1997; Alanara et al., 1998) and size heterogeneity (Alanara et al., 1998; Dibattista et al., 2005a)	Socio-biology, stocking density, and feeding technique have strong influences on the levels of social interactions (Alanara and Brannas, 1996; Adams et al., 1998; Andrew et al., 2002; Andrew et al., 2004)	Feeding technique should be species appropriate to avoid excess competition and aggression (Alanara and Brannas, 1996, 1997; Alanara et al., 1998; Andrew et al., 2002). The presence of a small number of larger fish may reduce aggression within groups of smaller fish (Adams et al., 2000). Increased dietary levels of L-tryptothan, has been shown to suppress aggressive activity (Winberg et al., 2001; Hoglund et al., 2005; Hseu et al., 2003). Substrate or background colour may be used to influence aggressive behaviour in some species (O'Connor et al., 1999; Hoglund et al., 2000, 2002; Merighe et al., 2004)

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Table 3 (*Continued*)

Area of welfare concern	Welfare issues involved	Sources of improved welfare
Abnormal behaviour and the freedom to express normal behaviour		
Abnormal behaviour includes repetitive behaviour and abnormal swimming activity/patterns	Understanding the functional origin of apparently abnormal behaviour is important. Empirical studies are required to establish whether abnormal behaviours represent diminished welfare or adaptive responses with no effect on welfare (Dawkins, 1998; FSBI, 2002; Dawkins, 2003, 2004)	Enriched rearing environments may improve welfare following release to augment wild populations (Maynard et al., 1996; Brown et al., 2003; Berejikian et al., 2000, 2001). Without empirical studies the importance of a given behavioural pattern to a given species is unclear. Studies of the mechanism of control and/or the behavioural and physiological consequences of denial of expression of key behaviours are required. Choice studies may allow assessment of the value associated with a given behaviour or resource

areas of welfare concern discussed do not exist in isolation but interact to affect the welfare of the individual. Stocking density, diet, feeding technique, and management procedures all have strong effects on stress levels, subsequent stress tolerance, health, and the presence of aggressive behaviour. In turn, these effects feedback to one another to further influence welfare. Research into the interaction between stress physiology and behaviour has also shown strong links between aggression and stress sensitivity (Overli et al., 2005). Future investigations into the neuroendocrine control mechanisms involved and the existence of ‘‘coping styles’’ may provide further insight into how multiple factors combine to effect welfare within a given situation.

The very nature of aquaculture demands high standards of management and stockmanship to avoid the adverse economic effects of stress and disease. However, knowledge of the effects of handling, transport, and food deprivation on stress physiology should be used to make species specific recommendations for best practice. Continued research into reducing stress response through diet, conditioning and perhaps selective breeding will help to improve welfare standards. Further research into the most effective treatment and/or management of sea lice, fin rot, viral diseases, and non-infectious production related problems, is crucial to future improvements of welfare. While it has been shown that humane slaughter is possible, the key goal now is to implement these techniques commercially.

A complex matrix of factors influences the effect of stocking density and the relative importance of these factors is case specific. Therefore, the use of research into how environmental parameters, physiology, and particularly behavioural factors play a role should be productive in improving welfare, and studies on the farm must continue to be carried out to put laboratory findings into context.

While behavioural observations are useful as early indicators of poor welfare and health, caution should be taken in the interpretation of abnormal behaviour and the lack of freedom to express natural behaviour. Investigation into the origin of apparent abnormal behaviour may improve welfare, and the use of choice test experiments within the aquaculture environment may help us to interpret physiological response to aquaculture stressors as well as the importance of expression of natural behaviours. Regardless of judgements on the ability of fish to suffer, these studies will help improve welfare. However, they may, in addition, provide further insight into the subjective experiences of fish, their capacity to suffer and subsequently the importance of fish welfare consideration.

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