WATER QUALITY AND WELFARE ASSESSMENT ON UNITED KINGDOM TROUT FARMS

THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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Declaration

I declare that this thesis has been composed in its entirety by myself. Except where specifically acknowledged, the work described in this thesis has been conducted by me and has not been submitted for any other degree.

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Abstract

Interest in the subject of fish welfare is continuing to grow, with increasing public awareness and new legislation in the UK. Water quality has long been recognised as being of prime importance for welfare: water provides the fish with oxygen and removes and dilutes potentially toxic waste metabolites. This thesis investigates the interactions between water quality and the welfare of farmed rainbow trout (*Oncorhynchus mykiss* Walbaum).

A literature review was undertaken to identify current recommended water quality limits for the health and welfare of farmed rainbow trout. Contradictions in the literature regarding suggested 'safe' water quality limits were also identified, as were deficiencies in some of the methods used to arrive at conclusions for recommended limits. The literature relating to the effects of poor water quality on welfare were also reviewed. The review ends with a discussion about water quality monitoring in the context of on-farm welfare assessment and how the information might be used in such a scheme.

A telephone survey of UK rainbow trout farmers was undertaken to ascertain the level of water quality monitoring currently conducted. Participants in this study accounted for over 80% of 2005 UK rainbow trout production. It was established that 54% of farmers monitored dissolved oxygen to some extent and 69% monitored temperature, the most commonly measured water quality parameters and among the most important for health, welfare and growth. Subsequent visits were made to a sample of the participants in the telephone survey to obtain more detailed information of the farming operations, such as frequency of water quality monitoring, retention of production data and slaughter methods. Monitoring water quality will be an integral part of any on-farm welfare assessment scheme, and while measuring some water quality parameters requires specialist equipment, farmers should be able to monitor the essential parameters, dissolved oxygen and temperature. Any on-farm welfare assessment scheme for rainbow trout should incorparate fish-based measures in addition to resource-based parameters in order to provide as complete an overview of trout welfare as possible.

An epidemiological study was undertaken to investigate the current status of welfare on UK rainbow trout farms and to identify risk factors for welfare. Forty-four trout farms from throughout the British Isles were visited between July 2005 and April 2007, sampling a total of 3700 fish from 189 different systems. Farms were visited twice, once in winter and once in summer, to account for any seasonal differences in fish physiology and environmental conditions. Data were collected on a range of fish parameters, together with background information on the batch from which the fish originated. Particular emphasis was placed on water quality due to the potential effects this can have on welfare. The water in each system sampled was monitored for 24 hours, with measurements of dissolved oxygen, temperature, pH, specific conductivity and ammonia taken every 15 minutes.

A welfare score was developed for each fish using a multifactorial method, combining data on the condition of the fins, the condition of the gills, the stress hormone cortisol, the splenosomatic index and the mortality levels for the population of fish in the system. Using this welfare score and the individual components of the score as response variables, multi-level models were developed using the water quality, system and husbandry data collected. The primary risk factor that was associated with deteriorating welfare was disease. The purpose for which the fish was being farmed was also important, as fish farmed for the table market had on average worse welfare than those farmed for restocking fisheries. Seasonal effects, linked to higher water temperatures in summer, were associated with poorer welfare scores.

Aside from seasonal effects, there is not much evidence that poor water quality is a major problem for the welfare of farmed rainbow trout in the UK. While deteriorating water quality certainly has the potential to affect the welfare of farmed rainbow trout, water quality measurements were within recommended ranges for the majority of farms visited. The results of this epidemiological study suggest that factors other than water quality may have a greater impact on trout welfare, such as exposure to diseases and production differences between farming for the table and restocking markets.

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1 General Introduction

“The greatness of a nation and its moral progress can be judged by the way its animals are treated.” (Mohandas Gandhi, cited in Appleby & Hughes 1997).

1.1 Why is fish welfare important?

1.1.1 Fish sentience

The primary argument why animal welfare is important is succinctly made by John Webster (2006), “Whenever we use a sentient animal for own (*sic*) purposes we assume responsibility for its welfare.” If this moral stance is accepted, and that therefore only sentient animals are worthy of welfare considerations, then establishing if fish are sentient is imperative (Lund *et al*. 2007). Duncan (1993 cited in Fraser 1999) stated that sentience was a “necessary prerequisite for welfare”.

Sentience can be defined as the ability to consciously experience emotions and feelings, and by extension the capacity to suffer. Dawkins (1997) defines consciousness as “a range of states in which there is an immediate awareness of thought, image, memory or sensation”, which is separate from self-awareness. Providing conclusive evidence of sentience in an animal is extremely difficult and requires specialised techniques. Chandroo *et al*. (2004a) discussed the use of motivational affective states, which encompass a range of conscious experiences such as pain, fear, thirst, hunger and pleasure. These motivational affective states have been shown to affect animal behaviour and, although they are subjective experiences, can be measured through indirect evidence, such as observing behaviour. Using these motivational affective states to understand welfare has advantages over attempting to compare animals' subjective experiences and neuroanatomy with that of humans, as has been attempted in the past (see Rose 2002, 2007, Iwama 2007 for a discussion). Using motivational affective states does not involve the inherent bias of anthropomorphism in attempting to compare human and animal subjective states, allowing such states to be classified as being negative or positive for the animal concerned (Chandroo *et al*. 2004a).

One method to determine if fish are capable of suffering is to consider whether fish experience physical injury as pain (Rose 2002). Pain has 2 components, the detection of noxious stimuli, or nociception, and the awareness of pain as a conscious experience (International Association for the Study of Pain, www.iasp-pain.org). It has been demonstrated that fish have nociceptors (Sneddon *et al*. 2003a, b) and are therefore capable of detecting noxious stimuli, and that they respond behaviourally and physiologically to the noxious stimuli (Ashley & Sneddon 2008). The use of analgesics was observed to ameliorate the effects of noxious stimuli in rainbow trout (*Oncorhynchus mykiss*) (Sneddon 2003), providing further evidence for pain perception in fish. However, to what extent, if at all, they are consciously aware of pain is open to debate (Chandroo *et al.* 2004b). Indirect evidence can be used to establish if fish can experience pain and other motivational affective states, such evidence including neuroanatomy, neurophysiology and behaviour (Chandroo *et al*. 2004a). While some studies have focused on neuroanatomy and neurophysiology (*e.g.* Rose 2002, Sneddon *et al.* 2003a, b), it has been argued that understanding the subjective states of animals can only be achieved through behavioural observations (Dawkins 2004, 2006b, Braithwaite & Boulcott 2008). Adaptive, flexible behaviour can be indicative of consciousness in animals, as opposed to behaviour that is rigid and automatic (Dawkins 2006a). Yue *et al*. (2004, 2008) used behaviour and affective states to investigate fear in rainbow trout, suggesting that trout are capable of fear and suffering and that their behaviour was indicative of a flexible response, rather than a rigid, reflexive one.

Certain behavioural actions are believed to indicate that an animal is able to form and act upon internal representations of its external environment (Ashley & Sneddon 2008). The ability to form these internal representations is believed to be present only in animals with a level of neural complexity that suggests a basic level of consciousness (Chandroo *et al*. 2004a). There is growing evidence that fish have some level of consciousness (see Chandroo *et al*. 2004a, Braithwaite & Boulcott 2008 for reviews). Portavella *et al.* (2002) demonstrated that different areas of the goldfish (*Carassius auratus*) brain were responsible for emotional, temporal and spatial activities, indicating that goldfish are capable of differentiated emotional learning and spatial and temporal learning. Additionally, areas of the goldfish brain were suggested to be homologous with areas of brains of higher vertebrates known to be involved with emotion and spatial behaviour (Braithwaite & Boulcott 2008). This complexity of behaviour and neuroanatomy is highly suggestive that fish are sentient and have some capacity for consciousness or awareness. It would be almost impossible to definitively prove that fish are sentient, however in light of the available evidence it is highly likely that they are sentient. On balance, fish fall within our moral compass and are deserving of welfare considerations.

1.1.2 UK legislation

In addition to the moral responsibilities that stewardship confers with respect to sentient animals, fish welfare is governed by a legal framework. In the UK over the last century, there have been many pieces of legislation enacted covering the welfare of farmed animals. The focus of legislation over that period has shifted from prevention of cruelty to promotion of good welfare in line with scientific advances in our understanding of the animals we farm. The first main pieces of legislation to cover the welfare of farmed animals was The Protection of Animals Act 1911 and the Protection of Animals (Scotland) Act 1912 (Voas 2008). This initial legislation was not intended to cover farmed fish, given the infancy of the fish farming industry at that stage, however, fish are specifically included under the latest legislation, the Animal Welfare Act 2006 and the Animal Health and Welfare (Scotland) Act 2006. These pieces of legislation provide a framework for the general welfare of animals in the UK, with more specific secondary legislation intended in the future (http://www.defra.gov.uk/animalh/welfare/act/secondary_legis.htm). Animal welfare legislation is based around the principles of the 'five freedoms', set out in the Brambell Committee Report (Brambell 1965, cited in Voas 2008). The five freedoms are:-

1. Freedom from thirst, hunger and malnutrition
2. Freedom from discomfort due to environment
3. Freedom from pain, injury and disease
4. Freedom to express normal behaviour for the species
5. Freedom from fear and distress

In addition to legislation covering general aspects of farming, there also exists legislation covering specific aspects, such as slaughter (The Welfare of Animals (Slaughter or Killing) Regulations 1995) and transport (The Welfare of Animals (Transport) (Scotland) Regulations 2006; The Welfare of Animals (Transport)

(England) Order 2006; The Welfare of Animals (Transport) (Wales) Order 2007). Slaughter regulations require that anyone killing farmed animals should do so humanely, without causing unnecessary pain or suffering, and have received sufficient training to do so. Transport regulations require that animals that are transported are done so in a way that does not cause unnecessary pain or suffering. Specific regulations for the transport of fish include the need to provide suitable containers with an adequate amount of water.

1.1.3 Public Awareness of Welfare

In recent years there has been an increase in the public's awareness and concern about farm animal welfare (Appleby & Hughes 1997, Haper & Makatouni 2002) including fish (Turnbull & Kadri 2007). It is not clear if public concern for fish welfare is translated as a concern for the well-being of the animals *per se*, or rather concern for the quality of the products they are consuming (Haper & Makatouni 2002, Blokhuis *et al*. 2003). Public concern for animal welfare has risen in tandem with concern about food health and safety in response to intensification of agricultrual animal production (Haper & Makatouni 2002). Welfare friendly products are perceived as being healthier and better quality, in line with a similar perception of organic products (http://www.food.gov.uk/news/newsarchive/2003/jun/cheltenham), with consumers often confusing organically grown products with 'welfare-friendly' products (Haper & Makatouni 2002). A Dutch study into consumer attitudes towards pig and fish welfare suggested that consumers were aware and concerned about fish welfare, however, they did not want to know details about husbandry and slaughter methods (Frewer *et al*. 2005). It appears that public concern for farmed animal welfare is a complicated issue, yet whatever the reasons for the concern, be it for the animals or for food safety and quality, concern exists and therefore welfare matters and requires investigation by the scientific and philosophical community. Serpell (2004) states that no improvements in animal welfare will ever be made, regardless of scientific and philosophical advances, unless there is public concern for the animals.

Governmental and non-governmental organisations have issued reports on farmed fish welfare, highlighting many of the welfare issues surrounding modern, intensive fish farming (*e.g.* Lymbery 2001, 2002, Stevenson, 2007) including the influential report by the Farm Animal Welfare Council (FAWC 1996). Additionally, the RSPCA have issued welfare standards for farmed Atlantic salmon (*Salmo salar*) under their Freedom Foods scheme (Anon 2007). These schemes and reports maintain public awareness of welfare issues and contribute to ongoing debate on farming fish and the conditions under which cultured fish should be reared.

1.1.4 Practical welfare concerns

From a practical perspective, fish welfare matters to the people who farm them. Many farmers have accepted that fish feel pain and have the capacity to suffer (Read 2008), and therefore work hard to provide their stock with optimal husbandry and environmental conditions. It is unlikely that fish farmers will form human-animal relationships similar to those found with larger livestock (Waiblinger *et al*. 2006), however, there is no doubt that fish farmers care about the welfare of their stock (North *et al.* 2008, Read 2008, fish farmers short course, Institute of Aquaculture, J. Turnbull, pers. comm.). Furthermore, fish that have good welfare will grow well, have less disease, high survival rates and therefore produce an increased rate of financial return for the farmer (Turnbull & Kadri 2007, Huntingford & Kadri 2008, Read, 2008).

The concern of the fish farmer towards welfare is reflected in the codes of practice generated by the industry trade associations. The Federation of European Aquaculture Producers have produced the Code of Conduct for European Aquaculture, while in the UK, the British Trout Association have their own Code of Practice (Anon 2002) as well as the Quality Trout UK standards (Anon 2006). For aquaculture in Scotland, a voluntary code of practice has been issued entitled ‘Scotland: a Code of Good Practice for Scottish Fin Fish Aquaculture’, which covers 95% of Scottish salmon production (www.scottishsalmon.co.uk). Each of these documents contains requirements relevant to the welfare of farmed fish, specifically relating to stockmanship and husbandry procedures and the minimisation of distress to the stock.

1.2 What is animal welfare?

Unfortunately, there is no universally agreed definition of animal welfare. Welfare can mean different things to different people, and these differences have resulted in disagreements, not only in how to define welfare, but how best to assess it. Fraser *et al*. (1997) categorised three ethical concepts that have allowed progress to be made towards an improved understanding of welfare and have facilitated discussion between proponents of different ethical stances. The three welfare concepts around which many welfare definitions are based are that the animal should be functioning well, that it should feel well and that it should be allowed to express its natural behaviour.

1.2.1 Function-based definitions of welfare

Definitions of welfare under this concept state that welfare is good if the animal's biological systems are functioning well (Duncan & Fraser 1997). Assessment of the welfare state of an animal is made by measuring physiological, morphological and in some cases production based indices, such as mortality rates. Welfare is indicated by the condition of the animal, the disease state, signs of physical injury, normal growth rates and normal functioning of physiological processes. A purely functional approach to welfare was adopted by McGlone (1993, cited in Duncan & Fraser 1997), who dismissed feelings-based definitions and held that welfare is only infringed when “physiological conditions are disturbed to the point that survival or reproduction is impaired.” This assumption in functional welfare definitions that if the animal is functioning well then it is in a good welfare state may not always be the case (Huntingford *et al.* 2006), as it is possible an animal that is functionally healthy may not experience good welfare, for example if a social animal is denied companionship (Huntingford & Kadri 2008). Fraser (1999) stated that welfare refers to what an animal is experiencing, therefore function alone cannot provide an entire welfare picture.

1.2.2 Feelings-based definitions of welfare

Feelings-based definitions are based on the subjective states of the animal. Good welfare is defined as the animal being free of negative experiences such as pain, fear, hunger and that it has access to positive experiences. These definitions are reliant upon the animal being sentient, as discussed above, and upon our ability to appreciate to some extent what the subjective experiences of the animal are. While function-based welfare definitions are useful and can be easy to measure, it has been argued that it is the subjective experience of the animal that is most important (Dawkins 1997, 2006a, Duncan & Fraser 1997, Fraser 1999, Duncan 2006).

1.2.3 Nature-based definitions

These definitions are based on the concept of an animal having an inherent biological nature, or *telos* (Rollin 1992, cited in Duncan & Fraser 1997), and the suggestion that this inherent nature should be taken into account when raising the animal under culture conditions (Duncan & Fraser 1997). The assumption here is that animals have evolved and adapted for life in their natural environment, and that therefore the animals should be allowed to perform all their natural behaviours under culture conditions in order to meet their 'behavioural needs' (Huntingford & Kadri 2008). These nature-based definitions are extensions of feelings-based definitions, as they refer to how an animal feels, for example if an animal is motivated to perform some behaviour, and is subsequently denied the opportunity to carry this out, then presumably the animal would experience a sense of frustration and suffering, resulting in poor welfare. Lawrence (2008) poses the issue in terms of a potential conflict between natural biological adaptation and artificial culture conditions and of how this might be expressed in the animal's emotional and functional responses. The example commonly used for fish is that of wild Atlantic salmon (*Salmo salar*) (Huntingford *et al.* 2006, Huntingford & Kadri 2008). In the wild, Atlantic salmon will migrate over long distances in the ocean. If the reason for this migration is to leave an area with low food supplies and to search for food, then raising salmon in cages with an abundant supply of food may not *per se* promote poor welfare. However, if salmon are motivated to migrate regardless of food supply, then keeping salmon in cages may well lead to poor welfare. To date the question over the reasons for salmon migration has not been answered.

With nature-based definitions, there is the assumption that all that is natural is good (Duncan & Fraser 1997). However, much natural behaviour has evolved in animals to enhance their chances of survival when faced with a threatening situation, yet such behaviour may be redundant under culture conditions, and may even prove harmful. Rainbow trout when startled have been observed to begin a ‘tidal wave’, with all individuals in a culture system moving rapidly towards one end of the system away from the perceived threat (pers. obs.), potentially causing injury as fish are crushed or even forced out of the water altogether. However, Špinka (2006) argues that while the full repertoire of natural behaviour is not necessary for animals held under culture conditions, animals should be encouraged to perform some natural behaviour in order to improve their welfare, as long as the behaviour is not harmful to the fish.

Welfare means different things to different people, and there are many different aspects to welfare that need to be taken into account. For the purposes of this study, welfare is taken to be the physical and mental state of the fish in relation to its environment (Appleby & Hughes 1997, Duncan & Fraser 1997); this working definition incorporates function and feelings based definitions, and will also extend to include nature-based definitions should these have positive or negative effects on the mental state of the fish. In a similar vein, Dawkins (2003) suggests welfare can be covered by asking two questions: ‘Are the animals healthy and do they have what they want?’ These questions also cover functional and feelings-based aspects of welfare.

1.2.4 The Stress Response

If welfare is defined as the physical and mental state of the fish, how the fish perceives its environment is central to welfare. If the fish' reaction to a situation is fear, distress or even excitement, then the stress response will be activated. The stress response evolved in animals to increase the animal's survival chances when faced with a threat, real or perceived (Pottinger 2008). The stress response is not welfare, although it has often been equated with it (*e.g.* Varsamos *et al.* 2006, Drew *et al.* 2007). However, the stress response is an integral component of welfare, and will be referred to throughout this thesis. It has been argued that the stress response in fish is not only a physiological reaction, but also has a psychological component too (see Chandroo *et al.* 2004a), and therefore has the potential to cover functional as well as feelings-based definitions of welfare. This section briefly outlines the mechanisms and different stages of the stress response. Traditionally, the stress response is classified into 3 stages.

1.2.4.1 Stage 1 –Primary Neuroendocrine Responses

The first stage comprises neuroendocrine components. The first of these is a very fast activation of the sympathetic nervous system that culminates in the release of catecholamines into the blood from chromaffin tissues (Mazeaud & Mazeaud 1981). At the same time, the second, slower endocrine response is a cascade of activation down the HPI axis – the hypothalamic, the pituitary and the interrenal tissue (Donaldson 1981). The endpoint of this is the release of the steroid hormone cortisol into the blood, elevated levels of which are detected up to a few minutes following exposure to the stressful event and can take up to one or more hours to return to normal levels (Wendelaar Bonga 1997).

1.2.4.2 Stage 2 – Secondary Responses

This stage of the stress response if characterised by increases in the fish' respiratory capacity, heart rate, opercular beat rate, blood flow to the gills, and the mobilisation of carbohydrate and lipid energy reserves (Pottinger 2008). These physiological changes facilitate behavioural changes that enhance the chances of survival, such as escape or freezing and a general increase in alertness.

1.2.4.3 Stage 3 – Tertiary Responses

The primary and secondary stress responses are not welfare concerns *per se*, as fish are well adapted to cope with acute stressors (Huntingford & Kadri 2008). However, continual or frequent intermittent activation of the stress response over extended periods of time result in chronic stress (Pottinger 2008). Fish are not well adapted to chronic stress, the consequences of which can be poor growth, loss of reproductive function and immunosupression, which can lead to increased susceptibility to disease (Wendelaar Bonga 1997).

1.3 Outline of the project

This study was funded by the British Trout Association under the Niall Bromage Studentship, in association with a Defra-funded project AW1205. The primary aim of the project was to investigate the relationships and interactions between water quality and the welfare of farmed rainbow trout in the UK. This thesis covers three of the scientific objectives of the AW1205 project.

Chapter 2. Scientific Objective 01. *A literature review of information relating to water quality and welfare.*

Chapter 3. Scientific Objective 02. *Description of current status of water quality monitoring and control on farms.* The scope of this objective was extended to investigate the structure of the industry and how that would influence any future on-farm welfare assessment scheme, and additionally to investigate other types of information recorded on UK trout farms that could be used to assist such a scheme.

Chapters 4/5. Scientific Objective 05. *Farm based epidemiological study of relationship between water quality and indicators of welfare.*

This thesis takes the format of a series of manuscripts for publication: chapter 2, the literature review, was published in the book 'Fish Welfare' (edited by E.J. Branson, Blackwell Publishing, Oxford), while chapters 3, 4 and 5 are draft manuscripts. The literature review was written in conjunction with T. Ellis (CEFAS), who wrote the majority of the discussion, while I wrote the body of the review. B. North and J. Turnbull co-edited the manuscript. The data collection for chapter 3 was done primarily by myself, with assistance from J. Nikolaidis with the telephone calls to farmers, and I. Hoyle (Bristol University) and C. Pond (CEFAS) with data collection in the South of England. The manuscript was written by me and edited by J. Turnbull. The majority of the data collection for the epidemiological study was conducted by myself, with B. North and I. Berrill carrying out some of the sampling. Data analysis and writing of the manuscripts was done by myself and edited by J. Turnbull and J Bron.

1.4 References

Anon. 2002. *British Trout Association Code of Practice*. British Trout Association. pp11.

Anon. 2006. *Quality Trout UK Farm & Hatchery Certification Standards*. Quality Trout UK. pp25.

Anon. 2007. *RSPCA Welfare standards for farmed Atlantic salmon*. RSPCA, Horsham, UK.

Appleby, M.C., Hughes, B.O. 1997. *Introduction*. In: Appleby, M.C., Hughes, B.O. (Eds). Animal Welfare. CABI, Oxfordshire. ppxi-xiii.

Ashley, P.J., Sneddon, L.U. 2008. *Pain and fear in fish*. In: Branson, E.J. (ed) Fish welfare. Blackwell Publishing, Oxford. pp49-77.

Blokhuis, H.J., Jones, R.B., Geers, R., Miele, M., Veissier, I. 2003. Measuring and monitoring animal welfare: transparency in the food product quality chain. *Animal Welfare*. **12**: 445-455.

Braithwaite, V.A., Boulcott, P. 2008. *Can fish suffer?* In: Branson, E.J. (ed) Fish welfare. Blackwell Publishing, Oxford. pp78-92.

Brambell, F.W.R. 1965. *Report of the technical committee to enquire into the welfare of livestock kept under intensive husbandry systems*. HMSO, London.

Chandroo, K.P., Duncan, I.J.H., Moccia, R.D. 2004a. Can fish suffer?: perspectives on sentience, pain, fear and stress. *Applied Animal Behaviour Science*. **86**: 225-250.

Chandroo, K.P., Yue, S., Moccia, R.D. 2004b. An evaluation of current perspectives on consciousness and pain in fishes. *Journal of Fish and Fisheries.* **5**:281-295.

Dawkins, M.S. 1997. Why has there not been more progress in animal welfare research? *Applied Animal Behaviour Science*. **53**: 59-73.

Dawkins, M.S. 2004. Using behaviour to assess animal welfare. *Animal Welfare.* **13**: S3-7.

Dawkins, M.S. 2006a. A user’s guide to animal welfare science. *Trends in Ecology & Evolution*. **21(2)**: 77-82.

Dawkins, M.S. 2006b. Through animal eyes: What behaviour tells us. *Applied Animal Behaviour Science*. **100**: 4-10.

Donaldson, E.M. 1981. *The pituitary-interrenal axis as an indicator of stress in fish*. In: Pickering, A.D. (Ed). Stress and Fish. Academic Press. pp11-48.

Drew, R.E., Schwabl, H., Wheeler, P.A., Thorgaard, G.H. 2007. Detection of QTL influencing cortisol levels in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. **272**: S183-S194.

Duncan, I.J.H. 1993. Welfare is to do with what animals feel. *Journal of Agricultural and Environmental Ethics*. **6(Suppl. 2)**: 8-14.

Duncan, I.J.H., Fraser, D. 1997. *Understanding animal welfare*. In: Appleby, M.C., Hughes, B.O. (Eds). Animal Welfare. CABI, Oxfordshire. pp19-32.

Duncan, I.J.H. 2006. The changing concept of animal sentience. *Applied Animal Behaviour Science*. **100**: 11-19.

FAWC. 1996. *Report on the welfare of farmed fish*. Farm Animal Welfare Council, Surbiton, UK.

Fraser, D., Weary, D.M., Pajor, E.A., Milligan, B.N. 1997. A scientific conception of animal welfare that reflects ethical concerns. *Animal Welfare*. **6**: 187-205.

Fraser, D. 1999. Animal ethics and animal welfare science: bridging the two cultures. *Applied Animal Behaviour Science*. **65**: 171-189.

Frewer, L.J., Kole, A., van de Kroon, S.M.A., de Lauwere, C. 2005. Consumer attitudes towards the development of animal-friendly husbandry systems. *Journal of Agricultural and Environmental Ethics*. **18(4)**: 345-367.

Harper, G.C., Makatouni, A. 2002. Consumer perception of organic food production and farm animal welfare. *British Food Journal.* **104**: 287-299.

Huntingford, F.A., Adams, C., Braithwaite, V.A., Kadri, S., Pottinger, T.G., Sandøe, P., Turnbull, J.F. 2006. Review Paper: Current issues in fish welfare. *Journal of Fish Biology*. **68(2)**: 332-372.

Huntingford, F.A., Kadri, S. 2008. *Welfare and fish*. In: Branson, E.J. (ed) Fish welfare. Blackwell Publishing, Oxford. pp19-31.

Iwama, G.K. 2007. The welfare of fish. *Diseases of Aquatic Organisms*. **75**:155-158.

Lawrence, A.B. 2008. *What is animal welfare?* In: Branson, E.J. (ed). Fish welfare. Blackwell Publishing, Oxford. pp7-18.

Lund, V., Mejdell, C.M., Röcklinsberg, H., Anthony, R., Håstein, T. 2007. Expanding the moral circle: farmed fish as objects of moral concern. *Diseases of Aquatic Organisms*. **75**: 109-118.

Lymbery, P. 2001. *Supermarkets and farm animal welfare*: 'Raising the standard'. A Compassion in World Farming report, Hampshire, UK.

Lymbery, P. 2002. *In too deep – the welfare of intensively farmed fish*. A Compassion in World Farming report, Hampshire, UK.

Mazeaud, M.M., Mazeaud, F. 1981. *Adrenergic responses to stress in fish*. In: Pickering, A.D. (Ed). Stress and Fish. Academic Press. pp49-76.

McGlone, J.J. 1993. What is animal welfare? *Journal of Agricultural and Environmental Ethics*. **6(Suppl. 2)**: 26-36.

North, B.P., Ellis, T., Knowles, T., Bron, J.E., Turnbull, J.F. 2008. *The use of stakeholder focus groups to identify indicators for the on-farm assessment of trout welfare.* In: Branson, E.J. (ed) Fish welfare. Blackwell Publishing, Oxford. pp243-267.

Portavella, M., Vargas, J.P., Torres, B., Salas, C. 2002. The effects of telencephalic pallial lesions on spatial, temporal, and emotional learning in goldfish. *Brain Research Bulletin*. **57**: 397-399.

Pottinger, T.G. 2008. *The stress response in fish – mechanisms, effects and measurement.* In: Branson, E.J. (ed). Fish welfare. Blackwell Publishing, Oxford. pp32-48.

Read, N. 2008. *Fish farmer's perspective of welfare*. In: Branson, E.J. (ed). Fish welfare. Blackwell Publishing, Oxford. pp101-110.

Rollin, B.E. 1992. *Animal rights and human morality*. Revised Edition. Prometheus Books, Buffalo, USA.

Rose, J.D. 2002. The neurobehavioural nature of fishes and the question of awareness and pain. *Reviews in Fisheries Science*. **10(1)**: 1-38.

Rose, J.D. 2007. Anthropomorphism and ‘mental welfare’ of fishes. *Diseases of Aquatic Organisms*. **75**: 139-154.

Serpell, J.A. 2004. Factors influencing human attitudes to animals and their welfare. *Animal Welfare*. **13**: s145-151.

Sneddon, L.U. 2003. The evidence for pain in fish: the use of morphine as an analgesic. *Applied Animal Behaviour Science*. **83**: 153-162.

Sneddon, L.U., Braithwaite, V.A., Gentle, M.J. 2003a. Do fish have nociceptors? Evidence for the evolution of a vertebrate sensory system. *Proceedings of the Royal Society of London, Series B*. **270**: 1115–1121.

Sneddon, L.U., Braithwaite, V.A.,Gentle M.J. 2003b. Novel object test: examining nociception and fear in the rainbow trout. *Journal of Pain*. **4**: 431-440.

Špinka, M. 2006. How important is natural behaviour in animal farming systems. *Applied Animal Behaviour Science*. **100**: 117-128.

Stevenson, P. 2007. *Closed waters: the welfare of farmed Atlantic salmon, rainbow trout, Atlantic cod and Atlantic halibut*. A Compassion in World Farming/WSPA report, UK.

Turnbull, J.F., Kadri, S. 2007. Safeguarding the many guises of farmed fish welfare. *Diseases of Aquatic Organisms*. **75**: 173-182.

Varsamos, S., Flik, G., Pepin, J.F.,. Bonga, S.E.W., Breuil, G. 2006. Husbandry stress during early life stages affects the stress response and health status of juvenile sea bass, *Dicentrarchus labrax. Fish & Shellfish Immunology.* **20(1)**: 83-96.

Voas, A. 2008. *Welfare legislation applying to farmed fish in the UK*. In: Branson, E.J. (ed) Fish welfare. Blackwell Publishing, Oxford. pp93-98.

Waiblinger, S., Boivin, X., Pedersen, V., Tosi, M.-V., Janczak, A.M., Visser, E.K., Jones, R.B. 2006. Assessing the human-animal relationship in farmed species: A critical review. *Applied Animal Behaviour Science*. **101(3-4)**: 185-242.

Webster, J. 2006. Animal sentience and animal welfare: What is it to them and what is it to us? *Applied Animal Behaviour Science*. **100**: 1-3.

Wendelaar Bonga, S.E. 1997. The stress response in fish. *Physiological Reviews*. **77**: 591-625.

Yue, S., Moccia, R.D., Duncan, I.J.H. 2004. Investigating fear in domestic rainbow trout, *Oncorhynchus mykiss*, using an avoidance learning task. *Journal of Applied Animal Behaviour Science*. **87**:343-354.

Yue, S., Duncan, I.J.H., Moccia, R.D. 2008. Investigating fear in rainbow trout (*Oncorhynchus mykiss*) using the conditioned- suppression paradigm. *Journal of Applied Animal Welfare Science*. **11**:14-27.

2 The influences of water quality on the welfare of farmed rainbow trout: a review.

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2.1 Introduction

For the welfare of farmed fish, the quality of the water is central. It is a primary environmental consideration, with the potential to markedly affect health. Fish exist in intimate contact with the water through the huge surface area of the gills and skin, and it is widely acknowledged that fish are vulnerable to inappropriate water quality. The water provides fish with the oxygen required to survive, dilutes and removes potentially toxic metabolites, as well as providing support against gravity.

Inappropriate levels of water quality parameters affect physiology, growth rate and efficiency, cause pathological changes and organ damage and, in severe cases, cause mortality. The sub-lethal effects of poor water quality are also commonly linked to increased disease susceptibility, although scientific evidence for direct relationships is lacking. At present, there is insufficient information to conclude if poor water quality has an adverse effect on the welfare of UK farmed trout.

Salmonids are recognised as being less tolerant of poor water quality, *e.g.* low oxygen (Wedemeyer 1996) and ammonia (Haywood 1983), than those species that have evolved to inhabit warmer, slower flowing and static waters. Inadequate water quality, as will be illustrated, has a direct impact on fish health, causing either chronic or acute effects. Although frequently considered as a complementary issue to welfare, health is in fact a central tenet of welfare. Inadequate water quality may also have an indirect effect on health by increasing susceptibility to disease.

It is important to consider water quality in terms of both the characteristics of the local catchment supply and the influence of farm management practices. On this basis, water quality parameters can be separated into three categories: the first category reflects the parameters that are largely affected by the biological loading and water treatment systems applied by the farmer and are therefore largely under their control, *i.e.* oxygen, ammonia, carbon dioxide, nitrite. The second category includes those parameters that relate to the local catchment water chemistry and are therefore largely outside the control of the farm manager, *i.e.* acidity, alkalinity, hardness, temperature, conductivity, heavy metal concentration. A third category includes those parameters that can reflect the characteristics of both the intake water and farm management practices, *i.e.* nitrate, suspended solids, supersaturation. These parameters are discussed in turn below in relation to physico-chemistry, effects on the fish and the practicalities of measurement. Discussion does not include those water quality parameters that may occur sporadically, originating either within the farm (*e.g.* disinfectants and chemotherapeutants such as ozone and salt), or originating outside the farm (*e.g.* pesticides or pollutants).

2.2 Dissolved Oxygen

Dissolved oxygen (DO) is the primary water quality consideration for any salmonid farmer. Oxygen passively diffuses into water from the atmosphere, and the maximum amount that will dissolve depends upon a number of variables including temperature, salinity and altitude. Fish extract oxygen from the water by passive diffusion through the gills. An adequate concentration of DO in the water is required to facilitate the passive diffusion down a concentration gradient from the water into the blood (Colt & Tomasso 2001). If DO concentrations fall below the requirements of the fish, then fish cannot convert energy as efficiently into a usable form, resulting in reduced growth rate, food conversion efficiency and swimming ability (Jones 1971). The opercular ventilation rate increases as DO concentrations decrease, and fish may show a gasping response (Wedemeyer 1996). It has been reported that salmonids show a behavioural avoidance of low oxygen levels (Levy *et al.* 1989) and there are observations that the distribution of fish changes, with fish moving towards the surface or water inflow where DO concentrations are higher (Wedemeyer 1996). There is a lack of information on the effects of a reduced DO concentration on relevant physiological measures of red blood cells (*e.g.* haemoglobin concentration, cell count, haematocrit). However, when DO approaches lethal levels effects such as anorexia, respiratory distress, tissue hypoxia, precede unconsciousness and death (Wedemeyer 1996).

2.2.1 Existing Recommendations

The DO requirements for rainbow trout (*Oncorhynchus mykiss*) have been well studied (Liao 1971, see Smart 1981 for a review). A minimum DO concentration of 5-6 mg/L is frequently recommended for the health of rainbow trout (Smart 1981, Colt &

Tomasso 2001). This figure is widely accepted within the industry based upon experience (*e.g.* Anon 2001). Colt & Tomasso (2001) stated that there are some basic points when considering a minimum DO level, *i.e.*:-

- Fish of a given species and size require more oxygen in warmer water than in cooler water, due to their increased metabolic rate in warmer water.
- Fish require a greater amount of oxygen after feeding than before, again due to an increased metabolic rate and the specific oxygen demand.
- Oxygen consumption is proportional to the size and number of fish in a given system.
- Smaller fish use more oxygen per unit weight than larger fish.
- Fish require more oxygen if they have impaired gill function, are exposed to stressors, or if their oxygen-carrying capacity is impaired.

Wedemeyer (1996) suggested that 5-6 mg/L is too low as there is no safety margin for temporary increases in DO requirements due to increased swimming activity, overfeeding and CO_2 increases. As higher water temperatures cause an increase in the metabolic rate and oxygen demand of fish, farmers may encounter problems during summer seasons when the capacity of the water to hold oxygen is reduced. In recognition of this, Wedemeyer (1996) suggests minimum oxygen levels as shown in table 2.1 to promote good health and physiological condition in the fish stock.

Therefore, even with a parameter as fundamental as dissolved oxygen, there is disagreement regarding a minimum concentration for rainbow trout culture, with recommendations ranging from 5 to 9 mg/L, depending on the temperature.

Table 2.1 Solubility of oxygen (mg/L) in fresh water in equilibrium with air at 101.325 kPa (Anon 1980) and minimum recommended DO concentrations for coldwater fish in aquaculture (from Wedemeyer, 1996).

Temperature °C	Oxygen Solubility, i.e. 100% saturation mg/L	Minimum DO Required	
		mg/l	% saturation
5	12.8	9.1	71
10	11.3	8.8	78
15	10.2	8.3	81
20	9.2	7.8	85
25	8.2	7.4	90
30	7.5	6.9	92

2.3 Hyperoxia

Oxygenation (*i.e.* the use of pure oxygen supplementation) is increasingly being used to raise the carrying capacity of intensive fish culture systems (Colt & Watten 1988, Warrer-Hansen 2003). Very little is known about the potential effects of hyperoxia (DO levels > 100% saturation) on fish welfare. Some physiological effects of hyperoxia have been recorded on erythrocyte size and numbers but this was not associated with effects on growth or mortality (Ritola *et al.* 2002). The physical effects of gas supersaturation are discussed elsewhere, but it should be stated that supersaturation is considered to be a less significant problem for oxygen than for nitrogen, and recommendations for maximum dissolved oxygen levels could not be found in the literature.

2.4 Ammonia

The literature relating to ammonia and its toxic effects on fishes is vast, however it is often contradictory and confusing. Several lethal values of ammonia have been reported for rainbow trout, along with many ‘safe’ levels. In his extensive review of ammonia in aquaculture, Meade (1985) suggests that the reasons for the contradictions in the literature are due to fluctuating ammonia levels caused by variations in diurnal ammonia excretion rates, making predictions about ammonia toxicity difficult; and that the effects of ammonia cannot be predicted based on the concentrations of un-ionised ammonia alone (see below).

2.4.1 Sources

Ammonia is a substance toxic to all vertebrates and is found in the aquatic environment. Sources of ammonia are: excretion by plants and animals; microbial decomposition of organic matter; volcanic emissions; and anthropogenic origins such as the release of fertilizers and industrial emissions (Randall & Tsui 2002).

In aquaculture practices, while ammonia may be present in incoming waters, the majority of ammonia found in a fish farm is produced by the fish. Ammonia is the primary waste metabolite produced by fish from the catabolism of protein contained within the feed. The ammonia is excreted from the fish via the gills (Evans *et al.* 2005). Ammonia can also come from the decomposition of uneaten food, although this is considered a relatively minor source (Hinshaw & Fornshell 2002).

2.4.2 Terminology and Chemistry

In the aquatic environment, ammonia exists in two forms in equilibrium; as un-ionised ammonia, NH_3, and as ionised ammonium, NH_4^+.

$$NH_3 + H^+ + OH^- \leftrightarrow NH_4^+ + OH^-$$

Thus, total ammonia concentration is the sum of the concentrations of un-ionised ammonia and ionised ammonium.

Total Ammonia = $[NH_3] + [NH_4^+]$

Methods for the measurement of ammonia do not differentiate between the two forms, the proportions of which vary depending upon the position of the equilibrium. The customary UK practice is therefore to express total ammonia concentration as just the amount of nitrogen present - *i.e.* total ammonia nitrogen (TAN) – rather than trying to include the variable hydrogen component (Anon 1981). The un-ionised ammonia fraction is referred to as NH_3 -N and the ionised ammonium as NH_4^+ -N.

The equilibrium between the NH_3 and NH_4^+ varies in relation to the various factors, most significantly the concentration of hydrogen ions (*i.e.* pH) and temperature. The ionisation constant, pK_a is temperature-dependant and can be estimated from temperature according to the following equation:

pKa = 10.055 – 0.0325(Temp °C)

The percentage of NH_3 can be calculated by entering the pH and pKa values into the following equation (Wedemeyer 1996):

.%NH_3 = 100/(1 + antilog (pK_a – pH)

The [NH_3-N] is then calculated by multiplying the measured [TAN] by the %NH_3. Finally, [NH_3-N] is multiplied by 1.22 to convert to [NH_3], thereby correcting for the molecular weight of hydrogen. It is important to recognise that ammonia concentrations are expressed in different ways in different studies, *e.g.* [NH_3], [NH_3-

N], [TAN]. Haywood (1983) recommended expressing ammonia concentrations as mg/L NH_3 rather than NH_3-N.

The percentage distribution of each form is therefore highly dependent upon the pH and to a lesser extent the water temperature (Colt & Tomasso 2001). The pK_a value is also affected by ionic strength, pressure and salinity (Colt & Tomasso 2001, Randall & Tsui 2002), although these factors have a minor effect on the distribution of total ammonia forms. The most important factor in determining the distribution of ammonia forms is the pH.

2.4.3 Nature of Ammonia Toxicity

The distribution of total ammonia between NH_3 and NH_4^+ is important, as the former is considered to be the toxic form to vertebrates, while the ammonium ion is considered to be essentially non-toxic at the levels experienced in aquaculture systems. Most biological membranes are permeable to un-ionised ammonia and relatively impermeable to ionised ammonium (Randall & Tsui 2002). Therefore, in fish, ammonia in the external medium either induces retention of endogenous ammonia in the fish, or the exogenous ammonia enters via the gills by passive diffusion down a concentration gradient (Haywood 1983). However, several authors have questioned the opinion that only un-ionised ammonia is toxic, suggesting that ammonium ions also contribute to the toxicity (Tomasso 1994, Linton *et al.* 1998a).

Acute ammonia toxicity affects the central nervous system of fish (Randall & Tsui 2002), and manifests as a neurological disorder (Haywood 1983). While the exact nature of ammonia toxicity is not known in fish, it appears that ammonia interferes with physiological processes that eventually result in death of cells in the brain.

Another theory is that excessive ammonia depolarises muscle fibres and neurons, again leading to cell death (Randall & Tsui 2002).

A suggested detoxification mechanism in fish is that ammonia in the blood is converted into glutamine through the action of glutamine synthetase, an enzyme that is found to be up-regulated during exposure to ammonia (Wicks & Randall 2002a). It is also thought that fish can, to some extent, convert ammonia to urea (Haywood 1983).

2.4.4 Acute toxicity levels

Many studies have been conducted into the acute toxicity of ammonia to rainbow trout. Most studies have investigated the LC_{50}, or the median concentration of ammonia required to kill 50% of the experimental fish within a given period of time, usually 96 hours.

In a series of 81 experiments, Thurston and Russo (1983) found the $96h\text{-}LC_{50}$ for rainbow trout ranged from 0.16 mg/L NH_3 -N to 1.1mg/L NH_3 -N. All experiments were conducted in similar water chemistry conditions and fish were from the same strain. Differences in acute toxicity tolerances were found to be due to different life stages of the test fish, which will be discussed in section 2.7. Meade (1985) quoted a $96h\text{-}LC_{50}$ of 0.32 mg/L NH_3 –N for rainbow trout.

Short term exposures of fish to high concentrations of ammonia result in increased ventilation rate, hyperexcitability, erratic swimming, loss of equilibrium, convulsions and death (Smart 1976, 1981, Haywood 1983, Russo & Thurston 1991).

2.4.5 About acute toxicity tests

Acute toxicity tests are used as indicators of concentrations of toxicants that will have an immediate affect on organisms, and are employed in drawing up standards for the control of ammonia concentrations in aquatic systems. In the absence of reliable chronic toxicity test results, a general rule-of-thumb for ‘safe’ levels for organisms is to use 10% of the 96h-LC_{50} values for maximum limits. Such methodology has led to a suggested maximal level of 0.02 mg/L (Haywood 1983). However, there are several considerations that must be taken into account with regard to toxicity tests. In order to standardise the tests as far as possible, the United States Environmental Protection Agency states that acute toxicity studies with ammonia should adhere to the following criteria (Randall & Tsui 2002): exposure of organisms to ammonia should be under static conditions, with the test organisms starved, rested and unstressed. While the rationale behind this allows comparisons to be made between tests, the test conditions only bear limited resemblance to conditions that farmed fish would encounter and therefore the relevance of such tests is questionable.

2.4.6 Chronic effects

Reported effects of chronic exposure to ammonia in the rainbow trout include gill damage (swelling, mucus production, epithelial lifting, hyperplasia, breakdown of the pillar cell structure of the secondary lamellae, fusion of gill lamellae), ion imbalances, impaired liver function, impaired renal function, decreased food intake, growth and food conversion, and increased fin erosion (Larmoyeux & Piper 1973, Smith & Piper 1975, Smart 1976, Alabaster & Lloyd 1982, Haywood 1983, Thurston *et al.* 1984, Tomasso 1994, Twitchen & Eddy 1994). In an extensive study into the chronic effects of ammonia on rainbow trout lasting 5 years and 3 generations of fish, Thurston *et al.* (1984) found evidence of gill and kidney damage at constant ammonia concentrations up to 0.07 mg/L NH_3 (0.06 mg/L NH_3 –N), however there was no evidence that growth or fecundity was affected. However, Daoust and Ferguson (1984) could find no evidence of gill damage in rainbow trout exposed to ammonia concentrations up to 0.4 mg/L NH_3 for 90 days. This led Meade (1985) to conclude that gill damage is probably not caused by ammonia toxicity, proposing a hypothesis that other metabolites and their interactions with water chemistry are possibly involved. With regard to the findings from Larmoyeux and Piper (1973) and Smith and Piper (1975), it should be noted that in both cases the dissolved oxygen concentration was substantially less than saturation and was possibly a factor in the findings of gill damage. It has also been suggested that while the gills may be primarily affected by the external concentration of NH_3 in the water, the internal physiology is affected by the total ammonia concentration (Haywood 1983).

2.4.7 Factors affecting ammonia toxicity

(1) *Dissolved Oxygen.* Many researchers have observed that the toxicity of ammonia increases with decreasing DO concentrations (see Russo & Thurston 1991 for a review). Thurston *et al.* (1981a) conducted acute toxicity experiments over a range of DO concentrations and found that tolerance to ammonia decreased with decreasing DO, as shown in Figure 2.1.

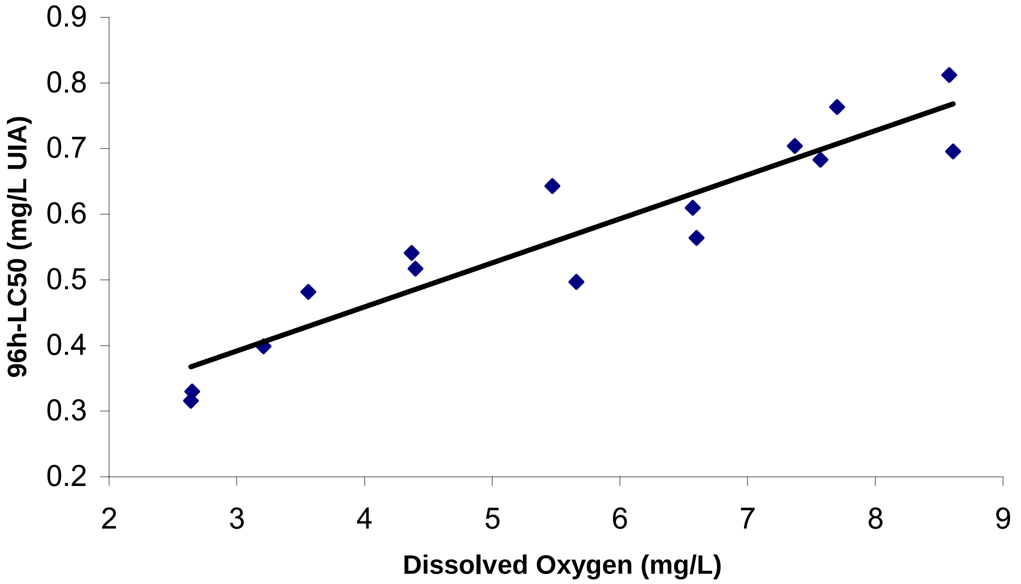

Figure 2.1 - Affect of dissolved oxygen on 96h-LC$_{50}$ ammonia toxicity to rainbow trout (from Thurston *et al.* 1981a)

The 96h–LC$_{50}$ for ammonia toxicity for rainbow trout fell by around 30% between DO concentrations of 8.5 and 5 mg/L. As discussed, a minimum recommendation for DO concentration is 5 mg/L. Whilst this figure may be adequate to maintain fish health when other water quality parameters are satisfactory, the literature demonstrates that if ammonia concentrations increase, then DO concentrations that were previously believed to be adequate may not be so.

(2) *pH*. The water pH affects the toxicity of ammonia by altering the distribution ratio of the total ammonia forms, as discussed in the section on the terminology and chemistry of ammonia, with an increase in pH resulting in an increase in the fraction of un-ionised ammonia. However, independent of the effect of pH on the equilibrium of ammonia species, Russo and Thurston (1991) found that the 96h-LC$_{50}$ value decreased with decreasing pH over a range of 9 to 6.5. As the lower pH figure is not considered to be toxic, it is possible that the toxic effects were due to the increasing concentration of ammonium ions (NH_4^+) (Tomasso 1994, Linton *et al.* 1998a).

(3) *Temperature*. The effects of temperature on the toxicity of ammonia are not clear; apart from the effect temperature has on the distribution of ammonia forms. Thurston and Russo (1983) observed a decrease in acute ammonia toxicity to rainbow trout as temperature increased over the range 12-19°C. However, some studies noted the reverse of this, or no effect due to temperature (Meade 1985).

(4) *Acclimation*. There is some evidence that prior exposure of rainbow trout to sublethal levels of ammonia increases their tolerance to environmental ammonia (Daoust & Ferguson 1984. Meade 1985, Russo & Thurston 1991). However Linton *et al.* (1998a) did not find any evidence of acclimation to ammonia, although they suggest that the very low levels of ammonia used during attempted acclimation were not sufficient to trigger an acclimation response. It has been suggested that acclimation can occur due to upregulation of the enzymes involved in detoxification of ammonia (Randall & Tsui 2002).

(5) *Fluctuating ammonia levels*. It has long been recognised that within culture systems, environmental ammonia levels fluctuate hourly due to variability in ammonia excretion levels (Smith & Piper 1975). Thurston *et al.* (1981b) reported that test fish tolerated constant concentrations of ammonia better than fluctuating levels. Given that fluctuating ammonia levels present a more realistic scenario in fish culture conditions, this finding brings into question all findings from ammonia toxicity tests where constant ammonia concentrations are used.

(6) *Exercise*. Shingles *et al.* (2001), Wicks *et al.* (2002) and McKenzie *et al.* (2003) reported that swimming increases the toxicity of ammonia to rainbow trout and that increasing levels of environmental ammonia decrease swimming ability. Wicks *et al.* (2002) found that the 96h-LC_{50} was 32 mg/L TAN (around 0.08 mg/L NH_3–N) for

exercised fish compared to 207 mg/L TAN (0.52 mg/L NH_3-N) for rested fish. This figure is significantly lower than other 96h – LC_{50} (see section on acute toxicity levels).

(7) *Feeding/fasting.* Within fish culture conditions, a primary source of ammonia is the metabolism of the fish. Therefore, it is unavoidable that feeding will have an impact on ammonia levels. There is also evidence that feeding affects the toxicity of ammonia - fed fish are less susceptible to environmental ammonia than unfed fish (Randall & Tsui 2002). This is thought to be due to a more efficient detoxification system in the fed fish. Wicks and Randall (2002b) report that fed fish can tolerate internal plasma ammonia levels on a par with lethal environmental concentrations, which is thought due to activation of the ammonia detoxification system (Wicks & Randall 2002a).

(8) *Stress*. There is some evidence that stress increases the toxicity of ammonia to fish (Randall & Tsui 2002), but this is not conclusive. Randall and Tsui (2002) also suggest that fish that are repeatedly stressed up-regulate the ammonia detoxification system, which may afford some protection against ammonia toxicity.

(9) *Ionic strength of water*. The ionic strength of water (as measured by dissolved solids) affects the equilibrium of the two forms of ammonia, albeit to a much lesser extent than pH and temperature (Messer *et al.* 1984). Meade (1985) reported that in freshwater, ammonia toxicity increases as the ionic strength of the water moves away from the ionic strength of the blood of the fish, which is roughly a third of the strength of sea water (Fevolden *et al.* 2003). Ammonia has a diuretic effect on rainbow trout, and therefore fish must replace the ions that are lost in the urine (Lloyd & Swift 1976); increasing the salinity of the water reduces the osmoregulatory cost of

increased ventilation that is incurred as a result of exposure to ammonia. Furthermore, some authors have suggested that ammonia is actively transported out of the body through a NH_4^+/Na^+ pump, therefore higher concentrations of Na^+ in the water will enhance this, reducing the concentration of ammonia in the fish and relieving some of the effects of toxicity (Soderberg & Meade 1992). However, some authors dispute the existence of the NH_4^+/Na^+ pump (Wilson *et al.* 1994), asserting that all ammonia excretion in freshwater rainbow trout is through passive diffusion. Calcium and other divalent cations in the water (*e.g.* Mg^{2+}) are known to decrease the gill membrane permeability and can increase sodium influx, which could also reduce the toxicity of ammonia (Soderberg & Meade 1992). In one study, an increase in the calcium ion concentration was shown to ameliorate ammonia toxicity in rainbow trout (Wicks *et al.* 2002).

(10) *Life stage and size*. In their series of acute toxicity experiments, Thurston and Russo (1983) found that tolerance to ammonia toxicity increased as fish developed from the larval stage, to a maximum tolerance as juveniles (around 1-4 g), following which tolerance to ammonia decreased. In his review of ammonia, Meade (1985) reports that tolerance of rainbow trout was up to 50 times greater in fish that had not fully absorbed the yolk than in adult trout.

2.4.8 Existing recommended levels

From the literature, there is widespread disagreement regarding safe levels of ammonia in culture systems for rainbow trout. Hampson (1976) recommends a maximum limit of 0.3 mg/L NH_3 –N, while Wedemeyer (1996) recommends no more than 0.02 mg/L NH_3. Following their 6 month trial on rainbow trout, Smith and Piper (1975) recommended a maximum ammonia concentration of 0.0125 mg/L NH_3, which was the ‘no observable effect concentration’ for growth. However, it should be noted that the dissolved oxygen in that experiment was low, with an average of around 6 mg/L. The recommended maximum of 0.0125 mg/L NH_3 –N was nonetheless echoed by Westers and Pratt (1977) and Soderberg *et al.* (1983). Following a review of various studies, Haywood (1983) recommended maximum levels of only 0.002 mg/L for salmonids and added that total ammonia levels should also be below 1 mg/L to account for uncertainty on the toxic action of ionised ammonia.

Meade (1985) contended that differences between different culture systems and water chemistry make recommending a maximum ‘safe’ level of ammonia for rainbow trout inappropriate, as ammonia concentrations in one system may affect fish health while the same concentration in another system may have no affect. Klontz (1991) differentiated between intermittent and constant concentrations of ammonia, recommending maxima of 0.05 mg/L and 0.03 mg/L NH_3 respectively.

2.4.9 Positive effect of ammonia

While ammonia is recognised as a toxicant and is detrimental to the health of fish, there is some evidence that low concentrations of ammonia can stimulate growth. Studies by Linton *et al.* (1998a) showed increased growth at low TAN levels of 1.96 mg/L (around 0.035 mg/L NH_3 –N), which agreed with earlier work at the same laboratory (Linton *et al.* 1997; 1998b) and subsequent work carried out by Wood (2004). Wood (2004) postulated that low ambient levels of ammonia either stimulate ammonia incorporation into amino acids and protein synthesis and/or reduce metabolic costs, as growth was improved without an alteration in food consumption by the fish. This, however, conflicts with prior studies where growth was suppressed at ammonia concentrations as low as 0.002 mg/L NH_3 -N (Russo & Thurston 1991).

2.5 Nitrite

Nitrite, NO_2^-, is formed from the oxidation of ammonium (NH_4^+) in the aquatic environment. Nitrifying bacteria, *Nitrosomonas* spp., oxidise ammonium into nitrite. The bacteria *Nitrobacter* spp. then convert nitrite into nitrate, NO_3^- (Lewis & Morris 1986).

Nitrite can be found in high concentrations naturally, such as in deep stratified lakes in the hypolimnetic layer (Boyd 1990). Within aquaculture systems, the primary source of nitrite is the oxidation of ammonium produced by the fish. Nitrite concentrations may increase if oxidation rates of ammonia exceed oxidation rates of nitrite (Colt & Tomasso 2001), or if the oxidation process is inhibited, *e.g.* by ammonia (Russo & Thurston 1991). However, in trout farming, nitrite produced within the farm is generally not problematic in flow-through systems predominant in the industry which constantly flush and remove organic wastes . An exception to this is in malfunctioning recirculation systems when biological filtration is relied upon to maintain water quality. However, the main sources of high nitrite concentrations are anthropogenic in origin, such as from sewage effluents and agricultural drainage (Wedemeyer 1996); these pose the main nitrite threat to trout farming by affecting the initial water intake.

In freshwater fish, nitrite enters through the gills. Nitrite ions are actively taken up through the chloride cells and can be pumped in against a concentration gradient

(Jensen 2003), which can result in blood plasma concentrations of nitrite up to ten times that of the ambient water concentration (Eddy *et al*. 1983).

Nitrite is toxic to fish as it diffuses from the blood plasma into the red blood cells, where it oxidises the Fe^{2+} in haemoglobin (Hb) to the Fe^{3+} oxidation state, converting haemoglobin into methaemoglobin (metHb). MetHb lacks the capacity to bind to oxygen, therefore the oxygen-transport system in the fish is disabled resulting in hypoxia. The build up of MetHb is known as methaemoglobinaemia, or more commonly brown blood disease, named after the characteristic colour of blood and gills of chronically nitrite-exposed fish or other animals. MetHb occurs naturally in the blood of fish, typically at levels of 1-3%, however levels in excess of 10% are detrimental to fish health, and clinical signs have been reported with levels over 25% (Lewis & Morris 1986). Nitrite exposure may also damage the gills (hypertrophy, hyperplasia, epithelial separation) and the thymus (haemorrhage and necrotic lesions) (Wedemeyer 1996). The thymus is located in the gill cavity and is involved in the production of lymphocytes (Bowden *et al.* 2005).

Nitrite induced metHb is a reversible condition, as the red blood cells of fish contain an enzyme, metHb reductase, that reduces metHb to Hb (Scott & Harrigan 1985). If nitrite levels in the water are reduced before metHb levels become lethal, the fish should fully recover (Jensen 2003).

Aside from the indicative brown blood found in exposed fish, gross signs of methaemoglobinaemia are lethargy as blood levels of metHb approach 70-80%, with disorientation and unresponsiveness reported at levels near 100% (Westin 1974). The lethargy and lack of activity reported in fish with methaemoglobinaemia may well be a behavioural response to cope with the condition, as this reduces their oxygen demand. However, should the fish be startled or forced to become active, they may then die from hypoxia (Huey *et al*. 1980).

2.5.1 Toxicity

96h-LC_{50} values for rainbow trout range from 0.19 to 12.6 mg/L NO_2^- -N (see reviews by Lewis & Morris 1986, Russo & Thurston 1977, 1991, Russo *et al.* 1981, Eddy & Williams 1994). There are several reasons for this range being over two orders of magnitude; however the primary reason is water chemistry, or more specifically the chloride ion concentration of the water. Nitrite is transported into the fish through chloride cells in the gills, and it appears that the presence of chloride ions in the water compete with the nitrite for transport; as the concentration of chloride ions increases, so the uptake of nitrite decreases. Table 2.2 demonstrates the effect of chloride ions on nitrite toxicity.

Table 2.2 Selected acute nitrite toxicity figures for rainbow trout (from Lewis & Morris 1986)

Nitrite-N 96h LC_{50} (mg/L)	Cl^- (mg/L)	pH	Ca^{2+} (mg/L)	Alkalinity ($CaCO_3$, mg/L)
0.24	0.35	7.9	60	176
3	10	7.7	52	171
8	20	7.7	52	171
11	40	7.7	52	171

Other anions in the water that affect nitrite toxicity are bromide and bicarbonate. Bromide was shown to have a greater effect on nitrite toxicity than chloride (Eddy *et al.* 1983), however as bromide is not typically present in freshwaters, this is of academic interest only. Bicarbonate inhibits the uptake of chloride from water, and appears to have the same effect on nitrite uptake, although it does not affect nitrite toxicity to the same degree as chloride (Lewis & Morris 1986). Sulphate, phosphate and nitrate have also been shown to affect nitrite toxicity (Russo & Thurston 1991). There is some evidence that calcium ions (Ca^{2+}) may increase the inhibitory effects of chloride on nitrite toxicity through its action on the gill membrane (Tomasso 1994).

Low concentrations of dissolved oxygen affect the toxicity of nitrite (Lewis & Morris 1986). As nitrite affects the ability of the blood to transport oxygen, a reduction in ambient water DO concentrations will exacerbate the effect of toxicity. The effect of temperature on the toxicity of nitrite to rainbow trout is not known; however there is inconclusive evidence from studies on the channel catfish (*Ictalurus punctatus*) that higher temperatures can increase nitrite toxicity (Lewis & Morris 1986). With regard to the size of fish, there is some evidence that smaller rainbow trout are more tolerant of nitrite than larger trout (Lewis & Morris 1986).

Aside from studies into the acute lethal effects of nitrite toxicity, there have been very few studies into the long-term chronic effects in rainbow trout. Wedemeyer and Yasutake (1978) exposed rainbow trout to nitrite concentrations up to 0.06 mg/L NO_2^- -N for 6 months in soft water with a low chloride content. There were no mortalities, growth was not significantly different between treatments and only mild methaemoglobinaemia was noted (around 5%). Hypertrophy of the gills was observed, with the most severe cases noted around 4 weeks into the trial; after 7 weeks hypertrophy was observed less frequently and at the conclusion of the trial no hypertrophy was recorded, indicating that the fish were able to acclimate to the nitrite concentrations.

From their review of the literature, Lewis & Morris (1986) concluded that lethal concentrations over 96 hours and concentrations showing minimal or negligible effects only differ by a few-fold, indicating that if the fish survive the initial exposure, then they can probably acclimate and survive ongoing exposure.

2.5.2 Existing recommendations

The recommended maximum concentration for nitrite is 0.1 mg/L NO_2^- (Wedemeyer 1996) (≡0.03mg/L NO_2^- -N). However the chloride concentration and, to some extent the concentration of other ions in the water, will have a major effect on the toxicity of any nitrite present.

2.6 Nitrate

Nitrate is produced from the oxidation of nitrite by the bacteria *Nitrobacter* spp. (Lewis & Morris 1986). The 96h LC_{50} for salmonids is in the range of 1000-3000 mg/L NO_3^--N (Wedemeyer 1996). Nitrate within flow-through aquaculture systems is generally dismissed as a threat to the health of older life stages of farmed rainbow trout (Russo & Thurston 1991, Tomasso 1994, Wedemeyer 1996). However a maximum value of 1 mg/L is suggested by Wedemeyer (1996) as a guideline, as exposure of eggs can result in developmental problems. Therefore nitrate exposure via inflow water poses a significant potential threat during the hatchery stages. Nitrate levels in many English waters, both ground and surface waters, are increasing due to agricultural run-off, with concentrations around 50 mg/L being found in a number of areas (Defra 2004).

2.7 Carbon Dioxide

Carbon dioxide (CO_2) is found naturally in most surface waters at levels of 1-2 mg/L and originates from diffusion from the atmosphere, microbial decomposition of organic matter in sediments and the respiration of micro-organisms, algae and aquatic plants (Wedemeyer 1996). Naturally higher levels of CO_2 can be found in well or spring water. Within aquaculture systems, the primary source of CO_2 is fish metabolism. CO_2 is considered to represent an increasingly important issue as more intensive production technologies, *i.e.* oxygen injection, are being introduced (Summerfelt 2002). As a rough guide, for each unit of oxygen that a fish respires, around 1.4 units of carbon dioxide are generated (Westers 2001).

Carbon dioxide reacts with water when it dissolves, forming a mixture of CO_2, carbonic acid (H_2CO_3), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions.

$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + CO_3^- \leftrightarrow H^+ + CO_3^{2-}$

The percentage distribution of each form is determined mainly by the pH. At a pH less than 5, the dominant form is dissolved CO_2; at pH between 7 and 9, the bicarbonate ion is the dominant form; while at pH 11, the carbonate ion has the greatest percentage. Carbonic acid is only present in water in very small quantities and is generally discounted (Wedemeyer 1996). As CO_2 dissolves, hydrogen ions are released, decreasing the pH of the water, and further increasing the proportion of CO_2 present in the dissolved form (Westers 2001).

2.7.1 Toxicity

Out of the forms of dissolved carbon dioxide in the water, CO_2 and carbonic acid are the toxic forms, while the bicarbonate and carbonate ions are not toxic (Wedemeyer 1996). Carbonic acid is generally discounted as having any influence on toxicity due to the small quantities present (<1%).

Carbon dioxide is toxic to fishes because increases in ambient CO_2 concentrations result in the fish being unable to excrete endogenous carbon dioxide, leading to CO_2 increases in the blood, known as hypercapnia. As a result of this, the blood pH decreases, leading to acidosis, reducing the oxygen carrying capacity of the blood in a process called the Bohr effect. The reduction in blood pH weakens the bond between haemoglobin and oxygen molecules, resulting in the release of oxygen molecules which then passively diffuse into cells that have a low partial pressure of oxygen. This effect has been observed in salmonids at water concentrations of CO_2 of around 20 mg/L (Westers 2001). Danley *et al.* (2001) recorded reduced growth in rainbow trout over a 90 day experiment with CO_2 concentrations up to 45 mg/L, but there was no report of significant mortalities at this level. Clinical signs of carbon dioxide toxicity include moribund fish, gaping mouths, flared operculae, and bright red gill lamellae (Summerfelt 2002).

A well known effect of CO_2 in conjunction with hard water, is nephrocalcinosis (Harrison 1979a, b, Smart 1981, Fikri *et al.* 2000). This chronic degenerative condition of the kidney is characterised by calcareous deposits (Harrison & Richards 1979; Smart *et al*. 1979). The white gritty kidney deposits consist of calcium salts, occur within the ureters on the surface of the kidneys, and the kidneys become swollen, sometimes with fluid-filled cysts (Harrison 1979a). The kidney is a major haemopoeitic organ in fish, and blood haematocrit values and haemoglobin content decrease in affected fish (Yurkowski *et al.* 1985). Severely affected fish become dark in colour, have a swollen abdomen and most of the functional kidney tissue is destroyed (Harrison 1979a; Yurkowski *et al.* 1985).

Nephrocalcinosis occurs when natural CO_2 levels in the water are high and/or when additional oxygenation is used to increase carrying capacity, and the total amount of metabolic CO_2 excreted is increased as a result (Harrison 1979b). CO_2 levels of 12 mg/L induce nephrocalcinosis, with higher concentrations increasing the prevalence and severity of the condition (Harrison 1979b, Smart *et al.* 1979). Although CO_2 level is a primary factor in the induction of the condition, a variety of physico-chemical factors associated with water chemistry, diet composition, strain and species of fish are involved in its development (Harrison & Richards 1979, Smart *et al.* 1979). Nephrocalcinosis was highlighted as an issue in farmed UK trout populations in the 1970s, but has received little attention since. Nephrocalcinosis has recently been reported in rainbow trout in Israel and Atlantic salmon smolts in Norway (Fikri *et al.* 2000, Fivelstad *et al.* 2003a). Possible methods to manage the condition include increased dietary magnesium, or avoiding susceptible strains and species or pre-disposed sites (Harrison 1979a,b).

2.7.2 Existing recommendations

Wedemeyer (1996) recommends CO_2 levels should not exceed 10 mg/L, although Smart (1981) reported that there was no reduction in growth or FCR at CO_2 levels of 24 mg/L. Heinen *et al.* (1996) recommend safe levels between 9 and 30 mg/L based upon their literature review. Noble & Summerfelt (1996) state that safe levels vary due to other water quality factors (such as DO, pH and alkalinity), which must all be taken into account when considering recommended safe levels for aquaculture.

2.8 Suspended Solids

The literature pertaining to the effects of suspended solids on fish is surprisingly sparse, considering the potentially severe impacts that high levels can have on aquaculture production. This is likely to be due to the wide variability in the nature of the particulate matter and the different effects that the various forms of suspended solids have on fish. There is however a wealth of literature relating to suspended solids in effluent waters from fish farms (*e.g.* Beveridge *et al.* 1991, Hinshaw & Fornshell 2002, Tucker *et al.* 2002) and the effects these have on the flora and fauna of receiving waters.

Suspended solids come in a wide variety of materials (clay, volcanic ash, pollen, uneaten food, faeces) in a variety of sizes and shapes (Klontz 1993, Wedemeyer 1996). Solids such as clay and soil sediments occur naturally (Boyd 1990), or through anthropogenic influences such as mining, logging or construction (Colt & Tomasso 2001). Such suspended solids will typically enter the farm in the inflow water. Within fish culture systems, uneaten food, faecal solids (Wedemeyer 1996), microfauna (Chen *et al.* 1994) and build-up from biofilters that have broken off in recirculation systems (Noble & Summerfelt 1996) contribute to total suspended solids.

Suspended solids are defined as particulate matter within the water with a diameter greater than 1 µm. Solids have organic and inorganic components, with the organic section known as volatile suspended solids (Chen *et al.* 1994). Solids can also be classified as settleable or non-settleable, with the larger settleable solids having a diameter greater than 100µm. Non-settleable solids tend to be the most problematic in culture systems; mortalities have been reported within an intensive rainbow trout farm associated with suspended solids with a diameter of 5-10µm (Chen *et al.* 1994). The construction of the culture system and the rate of water flow will influence the amount of suspended solids in the system at any one time. For example, self-cleaning raceways use water velocities in excess of 3 cm/sec to prevent solids such as uneaten food from settling (Wedemeyer 1996).

Suspended solids have been shown to affect fish health by physically abrading or clogging the gills, smothering eggs during incubation, abrading the skin and impairing visual feeding (Alabaster & Lloyd 1982, Wedemeyer 1996). Redding *et al.* (1987) showed that steelhead trout (the anadromous form of rainbow trout) exposed to suspended solids over 400 mg/L, suffered a classic stress response of increased blood cortisol. However they reported no gill damage despite exposing the test fish to suspended solids concentrations up to 3000 mg/L for up to 8 days. Magor (1988) reported gill damage such as lamellar oedema and telangiectasis (dilation of the capillaries) in coho salmon, *Oncorhynchus kisutch,* exposed to suspended solids as low as 44 mg/L. Alabaster & Lloyd (1982) reported that rainbow trout could survive for a day in suspended solid concentrations of 80,000 mg/L, and that they could survive for 10 months in suspended solid concentrations of 200 mg/L, although the type of solid (material, shape and size) affects the effect on fish.

In addition to the above direct effects of suspended solids, there may be indirect effects on fish health. Organic suspended solids have the potential to increase the biological oxygen demand of the culture system, thereby reducing the dissolved oxygen, (Chen *et al.* 1994) and some solids can mineralise to produce ammonia (Liao & Mayo 1974). Some microorganisms associated with suspended solids produce CO_2 through respiration resulting in a reduction of water pH, and some microorganisms can be facultative fish pathogens (Noble & Sommerfelt 1996).

2.8.1 Existing recommendations

Due to the potential variability in the size and type of suspended solids, few recommendations for maximum suspended solids exist. The shape, in particular the presence of irregular sharp edges, will affect the degree of abrasive impact (Wedemeyer 1996). Wedemeyer (1996) suggests 80-100 mg/L total suspended solids (TSS) as a guide for a reasonable maximum chronic exposure level, while Chen *et al.* (1994) suggest a maximum of 15 mg/L TSS. Alabaster & Lloyd (1982) state that there is no evidence of effects at concentrations under 25 mg/L.

2.9 Gas Supersaturation

Supersaturated water has been recognised as a problem for fish culturists for over 100 years (Garton & Nebeker 1977). Supersaturation occurs when the partial pressure of one or more of the gases dissolved in the water becomes greater than the atmospheric pressure. Under normal conditions, the partial pressures of the gases dissolved in water are in equilibrium with the atmospheric gases. However, this balance can be altered by natural means, such as large waterfalls, sudden temperature changes or through anthropogenic influences, such as from large dams (Garton & Nebeker 1977). Within aquaculture systems, supersaturation can be due to a variety of mechanisms: sudden increases in temperature; sudden decreases in pressure (*e.g.* when ground water comes to the surface via borehole pumping or natural springs); entrapment of air in piped supplies or in spillways of dams; and oxygen injection systems (Doulos & Kindschi 1992, Wedemeyer 1996).

Gas supersaturation becomes a fish health issue when it manifests as Gas Bubble Disease (GBD), which is similar to decompression sickness experienced by scuba divers. The blood and tissues of fish will equalise with the partial pressures of the ambient water, therefore if the ambient water is supersaturated, then the blood and tissues of the fish will also become supersaturated. Bubbles of gas, known as gas embolisms, may then form in the vascular system through a change in venous blood pressure, and are rapidly carried to the skin, mouth and fins (Wedemeyer 1996). Depending on the severity of the condition, tissue necrosis and death may result (McDonough & Hemmingsen 1985) and embolisms in the heart or other vital organs normally cause death (Wedemeyer 1996). Fish may recover if held under greater hydrostatic pressure (*i.e.* in deeper water) and the pressure gradually reduced, or if the temperature is reduced gradually (Wedemeyer 1996).

2.9.1 Recommendations

Wedemeyer (1996) noted that recommending a maximum figure for supersaturation is difficult; maximum chronic safe exposure limits vary with species, size and environmental conditions (*e.g.* depth affects hydrostatic pressure). He suggested that for salmonids, supersaturation should be 103% for hatchery stages and 105% for ongrowing stages.

2.10 Acidity

Acidity is the quantitative capacity of water to react with a strong base to a designated pH (APHA 1998). Acidity should be measured by titration with a standard base such as 0.02N NaOH to the phenolphthalein end point at a pH of 8.3, and expressed as milliequivalents per litre (meq/L) (APHA 1998). However, the process of titration is time consuming and requires specialist equipment, therefore acidity is often expressed as pH, which is a measure of the negative logarithm of the concentration of hydrogen ions (H^+) present at 25 °C. A pH of 7 is considered neutral, < 7 acidic, and > 7 is alkaline.

Waters can be naturally acidic, however within aquaculture systems, the pH can fall due to respiration and excretion of CO_2 from the fish (see section on carbon dioxide). If the inflow water to the culture system is soft or has low alkalinity, then the decrease in pH could become a problem for the fish, as the water has no buffering capacity to protect against the pH change.

Acidic waters have been shown to reduce the swimming capabilities of rainbow trout (Ye & Randall 1991); to affect the acid-base regulation (McDonald *et al.* 1980) and the regulation of ions (Ye *et al.* 1991); to interfere with the ability of fish to excrete ammonia (Wright & Wood 1985, Randall & Wright 1989), carbon dioxide and to transport oxygen (Randall 1991); and increase the toxicity of ammonia (see above).

2.10.1 Existing recommendations

Existing recommendations for fish health are for the water to have a pH no less than 6, as above this figure the effects of acidity are negligible (Randall 1991). Aside from the direct effects of acidity on fish, a reduction in the water pH affects other water chemistry parameters, for example the distribution of ammonia forms (see section on ammonia), or the solubility of toxic metals in the water, for example aluminium (Wedemeyer 1996).

2.11 Alkalinity

Alkalinity is a measure of the total concentration of alkaline substances dissolved in the water. It is the capacity of water to neutralise hydrogen ions (H^+) and is measured by titration with standardised acids to the methyl end point of pH 4.3 and expressed as milliequivalents/L (meq/L) or mg/L (as calcium carbonate, $CaCO_3$) (APHA 1998). The majority of waters with high alkalinity also have an alkaline pH and a high concentration of total dissolved solids.

As with water hardness (see next section), alkalinity has the potential to provide protection to the water system by buffering against large and sudden pH changes. However, while the properties of alkalinity are usually beneficial, highly alkaline waters can also be problematic for fish, as ammonia excretion and production can be inhibited (Wright & Wood 1985, Wilson *et al.* 1998), which can result in toxic levels of ammonia in the fish (Wedemeyer 1996).

2.11.1 Recommended level

Wedemeyer (1996) provides recommendations for upper and lower limits for alkalinity: >20 mg/L (to provide some capacity for buffering against pH extremes) and <100-150 mg/L (as $CaCO_3$) (to ensure that ammonia excretion is not inhibited).

2.12 Hardness

Hardness is primarily a measure of the amounts of calcium (Ca^{2+}) and magnesium (Mg^{2+}) salts that are present in the water (APHA 1998). Although other divalent dissolved metals such as iron (Fe), copper (Cu), lead (Pb) and zinc (Zn) also contribute to total water hardness, these elements are usually present in such small quantities that hardness is generally taken as a measurement of calcium and magnesium salts (Wedemeyer 1996).

As a method of classifying water for use in aquaculture, water hardness and alkalinity are probably the most useful measurements for biological systems. As with alkalinity, hardness is also used as a measure of the buffering capacity of the water. Soft water is usually acidic and hard water is usually alkaline. Water can be classified in terms of hardness as shown in table 2.3 (Wedemeyer 1996).

Table 2.3 Classification of water in terms of hardness, as shown in Wedemeyer (1996).

Soft	<75 mg/L (as $CaCO_3$)
Moderate	75-150 mg/L
Hard	150-300 mg/L
Very hard	>300 mg/L

In fresh water, rainbow trout must regulate the concentration of ions in their blood through active transport of ions from the water through the gills. The regulation and transport of these ions is a vital task to enable the fish to maintain homeostasis, as ions are lost from the blood by diffusion through the gills and through the copious amount of urine produced by freshwater fish. The active transport of ions into the fish requires energy and is carried out against a concentration gradient. In soft water, the concentration gradient is very large (up to 3000 times between blood and water (Wedemeyer 1996) and can use several percent of the energy provided by the diet. In harder water, the concentration gradient is far less and therefore less energy is required to regulate the blood ionic content (Klontz 1991, Wedemeyer 1996;).

Additionally, water hardness is important in aquaculture, as it provides an indication of the calcium and magnesium carbonate buffering capacity of a system, which controls changes in the pH (Howells 1994). Water pH affects the toxicity of various compounds (ammonia, carbon dioxide, heavy metals) and therefore water hardness will influence the effects that these compounds will have on fish through regulation of pH.

2.13 Temperature

Temperature is a vitally important physical property of the water in aquaculture systems. The temperature of the water regulates the amount of dissolved oxygen that a body of water can hold, the rate of decomposition and photosynthesis, which will affect the oxygen demand in pond systems and the ionisation of ammonia (see above) (Colt & Tomasso 2001). Additionally, increasing temperature increases the growth and infectiousness of many fish pathogens (Roberts 1975) and increases the toxicity of many dissolved contaminants (Wedemeyer 1996). All of these factors have the capacity to compromise the health of farmed fish.

As fish are exothermic, increasing the water temperature increases the metabolic rate and hence oxygen consumption. It has been calculated that raising the water temperature from 9°C to 15°C reduces the capacity of water to hold oxygen by 12.8%, while increasing the metabolic rate of a 100 g rainbow trout by 67.5% and increasing ammonia excretion by 98.6%, which leads to a 58.8% increase in environmental un-ionised ammonia (Klontz 1993).

2.13.1 Existing recommendations

Optimum temperatures for growth and spawning have been examined for many species important to aquaculture. For the rainbow trout, the optimum temperature range is suggested to be 16-17°C for growth and 10-13°C for spawning (Colt &

Tomasso 2001). However, these ranges should only be regarded as guidelines. Wild rainbow trout are exposed to a wide seasonal temperature range characteristic of high latitudes. Temperature optima are primarily determined by the genetic tolerance of the fish to temperature (Wedemeyer 1996), and therefore temperature optima will differ between strains originating from different areas. Other factors that will affect temperature optima are the length of acclimation time, the DO concentration and the ions present in the water (Wedemeyer 1996). Based on avoidance experiments, Neill and Bryan (1991) noted the specific temperature at which fish displayed avoidance behaviour varies by ±5°C. They also stated that preferred temperatures are size specific, and depend on previous temperature acclimation history. Lethal temperatures have been estimated for rainbow trout at 0°C and 26°C (Wedemeyer 1996); however again these maxima should be treated with caution. The recommended range for salmonid culture is 7-18°C for on-growing and 8-10°C for eggs and fry. Inappropriate rearing temperatures have been associated with a number of deformities in salmonids in both hard tissues (foreshortened maxillae, gill operculum shortening, vertebral abnormalities leading to “short tails” and “humpbacks”) and soft tissues (swimbladder torsion, missing septum transversum) (Fish Farming International 1999, Branson & Turnbull 2008).

2.14 Conductivity

The conductivity of water is a measure of its ability to convey an electrical current (Boyd 1990), which indicates the ionic activity and content of the water. While different ions have different abilities to conduct electricity, generally the higher the concentration of ions, the greater the conductivity. Conductivity of freshwaters is usually in the range of 20 to 1500 µmhos/cm (µS/cm; Boyd 1990) with brackish water and seawater having far greater conductivities due to the large number of ions present.

Conductivity does not directly affect the welfare of fish, however it is a good indicator of the general condition of the water. Taking conductivity measurements can assist in evaluating variations in mineral concentrations in water and can also assist in estimating the total dissolved solids present in water. Mineral concentrations and total dissolved solids have the capacity to affect other water chemistry parameters, such as pH. There are no recommendations for conductivity levels for fish health/welfare, as each body of water will have a range of conductivity levels, however once that range has been established then variations away from that range can indicate that there may be a potential problem.

2.15 Heavy metals

Heavy metals that may potentially cause fish health problems in aquaculture systems include copper, cadmium, lead and zinc. In addition to natural sources of these elements, heavy metals may be introduced in culture systems through industrial discharges, or from their use in weed control. While these metals are generally only present in surface waters in trace amounts, they can be very toxic to fish, including rainbow trout (Wedemeyer 1996). In soft water, heavy metal ions are highly soluble and highly toxic; however hard, alkaline waters result in precipitation of the metals with carbonates or hydroxides, which reduces their toxicity. Suspended solids may also alleviate the effects of heavy metals as the ions will adsorb on to the particles. High temperatures, low dissolved oxygen and high concentrations of dissolved carbon dioxide increase the toxicity of the metals. Acute exposure by rainbow trout to lethal levels of zinc or copper may not become evident until one or two days after exposure, at which time mortalities in the stock will start to occur. However, water chemistry analysis at that time may be too late to detect heavy metals as the cause of the mortalities, with only background levels showing (Wedemeyer 1996).

2.15.1 Existing recommendations

For maximum recommended concentrations of heavy metals, see table 2.7.

2.16 Water flow

The flow of water through a fish culture system greatly influences the water quality in the system, by replenishing dissolved oxygen and flushing out metabolites such as ammonia, nitrite and carbon dioxide. The flow of water can also assist in removing suspended solids. Recommendations have been made for flow rate in relation to the biomass, *i.e.* loading rate. However, such recommendations *e.g.* 1-4 kg/L/min vary widely; it has been suggested that it is dependant upon temperature and fish size (Anon 2001).

The flow rate of water through a system will affect the speed of the current, which may have a knock-on effect on fish welfare independent of water quality considerations. The relationship between water flow and current speed will be determined by the design of the system, *e.g.* raceways versus ponds. It has been suggested that a moderate current speed provides exercise, improves physiological performance and growth, and reduces physical damage to the fins through behavioural changes (Jobling *et al.* 1993). Jobling *et al.* (1993) recommended current speeds of 0.75 – 1.5 body lengths/sec for salmonids.

2.17 Discussion

It is clear from this review that numerous different water quality parameters have the potential to have an adverse impact on the health (and hence welfare) of farmed rainbow trout. The various water quality parameters can be classed, albeit subjectively, into tiers of importance with regard to potential impact on the fish:

Tier 1: Oxygen

Tier 2: Ammonia, carbon dioxide, gas supersaturation

Tier 3: Nitrate, suspended solids, temperature

Tier 4: Nitrite, acidity, alkalinity, hardness, conductivity, heavy metals

This ranking is believed to be in line with opinions prevalent within the UK trout industry, reflects the facts that trout are typically farmed in agricultural rather than industrial areas, and that the use of low flow, static or recirculation systems is limited. Although water reuse is common (*i.e.* units receiving the outflow from upstream units) the flushing rate and lack of biofiltration mean that ammonia is of greater significance than its oxidation products.

2.17.1 Is poor water quality a cause for concern?

Fish may theoretically be exposed to inadequate water quality during routine rearing and/or during sporadic events such as transport, handling, grading, and harvest. However, there is insufficient information available at present to conclude whether poor water quality is affecting the health and welfare of trout currently farmed in the UK. It must be acknowledged that financial considerations in intensive fish farming lead to the temptation to push the carrying capacity of water flow to the limit.

Nevertheless, it must be stressed that it is in the farmer’s own economic interests to ensure that water quality does not have an impact on production, and farmers use their experience to avoid adverse impacts of water quality deterioration. Growth rate, feed conversion efficiency and disease incidence will be sensitive to water quality (see below) and adverse effects will impinge on profit margins.

2.17.2 Monitoring welfare in relation to water quality

Farmers have a duty of care to prevent or minimise the impact of poor environmental conditions on their animals. Quality assurance schemes and legislators also have a responsibility to ensure that fish are not exposed to adverse water quality if this leads to suffering. So how can this be achieved? Animal welfare can be monitored through either environment-based (*i.e.* requirements for good welfare) or animal-based (the responses to the environment) parameters (Mollenhurst *et al.* 2005).

2.17.2.1 Environment-based parameters

Prescription of water quality limits is an attractive option for safeguarding fish welfare. However, there are two main problems with such an approach, namely the standardisation of measurement and the setting of appropriate limits.

If water quality limits were introduced farmers would need the capacity to self-monitor the parameters, and such measurement would have to be standardised. Standardisation would have to take into account:

(1) The timing and frequency of sampling. These would need to be prescribed, and be appropriate for the anticipated fluctuations and cycles of each parameter. Oxygen, total ammonia, CO_2, pH and temperature can all vary markedly over a 24 h cycle (Wagner *et al.* 1995, Wurts 2003).

(2) The methodology of sampling. The method for taking the sample (*e.g.* to avoid aeration), the site from within the unit and any treatments (*e.g.* pre-filtering, chemical fixation) would need to be specified.

(3) The actual method for measurement. Most of the methods recommended (*e.g.* by the UK Standing Committee of Analysts: in the series of “Blue Books”; and by the American Public Health Association) require a scientific capacity and equipment beyond the scope of most farmers. On-farm monitoring would therefore be dependent upon the availability of suitable probes and portable spectrophotometric kits. With appropriate guidance, fish farmers could reasonably be required to monitor temperature, DO and pH using probes, total ammonia nitrogen, alkalinity, nitrate and nitrite using field spectrophotometers; and CO_2 and un-ionised ammonia levels via computer packages after input of the required measurements. Farm measurement of gas supersaturation and suspended solids does not appear to be practicable. The water quality parameters that a farm manager could reasonably be expected to measure may reflect the level of intensity of the operation.

(4) Any additional calculation methods (if required).

(5) The unit for concentration (particularly important for nitrogenous compounds).

The second problem is that the setting of appropriate limits is inherently difficult. The numerous toxicological studies assessing the physiological tolerance of farmed fish to various water quality parameters often give disparate or conflicting recommendations for safe levels. This is the result of complex interactions of water chemistry affecting the actions of fish, and the numerous endogenous factors that affect the response of fish. For example, common minimum recommendations for DO are 5-6 mg/L, yet no mention is made of temperature and the effect that has on the capacity of the water to retain DO. Similarly, recommendations for maximum concentrations of ammonia make no allowance for reduced DO concentrations, and safe levels of nitrite must consider the chloride concentration of the water. It appears that the inconsistencies in reported toxic levels of metabolites in fish are primarily due to differences in water quality between tests, as concluded by Meade (1985). Furthermore, acute toxicity tests follow guidelines that attempt to standardise results (Randall & Tsui 2002). Such highly controlled experimental studies will also be highly biosecure, so effects of water quality on disease susceptibility will not become apparent. While the need to standardise test results is understandable, the conditions under test bear little resemblance to those found in commercial aquaculture practices. The relevance of using the test results in guidelines for water quality recommendations to protect fish welfare on commercial farms must therefore be questioned.

The imposition of single all-encompassing water quality limits derived from highly controlled experimental results is therefore problematic, as it could not be considered to have a strong scientific basis. An additional consideration is that a safe limit will depend upon the duration of exposure. Hence it would be appropriate for tolerable levels during short term events such as handling and transport to be different to those during routine rearing.

2.17.2.2 Animal-based parameters

Animal based parameters represent the response of the animal to the environment and therefore have the potential to circumvent uncertainties in relation to appropriate parameter limits. Such 'welfare indicators' have great potential as they provide a direct assessment of how the animal is coping with the environment, avoid problems associated with water quality monitoring, and represent an integral part of good stockmanship. The animal-based parameters brought up in the above review can be categorised into behavioural, morphological and production indicators (table 2.4).

These indicators differ in their response time, sensitivity and specificity to a particular parameter. The behavioural and production indicators are non-specific responses, and may have other possible causes than poor water quality. The morphological indicators are more specific for water quality problems, although various gill abnormalities are non-specific responses (Fivelstad *et al.* 2003a). Although providing a clear signal that the fish have been exposed to poor water, they are only apparent after adverse exposure.

Table 2.4: Animal-based indicators of poor water quality

Category	Indicator	Water quality problem
Behaviour	Aggregation near surface or inlet	Low DO
	Increased ventilation rate	Low DO; High ammonia
	Gaping mouths, flared operculae	High CO_2, Low DO
	Decreased food intake	High ammonia
	Hyper-excitability	High ammonia
	Violent erratic swimming	High ammonia
	Loss of equilibrium	High ammonia
	Moribund, lethargy, unresponsiveness, disorientation	High CO_2 High nitrite
Morphology	Bright red gill lamellae	High CO_2
	Gill damage	High ammonia High nitrite High suspended solids
	Brown blood	High nitrite
	Thymus damage	High nitrite
	Developmental abnormalities	High nitrate; Temperature
	Nephrocalcinosis	High CO_2
	Gas bubble disease	Supersaturation
Production	Decreased growth	Low DO High ammonia
	Increased food conversion ratio	Low DO High ammonia
	Mortality	Lethal levels of any parameter

Upon initial water quality deterioration, it is the physiology of the animal that is likely to respond first. However, physiological measures have not typically been examined in studies of water quality, and it has been suggested that “normal haematological status in fishes may represent a value range so broad as to be meaningless” (Houston 1990). Physiological measures are therefore of little value as predictors.

If poor water has sub-lethal effects on fish physiology, behaviour and morphology, then it is highly probable that these will manifest in a reduced growth rate and increased food conversion ratio, as documented for dissolved oxygen and ammonia. These are perhaps the most sensitive animal-based indicators of poor water quality. However, growth is highly dependant upon temperature and photoperiod (as well as *inter alia* fish size, strain, diet quality, life stage) and baseline ’normal values’ have yet to be established for many fish species.

Diseases, both non-infectious and infectious, are very good indicators of environmental quality in relation to health. Some non-infectious diseases are specific to particular parameters, *e.g.* gas bubble disease (supersaturation), methaemoglobinaemia (nitrite), and nephrocalcinosis (CO_2 and hardness). Environmental gill disease is acknowledged to be due to poor water quality, but the contributory parameters are not well defined (Wedemeyer 1996). Fin erosion has been linked to various water quality parameters, *i.e.* low dissolved oxygen and alkalinity, and high ammonia and suspended solids (Bosakowski & Wagner 1994, Wedemeyer 1996). The gills are recognised as a primary route of antigen uptake (Zapata *et al*. 1987, Moore *et al.* 1998). An increase in ventilation rate due to reduced water quality will increase the volume of water (and number of water-borne pathogens) passing through the opercular cavity and damage to the gill epithelium will increase the risk of uptake of pathogens. Poor water quality has been implicated in the development of a variety of facultative (*e.g. Saprolegnia*, Carballo *et al.* 1995) and obligate fungal, bacterial and viral diseases (table 2.5).

Table 2.5 Infectious diseases of fish and predisposing water quality parameters (from Wedemeyer 1996).

Disease	Predisposing water quality parameter
Bacterial gill disease (*Flavobacterium* spp.)	Low oxygen (<4 mg/L), elevated ammonia (>0.02 mg/L)
Furunculosis (*Aeromonas salmonicida*)	Low oxygen (< 5 mg/L),
Bacterial kidney disease BKD (*Renibacterium salmoninarum*)	Water hardness < 100 mg/L
Infectious pancreatic necrosis (IPN)	Water hardness

Although effects of water quality on disease susceptibility are frequently cited, hard scientific evidence is lacking. A notable exception is an epidemiological survey of *Aeromonas* spp. infection in trout hatcheries in northeast Spain (Ortega *et al.* 1996). This study found an association between the prevalence of *Aeromonas* spp. infection and dissolved oxygen and ammonia levels. These water quality parameters were suggested to act as risk factors at respective concentrations of < 7 and > 0.05 mg/L. Such determination of risk of disease on farms provides a possible method for determining appropriate levels for water quality parameters.

Mortality rate can be used as an indicator of the nature of a problem (Wedemeyer 1996). Very high mortalities within a short period (*e.g.* >50% in <1 day) indicate oxygen depletion or acute toxicity; high mortalities over a longer period (*e.g.* 50% in 5 days) indicate a virulent disease, and low mortality over an extended period (10% in 7 days) indicates poor environmental conditions (Wedemeyer 1996). Mortality can therefore be used as an indicator of water quality problems.

2.17.3 Safeguarding trout welfare

If fish farmers identify a problem with water quality, then remedial management options should be available (table 2.6). For sudden acute problems due to system or supply failures this may include back-up supplies for oxygenation, aeration, water pumping etc. Operating near maximum production capacity equates to operation at maximum risk, so monitoring and back-up systems should be related to intensity of production.

Table 2.6 Possible management options to alleviate water quality problems (after Masser *et al.* 1999)

Water quality problem	Remedial management option
Low dissolved oxygen	Increase aeration Increase oxygenation Increase water exchange Feed daily ration over longer period Stop feeding Reduce stocking density
High carbon dioxide	Increase aeration Increase water exchange Add stripping column
High ammonia	Increase water exchange Reduce feeding rate
High nitrite	Increase water exchange Reduce feeding rate Add chloride

There have been calls for the introduction of legislation to preserve fish welfare (FAWC 1996, Lymbery 2002). The apparent simplicity of setting prescriptive limits for environmental quality is tempered by the problems of arriving at appropriate limits and the ability of farmers to self-monitor as discussed above. Wedemeyer (1996) recognised the complexity of setting limits, but nevertheless set out recommendations for water quality limits as a guideline to fish culturists (table 2.7). These limits were intended as a framework for the prevention of disease in aquaculture, and were based upon a combination of experimental toxicological studies and experience of farm environments. Although the latter basis may be scientifically questionable, it does represent a huge resource of information derived from the real world.

The introduction of legislation for within-unit water quality would represent another legislative imposition on farmers. However, it could not be considered too different from existing legislation regulating discharges. Currently fish farm effluents are monitored for dissolved oxygen level, increases in biological oxygen demand, and levels of ammonia and suspended solids.

The parameters over which the farmer can exert a degree of control, and could potentially be expected to monitor are dissolved oxygen, carbon dioxide, ammonia and nitrite. Parameters that are largely outwith the control of the farmer include acidity, alkalinity, temperature, nitrate, hardness and heavy metals. These water quality parameters could therefore be considered during registration of a new fish farm, but would not need routine monitoring (unless required for calculation of other parameters). If water quality parameters were to be introduced, the experience of the UK's Environment Agency and Scottish Environment Protection Agency (SEPA) in monitoring and enforcement would prove useful.

Table 2.7 Recommended water chemistry limits to protect the health of salmonid fishes (from Wedemeyer 1996)

Parameter	Recommended Limits
Acidity	pH 6-9
Alkalinity	>20 mg/L (as $CaCO_3$)
Aluminium	<0.075 mg/L
Un-ionised ammonia	<0.02 mg/L
Calcium	>5 mg/L
Carbon Dioxide	<5-10 mg/L
Chloride	>4 mg/L
Chlorine	<0.003 mg/L
Copper	<0.006 mg/L (soft water) <0.03 mg/L (hard water)
Dissolved Oxygen	6 mg/L (coldwater fish) 4 mg/L (Warmwater fish)
Gas supersaturation	<110% total gas pressure
	(<103% salmonid eggs)
Nitrate	<1 mg/L
Nitrite	<0.1 mg/L
Total Dissolved Solids	<200 mg/L
Total Suspended Solids	<80 mg/L

However, it must be stressed that these limits are a guideline, and rigid implementation would represent a highly precautionary approach. Also, such limits do not provide a guarantee of good fish health, as prediction is difficult due to complex interactions. This is well illustrated by a complex water quality problem that is emerging in Norwegian Atlantic salmon smolts (Fivelstad *et al.* 2003b). The increasing use of oxygenation has allowed more intensive rearing, with the result that carbon dioxide levels are higher. This has had a knock-on effect of lowering the pH (due to the poor buffering capacity of the low alkalinity water). The carbon dioxide and reduced pH are then thought to combine with low levels of aluminium to adversely affect the fish (reduction of growth rate and increased mortality, ventilation rate and incidence of gill lesions).

Due to the complex interactions between water quality parameters, it would be extremely difficult to introduce across the board limits. A possible solution to this would be to characterise farms on the basis of the water chemistry of the inflow water, and then set water quality guidelines based on the chemistry and the known interactions: for example intensive farms with high stocking densities with hard, alkaline water would require strict ammonia regulations to protect the welfare of fish, while a similar farm with soft, acidic water would require stringent CO_2 regulations.

A possible scheme for practical monitoring of fish welfare would be for farmers to introduce their own routine diagnostic screening using animal-based parameters in addition to water quality monitoring, although monitoring such parameters would place an additional onus on the farmer. Routine morphological screening could be incorporated into a farm management plan, and be restricted to a macroscopic examination of the skin, gills and fins. Some animal–based parameters are already monitored by many farmers, for example growth, food conversion ratio and mortalities, however the development of practical, on-farm welfare indicators is an area that requires further investigation. The gathering and management of such data could be considered part of best practice, would allow greater traceability of the product and have quality assurance benefits for the farmer.

2.18 Conclusions

There is a lack of strong scientific data on appropriate levels for water quality parameters from commercial aquaculture situations. Water quality limits could be introduced for some parameters, but these would have to be ranges rather than single limits, and standardised protocols for measurement would need to be developed.

Farmers should be made aware of fish-based indicators of poor water quality, and should periodically conduct health screening. They should also be encouraged to record incidences of fish based indicators and disease that relate to poor water quality, and use the experience to introduce and adapt farm-based management plans that apply to their local inflow systems and water.

Further on-farm research into the role of water quality in fish welfare is required.

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2.20 References

Alabaster, J.S., Lloyd, R. 1982. *Water quality criteria for freshwater fish, 2nd Ed*. Butterworth Scientific, London, UK.

American Public Health Association. 1998. *Standard Methods for the Examination of Water and Wastewater, 20th Edition*. American Public Health Association, Washington DC., USA.

Anon. 1980. *Methods for the examination of waters and associated materials: Dissolved oxygen in natural and waste waters 1979*. Standing Committee of Analysts, Blue Book. HMSO, London, UK.

Anon. 1981. *Ammonia in water 1981*. Standing Committee of Analysts, Blue Book. HMSO, London, UK.

Anon. 2001. *BTA Code of Practice, revision 2001*. British Trout Association, Ingleston, UK..

Beveridge, M.C.M., Phillips, M.J., Clarke, R.M. 1991. *A quantitative and qualitative assessment of wastes from aquatic animal production*. In: Brune, D.E., Tomasso, J.R. (Eds). Aquaculture and water quality. World Aquaculture Society, Baton Rouge, USA. pp 506-533.

Bosakowski, T., Wagner, E.J. 1994. Assessment of fin erosion by comparison of relative fin length in hatchery and wild trout in Utah. *Canadian Journal of Fisheries and Aquatic Sciences*. **51**: 636-641.

Bowden, T.J., Cook, P., Rombout, J.H.W.M. 2005. Development and function of the thymus in teleosts. *Fish and Shellfish Immunology*. **19**: 413-427.

Boyd, C.E. 1990. *Water quality in ponds for aquaculture*. Auburn University, Alabama, USA.

Branson, E.J., Turnbull, T. 2008. Welfare and deformities. In: Branson, E.J. (Ed). *Fish Welfare.* Blackwell Publishing, UK. pp 202-216.

Carballo, M., Munoz, M.J., Cuellar, M., Tarazona, J.V. 1995. Effects of waterborne copper, cyanide, ammonia, and nitrite on stress parameters and changes in susceptibility to saprolegniosis in rainbow trout (*Oncorhynchus mykiss*). *Applied and Environmental Microbiology*. **61**: 2108-2112.

Chen, S., Stechey, D., Malone, R.F. 1994. *Suspended solids control in recirculating aquaculture systems*. In: Timmons, M.B., Losordo, T.M. (Eds). Aquaculture water reuse systems: engineering design and management. Elsevier, Oxford, UK. pp 61-100.

Colt, J., Watten, B. 1988. Applications of pure oxygen in fish culture. *Aquacultural Engineering*. **7**: 397-441.

Colt, J.E., Tomasso, J.R. 2001. *Hatchery water supply and treatment*. pp 91-186. In: Wedemeyer, G.A., (Ed). Fish Hatchery Management*, 2nd Edition*. American Fisheries Society, Maryland, USA.

Danley, M., Mazik, P., Kenney, P.B., Kiser, R., Hankins, J. 2001. Chronic exposure to carbon dioxide: Growth, physiological stress responses, and fillet quality of rainbow trout. *Aquaculture 2001: Book of Abstracts*.. World Aquaculture Society, Baton Rouge, USA. p 161.

Daoust, P-Y., Ferguson, H.W. 1984. The pathology of chronic ammonia toxicity in rainbow trout, *Salmo gairdneri*, Richardson. *Journal of Fish Diseases*. **7**: 199-205.

Defra. 2004. *Nitrates- reducing water pollution from Agriculture*. http://www.defra.gov.uk/environment/water/quality/nitrate/default.htm

Doulos, S.K., Kindschi, G.A. 1992. Effects of oxygen supplementation on lake trout brood stock. *Journal of Applied Aquaculture*. **1(3)**: 89-96.

Eddy, F.B., Kunzlik, P.A.., Bath, R.N. 1983. Uptake and loss of nitrite from the blood of rainbow trout, *Salmo gairdneri* Richardson, and Atlantic salmon, *Salmo salar* L., in fresh water and in dulite sea water. *Journal of Fish Biology*. **23**:105-116.

Eddy, F.B., Williams, E.M. 1994. *Freshwater fish and nitrite*. In: Howells, G. (Ed). Water quality for freshwater fish. Gordon and Breach Science Publishers. pp 117-144.

Evans, D.H., Piermarini, P.M., Choe, K.P. 2005. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiological Reviews*. **85**: 97-177.

FAWC. 1996. *Report on the welfare of farmed fish*. Farm Animal Welfare Council, Surbiton, Surrey, UK.

Fevolden, S-E., Røed, K.H., Fjalestad, K. 2003. A combined salt and confinement stress enhances mortality in rainbow trout (*Oncorhynchus mykiss*) selected for high stress responsiveness. *Aquaculture*. **216**: 67-76.

Fikri, A., Recai, T., Hijran, Y.Y., Oquz, K. 2000. Nephrocalcinosis in intensively reared rainbow trout (*Oncorhynchus mykiss*). *Israeli Journal of Aquaculture*. **52**: 111-117.

Fish Farming International. 1999. Malformations related to heat. *Fish Farming International.* **26**: 22

Fivelstad, S., Olsen, A.B., Asgard, T. 2003a. Long-term sublethal effects of carbon dioxide on Atlantic salmon smolts (*Salmo salar* L.): ion regulation, haematology, element composition, nephrocalcinosis and growth parameters. *Aquaculture*. **215**: 301-319.

Fivelstad, S., Waagbø, R., Zeitz, S.F., Hosfled, A.C.D, Olsen, A.B., Stefansson, S. 2003b. A major water quality problem in smolt farms: combined effects of carbon dioxide, reduced pH and aluminium on Atlantic salmon (*Salmo salar* L.) smolts: physiology and growth. *Aquaculture*. **215**: 339-357.

Garton, G.A., Nebeker, A.V. 1977. *A multiple approach to solving the gas supersaturation problem*. In: Tubb, R.A. (Ed). Recent advances in fish toxicology. U.S. Environmental Protection Agency, Ecological Research Series EPA-600/3-77-085, Corvallis, Oregon, U.S.A. pp 4-19.

Hampson, B.L. 1976. Ammonia concentration in relation to ammonia toxicity during a rainbow trout rearing experiment in a closed freshwater-seawater system. *Aquaculture*. **9**: 61-70.

Harrison, J. 1979a. High CO_2 levels hit hard-water trout. *Fish Farmer*. **2**: 29.

Harrison, J.G. 1979b. *Nephrocalcinosis of rainbow trout (*Salmo gairdneri *Richardson) in freshwater; a survey of affected farms*. Presentation at Session on Cooperative Programme of Research on Aquaculture (COPRAQ), Munich (GFR), 23 Oct 1979.

Harrison, J .G., Richards, R.H. 1979. The pathology and histopathology of nephrocalcinosis in rainbow trout *Salmo gairdneri* Richardson in fresh water. *Journal of Fish Diseases*. **2**: 1-12.

Haywood, G.P. 1983. Ammonia toxicity in teleost fishes: a review. *Canadian Technical Report of Fisheries and Aquatic Science*. **1177**: iv + 35 p.

Heinen, J.M., Hankins, J.A., Weber, A.L., Watten, B.J. 1996. A semi-closed recirculating water system for high density culture of rainbow trout. *Progressive Fish-Culturist*. **58**: 11-22.

Hinshaw, J.M., Fornshell, G. 2002. Effluents from raceways. In: Tomasso, J.R. (Ed). *Aquaculture and the environment in the United States*. U.S. Aquaculture

Society, A Chapter of the World Aquaculture Society, Baton Rouge, USA. pp 77-104.

Houston, A.H. 1990. *Blood and circulation*. In Schreck, C.B., Moyle, P.B. (Eds). Methods for Fish Biology. American Fisheries Society, Maryland, USA. pp 273-334.

Howells, G. 1994. *Water quality for freshwater fish: further advisory criteria*. Gordon and Breach Science Publishers, Reading, UK.

Huey, D.W., Simco, B.A., Criswell, D.W. 1980. Nitrite-induced methemoglobin formation in channel catfish. *Transactions of the American Fisheries Society*. **109**: 558-562.

Jensen, F.B. 2003. Nitrite disrupts multiple physiological functions in aquatic animals. *Comparative and Biochemistry Physiological Part A*. **135**: 9-24.

Jobling, M., Baardvik, B.M., Christiansen, J.S., Jørgensen, E.H. 1993. The effects of prolonged exercise training on growth parameters and production parameters in fish. *Aquaculture International*. **1**: 95-111.

Jones D.R. 1971. The effect of hypoxia and anemia on the swimming performance of rainbow trout (*Salmo gairdneri*). *Journal of Experimental Biology*. **55**: 541–551.

Klontz, G.W. 1991. *Manual for rainbow trout production on the family-owned farm*. Nelson and Sons, Inc, Utah, USA.

Klontz, G.W. 1993. *Environmental requirements and environmental diseases of salmonids*. In: Stoskopf, M. (Ed). Fish Medicine. W.B.Saunders, Philadelphia, USA. pp 333-342.

Larmoyeux, J.D., Piper, R.G. 1973. Effects of water reuse on rainbow trout in hatcheries. P*rogressive Fish-Culturist*. **36**: 2-8.

Levy, D.A., Northcote, T.G., Hall, K.J., Yesaki, I. 1989. Juvenile salmon response to log storage in littoral habitats of the Fraser River estuary and Babine Lake. *Canadian Special Publication of fisheries and Aquatic Science*. **105**: 82-91.

Lewis, W.M. Jr., Morris, D.P. 1986. Toxicity of nitrite to fish: A review. *Transactions of the American Fisheries Society*. **115**: 183-195.

Liao, P.B. 1971. Water requirements of salmonids. *Progressive Fish-Culturist*. **33**: 210-215.

Liao, P.B., Mayo, R.D. 1974. Intensified fish culture combining water reconditioning with pollution abatement. *Aquaculture*. **3**: 61-85.

Linton, T.K., Reid, S.D., Wood, C.M. 1997. The metabolic costs and physiological consequences to juvenile rainbow trout of a simulated summer warming scenario in the presence and absence of sublethal ammonia. *Transactions of the American Fisheries Society*. **126**: 259-272.

Linton, T.K., Morgan, I.J., Walsh, P.J., Wood, C.M. 1998a. Chronic exposure of rainbow trout (*Oncorhynhcus mykiss*) to simulated climate warming and sublethal ammonia: a year-long study of their appetite, growth, and metabolism. *Canadian Journal of Fisheries and Aquatic Science*. **55**: 576-586.

Linton, T.K., Reid, S.D., Wood, C.M. 1998b. The metabolic costs and physiological consequences to juvenile rainbow trout of a simulated winter warming scenario in the presence and absence of sublethal ammonia. *Transactions of the American Fisheries Society*. **127**: 611-619.

Lloyd, R., Swift, D.J. 1976. *Some physiological responses by freshwater fish to low dissolved oxygen, high carbon dioxide, ammonia and phenol with particular reference to water balance*. In: Effects of pollutants on aquatic systems. Lockwood, A.P.M. (Ed). Cambridge University Press, Cambridge, UK. pp 47-70.

Lymbery, P. 2002. *In too deep – The welfare of intensively farmed fish*. A Compassion In World Farming Report. Petersfield, UK .

Magor, B.G. 1988. Gill histopathology of juvenile *Oncorhynchus kisutch* exposed to suspended wood debris. *Canadian Journal of Zoology*. **66**: 2164-2169.

Masser, M.P., Rakocy, J., Losordo, T.M. 1999. *Recirculating aquaculture tank production systems: Management of recirculating systems*. Southern Regional Aquaculture Centre Publication No 452.

McDonald, D.G., Hobe, H., Wood, C.M. 1980. The influence of calcium on the physiological responses of the rainbow trout, *Salmo gairdneri*, to low environmental pH. *Journal of Experimental Biology*. **88**: 109-131.

McDonough, P.M., Hemmingsen, E.A. 1985. Swimming movements initiate bubble formation in fish decompressed from elevated gas pressures. *Comparative Biochemistry and Physiology Part A*. **81**: 2009-212.

McKenzie, D.J., Shingles, A., Taylor, E.W. 2003. Sub-lethal ammonia accumulation and the exercise performance of salmonids. *Comparative Biochemistry and Physiology Part A*. **135**: 515-526.

Meade, J.W. 1985. Allowable ammonia for fish culture. *Progressive Fish-Culturist*. **47**: 135-145.

Messer, J.J., Ho, J., Grenney, W.J. 1984. Ionic strength correction for the extent of ammonia ionization in freshwater. *Canadian Journal of Fisheries and Aquatic Science*. **41**: 811-815.

Mollenhurst, H., Rodenburg, T.B., Bokkers, E.A.M., Koene, P., de Boer, I.J.M. 2005. On-farm assessment of laying hen welfare: a comparison of one environment-based and two animal-based methods. *Applied Animal Behaviour Science*. **90**: 277-291.

Moore, J.D., Ototake, M., Nakanishi, T. 1998. Particulate antigen uptake during immersion immunisation of fish: The effectiveness of prolonged exposure and the roles of skin and gill. *Fish and Shellfish Immunology*. **8**: 393-407.

Neill, W.H., Bryan, J.D. 1991. *Responses of fish to temperature and oxygen, and response integration through metabolic scope*. In: Brune, D.E., Tomasso, J.R. (Eds). Aquaculture and water quality. World Aquaculture Society, Baton Rouge, USA. pp 30-57.

Noble, A.C., Summerfelt, S.T. 1996. Diseases encountered in rainbow trout cultured in recirculating systems. *Annual Review of Fish Diseases*. **6**: 65-92.

Ortega, C., Muzquiz, J.L., Fernandez, A., Ruiz, I., De Blas, I., Simon, M.C., Alonso, J.L. 1996. Water quality parameters associated with *Aeromonas* spp.affected hatcheries. *Veterinary research.* **27**: 553-60.

Randall, D.J. 1991. *The impact of variations in water pH on fish*. In: Brune, D.E., Tomasso, J.R. (Eds). Aquaculture and water quality. World Aquaculture Society, Baton Rouge, USA. pp 90-104.

Randall, D.J., Wright, P.A. 1989. The interaction between carbon dioxide and ammonia excretion and water pH in fish. *Canadian Journal of Zoology*. **67**: 2936-2942.

Randall, D.J., Tsui, T.K.N. 2002. Ammonia toxicity in fish. *Marine Pollution Bulletin*. **45**: 17-23.

Redding, J.M., Schreck, C.B., Everest, F.H. 1987. Physiological effects on coho salmon and steelhead of exposure to suspended solids. T*ransactions of the American Fisheries Society*. **116**: 737-744.

Ritola, O., Tossavainen, K., Kiuru, T., Lindström-Seppä, P., Mölsä, H. 2002. Effects of continuous and episodic hyperoxia on stress and hepatic glutathione levels in one-summer-old rainbow trout (*Oncorhynchus mykiss*). *Journal of Applied Ichthyology*. **18**:159-164.

Roberts, R.J. 1975. *The effects of temperature on diseases and their histopathological manifestations in fish*. In: The Pathology of Fishes. Ribelin, W.E., Migaki, G. (Eds). The University of Wisconsin Press, USA. pp 477-496.

Russo, R.C., Thurston, R.V. 1977. *The acute toxicity of nitrite to fishes*. In: Tubb, R.A. (Ed). Recent advances in fish toxicology. U.S. Environmental Protection Agency, Ecological Research Series EPA-600/3-77-085, Corvallis, Oregon, U.S.A. pp 118-131.

Russo, R.C., Thurston, R.V., Emerson, K. 1981. Acute toxicity of nitrite to rainbow trout (*Salmo gairdneri*): effects of pH, nitrite species, and anion species. *Canadian Journal of Fisheries and Aquatic Science*. **38**: 387-393.

Russo, R.C., Thurston, R.V. 1991. *Toxicity of ammonia, nitrite, and nitrate to fishes*. In: Brune, D.E., Tomasso, J.R. (Eds). Aquaculture and water quality. World Aquaculture Society, Baton Rouge, USA. pp 58-89.

Scott, E.M., Harrigan, J.P. 1985. Methemoglobin reductase activity in fish erythrocytes. *Comparative Biochemistry and Physiology Part B*. **82**: 511-513.

Shingles, A., McKenzie, D.J., Taylor, E.W., Moretti, A., Butler, P.J., Ceradini, S. 2001. The effects of sublethal ammonia on swimming performance in rainbow trout, *Oncorhynchus mykiss*. *Journal of Experimental Biology*. **204**: 2691-2698.

Smart, G. 1976. The effect of ammonia exposure on gill structure of the rainbow trout (*Salmo gairdneri*). *Journal of Fish Biology*. **8**: 471-475.

Smart, G.R., Knox, D., Harrison, J.G., Ralph, J.A., Richards, R.H., Cowey, C.B. 1979. Nephrocalcinosis in rainbow trout *Salmo gairdneri* Richardson; the effect of exposure to elevated CO2 concentrations. *Journal of Fish Diseases*. **2**: 279-289.

Smart, G.R. 1981. *Aspects of water quality producing stress in intensive fish culture*. In: Pickering, A.D. (Ed). Stress and fish. Academic Press, London, UK. pp 277-293.

Smith, C.E., Piper, R.G. 1975. *Lesions associated with chronic exposure to ammonia*. In: Ribelin, W.E., Migaki, G. (Eds). The Pathology of Fishes. The University of Wisconsin Press, USA. pp 497-514.

Soderberg, R.W., Flynn, J.B., Schmittou, H.R. 1983. Effects of ammonia on growth and survival of rainbow trout in intensive static-water culture. *Transactions of the American Fisheries Society*. **112**: 448-451.

Soderberg, R.W., Meade, J.W. 1992. Effects of sodium and calcium on acute toxicity of un-ionized ammonia to Atlantic salmon and lake trout. *Journal of Applied Aquaculture*. **1**: 83-92.

Summerfelt, S.T. 2002. Understanding and treating carbon dioxide problems. *Aquacaulture Magazine*, **July/August**: 30-33.

Thurston, R.V., Phillips, G.R., Russo, R.C. 1981a. Increased toxicity of ammonia to rainbow trout (*Salmo gairdneri*) resulting from reduced concentrations of dissolved oxygen. *Canadian Journal of Fisheries and Aquatic Sciences.* **38**: 983-988.

Thurston, R.V., Chakoumakos, C., Russo, R.C. 1981b. Effect of fluctuating exposures on the acute toxicity of ammonia to rainbow trout (*Salmo gairdneri*) and cutthroat trout (*S. clarki*). *Water Research*. **15**: 911-917.

Thurston, R.V., Russo, R.C. 1983. Acute toxicity of ammonia to rainbow trout. *Transactions of the American Fisheries Society*. **112**: 696-704.

Thurston, R.V., Russo, R.C., Luedtke, R.J., Smith, C.E., Meyn, E.L. 1984. Chronic toxicity of ammonia to rainbow trout. *Transactions of the American Fisheries Society*. **113**: 56-73.

Tomasso, J.R. 1994. Toxicity of nitrogenous wastes to aquaculture animals. *Reviews in Fisheries Science*. **2**: 291-314.

Tucker, C.S., Boyd, C.E., Hargreaves, J.A. 2002. *Characterization and management of effluents from warmwater aquaculture*. In: Tomasso, J.R. (Ed). Aquaculture and the environment in the United States. U.S. Aquaculture Society. A Chapter of the World Aquaculture Society, Baton Rouge, USA.. pp 35-76.

Twitchen, I.D., Eddy, F.B. 1994. Effects of ammonia on sodium balance in juvenile rainbow trout *Oncorhynchus mykiss* Walbaum. *Aquatic Toxicology*. **30**: 27-45.

Wagner, E.J., Miller, S.A., Bosakowski, T. 1995. Ammonia excretion by rainbow trout over a 24-hour period at two densities during oxygen injection. *Progressive Fish-Culturist*. **57**: 199-205.

Warrer-Hansen, I. 2003. Oxygen and aeration systems. *Trout News*. **35**: 24-26.

Wedemeyer, G.A., Yasutake, W.T. 1978. Prevention and treatment of nitrite toxicity in juvenile steelhead trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada*. **35**: 822-827.

Wedemeyer, G.A. 1996. *Physiology of fish in intensive culture systems*. Chapman & Hall, London, UK.

Westers, H. 2001. *Production.* In: Wedemeyer, G.A. (Ed). Fish Hatchery Management, 2nd Ed. American Fisheries Society, Maryland, USA. pp 31-89.

Westers, H., Pratt, K.M. 1977. Rational design of hatcheries for intensive salmonid culture, based on metabolic characteristics. *Progressive Fish-Culturist*. **39**: 157-165.

Westin, D.T. 1974. Nitrate and nitrite toxicity to salmonid fishes. *Progressive Fish-Culturist*. **36**: 86-89.

Wicks, B.J., Randall, D.J. 2002a. The effect of sub-lethal ammonia exposure on fed and unfed rainbow trout: the role of glutamine in regulation of ammonia. *Comparative Biochemistry and Physiology, Part A*. **132**: 275-285.

Wicks, B.J., Randall, D.J. 2002b. The effect of feeding and fasting on ammonia toxicity in juvenile rainbow trout, *Oncorhynchus mykiss*. *Aquatic Toxicology*. **59**: 71-82.

Wicks, B.J., Tang, Q., Joensen, R., Randall, D.J. 2002. Swimming and ammonia toxicity in salmonids: the effect of sub lethal ammonia exposure on the swimming performance of coho salmon and the acute toxicity of ammonia in swimming and resting rainbow trout. *Aquatic Toxicology*. **59**: 55-69.

Wilson, R.W., Wright, P.M., Munger, S., Wood, C.M. 1994. Ammonia excretion in freshwater rainbow trout (*Oncorhynchus mykiss*) and the importance of gill boundary layer acidification: Lack of evidence for Na+/NH4+ exchange. *Journal of Experimental Biology*. **191**: 37-58.

Wilson, J.M., Iwata, K., Iwama, G.K., Randall, D.J. 1998. Inhibition of ammonia excretion and production in rainbow trout during severe alkaline exposure. *Comparative Biochemistry and Physiology Part B*. **121**: 99-109.

Wood, C.M. 2004. Dogmas and controversies in the handling of nitrogenous wastes: is exogenous ammonia a growth stimulant in fish? *Journal of Experimental Biology*. **207**: 2043-2054.

Wright, P.A., Wood, C.M. 1985. An analysis of branchial ammonia excretion in the freshwater rainbow trout: Effects of environmental pH change and sodium uptake blockade. *Journal of Experimental Biology*. **114**: 329-353.

Wurts, W.A. 2003. Daily pH cycle and ammonia toxicity. *World Aquaculture.* **June**: 20-21.

Ye, X., Randall, D.J. 1991. The effect of water pH on swimming performance in rainbow trout (*Salmo gairdneri*, Richardson). *Fish Physiology and Biochemistrry*. **9**: 15–21.

Ye, X., Randall, D.J., He, X. 1991. The effect of acid water on oxygen consumption, circulating catecholamines and blood ionic and acid-base status in rainbow trout (*Salmo gairdneri*, Richardson). *Fish Physiology and Biochemsitry*. **9**: 23-20.

Yurkowski, M., Gillespie, D.C., Metner, D.A., Lockhart, W.L. 1985. Nephrocalcinosis and blood chemistry in mature rainbow trout (*Salmo gairdneri*). *Canadian Technical Report on Fisheries and Aquatic Science*. **1348**: 11pp.

Zapata, A.G., Torroba, M., Alvarez, F., Anderson, D.P., Dixon, O.W., Wisnieswski, M. 1987. Electron microscopical examination of antigen uptake by salmonid gill cells after bath immunization with a bacterin. *Journal of Fish Biology*. **31, Supplement A**: 209-217.

3 Rainbow trout farming in the UK and potential for monitoring welfare.

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3.1 Abstract

An important step in improving the welfare of farmed rainbow trout is to assess the welfare on-farm. No welfare assessment schemes currently exist for farmed rainbow trout, and this paper discusses the type of information that might be required for such a scheme. For the development of an on-farm welfare assessment scheme, it is important to know the structure of the industry the scheme will apply to, and also what information is currently being retained by fish farmers. We contacted 109 UK rainbow trout farmers in 2005, who accounted for over 80% of UK rainbow trout production, and visited 58 of these farmers to obtain more detailed information about the farm operation. Information was collected on water quality monitoring, current farm standards or codes of practice adhered to, slaughter methods and record keeping. Monitoring water quality will be an integral part of any on-farm welfare assessment scheme, and while measuring some water quality parameters requires specialist equipment, farmers should be able to monitor the essential parameters, dissolved oxygen and temperature. Any on-farm welfare assessment scheme for rainbow trout should incorparate fish-based measures in addition to resource-based parameters in order to provide as complete an overview of trout welfare as possible.

3.2 Introduction

On-farm animal welfare assessment schemes (WAS) have been developed for many species of farmed animals, (*e.g.* Ekstrand *et al*. 1997, Amon *et al*. 2001, Bartussek 2001, Bracke *et al*. 2001, Johnsen *et al*. 2001, Whay *et al*. 2003, Smulders *et al*. 2006), however, to date only one scheme applies to farmed fish (Anon 2007a). With research into fish welfare gaining momentum within the UK and across Europe (BENEFISH (www.benefish.eu), COST Action 867 (www.cost.esf.org), Defra projects AW1202, AW1203, AW1204, AW1205, AW1206, AW1208 (www.randd.defra.gov.co.uk), FASTFISH (fastfish.imr.no), wealth (www.wealth.imr.no)), it is likely that welfare assessment schemes will emerge, as is currently happening with other farmed animals (COST Action 846 (www.cost.esf.org), Johnsen *et al*. 2001, Main *et al.* 2001). At the time of writing, there were no schemes for the assessment of farmed trout welfare, although it was understood that the RSPCA were preparing welfare standards for trout welfare through the Freedom Foods scheme (J. Avinezious, RSCPA, pers.comm).

Welfare assessment schemes (WAS) can have different applications and different purposes. Main *et al.* (2003) listed four categories of application for WAS; these are 1) research tool; 2) legislative requirements (non-voluntary); 3) certification schemes (voluntary); 4) advisory/management tool. Within these four applications, information gathered for a WAS could be used for daily monitoring or for retrospective analysis of welfare.

The development of a WAS, for whatever application, requires certain information if the scheme is to be effective. 1) the structure of the industry; 2) what motivates the industry (apart from profit)?; 3) what kind of information is currently retained by the farmer that could inform a WAS?; 4) what information needs to be in the scheme?; 5) what aspects of welfare the scheme is intended to assess?.

As part of a series of focus groups, North *et al.* (2008) discussed on-farm welfare assessment with stakeholders in the UK trout industry. During these meetings it was recognised that the selection of indicators included in any scheme would depend on the purpose or application of the scheme. There were high levels of agreement between the stakeholders on the type of information that could be collected to enable an assessment of welfare to be made. The range of information was grouped under the headings; Operational Welfare Indicators (OWIs), environmental quality, farm records, targeted stock sampling, demonstration of good stockmanship and (post-) harvest based measures.

On-farm welfare can be assessed through animal-based or resource-based measures (Main *et al.* 2003). Animal-based measures focus on the condition of the animal and how it responds to its environment. In the case of fish, these measures could include the condition factor (K), the condition of the fins, physiological measurements such as cortisol or lysosyme, and disease state. These measures might also include production statistics that directly or indirectly reflect the welfare state of the fish, such as mortality rates, specific growth rates and food conversion ratios. Fish behaviour has been identified as a very useful measure of welfare (Dawkins 2006), although there are currently no behavioural indicators of welfare available for use in on-farm welfare assessment. There is a need for these to be developed and they could be incorporated into welfare assessment in the future. One of the major disadvantages with animal-based measures, as applied to fish, is the need to sacrifice fish in order to obtain certain morphological or physiological measurements. Assessment of fish behaviour and gross morphological measures, such as condition factor, fin condition and gross pathological symptoms of disease, do not require fish to be killed and can be assessed using anaesthetised fish.

Resource-based measures for welfare assessment prescribe requirements for good welfare. These measures would include environmental parameters and husbandry and management practices that affect or have the potential to affect welfare. Monitoring of water quality would be central to any WAS, given the potential effects that poor water quality can have on fish welfare (see MacIntyre *et al.* 2008 for a review). Management practices have the potential to affect fish welfare in all areas of production, from stocking strategies, grading fish, maintenance of equipment and staff training to slaughter methods. Best practice methods are laid out in industry codes of practice and quality assurance scheme standards. In the UK trout industry, the British Trout Association (BTA) Code of Practice (Anon 2002) and Quality Trout UK (QTUK) Certification Standards (Anon 2006a) provide members with advice on best farming practice and also on management procedures that can affect the welfare of the fish. All members of the BTA and QTUK scheme must comply with the respective standards (D Bassett, BTA, pers. comm.). Veterinary health plans (VHP) are another resource-based measure and are a stipulation under the QTUK standards. VHPs are developed by individual farmers in partnership with veterinarians with the aim of agreeing best practices for the health and welfare of the fish. Areas covered under a VHP include biosecurity, health and disease monitoring and staff responsibilities. Welfare assessment can be achieved through animal-based measures only or resource-based measures only, however, to attain as broad a perspective as possible of the state of the animal’s welfare, a mixture of the two approaches is recommended (Johnsen *et al.* 2001).

The UK trout industry is over 100 years old, and in 2005 there were 270 registered rainbow trout (*Oncorhynchus mykiss*) farms, with 151 farms in England, 25 farms in Wales, 62 farms in Scotland and 32 farms in Northern Ireland (Tyson *et al.* 2007, R. Smith, FRS, pers. comm., R. Russell, DARD, pers. comm.). This number is down on the 373 registered farms recorded in 1994 (FAWC 1996), with the trend moving towards fewer and larger fish farms (Read 2008). Annual production in the UK for 2005 was 16,203 tonnes (Anon 2007b), with 12,482 tonnes produced for the table market and 3,721 tonnes of restocking fish for sports fisheries. Fish produced for the table market are usually harvested at around 400g (FAWC 1996), although in some marine sites rainbow trout are produced for fillets and harvest weight can be as much as 3kg. There is considerable variability in the weight of fish leaving restocking farms, with many fish stocked in the 500-800g range and occasionally fish are stocked in excess of 5kg. In England and Wales in 2005, there were 26 farms producing for the table market, 102 farms producing fish for restocking and 48 farms producing for both markets, with annual production of 5,900 tonnes for the table and 2,805 tonnes of restocking fish (Tyson *et al*. 2007). In Scotland the industry is dominated by the table market: in 2005 31 farms produced for fish for the table, 16 farms for restocking and 15 produced for both markets (R. Smith, FRS, pers.comm.). Annual production was 6,170 tonnes for the table and 819 tonnes for restocking (Anon 2006b). In Northern Ireland, 412 tonnes were produced for the table in 2006 and 97 tonnes for restocking. The UK trout industry therefore appears to differ geographically, with England and Wales having a large percentage of relatively small production restocking farms, while in Scotland the majority of farms and production is for the table market. In recent years, the UK trout industry has been actively involved in research directed towards farmed trout welfare in areas such as humane slaughter

(Lines *et al.* 2003, Lines & Kestin 2004, 2005), malformations and mortality in hatcheries (Read 2008), fin erosion (Hoyle *et al.* 2007, Ellis *et al.* 2008), and stocking density (North *et al*. 2006a, b) and are also the primary sponsors of this study.

Data for this study were collected as part of a larger Defra-funded project (AW1205), in association with the BTA, into water quality interactions with fish welfare. This study was undertaken to ascertain what information was being retained and recorded by UK trout farmers that could be useful to a WAS, with particular emphasis placed on water quality monitoring. Information that would assist with investigating the structure of the industry was also collected. It is envisaged that the data collected could also inform any future WAS regarding what was practically possible. Additionally, during discussion with farmers, the opportunity was taken to inform them of a companion epidemiological study into water quality interactions with fish welfare, and to ask if they would be willing to participate in this study. Informing potential participants in a study of the reasons for undertaking the study, and the potential benefits of the study, can enhance cooperation with the target group (Thursfield 1995).

The purposes of this study were therefore four-fold; 1) to determine the structure of the UK trout industry, 2) to investigate what information was currently collected by farmers that could assist with a welfare assessment scheme, 3) to determine what information could be collected for such a scheme, 4) to enhance cooperation for an epidemiology study into the welfare of farmed rainbow trout.

3.3 Method

The reference population for this survey comprised all farmers of rainbow trout in the United Kingdom including hatchery operators, table farmers and restocking farmers. This population was compiled from a list of trout farms from a previous Defra project (AW1203), from the British Trout Association membership list, Intrafish (a media house for the international fisheries and aquaculture industry) and the Yellow Pages (a business directory in the UK). Fishery only operators were excluded from the study due to the lack of fish rearing facilities in their operation.

3.3.1 Stage 1 Telephone contact with farmers

Data were collected for this study in 2 stages. The first stage involved contacting farmers by telephone, describing details of the project and completing a brief questionnaire. This was conducted from January until March 2005. Information was gathered on the type of production carried out on the site (hatchery, for the table market or restocking), annual production, the types of system on farm (tanks, cages, ponds, raceways), the source of the water supply, if the water was reused, recirculated or aerated, the characteristics of the water (hard, soft, acid, alkaline) and which water quality parameters were regularly measured on the site. Data collection was completed in line with Thrusfield (1995), who stated that the cooperation and participation of farmers would be best achieved if questionnaire was as simple as possible while still realising the aims of the study. The confidentiality of the participants was also assured.

A total of 109 farmers were contacted and agreed to participate in this stage of the study. All farmers within the reference population were telephoned at least once, with a total of over 1000 telephone calls. Figure 3.1 shows the locations of the farms involved in the study. Several farmers did not wish to participate in the study and many farms had closed down or were in the process of closing down at the time of calling. Many other farmers were unable to be contacted as they were unavailable at the time of telephoning.

3.3.2 Stage 2 Farm visits

The second stage of the study involved visiting farms from stage one and obtaining more detailed information. A total of 58 farms were randomly selected from the sample population for the second stage and were visited from May until July 2005.

Information was gathered on the water quality parameters that the farmer monitored and the frequency of the monitoring, any previous serious problems with water quality that impaired the welfare of the fish, the frequency of water quality monitoring by the Environment Agency or Scottish Environment Protection Agency and if the farmer received the results of that monitoring. Farmers were asked if they belonged to a QA scheme or adhered to a CoP and if they had a VHP, if they regularly monitored the fish and what production data was retained. Finally, information was collected on the staffing levels on the farm, the market the fish were produced for and the annual production of the farm.

Out of a reference population of 270 UK rainbow trout farms, the first stage of the study had 109 participants (43%). In England and Wales, there were 78 participants, with 13 table producers, 40 restockers and 26 producers for both markets. In Scotland, there were a total of 28 participants, with 16 table producers, 5 restockers and 7 participants producing for both markets, while in Northern Ireland there were 3 participants, all both table and restocking producers. Comparing the sample population for the telephone survey with the reference population indicates that the sample was indeed representative of the industry, however it appears that table farms were slightly overrepresented in the farm visit population. Figure 3.2 compares the trout industry with the sample populations for both stages of this study by the types of farming.

3.3.3 Data analysis

Data were analysed using Epi Info (Version 3.3.2, CDC, USA).

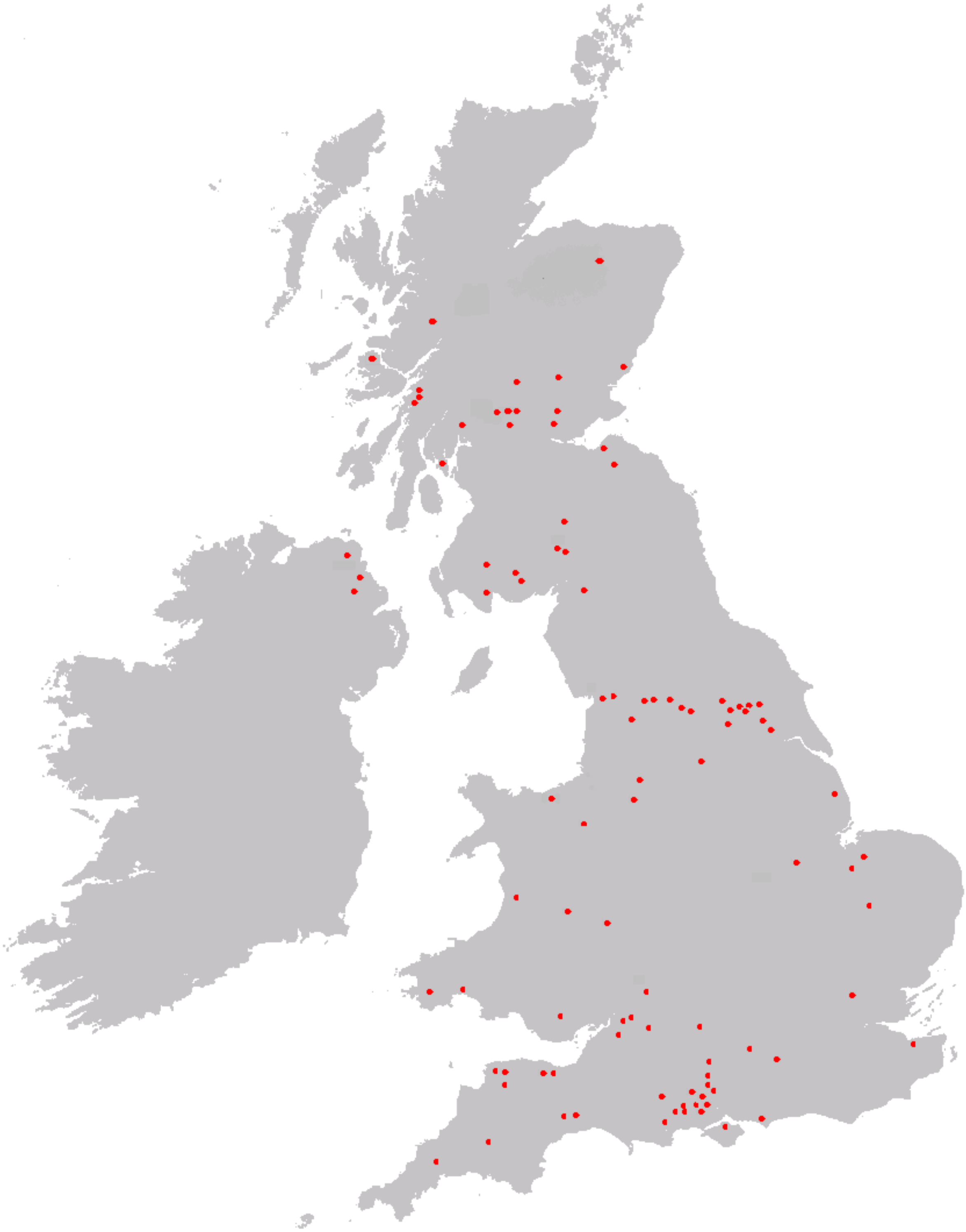


Figure 3.1 Locations of participating fish farms for stage 1 of study.

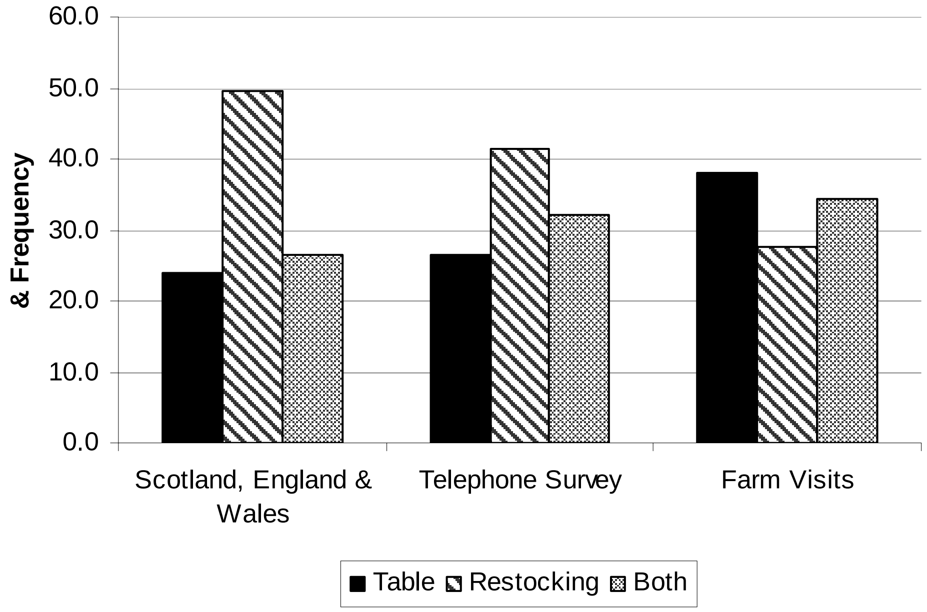


Figure 3.2 Division of farms into type of production for Scotland, England & Wales (Tyson et al. 2007), telephone survey participants and participants to farm visits.

3.4 Results

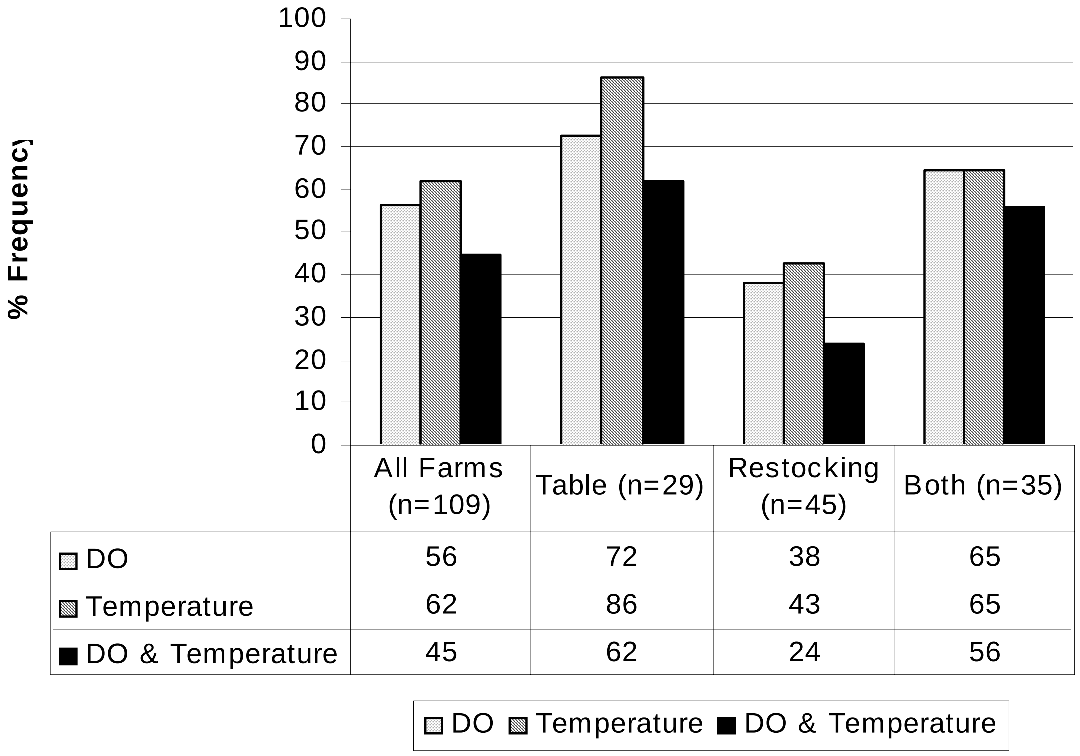
3.4.1 Telephone Survey

Of the 109 participants in the telephone survey, 29 farms were table producers, 45 produced fish for restocking and 35 farms produced for both the table and restocking market (table 3.1). The estimated number of fish farmed by the participants in this study is estimated at 27.5 millions. This was calculated using the annual production in tonnes and an average harvest weight of 400g for table market fish, 1500g for fillet sized fish, 600g for the average farm gate weight of restocking fish and a median weight of 500g for table and restocking farms, as the production split for these farms was not available. A total of 68 farms were members of the BTA.

Table 3.1 Number of farms, annual production (tonnes), size of farms by annual production and membership of British Trout Association for telephone survey participants (annual production figures for 8 restocking and 2 table and restocking farms were not available).

Farm Type	No. of Farms	Annual Production	Size of farms by annual production				BTA Members
			<50	50-100	100-500	500+	
Table	29	8637	5	1	17	6	26
Restocking	45	1583	24	11	2	0	25
Both	35	3231	18	7	8	0	17
Total	109	13451	47	19	27	6	68

Participants in the telephone survey were asked if they measured dissolved oxygen (DO) and temperature (figure 3.3). The highest percentage of farms that recorded these water quality parameters were table farmers, with 72% of table farmers measuring DO, 86% measuring temperature and 62% measuring both DO and temperature. Significantly fewer restocking farmers measured DO and temperature (Fisher's Exact Test, $p<0.005$), with only 38% measuring DO, 43% measuring temperature and 24% measuring both DO and temperature. For farmers that provide for both the table and restocking markets, 65% measured DO, 65% measured temperature and 56% measured both DO and temperature. More farmers that provide for both markets measured DO and temperature than restocking farms (Fisher's Exact Test, $p<0.05$), and compared with table farmers, there was no statistical difference for measuring DO ($p=0.352$), but more table farmers measured temperature ($p<0.05$). Of the 6 table farms that did not measure DO, 5 were marine loch and freshwater loch cage sites: the farm managers of these farms reported that DO did not vary much, were at a consistently high concentration and monitoring of DO was not warranted.



	All Farms (n=109)	Table (n=29)	Restocking (n=45)	Both (n=35)
□ DO	56	72	38	65
▨ Temperature	62	86	43	65
■ DO & Temperature	45	62	24	56

Figure 3.3 Percentage of farms by category that measure dissolved oxygen, temperature and both DO and temperature, from telephone survey.

3.4.2 Farm Visits

A total of 58 farms were visited in the second stage of the study; 22 farms produced for the table market, 15 farms were for restocking fisheries and 21 produced for both markets (table 3.3). The majority of table farms produced in the range of 100 to 500 tonnes per annum, while all restocking farms were under 100 tonnes per annum. In terms of annual production, this stage of the study was dominated by table producers, who accounted for almost 80% of all production. Restocking farms, while accounting for 26% of the number of farms, only contributed 6% to the total annual production. Farms producing for both markets accounted for 36% of the number of farms and 14.5% of annual production.

Table 3.2 Number of farms, annual production (tonnes) and size of farms for farms visited in second stage of study.

Farm Type	No. of Farms	Annual Production	Size of farms by annual production			
			<50	50-100	100-500	500+
Table	22	7888	1	1	16	4
Restocking	15	596.5	8	7	0	0
Both	21	1443	11	5	5	0
Total	58	9927.5	20	13	21	4

Participants in the study who were visited were asked if they were members of a QA scheme and thus complied with the scheme's standards (table 3.4). Of the 31 farms that were members of a quality assurance scheme, 29 were members of the Quality Trout UK scheme, 1 farm complied with the Tesco farm standards and 1 farm with The Soil Association organic standards. All farms that followed a CoP were members of the BTA and followed their CoP. Only 1 restocking farm had a written VHP.

Participants were also asked if they measured DO and temperature and the frequency of measuring (figure 3.4). Of the 20 table farms which measured DO, 17 measured it daily, while all table farms measured temperature daily. Only 5 restocking farms measured DO daily and 9 farms measured temperature daily, while of the farms producing for both markets, 7 farms measured DO daily and 14 farms measured temperature daily.

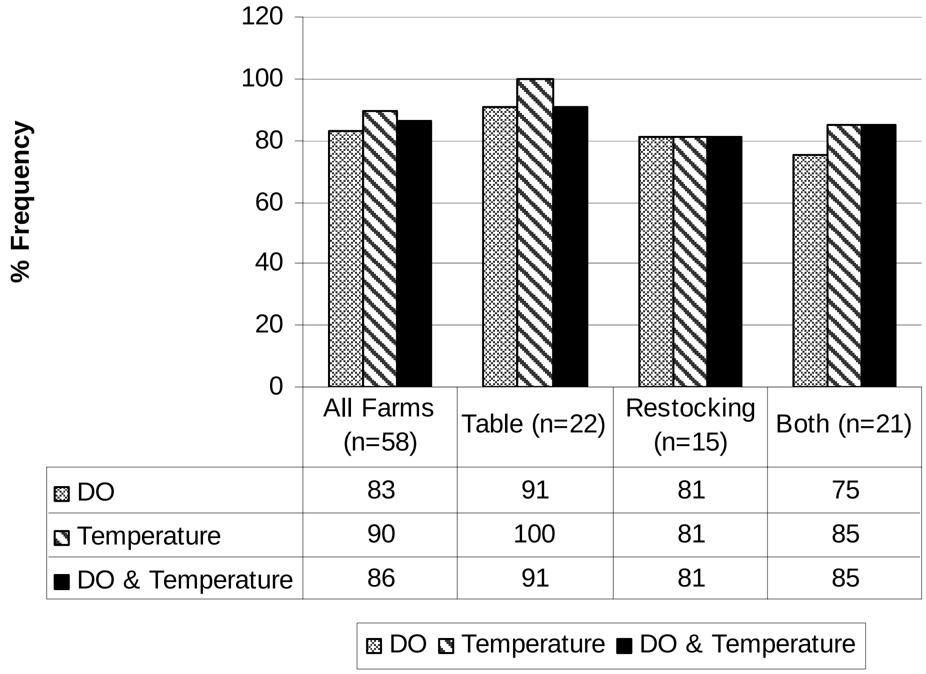


Figure 3.4 Percentage of farms by category that measure dissolved oxygen, temperature and both DO and temperature, from farm visit stage of study.

Table 3.3 Number and percentage, in parentheses, of farms from farm visits that abide by standards, maintain production records and methods of slaughter.

	Table	Restocking	Both
QA/CoP/VHP			
QA Scheme	21 (95.5)	0	10 (47.6)
Code of Practice	21 (95.5)	8 (53.3)	15 (71.4)
Veterinary Health Plan	20 (90.9)	1 (6.3)	14 (57.1)
QA/CoP & VHP	20 (90.9)	0	12 (57.1)
Records			
Track performance	21 (95.5)	12 (75.0)	17 (85.0)
Mortality records	22 (100)	13 (81.3)	16 (80.0)
FCR	19 (86.4)	10 (62.5)	13 (65.0)
Disease treatment	22 (100)	13 (81.3)	17 (85.0)
Biomass	22 (100)	9 (56.3)	13 (65.0)
Slaughter method *			
Percussive stun	0	n/a	5 (25.0)
Air asphixiation	4 (18.2)	n/a	4 (20.0)
Electric stun	15 (68.2)	n/a	6 (30.0)
Ice slurry	6 (27.3)	n/a	2 (10.0)
Carbon Dioxide	1 (4.5)	n/a	0

* 7 farmers for both table and restocking market did not slaughter fish on site - fish were transferred to another farm for final ongrowing prior to slaughter

All farmers were asked which farm records they kept and if they were able to track the performance of individual batches of fish (table 3.3). All table farmers maintained records of mortalities, disease treatments and the current biomass of their stock, while 1 table farmer did not track the performance of individual batches and 3 table farmers did not record the Food Conversion Ratio (FCR). For maintenance of records, there was no significant differences between restocking farmers and table farmersfor records of mortalities (p=0.158), FCR (p=0.154), and ability to track the performance of a batch (p=0.172), but table farmers did record biomass on more occasions (p=0.002). Comparing farmers providing for both markets with table farmers, no statistical difference was observed for maintenance of records for FCR (p=0.068) and tracking the performance of a batch (p=0.158), however significantly more table farmers recorded biomass (p=0.001), disease treatments (p=0.048) and maintained mortality records (p=0.02).

Restocking farms do not slaughter fish routinely. Out of the 43 farms that produced fish for the table market, 7 farms did not slaughter fish as fish were transferred off their farm to another farm for final ongrowing and harvest. There were 5 methods of slaughter carried out at the time of the visits; percussive stun to the head of the fish, asphyxiation in air, electric stun, submersion in ice slurry resulting in asphyxiation and use of carbon dioxide. The most common method of slaughter was electric stun, with 48% of farms that harvest fish using this method. After electric stun, air asphyxiation and ice slurry were used on 8 farms each and percussive stun was used on 5 farms. Only one farm slaughtered fish using carbon dioxide. Some farms used more than one method of slaughter.

All trout farms in this study had their outflow monitored by either the Environment Agency for England and Wales (EA) or the Scottish Environment Protection Agency (SEPA), although the frequency of such inspections varied. Most farms reported that inspections took place on average every month, although freshwater loch sites in Scotland reported inspections every 6 months and for marine loch sites annually. All but 3 farms received copies of the inspection report from the EA or SEPA.

For processing of the fish for table and both table and restocking farms, 18 farms sent their fish to large central processors, 15 farms had facilities for processing their own fish on site and 3 farms slaughtered the fish and sent them to another farm for processing. As mentioned above, 7 farms that produced fish destined for the table market did not slaughter fish.

Although the UK trout industry is often taken as a whole, it is perhaps most relevant to consider the industry in terms of table market producers and those who produce for restocking sports fisheries. The greatest number of farmers produce for restocking sports fisheries, although in terms of annual production, in 2005 only 23% of the total tonnage of fish produced in the UK were for restocking fisheries (Anon 2007b). As this statistic suggests, the majority of restocking farms are smaller producers than table farmers, with most farmers producing less than 100 tonnes per annum (table 3.1). One of the primary objectives of farming fish for restocking is to produce good quality fish in good condition and no damage to fins; qualities prized by sports fishermen. The fish are sold live and therefore no slaughter occurs on restocking only farms. Generally, restocking farms are less intensive than table market farms and fish are not stocked at as high a density as table market producers (North *et al*. 2006b).

Nationally there are fewer table market producers than restocking producers, however, the majority of annual production is produced for the table market. Table market producers generally farm fish to a portion size of 350-400g, although fillet portions require larger fish up to 3kg. These larger fish are produced in salmon-style cages in Scottish lochs. Table producers aim to farm fish with good flesh quality and they are not necessarily motivated to produce fish with good fins. Other physical damage to the body of the fish can result in rejection by the processor and is avoided where possible. Table farms generally produce more fish than restocking farms (table 3.1) and stocking densities are also higher (North *et al.* 2006b). Farms that were members of the BTA in 2005 accounted for 90% of table farmers who participated in the telephone survey for this study. The BTA is the main industry association for trout farming in the UK and have produced a CoP for its members to follow (Anon 2002). In addition to the BTA CoP, table producers can also join the Quality Trout UK scheme which has its own certification standards, including a section on welfare (Anon 2006a).

Several farms produced for both the table and restocking markets, in 2005 these farms accounted for 26.5% of the total number of farms in Scotland, England & Wales (Tyson *et al*. 2007, R. Smith, FRS., pers.comm.). While these farms produce for both markets, they tend to be predominantly either table or restocking farms, with a small percentage of fish produced for the other market.

3.5 Discussion

3.5.1 Validity of survey

The telephone survey for this study included 40% of all trout farmers in the UK who accounted for 83% of UK rainbow trout production in 2005. This accounts for a significant percentage of the UK trout industry and we are confident that the results of this study are representative of the industry. The farmers who participated were visited in the second stage of the study accounted for 21% of the number of farmers and 61% of UK production in 2005: although this is a low percentage of the total number of farmers, it still amounts to a significant percentage of total production. It appears that the results in the second stage are biased towards table farmers, however

3.5.2 Water Quality

Water quality was consistently mentioned by stakeholders to the UK trout industry as being fundamental to the welfare of fish (North *et al*. 2008). The water quality parameters that were mentioned as being of most importance were dissolved oxygen, temperature, suspended solids, ammonia and the flow of water. The measurement of suspended solids and ammonia require specialist equipment that would not be expected to be found on a trout farm, however, DO meters with thermometers are readily available and can be purchased for under £300. Fewer farmers reported measuring both DO and temperature than measured DO: this is perhaps surprising as most modern DO probes have the capacity to record temperature as well. However, the data are an indication that most table farmers have the capacity to measure and record DO and temperature. The proportion of table farmers measuring DO and temperature was greater for the farms visited than those contacted by telephone. The reason for this discrepancy is not known, although it is possible that farmers were more forthcoming about their operations in a face-to-face meeting rather than to a researcher over the telephone.

Restocking farms and those providing for both the table and restocking market again showed the same trend as for table only producers, with a greater proportion of farmers reporting that they measured DO and temperature during the farm visits. However, it appears that as a percentage, more table farms measure DO and temperature than restocking farms, while those farms that produce for both markets fall in between.

Water quality parameters such as ammonia, pH, water hardness and suspended solids are monitored by the EA and SEPA, and farmers may be able to utilise this information as an advisory tool. The measurements that the EA and SEPA take are applicable to the farm as a whole and are not specific to a particular unit on the farm; therefore their use as a resource-based measure is limited. Whether or not any of the data could be used in a welfare assessment scheme is open to debate.

Given the importance of water quality to the welfare of farmed fish (FAWC 1996, Wedemeyer 1996, North *et al*. 2006a) and the emphasis that stakeholders in the trout industry place on it (North *et al.* 2008), especially farmers (Read 2008), water quality will be an integral part of any on-farm welfare assessment scheme and will have to be measured and recorded by farmers and/or welfare inspectors. During a focus group, trout farmers agreed that if a farmer could provide records showing that key water quality parameters were maintained within specified limits, then they would be demonstrating that they were safeguarding welfare (North *et al*. 2008). The use to which a given category of information is put, will depend on the purpose and context of the assessment. For example, water quality records could be examined as part of a retrospective analysis of welfare or alternatively a range of water quality parameters could be measured for a point inspection of welfare by a welfare assessor.

The flow of water through a system was raised as important by the contributors to the focus groups (North *et al*. 2008). The flow of water determines the exchange rate in a system, removing waste metabolites and replenishing DO. Additionally, the flow of water can also affect the behaviour and distribution of fish in a system (Ross *et al*. 1995). However, aside from maintenance of good water quality, the effects of flow on the welfare of fish is poorly understood, for example if the water flow is low, yet DO is maintained, are the fish subject to poor welfare? It has been suggested that a moderate current speed provides exercise, improves physiological performance and growth, and reduces physical damage to the fins through behavioural changes (Jobling *et al*. 1993). There are also practical considerations with measuring water flow on-farm. Although using a flow meter to obtain the rate of water entering a system is straightforward, precise measurements of the volume of water entering the system are necessary if the flow rate is to be meaningful. Calculating the volume of water entering a system is not always a simple task on farms, as it will be affected by rainfall, rivers in spate or periods of drought. Additionally, calculating exchange rates on cage farms in freshwater lochs is time-consuming and impractical. Given that water quality is often a function of the flow rate, and that welfare effects of flow rate are not currently known, the measurement of water quality in a WAS would be sufficient for welfare purposes and the flow rate would probably not be necessary.

3.5.3 Farm Records

Farms records are important resource-based measures of welfare and can be used to provide traceability for the history of fish on a farm and demonstrate that certain welfare standards were adhered to (North *et al*. 2008). In addition to maintaining records of water quality measurements, mortality records, disease treatments and production data, such as biomass, stocking density, growth and FCR, were seen as potentially useful for assessing welfare.

Although maintenance of mortality records for trout farms are required by law (Registration of Fish Farming and Shellfish Farming Businesses Order 1985 (as amended)), only 80% of restocking farms and both table and restocking farms reported that they kept such records. Within a welfare assessment scheme, mortality records are useful for retrospective welfare analysis and may also be useful as an advisory management tool, as changes in ‘normal’ mortality levels may indicate a welfare problem that requires addressing.

Maintenance of records pertaining to disease treatments were seen by stakeholders as being important for welfare assessment, however, they opined that interpretation of such records need care (North *et al.* 2008). Treating fish for diseases could be an indication that the farmer was doing everything in his power to safeguard the health and welfare of his stock. Alternatively, a high incidence of disease on a farm and the subsequent treatment of disease could indicate that the farmer was failing in other areas of disease prevention, or that the farm was in an area with a high incidence of endemic disease. The use of prophylactic disease treatment would also need to be taken into account during any welfare assessment.

Recording and maintaining accurate production data, in addition to being good management practice and useful for retrospective welfare analysis, can also be used as an advisory tool for farm managers. Up-to-date knowledge of the biomass of fish in a unit provides the farmer with the current stocking density, growth and FCR, all of which can be a useful welfare tool. Stocking density is not a good predictor of welfare (North *et al*. 2006a), however, reduction in stocking density can be a useful tool to improve poor water quality, such as low DO or high ammonia. Maintenance of growth and FCR records could provide farmers with an early warning system that something is wrong with the fish. Additionally, for retrospective analysis, growth records could highlight if the fish have been ‘held back’ or ‘pushed on’, both of which are potential welfare issues.

3.5.4 Slaughter and Harvest Measures

Given the potential for poor welfare at the time of slaughter, it is likely that the slaughter method will be a part of any welfare assessment scheme. There were 5 methods of slaughter reported by farmers during the farm visits; percussive stun to the head destroying the brain, electric stunning, asphyxiation in air, asphyxiation in ice slurry and immersion in carbon dioxide saturated water. The most common method of slaughter recorded was electric stunning.

Asphyxiation, whether in air or ice, and the use of carbon dioxide were considered as unacceptable by the Farm Animal Welfare Council (FAWC 1996) and this opinion is echoed by Compassion in World Farming (Stevenson 2007). Since the FAWC report of 1996, much research has been conducted into electric stunning (Robb *et al*. 2002, Robb and Kestin 2002, Lines *et al*. 2003, Lines & Kestin, 2004, 2005.) and this method, along with a percussive stun to the head, are seen as acceptable and humane methods of slaughtering fish. Due to the small size at which table portion fish are harvested (350-400g), a percussive stun to the head is not an economical method of killing fish (Robb *et al*. 2002) and therefore for large scale table producers the only viable method is by electric stun. For the slaughter method to be a part of a welfare assessment scheme, it is foreseeable that the only acceptable slaughter methods will be by percussive or electric stun.

The stakeholders that participated in the welfare focus groups (North *et al*. 2008) raised the possibility of using post-harvest information to assist with welfare assessment. Feedback from processors was suggested as a source of objective and quantifiable data. It could provide information on deformities, fin damage, physical damage, cataracts, scale loss, size variability within a batch of fish and the presence of ectoparasites. The information that would be generated from processors could be used as part of a retrospective analysis of welfare and as an advisory tool for the farm management. However, the practicalities of obtaining information from processors may be restrictive. Although farms that have their fish processed at a large processing factory often receive reports on the number of fish rejected and carcass quality, extending the scope of information collected to include indicators of welfare may not be economical. The cooperation of processors would be necessary and would probably require an incentive to contribute time and effort to welfare assessment, unless their cooperation was part of a legislative requirement. Within the UK trout industry, many farms process their own fish. If post-harvest information was to be used in a welfare assessment scheme, it would be difficult to argue that fish processed by the farmer would be entirely objective. It remains to be seen if post-harvest measures could form part of a welfare assessment scheme.

3.6 Conclusions

The purpose of this study was to investigate the structure of the UK trout industry, to establish what information was being recorded on farms that could be useful to a WAS and to examine what could be done by the farmer for a WAS. The type of information required from the farmer and the method of welfare assessment will depend on the purpose of the assessment. For on-farm welfare assessment, the parameters that will form part of the assessment must be feasible, valid and repeatable (Spoolder *et al*. 2003). Additionally, the cooperation and enthusiasm of farmers is essential for the success of a WAS, and therefore the collection of information for such a scheme must not be overly burdensome or time-consuming (Mellor & Stafford 2001). As discussed by Turnbull and Kadri (2007), if participation of a WAS is competitively disadvantageous, then those farmers not members of the scheme will succeed and the original aims of the WAS will have been lost, therefore any scheme must act as an incentive to farmers to improve welfare. The aim of any farmed fish WAS has to be to maximise the welfare of farmed fish within a sustainable, economically viable industry.

While primarily resource-based only WAS' have been developed for some farmed animals (Amon *et al.* 2001, Bartussek 2001, Johnsen *et al*. 2001) including farmed Atlantic salmon (Anon 2007a), welfare assessment of farmed rainbow trout would require animal-based parameters in order to be meaningful. The effects on the welfare of farmed trout of many environmental conditions and husbandry practices are not sufficiently well understood to enable conclusions to be drawn about the welfare status of fish through resource-based parameters alone. An example of this is stocking density, where many studies have failed to establish the link between welfare in rainbow trout and stocking density (see Ellis *et al.* 2002, for a review); however Turnbull *et al*. (2005) did find an effect of stocking density on the welfare of farmed Atlantic salmon in sea cages in densities greater than 22 kg/m^3. Measuring animal-based parameters on a farm limits what can be practically recorded. For example, while fish behaviour could be a genuine indicator of welfare (Dawkins 2006, Turnbull & Kadri 2007), the use of behavioural indicators in on-farm welfare assessment is problematic. Aside from any confounding effects of the observer on the batch of fish being assessed, recording behaviour is time-consuming (Johnsen *et al*. 2001). Sørensen *et al*. (2001) state that successful welfare indicators should be measurable in a relatively simple and cost effective manner.

If the scheme has an element of retrospective welfare assessment, then information that will be required will be the water DO and temperature. Water flow is not a practical parameter to measure, however given the cost of DO and temperature probes, it is arguable that all farmers should have a DO/temperature probe and record the results, at least daily.

Codes of practice and certification standards have sections relevant to the health and welfare of fish, and therefore can assist welfare assessors as certain management and husbandry practices are detailed. Complying with codes of practice or certification standards does demonstrate a certain degree of good management practice and stockmanship, as does the provision of a Veterinary Health Plan. The aims of table farmers and restocking farmers are different and this needs to be taken into account when a WAS is being developed. It appears that fish are farmed less intensively for restocking than those farmed for the table, and therefore the level of monitoring may not need to be high as for table fish. For example, if restocking fish have demonstrably better welfare than table fish, then standards that are less onerous could be applied. For participation in a WAS, there must be minimum standards adhered to, and a VHP is good management practice for any farm.

3.7 Acknowledgements

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3.8 References

Amon, T., Amon, B., Ofner, E., Boxberger, J. 2001. Precision of assessment of animal welfare by the TGI 35L Austrian Animal Needs Index. *Acta Agriculturæ Scandinavica Section A-Animal Science*. **Suppl. 30**: 114-117.

Anon. 2002. *British Trout Association Code of Practice*. British Trout Association. pp11.

Anon. 2006a. *Quality Trout UK Farm & Hatchery Certification Standards*. Quality Trout UK. pp25.

Anon. 2006b. *Scottish Fish Farms Annual Production Survey, 2005*. Fisheries Research Services, UK. (www.marlab.ac.uk).

Anon. 2007a. *RSPCA Welfare standards for farmed Atlantic salmon*. RSPCA, Horsham, UK. pp67.

Anon. 2007b. Summary of UK rainbow trout production in 2005. *Finfish News 3*, Winter/Spring 2007. p60.

Bartussek, H. 2001. An historical account of the animal needs index ANI-35L as part of the attempt to promote and regulate farm animal welfare in Austria: an example of the interaction between animal welfare science and society. *Acta Agriculturæ Scandinavica Section A-Animal Science*. **Suppl. 30**: 34-41.

Bracke, M.B.M., Metz, J.H.M., Spruijt, B.M. Development of a Decision Support System to assess farm animal welfare. *Acta Agriculturæ Scandinavica Section A-Animal Science*. **Suppl. 30**:17-20.

Dawkins, M.S. 2006. A user's guide to animal welfare science. *Trends in Ecology & Evolution*. **21(2)**: 77-82.

Ekstrand, C., Algers, B., Svedberg, J. 1997. Rearing conditions and foot-pad dermatitis in Swedish broiler chickens. *Preventative Veterinary Medicine*. **31**: 167-174.

Ellis, T.E., North, B.P., Scott, A.P., Bromage, N.R., Porter, M., Gadd, D. 2002. The relationships between stocking density and welfare in farmed rainbow trout. *Journal of Fish Biology*. **61**: 493-531.

Ellis, T., Oidtmann, B., St-Hilaire, S., Turnbull, J.F., North, B.P., MacIntyre, C.M., Nikolaidis, J., Hoyle, I., Kestin, S.C., Knowles, T.G. 2008. *Fin erosion in farmed fish*. In: Branson, E.J. (Ed). Fish Welfare. Blackwell Publishing, Oxford. pp 121-149.

FAWC. 1996. *Report on the welfare of farmed fish*. Farm Animal Welfare Council, Surbiton, Surrey.

Hoyle, I., Oidtmann, B., Ellis, T., Turnbull, J., North, B., Nikolaidis, J., Knowles, T.G. 2007. A validated macroscopic key to assess fin damage in farmed rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. **270**: 142-148.

Jobling, M., Baardvik, B.M., Christiansen, J.S., Jørgensen, E.H. 1993. The effects of prolonged exercise training on growth parameters and production parameters in fish. *Aquaculture International*. **1**: 95-111.

Johnsen, P.F., Johannesson, T., Sandøe, P. 2001. Assessment of farm animal welfare at herd level: Many goals, many methods. *Acta Agriculturæ Scandinavica Section A-Animal Science*. **Suppl. 30**: 26-33.

Lines, J.A., Robb, D.H., Kestin, S.C., Crook, S.C., Benson, T. 2003. Electric stunning: a humane slaughter method for trout. *Aquacultural Engineering*. **28(3-4)**:141-154.

Lines, J., Kestin, S. 2004. Electrical stunning of fish: the relationship between the electric field strength and water conductivity. *Aquaculture*. **241**: 219-234.

Lines, J., Kestin, S. 2005. Electric stunning of trout: power reduction using a two-stage stun. *Aquacultural Engineering*. **32**: 483-491.

MacIntyre, C.M., Ellis, T., North, B.P., Turnbull, J.F. 2008. *The influences of water quality on the welfare of farmed rainbow trout: a review*. In: Branson, E.J. (ed) Fish welfare. Blackwell Publishing, Oxford. pp150-184.

Main, D.C.J., Kent, J.P., Wemelsfelder, F., Ofner, E., Tuyttens, F.A.M. 2003. Applications for methods of on-farm welfare assessment. *Animal Welfare*. **12**: 523-528.

Mellor, D.J., Stafford, K.J. 2001. Integrating practical, regulatory and ethical strategies for enhancing farm animal welfare. *Australian Veterinary Journal*. **79**: 762-768.

North, B.O., Turnbull, J.F., Ellis, T, Porter, M., Migaud, H., Bron, J., Bromage, N.R. 2006a. The impact of stocking density on the welfare of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. **255**: 466-479.

North, B.P., Ellis, T., Turnbull, J.F., Davis, J., Bromage, N.R. 2006b. Stocking density practices of commercial UK rainbow trout farms. *Aquaculture*. **259**: 260-267.

North, B.P., Ellis, T., Knowles, T., Bron, J.E., Turnbull, J.F. 2008. *The use of stakeholder focus groups to identify indicators for the on-farm assessment of trout welfare*. In: Branson, E.J. (ed) Fish welfare. Blackwell Publishing, Oxford. pp243-267.

Read, N. 2008*. Fish farmer's perspective of welfare*. In: Branson, E.J. (Ed). Fish Welfare. Blackwell Publishing, Oxford. pp 101-110.

Robb, D.H.F., O'Callaghan, M., Lines, J.A., Kestin, S.C. 2002. Electrical stunning of rainbow trout (*Oncorhynchus mykiss*): factors that affect stun duration. *Aquaculture*. **205(3-4)**:359-371.

Robb, D.H.F, Kestin, S.C. 2002. Methods used to kill fish: field observations and literature reviewed. *Animal Welfare*. **11**: 269-282.

Ross, R.M., Watten, B.J., Krise, W.F., DiLauro, M.N., Soderberg, R.W. 1995. Influence of tank design and hydraulic loading on the behavior, growth, and metabolism of rainbow trout (*Oncorhynchus mykiss*). *Aquacultural Engineering*. **14(1)**: 29-47.

Smulders, D., Verbeke G, Mormede P, Geers R. 2006. Validation of a behavioral observation tool to assess pig welfare. *Physiology & Behaviour*. **89(3)**: 438-447.

Sørensen, J.T., Sandøe, P., Halberg, N. 2001. Animal welfare as one among several values to be considered at a farm level: the idea of an ethical account for livestock farming. *Acta Agriculturæ Scandinavica Section A-Animal Science*. **Suppl. 30**: 11-16.

Spoolder, H., De Rosa, G., Hörning, B., Waiblinger, S., Wemelsfelder, F. 2003. Integrating parameters to assess on-farm welfare. *Animal Welfare*. **12**: 529-534.

Stevenson, P. 2007. *Closed waters: the welfare of atlantic salmon, rainbow trout, atlantic cod and atlantic halibut*. Compassion in World Farming, Surrey, UK. pp79.

Thrusfield, M. 1995. *Veteriary Epidemiology* 2nd Ed. Blackwell Science. pp 483.

Turnbull, J.F., Bell, A., Adams, C., Bron, J., Huntingford, F.A. 2005. Stocking density and welfare of cage farmed Atlantic salmon: application of a multivariate analysis. *Aquaculture*. **243**: 121-132.

Turnbull, J.F., Kadri, S. 2007. Safeguarding the many guises of farmed fish welfare. *Diseases of Aquatic Organisms*. **75**: 173-182.

Tyson, P, Dunn P, Mewett, J, Crane, C. 2007. 2005 Survey of trout production in England and Wales. *Finfish News 3, Winter/Spring 2007*. p53-56.

Wedemeyer, G.A. 1996. *Physiology of fish in intensive culture systems*. Chapman & Hall. pp 232.

Whay, H.R., Main, D.C.J., Green, L.E., Webster, A.J.F. 2003. An animal-based welfare assessment of group-housed calves on UK dairy farms. *Animal Welfare.* **12**: 611-617.

4 An epidemiological study of welfare in farmed rainbow trout: current status of fish and water quality.

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4.1 Abstract

The welfare of farmed fish is receiving much attention, however there is little published information available on the current state of fish welfare or environmental conditions on UK rainbow trout farms. This study examined the welfare of rainbow trout (*Oncorhynchus mykiss* Walbaum) on commercial farms. Forty-four trout farms from throughout the British Isles were visited between July 2005 and April 2007, sampling a total of 3699 fish from 189 different systems. Farms were visited twice, once in winter and once in summer, to account for any seasonal differences in fish physiology and environmental conditions. Data were collected on a range of morphological and physiological parameters, together with background information on the batch which the fish came from. Particular emphasis was placed on water quality due to the known effects this has on welfare. For each system sampled from, water was monitored for 24 hours, with measurements of dissolved oxygen, temperature, pH, specific conductivity and ammonia taken every 15 minutes.

Over 95% of fish sampled had plasma cortisol concentrations less than that commonly regarded as being acutely stressed (40ng/mL), and very few fish had major gill pathologies. Fin condition was generally good throughout the study, with restocking fish having significantly better fins than those farmed for the table market. Water quality was generally good, although at the time of sampling, 14% of systems sampled from had dissolved oxygen concentrations less than the industry-

recommended minimum of 6mg/L. Unionised ammonia concentrations were low throughout the study, with the maximum concentration at any time being less than a recommended concentration of 0.02mg/L. It was concluded poor welfare on UK trout farms were unlikely to be linked to poor water quality.

4.2 Introduction

While much has been written on the welfare of farmed rainbow trout (*Oncorhynchus mykiss* Walbaum) (*e.g.* Ellis *et al.* 2002, North *et al.* 2006a, b, St-Hilaire *et al.* 2006, Stevenson 2007, MacIntyre *et al.* 2008), there is limited information available on the current welfare status of farmed rainbow trout in the UK, and under what conditions the fish are being reared (St-Hilaire *et al.* 2006). It is well appreciated that poor water quality can have a detrimental effect on the welfare of farmed rainbow trout (Wedemeyer 1996, North *et al.* 2006a, MacIntyre *et al.* 2008). Inappropriate levels of water quality parameters affect physiology, growth rate and efficiency, cause pathological changes and organ damage and, in severe cases, cause mortality (Wedemeyer 1996). The sub-lethal effects of poor water quality are also commonly linked to increased disease susceptibility (Wedemeyer 1996), although scientific evidence for direct relationships is lacking (MacIntyre *et al.* 2008). At present, there is insufficient information to conclude if poor water quality has an adverse effect on the welfare of UK farmed trout. Often contradictory recommendations exist for limits for many water quality parameters (MacIntyre *et al.* 2008), however for arguably the most important parameter, dissolved oxygen, a minimum of 6mg/L is generally accepted within the industry (Wedemeyer 1996, Anon. 2002). For unionised ammonia, a toxic metabolite generally produced from fish waste within the aquaculture system (Evans *et al.* 2005), recommended maximum concentrations vary by 2 orders of magnitude, from 0.002 mg/L to 0.36 mg/L NH_3 (see MacIntyre *et al.* 2008), however the figure quoted by Wedemeyer (1996) of 0.02 mg/L is frequently accepted as being a reasonable maximum concentration to maintain fish welfare.

This study was part of a larger project funded by Defra (AW1205) in association with the British Trout Association, with the aim of examining the relationship between water quality and the welfare of farmed rainbow trout. A previous study as part of the same project (Chapter 3) contacted UK trout farmers to determine the level of water quality monitoring, with the majority of trout farmers monitoring dissolved oxygen (DO) and temperature. Data were available to the farmers on ammonia levels and suspended solids from the Environment Agency/SEPA branch responsible for that region, however the frequency of reports from these agencies were variable between regions. The purpose of this study was to take an epidemiological approach to welfare to determine the relationships between welfare and water quality and describe risk factors for farmed trout welfare. Epidemiological studies have been suggested as a suitable strategy for investigating animal welfare (Dawkins 2006) and have been used previously for fish welfare (Juell & Fosseidengen 2004, Turnbull *et al.* 2005). In a study of this type, where a large number of data parameters are collected, there is the potential to conduct many types of analyses on the data. This paper describes the condition of rainbow trout farmed in the UK, as assessed through morphological and physiological parameters, and water quality conditions on farms. Chapter 5 describes the development of a welfare score and subsequent multi-level analysis of the data to investigate risk factors for the welfare of farmed rainbow trout.

4.3 Materials and Methods

A total of 44 farms were visited for this study, sampling for which commenced in July 2005 and ended in April 2007. Participating farms for the study were randomly selected from a list of trout farms prepared for a previous study (see Chapter 3). To account for seasonal variability in welfare and farm conditions, farms were visited twice, once in summer and once in winter. A previous study by North *et al.* (2006b) found that farmers will stock at greater densities during winter months. Of the 44 farms that participated in the study, 39 were visited twice: due to adverse weather conditions, the 2006 Viral Haemorrhagic Septicaemia (VHS) outbreak and other circumstances outwith our control, 5 farms were only visited once.

Up to 4 different systems were selected during each visit and both small (<100g) and large (>100g) fish were sampled. Either 12 or 24 fish were sampled from each system, with up to 4 systems and a maximum of 48 fish sampled per visit. In total, 3699 fish were sampled from 189 systems. The variability in the number of systems sampled from was due to availability of suitable fish on the farm.

4.3.1 Fish Measures

Fish were netted from the systems by farm staff, put immediately into water with a 1:5000 concentration of 2-phenoxy ethanol (Sigma, Dorset, UK) and killed with a percussive stun to the head. Immediately following death, a blood sample was taken from the caudal vena cava using either 1ml or 2ml syringes, depending on the size of the fish, and a heparinised 23 gauge hypodermic needle. Blood samples were stored in eppendorf tubes on crushed ice prior to processing on-site.

4.3.1.1 Haematocrit

Approximately 100µl of blood was put into a non-heparinised capillary tube and spun for 3 mins at a relative centrifugal force (RCF) of 14,000g. Haematocrit was calculated by measuring the length of red blood cells (RBCs) in the capillary tube to the total blood length using a ruler: haematocrit was expressed as the percentage of RBC to total blood (% packed red cell volume). Blood was processed for haematocrit values immediately following extraction from 12 fish, with time from first extraction to centrifugation was <10 min.

4.3.1.2 Plasma cortisol

The remaining blood was centrifuged on site for 12 min. at a RCF of 600*g* in a Labnet Microcentrifuge (Denver Instrument Company, USA). The plasma was then removed and stored in cryovials. If travel to the Institute of Aquaculture was possible on the day of sampling, samples were stored on crushed ice and transferred to -70°C upon arrival at the Institute. Otherwise, samples were stored in liquid nitrogen for up to 3 days before transfer to -70°C. Concentrations of plasma cortisol were determined by radioimmunoassay using the method described in Ellis *et al.* (2004) as adapted by North *et al.* (2006a). Concentrations were calculated to ng/mL.

4.3.1.3 Condition Factor

The fork length and weight of each fish was measured and used to calculate the condition factor as follows:

Condition Factor (K) = [weight(g) x 100]/ fork length(cm)^3

4.3.1.4 Hepatosomatic Index

The liver was removed from each fish, with the gall bladder separated from the liver before weighing (±0.01g) using Fisherbrand SG202 Microscales (Fisher Scientific, UK). The hepatosomatic index (HSI) was calculated as follows:

HSI = (Liver weight / total body weight) x 100

4.3.1.5 Splenosomatic Index

The spleen was removed and weighed (±0.01g). The splenosomatic index (SSI) was calculated as follows:

SSI = (Spleen weight / total body weight) x 100

In some cases, more than one spleen was observed in the fish, not uncommonly (Noga 2006), in which case both were added together as the spleen weight.

4.3.1.6 Gill condition

The second anterior gill arch of the left gill was removed and stored in 10% neutrally buffered formalin. The gills were dehydrated, embedded in paraffin wax, sectioned at 5μm and stained with haematoxylin and eosin (H&E). The sections were viewed under a light microscope (Olympus Optical Co (UK) Ltd, UK).

Gill samples were collected from every fish in this study, a total of 3699. This was not a realistic number of gills to analyse, given the time required to prepare histology slides, and furthermore, there are many pathologies that can be affect the gills (Sanchez *et al.* 1997, Speare & Ferguson 1989). Therefore a practical solution to the problem was required.

Mettam (2005) investigated gill pathologies in rainbow trout primarily relating to poor water quality. As part of this investigation, 3 primary lamellae (1° lamellae) on the second gill arch left side were examined and the following gill pathologies were recorded: 1) total number of lamellae; 2) number of lamellae with epithelial lifting; 3) number of lamellae with hyperplasia; 4) number of fused lamellae; 5) number of inflamed lamellae; 6) number of inflamed interlamellar spaces; 7) number of clavate lamellae (clubbing); 8) number of lamellae with thrombi; 9) number of lamellae with epithelial separation; 10) number of mucous cells; 11) number of chloride cells; 12) presence and number of parasites; 13) number of lamellae with epithelial hypertrophy; 14) number of necrotic epithelial cells; 15) number of cells with congestion. For each gill sample, 3 primary lamellae (1° lamellae) on the second gill arch left side were examined.

The outcome of the above investigation demonstrated that three pathologies were potentially useful as quantitative welfare indicators related to deteriorating water quality: 1) epithelial separation; 2) epithelial hyperplasia and 3) lamellar fusion. However, epithelial separation can be an artefact of the euthanasia method employed in this epidemiology study (anaesthesia and concussion) (Mettam 2005). It was therefore decided that only epithelial hyperplasia and lamellar fusion would be recorded.

In addition to epithelial hyperplasia and lamellar fusion, telangiectasia were also recorded as a possible pathology associated with poor water quality. While telangiectasis is commonly found in lamellae from fish killed by a percussive stun to the head (Mettam 2005), in some cases it may be as a result of poor water quality (J. Turnbull, pers. comm.) and can be identified by the level of healing, which would not be present if telangiectasis formed peri-mortem. From each gill that was processed, 3 primary lamellae were examined and pathologies recorded.

The gills of all fish sampled from 4 farms were analysed for epithelial hyperplasia, lamellar fusion and pre-mortem telangiectasia. Little variation was recorded in the types or severity of the pathologies observed where they were present in a batch of fish. Following this, the gills of 6 fish per batch from 30 farms were analysed with the same result; little variation in type or severity of pathology. For the final 10 farms, the gills from 3 fish per batch were analysed with the same result. The method of assessment for the gills was simply to apply a dichotomous variable, 'not affected or slightly affected' and 'moderately to severely affected'. Very mild hyperplasia with no lamellar fusion was assessed as 'slightly affected', while moderate, diffuse hyperplasia with any fusion was assessed as 'moderately to severely affected'. This approach is similar to that taken by Fivelstad *et al.* (2003) and Lang *et al.* (1987).

4.3.1.7 Fin Condition

All fins were assessed using a ranking scale from a modified photographic key developed by Hoyle *et al.* (2007). Fins were given a score from 1 to 5 based on the degree of splitting, erosion, thickening, kinking and presence of blood, with a score of 1 representing little if any damage and 5 representing virtually total loss of the fin. Separate keys for small (<50g) and large (>50g) were used, (figures 4.1 and 4.2). Although Hoyle *et al.* (2007) used a score of '0' to indicate undamaged fins, In this study, scores of 0 were not used for larger fish, since, as indicated by Hoyle *et al.* (2007), this score was intended as a reference point applicable to wild fish only. The total fin score for each fish is the sum of each individual fin score for the 7 fins assessed (dorsal, caudal, anal, left and right pelvic, left and right pectoral).

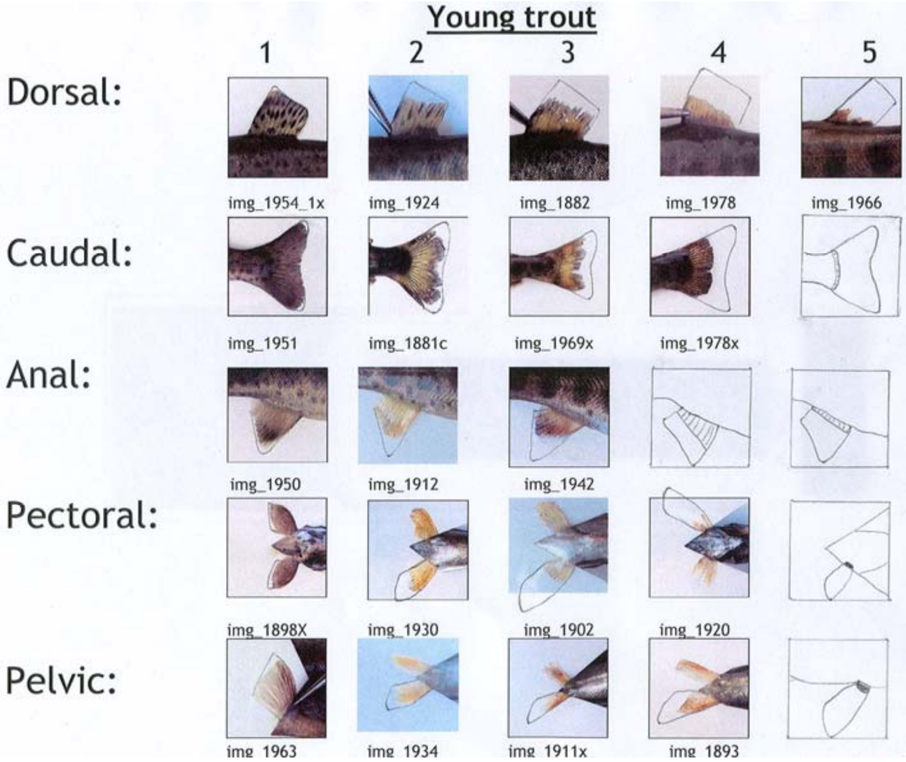

Figure 4.1 Fin photographic identification key used for small trout (<50g). From Hoyle *et al.* 2007.

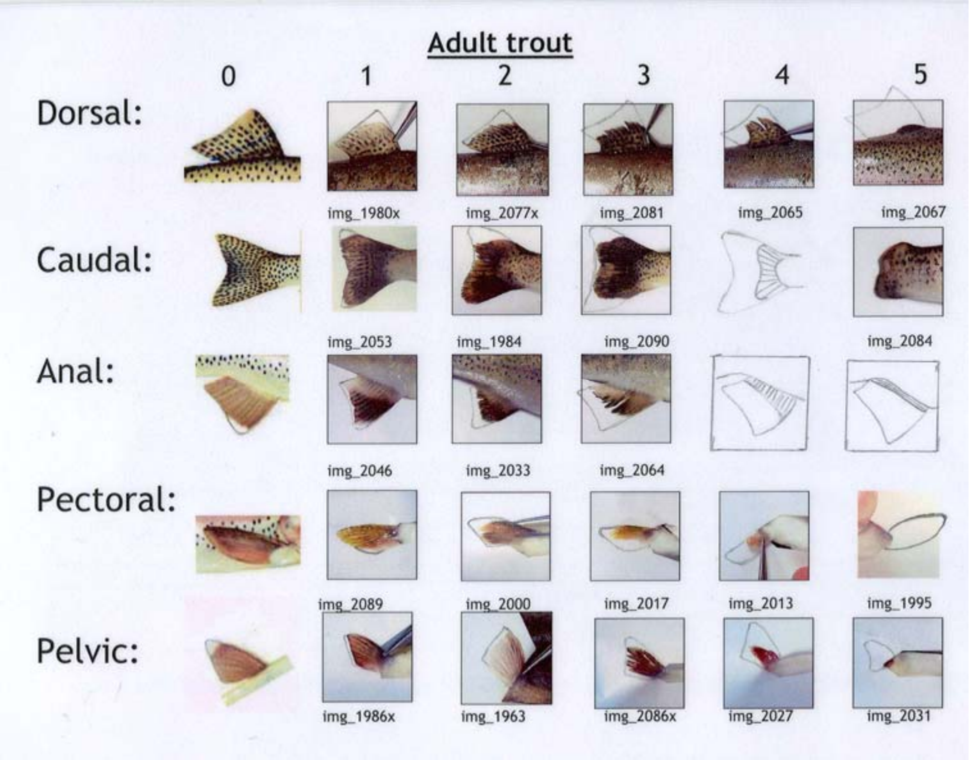

Figure 4.2 Fin photographic identification key used for large trout (>50g). From Hoyle *et al.* 2007.

4.3.2 Water Quality

Water hardness, alkalinity and nitrite were measured using a Palintest 5000 (Palintest, Gateshead, UK), a multiparameter photometer that measures colour changes in test water following addition of a reagent. Detection limits for hardness and alkalinity were 0-500 mg/L as $CaCO_3$ at 570nm and 0-0.5 mg/L NO_2-N (0-1.6 mg/L NO_2) at 520nm.

Dissolved oxygen (DO) (mg/l), temperature (°C), pH, specific conductivity (mS/cm, formerly mho/cm) and ammonium (NH_4+-N) (mg/l) were measured using a YSI 6600 multi-parameter sonde (YSI Hydrodata Ltd, Herts, UK). A sonde was placed in the outflow of each system sampled, and measurements taken every 15 minutes..

Unionised ammonia (NH_3 mg/L) was calculated from ammonium (mg/L NH_4+-N) using the temperature and pH measurements and the following equations as described by Wedemeyer (1996):-

$\%NH_3\text{-}N = 100/(1 + \text{antilog}(pKa - pH))$

Where $pKa = 10.055 - 0.0325(\text{Temp °C})$

Total ammonia is the sum of the concentrations of un-ionised ammonia and ionised ammonium.

$\text{Total Ammonia} = [NH_3] + [NH_4^+]$

The percentage of ionised ammonium (%NH_4+-N) is 100 - %NH_3-N. Using this percentage and the measurement of NH_4-N recorded by the YSI sonde, the concentration of total ammonia nitrogen (TAN) was established. The concentration of NH_3-N was calculated by applying the known percentage of NH_3-N to the concentration of TAN. To establish the concentration of NH_3 (mg/L), a correction factor of 1.22 was applied to the concentration of NH_3-N, which corrects for the molecular weight of hydrogen.

In order to describe the variability of the data collected by the sondes, the following parameters were calculated for each measurement recorded over 24 hours: mean, standard deviation, minimum, maximum, range, average change per 15 minutes over sample period, maximum change per 15 minutes recorded over sample period.

Carbon dioxide concentrations were calculated using the following equation, adapted from Anon. (1986), where T = temperature (°C):

$$[CO_2] \text{ in mg/L} = \exp(\log \text{Alkalinity} + 3404.71/(273+T) + 0.032786T - \text{pH} - 5.93994)$$

4.3.3 Rearing System Information

The type of holding unit was recorded (cage, pond, raceway, tank), as was the construction material of the unit, the volume (m^3) and whether the water was supplemented by aeration or oxygenation.

4.3.4 Farm Records

Through discussion with the farmer and examination of available farm records, the following details were collected for each batch of fish that was sampled:

Biomass (kg), number of fish in the unit, growth rate for the month prior to sampling, current food conversion ratio (FCR), mortalities for the month prior to sampling, whether the fish were diploid or triploid, whether fish were farmed for table market or restocking, the disease history, feeding method and frequency of feeding, the hatchery of origin and source of eggs, the hatch date, the number of days the fish had been on the farm and the number of days since the fish had been moved.

4.3.5 Statistical Analysis

The data were described and preliminary analysis was conducted with Minitab V.13 (Minitab Inc., USA). Due to postiviely skewed distribution, natural log transformations were conducted on the data for cortisol, HSI and SSI. The data for percentage mortalities per month were transformed by log arcsine transformation. Anova was used on condition factor, cortisol, SSI, haematocrit and mortalities. Differences between system type for the ordered categorical fin score were analysed using the Kruskal-Wallis test, and differences between individual fin scores were analysed using Mann-Whitney U-test. Subsequent multilevel modelling was conducted with MlwiN version 2.02 (Multilevel Models Project, UK): multilevel modelling analysis is described in Chapter 5.

4.4 Results

4.4.1 Morphological fish measurements

Fish sampled for this study ranged from 17g to 1495g. The condition factor, K, ranged from a low of 0.84 to a maximum of 2.2, with a mean ±SD of 1.31 ±0.15. Differences were found between fish farmed for restocking compared with fish farmed for the table market, with restocking fish having significantly lower K with a mean ±SD of 1.29 ±0.14 compared with table fish 1.35 ±0.15 ($F=126.68$, $p<0.001$) (figure 4.3). K was significantly influenced by the type of system the fish were in (figure 4.4), with significantly greater condition factors found in cages compared with ponds, raceways and tanks ($F=122.87$, $p<0.001$). A significant size effect was observed for K ($p<0.001$), however the effect was small, with an r^2 of 0.086 and regression equation of $K = 1.21 + 0.000267x$ Weight(grams), suggesting that any size effect on K was minimal.

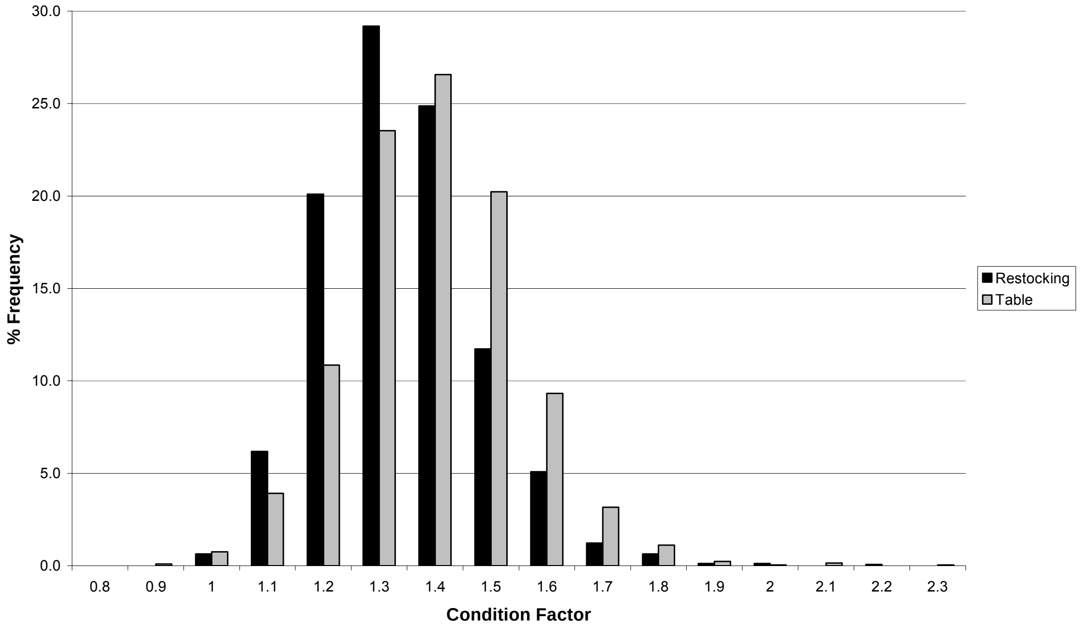

Figure 4.3 Frequency distribution of condition factors for all rainbow trout sampled in study (n=3699), divided by fish farmed for restocking (n=1553) and fish farmed for table market (n=2146).

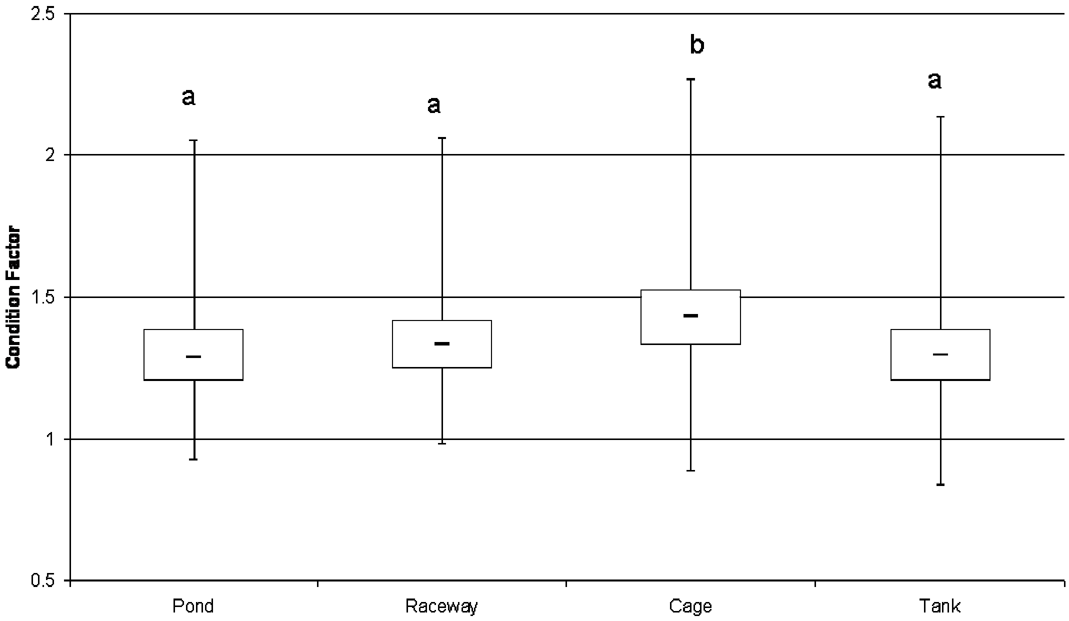

Figure 4.4 Condition factor scores for fish by system type (n=3699). Different letters denote significant differences ($p<0.05$). Pond systems 1.29 ±0.14 (mean ±SD), raceways 1.33 ±0.14, cages 1.43 ±0.15, tanks 1.30 ±0.15.

The condition of the fins, as measured using the aggregate fin score, differed by system type. The lowest fin scores, and thus the best fins, were found from fish from pond systems, with a median of 11 ($p<0.001$), with the highest scores, and thus worst fins found from fish from raceways with a median of 14 ($p<0.001$), with fins from fish from cages and tanks statistically undistinguishable ($p=0.581$) with a median of 12.

The fin condition of fish was also associated with the purpose of farming: fins from fish farmed for restocking had significantly better fin condition scores than fish farmed for the table market ($p<0.001$) (figure 4.5). Fins with the best condition scores were consistently the anal and pelvic fins, and the most damaged fin observed consistently throughout this study was the dorsal fin (Mann Whitney U-test, $P<0.001$), with a median (Q1, Q3) of 2 (2, 3) (figure 4.6). Median (Q1, Q3) for the remaining fins were: caudal fin 2 (1, 2), anal fin 1 (1, 2), left and right pectoral 2 (1, 2), left and right pelvic 1 (1,2).

The overall plasma cortisol concentration was 8.29 ±13.36 ng/mL (mean ±S.D, $n=3311$), with differences noted between system type. Fish from cage systems had the highest cortisol concentrations ($F=54.74$, $p<0.001$) of 15.4 ±19.62 ng/mL (mean ±SD), compared with the lowest concentration found in raceways of 5.89 ± 7.81 ng/mL (figure 4.7). The mean ±SD for cortisol concentrations from ponds and tanks were 7.51 ±12.34 and 8.25 ±14.31 ng/mL respectively. Fish with cortisol concentrations less than 10ng/mL accounted for 78% of all samples, while concentrations less than 20ng/mL accounted for 90% of all samples. Cortisol concentrations greater than 40ng/mL were found in 3.6% of instances, and the maximum cortisol concentration recorded was 109.22ng/mL

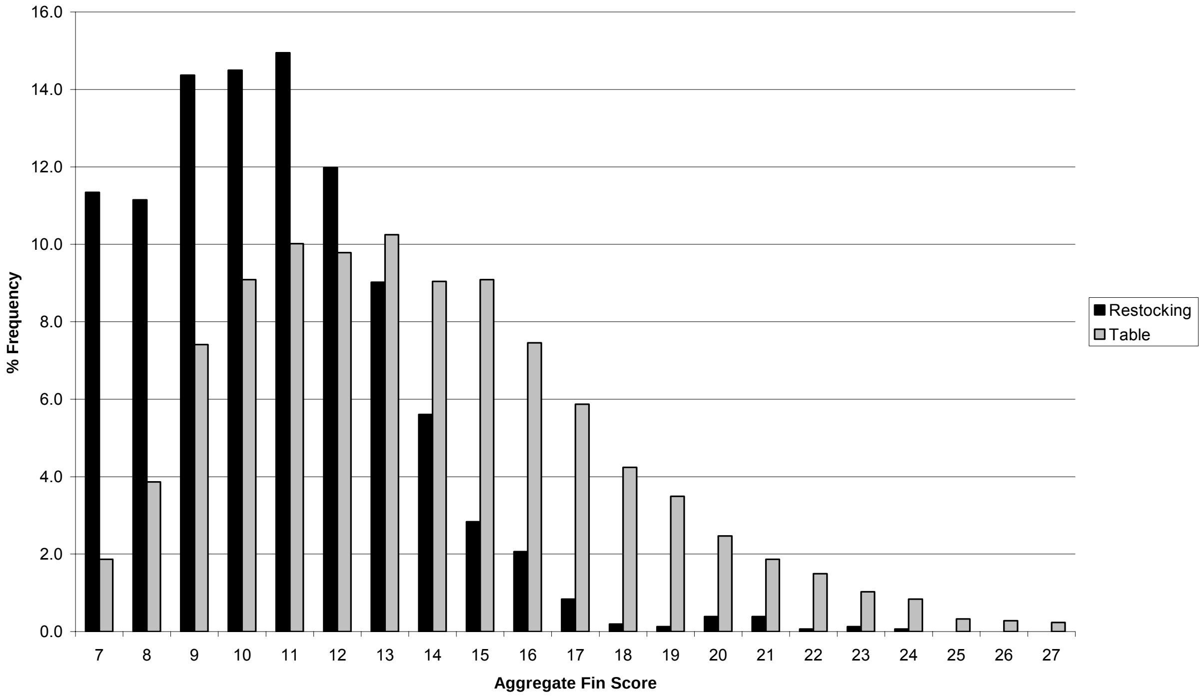

Figure 4.5 Percentage frequencies for fin condition scores by farming purpose (restocking fish n=1553, table market fish n=2146). Aggregate fin scores are the sum of the fin condition scores given to individual fins (dorsal, caudal, anal, left and right pectoral and left and right pelvic).

Haematocrit values were 37.02 ±7.05% (mean ±SD, n=3623), which is in agreement with the reported natural range for healthy fish for haematocrit of 24-43% (Wedemeyer, 1996). Haematocrit was not affected by the size of fish (p=0.623), however differences were noted between system types, with the lowest values found in fish from tank systems (35.8 ±7.6 mean ±SD) significantly different (p<0.001) to values found in ponds (36.9 ±6.9), raceways (39.6 ±7.0) and cages (37.9 ±7.0).

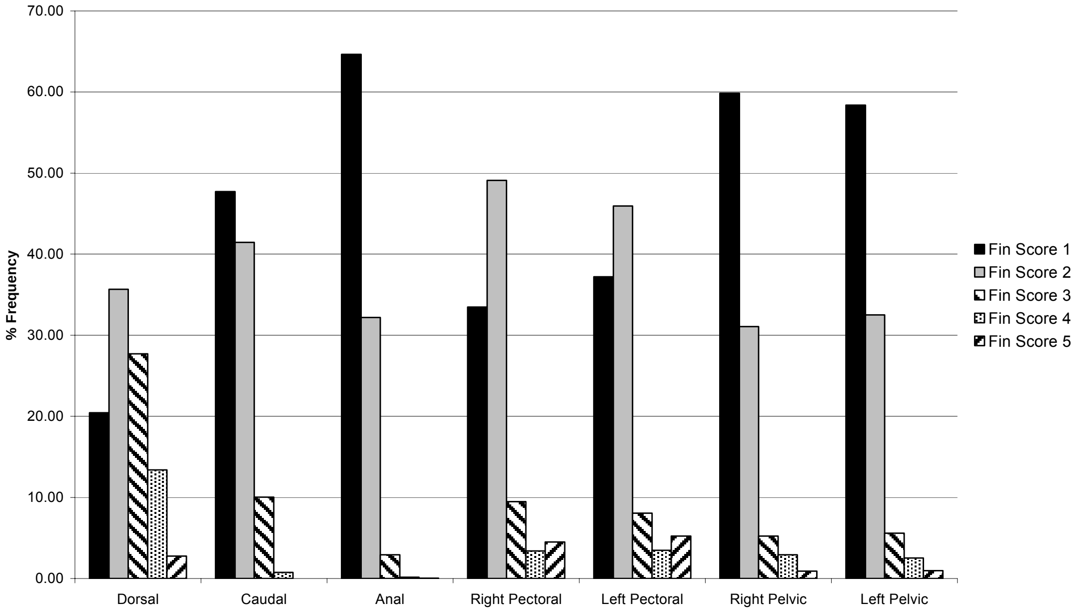

Figure 4.6 Percentage frequencies of fin condition scores by fin type (n=3699). A fin condition score of 1 represents no or very little damage, while a score of 5 represents almost complete loss of fin (see figures 4.2 and 4.3 for explanation of scores).

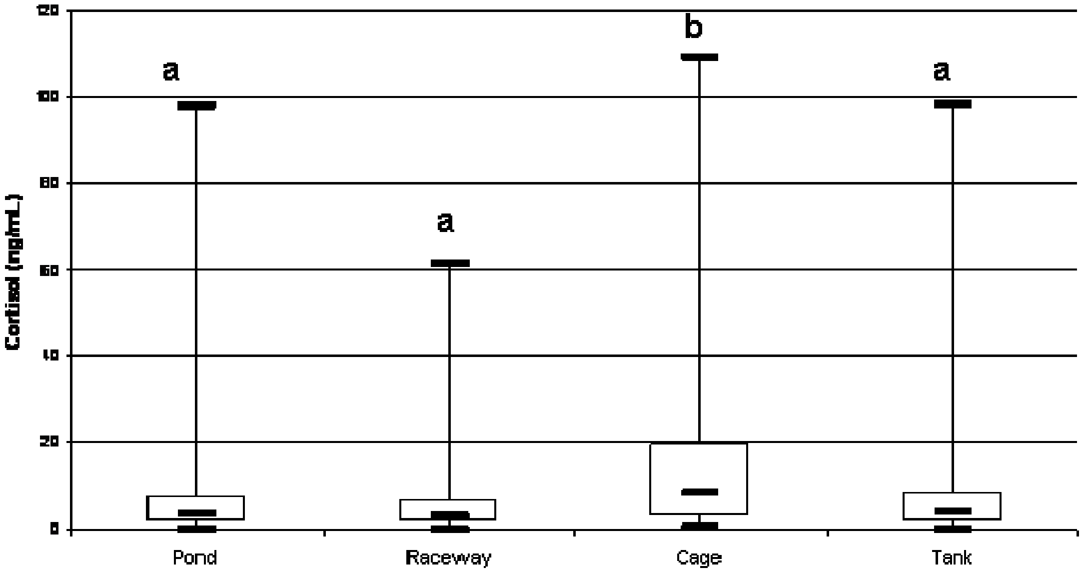

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Figure 4.7 Cortisol concentrations (mean ±S.D.) by system type. Letters denote significant differences (p<0.05).

The splenosomatic index (SSI) showed variation between system type. Fish from raceways had significantly larger spleens than fish from other system types

($p<0.001$), with and SSI of 0.30 ±0.48 (mean ±SD). No significant differences were noted among ponds, cages or tanks for SSI, with SSI scores of 0.18 ±0.13, 0.17 ±0.08 and 0.19 ±0.1 respectively. The mean ±SD for the hepatosomatic index was 1.26 ±0.31.

4.4.2 Water Quality

A minimum recommended safe DO limit of 6mg/L is generally accepted within the UK trout industry (Wedemeyer 1996, Anon. 2002); 165 systems (85.7%) had mean DO values in excess of 6mg/L, while 175 systems (92.1%) had mean DO values in excess of 5mg/L (Fig. 4.8). Of the 24 systems with DO concentrations <6 mg/L, 3 (1.6%) used aeration, while 12 (6.3%) used oxygenation.

The range of mean temperatures was 2.47°C and 18.91°C, with the lowest and highest temperatures recorded throughout the study being 1.47°C and 20.70°C (figure 4.8).

The maximum mean UIA value recorded over a 24 hour period was 0.0097mg/L (figure 4.9), while the maximum UIA concentration recorded throughout the entire study was 0.016mg/L UIA. Carbon dioxide measurements ranged from 0.09 to 59.2 mg/L, with a mean ±SD of 8.6 ±9.2 mg/L (figure 4.10).

Alkalinity and hardness were both measured as mg/L as $CaCO_3$, and were highly correlated (Pearson correlation 0.873, $p<0.001$). The lowest values recorded for these parameters were below the detectable limit for the Palintest photometer used for this study, and these values were expressed as 0. The water hardness for farms sampled from ranged from 0 to 290 mg/L, while alkalinity ranged from 0 to 305 mg/L.

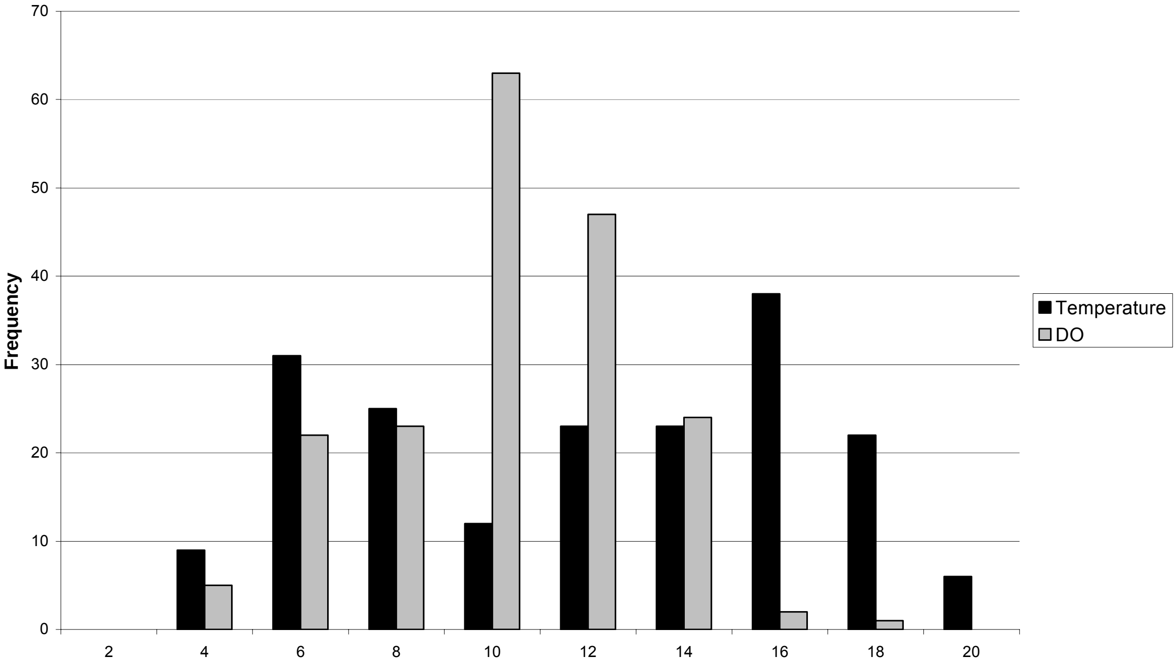

Figure 4.8 Frequencies of 24 hour mean DO (mg/L) and temperature (°C) values for all 189 systems sampled.

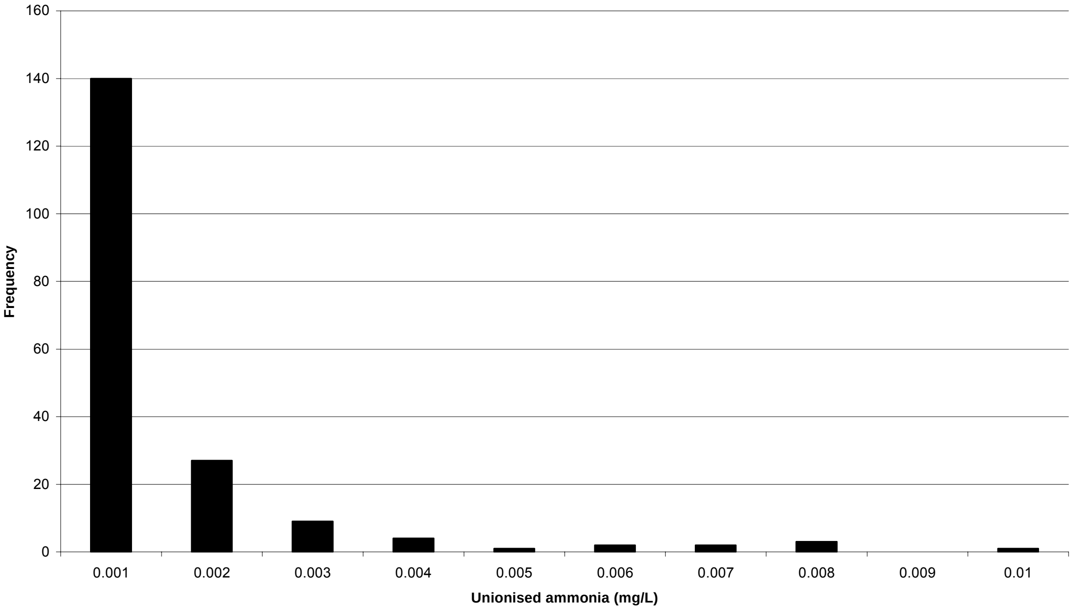

Figure 4.9 Frequency of 24 hour mean UIA (mg/L) values for 189 systems. Mean ±SD 0.001 ±0.001 mg/L, maximum mean concentration over 24 hour period sampled was 0.0097mg/L..

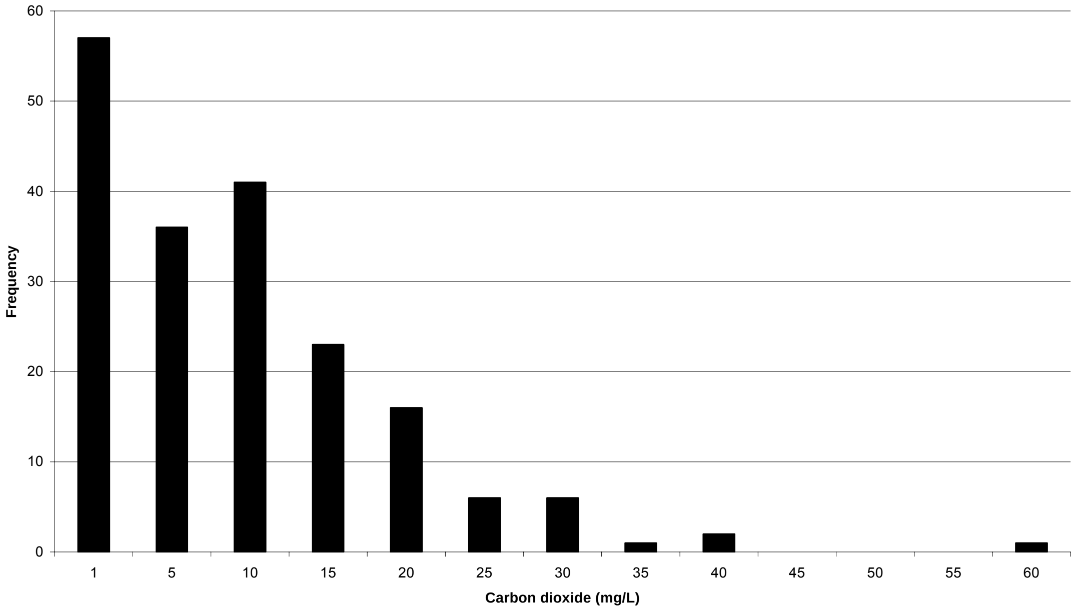

Figure 4.10 Frequency of carbon dioxide values recorded (mg/L) .Mean ±SD was 8.6 ±9.2mg/L, with maximum concentration measured 59.2 mg/L.

4.4.3 Analysis

The data were analysed for relationships between the condition of the fish and water quality parameters. A weak regression was observed between CF and mean UIA values, however while the regression was statistically significant, the r^2 value was only 0.01 and the equation was K= 1.33 – 9.35 x UIA. As the mean UIA was <0.001mg/L, UIA had a very slight effect on the K of fish. No significant correlations were observed between the fin score and any water quality parameters, and no significant regressions were found. For cortisol measurements, a significant relationship was observed for water hardness and alkalinity (p<0.001) with a regression equation of ‘Cortisol (ng/ml) = 9.65 - 0.0156 x hardness’. No other relationships were observed between the individual fish measurements and water quality parameters.

The percentage mortality of the batch of fish for the month prior to sampling was calculated for each system, although data were missing for 12 systems (6%) (figure 4.11). The mean ±SD of percentage mortality was 1.05 ±1.83%/month. Significantly greater mortalities were found in fish farmed for the table market than those for restocking (F=9.76, p=0.002). Fourteen different diseases were recorded in this study (table 4.1), with the most common diseases being Rainbow Trout Fry Syndrome (*Flavobacterium psychrophilum*) and White Spot (*Ichthyophthirius multifiliis*), which occurred in 28% and 32% of batches respectively.

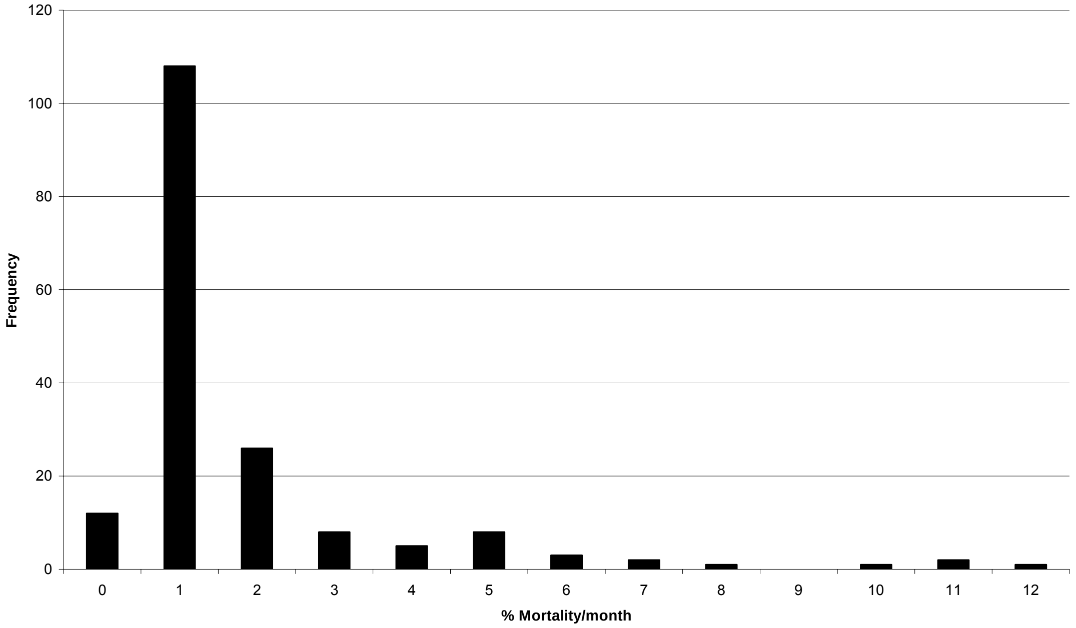

Figure 4.11 Frequency of percentage mortality for one month prior to sampling (n= 177).

Table 4.1. List of diseases and prevalence recorded from 181 batches of fish sampled. Disease history was not available for 8 batches.

Disease	Prevalence
No Diseases	37.6%
Rainbow Trout Fry Syndrome	28.2%
White Spot (Ich)	32.0%
Proliferative Kidney Disease	7.2%
Bacterial Gill Disease	2.2%
Red Mark Syndrome	3.9%
Enteric Red Mouth	6.1%
Bacterial Kidney Disease	2.2%
Furunculosis	6.1%
Gyrodactylus	2.2%
Sleeping Disease	3.9%
Costia	11.6%
Chilonodella	1.1%
Eye fluke	1.7%
Undiagnosed heart disease	0.6%
Nephrocalcinosis	1.1%

4.5 Discussion

4.5.1 Water Quality

This chapter is essentially a description of the data collected for this epidemiological study, with the detailed analysis is conducted in Chapter 5. Poor water quality has the potential to adversely affect the welfare of farmed rainbow trout (Wedemeyer 1996, North *et al.* 2006a, MacIntyre *et al.* 2008). However, it appears that for the majority of farms sampled for this study, water quality levels were within the ranges for what is currently considered to be safe. In a few systems, DO was below the current recommended minimum of 6 mg/L, and this will need to be addressed by the farmers of these systems to safeguard the welfare of their fish. Half of the systems with DO concentrations less than 6 mg/L used supplementary oxygenation systems, suggesting that the loading levels in the system may have been too high for the water quality conditions and that the farmer should have taken action to reduce the burden on the system. The vast majority of systems had UIA concentrations less than even the most conservative maximum recommended level (see MacIntyre *et al.* 2008 for discussion on recommended UIA levels), while no systems had UIA concentrations greater than the generally accepted maximum of 0.02 mg/L (Wedemeyer 1996). Therefore it does not appear that UIA is a major problem on UK trout farms, however the multi-level analysis in Chapter 5 investigates the relationship between UIA and welfare in greater detail. From the literature for carbon dioxide, it is not clear what constitutes a safe level (see MacIntyre *et al.* 2008), with recommended maximum concentrations varying from 9 to 30 mg/L. However, the upper quartile value recorded for this study was below most of the recommended maxima concentrations. Based on the literature and the water quality measurements taken during this study, water quality is generally good on UK trout farms, although for those farms with DO less than the recommended 6 mg/L, efforts should be made by farmers to increase DO.

4.5.2 Morphological and physiological measurements

Cortisol levels for unstressed rainbow trout are generally quoted as being < 10 ng/mL (Pickering & Pottinger 1989, Pottinger *et al.* 1999), with acutely stressed rainbow trout typically having cortisol concentrations of 40-200 ng/mL (Pickering & Pottinger, 1989). Fish that are chronically stressed are reported to have plasma cortisol concentrations that remain elevated but are well below peak, acute concentrations (Wendelaar Bonga 1997). In this study, where a point sample was taken from fish with no reference sample obtained from unstressed fish, making inferences about the stressed state of fish from cortisol samples is inherently difficult. However, the cortisol concentrations taken throughout this study suggest that farmed rainbow trout in the UK are not generally in a stressed condition, as 4 out of every 5 of samples were below the 10ng/mL quoted for unstressed fish (Pickering & Pottinger 1989, Pottinger *et al.* 1999). While low cortisol concentrations might not indicate an unstressed fish, as the capacity of the interrenal tissue to produce cortisol may be exhausted (Huntingford *et al.* 2006), in a study of this size, it is extremely unlikely that depleted interrenal tissue would be the primary cause for low cortisol concentrations, and it is more likely that the fish simply do not perceive normal husbandry conditions found on UK trout farms as stressful. The relationship between high cortisol and low water hardness may be associated with the relationship between higher cortisol concentrations found in cage systems. The majority of cage systems sampled in this study were situated in freshwater Scottish lochs, where water hardness was below the detection limit of the Palintest photometer and recorded as 0. Analysis of the cortisol data by multi-level analysis is described in more detail in Chapter 5.

Fin damage can be assessed in many different ways, such as assigning a qualitative score to each fin, measuring the relative fin length or assessing different types of damage individually (Kindschi 1987, Goede & Barton 1990, Bosakowski & Wagner 1994, Turnbull *et al.* 1998, MacLean *et al.* 2000, North *et al.* 2006a, St-Hilaire *et al.* 2006, Hoyle *et al.* 2007), each with their own merits and drawbacks. The method used in this study (from Hoyle *et al.* 2007) was selected as it takes into account all types of damage. The method also allowed for a large number of fins to be assessed within the time limitations placed on the researcher on a farm, compared with measuring the relative fin length for each fin or assessing other types of damage individually. Any damage to fins is a physical injury to the fish, as fins are living tissue, containing nerves, nociceptors and a blood supply (Becerra *et al.* 1983). Damage to fins may result in reduced locomotion or manoeuvrability, reduction in some communication with cohorts possibly leading to increased aggressive attacks.

Fin damage might also cause fish to protect damaged fins by adopting certain body positions, possibly lead to increased susceptibility of predation and reduce feeding efficiency (Ellis *et al.* 2008). Fins from fish that were farmed for restocking purposes were in a better condition than those farmed for the table market, and this issue is discussed in greater detail in Chapter 5. A previous epidemiological study of fin damage on UK trout farms in 2003 found that the most damaged fins were the dorsal and pectoral fins (St-Hilaire *et al.* 2006). The findings of this present study agree concur with those of St-Hildaire *et al.* (2006), even though the method used to assess fin damage was different, where the 2003 study used relative fin length to assess fin damage. Evaluation of the different methods for assessing fin damage is discussed by Hoyle *et al.* (2007).

It is difficult to assess the general condition of fish from the somatic indices measured, as normal values can vary greatly due to the age of the fish and seasonal cycles (Goede & Barton 1990, Boujard & Leatherland 1992). However, splenomegaly, a marked enlargement of the spleen, is a useful indicator of certain diseases, for example Proliferative Kidney Disease and Rainbow Trout Fry Syndrome (Noga 2006), as there is little ambiguity about the results. Splenomegaly may also occur in healthy salmonids during spawning, however, as the majority of farmed rainbow trout do not reach maturity, this is not a major concern when assessing the condition of fish.

The condition factor, K, is an indication of the body lipid content (Herbinger & Friars 1991), the reproductive state (Barnham & Baxter 1998), the nutritional state and the general condition of the animal (Goede & Barton 1990), and can be affected by life stage (Goede & Barton 1990) and by season (Nordgarden *et al.* 2003). Assessing what is a normal condition factor is problematic. It is generally accepted that fish with a condition factor <1 are in poor condition but it can equally be argued that fish with a high condition factor (>1.8) are also in poor condition (Turnbull & Kadri 2007). In this study, the condition factors measured followed a normal distribution with a mean of 1.31 with a standard deviation of 0.15, which can be argued to be within the normal, healthy range for farmed rainbow trout.

4.5.3 Conclusions

Given the generally good water quality conditions encountered throughout this study, it is not possible to conclude that poor welfare in UK rainbow trout farms is caused as a result of poor water quality. Dissolved oxygen was generally above the industry recommended minimum of 6mg/L (Anon 2002), while unionised ammonia was always under the recommended limit of 0.02mg/L cited by Wedemeyer (1996). The lack of strong associations between the condition of the fish and water quality parameters also support the conclusion that poor welfare is not caused as a result of water quality. Generally, the condition of the fish sampled for this study was also good, with few fish having major loss of fins or moderate/severe gill pathologies. Ninety-six percent of fish had plasma cortisol concentrations below that considered as the level for acutely stressed fish, suggesting that farmed rainbow trout were able to adapt to conditions found on UK farms and that the majority of fish were not subjected to chronic stressors.

4.6 References

Anon. 1986. *The determination of carbon dioxide in natural, treated and beverage waters with a supplement on sampling bottled and canned waters, 1986*. Standing Committee of Analysts, Blue Book. HMSO.

Anon. 2002. *British Trout Association Code of Practice*. British Trout Association. pp11.

Barnham, C., Baxter, A. 1998. *Fisheries Notes: Condition Factor, K, for Salmonid Fish*. State of Victoria, Department of Primary Industries. ISSN 1440-2254.

Becerra, J., Montes, G.S., Bexiga, S.R.R., Junqueira, L.C.U. 1983. Structure of the tail fin in teleosts. *Cell and Tissue Research*. **230**: 127-137.

Bosakowski, T., Wagner, E.J. 1994. Assessment of fin erosion by comparison of relative fin length in hatchery and wild trout in Utah. *Canadian Journal of Fisheries and Aquatic Sciences.* **51**: 636-641.

Boujard, T., Leatherland, J.F. 1992. Circadian pattern of hepatosomatic index, liver glycogen and lipid content, plasma non-esterified fatty acid, glucose, T_3, T_4, growth hormone and cortisol concentrations in *Oncorhynchus mykiss* held under different photoperiod regimes and fed using demand-feeders. *Fish Physiology and Biochemistry.* **10(2)**: 111-122.

Dawkins, M.S. 2006. A user’s guide to animal welfare science. *Trends in Ecology & Evolution*. **21(2)**: 77-82.

Ellis, T.E., North, B.P., Scott, A.P., Bromage, N.R., Porter, M., Gadd, D. 2002. The relationships between stocking density and welfare in farmed rainbow trout. *Journal of Fish Biology*. **61**: 493-531.

Ellis, T, James, J.D., Stewart, C., Scott, A.P. 2004. A non-invasive stress assay based upon measurement of free cortisol released into the water by rainbow trout. *Journal of Fish Biology*. **65(5)**: 1233-1252.

Ellis, T., Oidtmann, B., St-Hilaire, S., Turnbull, J.F., North, B.P., MacIntyre, C.M., Nikolaidis, J., Kestin, S.C., Knowles, T., Hoyle, I. 2008. *Fin erosion in farmed fish*. In: Branson, E.J. (ed). Fish welfare. Blackwell Publishing, Oxford. pp121-149.

Evans, D.H., Piermarini, P.M., Choe, K.P. 2005. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiological Reviews*. **85**: 97-177.

Fivelstad, S., Waagbø, R., Zeitz, S.F., Hosfled, A.C.D, Olsen, A.B., Stefansson, S. 2003. A major water quality problem in smolt farms: combined effects of carbon dioxide, reduced pH and aluminium on Atlantic salmon (*Salmo salar* L.) smolts: physiology and growth. *Aquaculture*. **215**: 339-357.

Goede, R.W., Barton, B.A. 1990. Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. *American Fisheries Society Symposium*. 8: 93-108.

Herbinger, C.M., Friars G.W. 1991. Correlation between condition factor and total lipid content in Atlantic salmon, (*Salmo salar* L.) parr. *Aquaculture and Fisheries Management*. **22**:527-529.

Hoyle, I., Oidtmann, B., Ellis, T., Turnbull, J., North, B., Nikolaidis, J., Knowles, T.G. 2007. A validated macroscopic key to assess fin damage in farmed rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. **270**: 142-148.

Huntingford, F.A., Adams, C, Braithwaite, V.A., Kadri, S., Pottinger, T.G., Sandøe, P., Turnbull, J.F. 2006. Review Paper: Current issues in fish welfare. *Journal of Fish Biology*. **68(2)**:. 332-372.

Juell, J., Fosseidengen, J.E. 2004. Use of artificial light to control swimming depths of Atlantic salmon (*Salmo salar*) in production cages. *Aquaculture*. **233**: 269-282.

Kindschi, G.A. 1987. Method for quantifying degree of fin erosion. *Progressive Fish Culturist*. **49**: 314-315.

Lang, T., Peters, G., Hoffman, R., Meyer, E. 1987. Experimental investigations on the toxicity of ammonia: Effects on ventilation frequency, growth, epidermal mucous cells, and gill structure of rainbow trout *Salmo gairdneri. Diseases of Aquatic Organisms*. **3(3)**: 159-165.

MacIntyre, C.M., Ellis, T., North, B.P., Turnbull, J.F. 2008. *The influences of water quality on the welfare of farmed rainbow trout: a review*. In: Branson, E.J. (ed) Fish welfare. Blackwell Publishing, Oxford. pp150-184.

MacLean, A., Metcalfe, N.B., Mitchell, D. 2000. Alternative competitive strategies in juvenile Atlantic salmon (*Salmo salar*): evidence from fin damage. *Aquaculture*. **184**: 291-302.

Mettam, J. 2005. An investigation into the use of gill pathologies in rainbow trout (*Oncorhynchus mykiss*) as a welfare score relecting water quality. MSc Thesis. University of Stirling.

Noga, E.J. 2006. *Spleen, thymus, reticulo-endothelial system, blood*. In: Systematic Pathology of Fish, 2nd Ed. Ferguson, H.W. (Ed). Scotian Press.

Nordgarden, U., Oppedal, F., Taranger, G,. Hemre, G,. Hansen, T, 2003. Seasonally changing metabolism in Atlantic salmon (*Salmo salar* L.) **I**. Growth and feed conversion ratio. *Aquaculture Nutrition*. **9(5)**: 287-293.

North, B.P., Turnbull, J.F., Ellis, T., Porter, M.J., Migaud, H., Bron, J., Bromage, N.R. 2006a. The impact of stocking density on the welfare of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. **255**: 466-479.

North, B.P., Ellis, T.,Turnbull, J.F.,Davis, J.,Bromage, N.R. 2006b. Stocking density practices of commercial UK rainbow trout farms. *Aquaculture*. **259**: 260-267.

Pickering, A. D., Pottinger, T. G. 1989. Stress responses and disease resistance in salmonids fish: Effect of chronic elevation in plasma cortisol. Fish Physiology and Biochemistry. **7**: 253-258.

Pottinger, T. G., Yeomans, W. E., Carrick, T. R. 1999. Plasma cortisol and 17β-oestradiol levels in roach exposed to acute and chronic stress. Journal of Fish Biology. **54**: 525-532.

Sanchez, J.G., Speare, D.J., Johnson, G.J. 1997. Morphometric and histochemical assessment of the branchial tissue of rainbow trout, *Onchorhynchus mykiss* (Walbaum), associated with chloramine-T treatment. *Journal of Fish Diseases*. **20**: 375-381.

Speare, D.J., Ferguson, H.W. 1989. Fixation artefacts in rainbow trout (*Salmo gairdneri*) gills: a morphometric evaluation. *Canadian Journal of Fisheries and Aquatic Science*. **46**: 780-785.

St-Hilaire, S., Ellis, T., Cooke, A., North, B.P., Turnbull, J.F., Knowles, T., Kestin, S. 2006. Fin erosion on commerical rainbow trout farms in the UK. *Veterinary Record*. **159**: 446-451.

Stevenson, P. 2007. *Closed waters: the welfare of farmed Atlantic salmon, rainbow trout, Atlantic cod and Atlantic halibut*. A Compassion in World Farming/WSPA report, UK.

Turnbull, J.F., Adams, C.E., Robertson, D.A. 1998. Attack site and resultant damage during aggressive encounters in Atlantic salmon (*Salmo salar*) parr. *Aquaculture*. **159**: 345-353.

Turnbull, J.F., Bell, A., Adams, C., Bron, J., Huntingford, F.A. 2005. Stocking density and welfare of cage farmed Atlantic salmon: application of a multivariate analysis. *Aquaculture*. **243**: 121-132.

Turnbull, J.F., Kadri, S. 2007. Safeguarding the many guises of farmed fish welfare. *Diseases of Aquatic Organisms*. **75**: 173-182.

Wedemeyer, G.A. (1996). *Physiology of fish in intensive culture systems*. Chapman & Hall. pp 232.

Wendelaar Bonga, S.E. 1997. The stress response in fish. *Physiological Reviews*. **77**: 591-625.

5 Identification of risk factors for the welfare of farmed rainbow trout in the UK.

MacIntyre, C.M., Berrill, I.K., North, B.P., Knowles, T., Turnbull, J.F.

5.1 Abstract

Water quality has been identified as having the potential to affect fish welfare, however it was not known if water quality was a welfare problem on UK trout farms. This paper presents and discusses the results of a cross-sectional study of the welfare of farmed rainbow trout (*Oncorhynchus mykiss* Walbaum) on UK farms. We visited 44 farms, sampling 3699 fish from 189 different systems over 2 seasons. An aggregate welfare score was developed using plasma cortisol concentrations, spleen size, fin condition, gill condition and population mortality levels, which combined different aspects of welfare. Multilevel models were developed to identify risk factors for the aggregate welfare score and each of its constituents. The primary finding of this study was that disease had a major effect on the welfare of farmed rainbow trout, not just during an outbreak, but with the effects of disease persisting after the outbreak had ended, irrespective of the disease involved. Fish farmed for restocking fisheries generally had better welfare than those farmed for the table market. There was no systematic evidence that poor water quality was a major risk factor for farmed UK rainbow trout.

5.2 Introduction

The body of literature relating to fish welfare is increasing rapidly, and as a result so is our understanding of what welfare actually is and what it means to fish (*e.g.*

Chandroo *et al.* 2004, Huntingford & Adams 2005, Huntingford *et al.* 2006, Braithwaite & Boulcott 2007, Iwama, 2007, Turnbull & Kadri, 2007). Fish welfare is a multifaceted, multifactorial subject that encompasses every aspect of an individual's life, from health and well-being to quality of life and an absence of suffering. While there is no universally accepted definition of welfare (Fraser 1999), it can be regarded as the physical and mental state of an animal in relation to its environment (Appleby & Hughes 1997, Duncan & Fraser 1997). The physical state, as applied to welfare, of an animal can be measured by morphological, physiological and certain production-based indices of welfare (North *et al.* 2006a, Turnbull *et al.* 2008, chapter 4) however assessing the subjective, mental state of an animal is a more difficult prospect. It has been suggested that certain behaviours may provide an honest representation of the mental state of animals (Dawkins 2004, 2006, Braithwaite & Boulcott 2008), however, to date no on-farm behavioural welfare indicators for fish have been developed (Turnbull *et al.* 2008).

This study was conducted as part of a larger project examining water quality interactions with the welfare of farmed rainbow trout (AW1205), funded by Defra and the British Trout Association. The aim of the study was to investigate the relationship between water quality and functional welfare indicators for farmed rainbow trout (*Oncorhynchus mykiss* Walbaum), although other aspects of husbandry were included. The project followed on from a previous Defra project (AW1203) which investigated the relationship between stocking density and welfare (North *et al.* 2006a, b). One of the main findings from that study was that water quality was a better predictor of welfare indicators than stocking density.

Water quality was identified as a major factor for the welfare of farmed rainbow trout (FAWC 1996), and while poor water quality certainly has the capacity to cause poor welfare (MacIntyre *et al.* 2008), it is not known if poor water quality is a welfare concern on UK trout farms. This study aimed to determine risk factors for welfare using an epidemiological approach, as used for welfare in poultry and Atlantic salmon (*Salmo salar*) (Jones *et al.* 2005, Juell & Fosseidengen 2004, Turnbull *et al.* 2005).

5.3 Method

5.3.1 Sample Population

Forty four farms were visited for this study between July 2005 and April 2007. Farms were randomly selected from a database of trout farms prepared for a previous study (see chapter 3). To account for any seasonal variability in welfare and farm conditions, farms were visited twice, once in summer and once in winter. We were unable to make the second visit to 5 farms due to adverse weather conditions, the 2006 Viral Haemorrhagic Septicaemia (VHS) outbreak and other circumstances outwith our control. A total of 3699 fish were sampled from 189 systems. Either 12 or 24 fish were sampled from each system, from up to 4 systems per visit.

5.3.2 Examination of Fish

The length and weight of each fish was recorded and converted into a condition factor (K). In many studies, an increasing K is considered as an improving K, *i.e.* the condition of the fish is thought to improve the larger K gets (Barnham & Baxter 1998). While it is recognised that a very low condition factor (<1) is an indication of poor welfare, it can also be argued that a high condition factor is similarly a sign of poor welfare (Turnbull & Kadri 2007). To take this into account, the distance of the condition factor for each fish from an idealised mean was calculated. The overall mean of K for the entire dataset was 1.3, and this was taken to be a reasonable figure for an idealised mean. The deviation of K from 1.3 was calculated for each fish. Each fin (except the adipose fin) was assessed using a 5-point scale (Hoyle *et al.* 2007) and the individual fin score summed to give a total fin condition score. The liver and spleen of each fish was weighed and converted into the Hepatosomatic Index (HSI) and Splensomatic Index (SSI) (Chapter 4). The second anterior gill arch of the left gill was removed, embedded in paraffin wax, sectioned at 5µm and stained with haematoxylin and eosin. Gills were allocated a dichotomous variable based on if they were "not affected/slightly affected" or "moderately/severely affected" for the pathologies lamellar hyperplasia and lamellar fusion. Blood samples were taken from the caudal vena cava and analysed for haematocrit and cortisol. Haematocrit was measured as the percent packed red cell volume and concentrations of plasma cortisol were determined to ng/mL by radioimmunoassay using the method described in Ellis *et al.* (2004) as adapted by North *et al.* (2006a). A full description of materials and methods can be found in chapter 4.

5.3.3 Environmental and Husbandry Parameters

Water quality data were collected for dissolved oxygen (DO) (mg/L), temperature (°C), pH, specific conductivity (mS/cm) and ammonium (NH4+-N mg/L) using a YSI 6600 multi-parameter sonde (YSI Hydrodata Ltd, Herts, UK). A sonde was placed in the outflow of each system sampled, and measurements taken every 15 minutes for 24 hours. Output from the sondes was converted to the mean, standard deviation, maximum, minimum, range, average difference between the 15 minute measurements over the course of 24 hours (Δ/15min/24 hour) , and the maximum change in measurements over the same period (Max Δ/15min/24 hour). Water alkalinity, hardness and nitrite were measured using a Palintest 500 (Palintest, Gateshead, UK), a multiparameter photometer that measures colour changes in test water following addition of a reagent.

Through observation and interview with the farmer, data were collected on the system and history of the batch of fish sampled from. Table 5.1 lists the variables collected.

Table 5.1 List of environmental and husbandry variables collected for each system. The 24 hour measurements of temperature, DO, pH and NH3 were each converted to the 7 variables shown.

WATER QUALITY		
Alkalinity Hardness Nitrite CO_2 Specific Conductivity	Temperature Dissolved Oxygen pH NH_3	Mean Standard deviation Maximum Minimum Range Δ/15min/24hour Max Δ/15min/24hour
SYSTEM	PRODUCTION INFORMATION	
Type (e.g. pond, raceway, cage, tank) Construction material Volume Aeration Oxygenation	Biomass Stocking density Number of fish in system Egg source Hatchery Hatch date Growth/month Mortality/month	FCR Time on farm Time in current system Ploidy Table or restocking Feeding method Time since last grading Disease history

5.3.4 Welfare Score

The data were initially analysed using principal components analysis (PCA) to establish if any biologically relevant groupings within the fish parameters were present that could be utilised as a welfare score. This approach was used in previous studies (Turnbull *et al.* 2005, North *et al.* 2006a, Adams *et al.* 2007). No such groupings were present in the data and therefore a different approach for assessing welfare was required. Chapter 4 discussed the measurements recorded from fish that were suitable for use as on-farm welfare indicators in this study. The parameters selected as being most appropriate and relevant to welfare were the fin condition score, gill condition score, plasma cortisol concentration, SSI, and population mortality levels.

An aggregate welfare score was calculated for each fish using the selected parameters. Values greater than the 75% quartile were adjudged to indicate poor welfare for the SSI, plasma cortisol concentrations and batch mortality levels. This rule was not applied to the fin or gill conditions scores, as any loss of fin condition is a sign of physical injury, and the 75% quartile is not applicable to a dichotomous variable. Descriptive statistics for the welfare parameters are given in table 5.2. The results for each welfare indicator were ranked, converted to a percentile and standardised so that all had a mean=1. The signs of the welfare indicators were reversed, so that increases in any of the indicators represented improving welfare, and summed to give an aggregate welfare score for each fish.

Table 5.2 Descriptive statistics for the welfare indicators selected for the welfare score.

Variable	Median	StDev	Min	Max	25% Quartile	75% Quartile
Fin Score	12	3.67	7	27	10	15
SSI	0.16	0.26	0.01	4.83	0.12	0.22
Cortisol	3.63	11.78	0.11	149.66	1.64	8.49
Mortality	0.29	1.63	0	11.57	0.1	1

5.3.5 Data Analysis

The 4 welfare indicators and total welfare score were analysed in multilevel models using MLwiN (v2.02) (Centre for Multilevel Modelling, University of Bristol). All models constructed were random intercept variance components models and took the following form:

$$y_{ijk} = \beta_0 + \sum\beta X_{ijk} + \sum\beta X_{jk} + v_{0k} + u_{0jk} + e_{0ijk}$$

where y_{ijk} is the welfare indicator or aggregate welfare score for individual fish in a system within a farm, β_0 is a constant, and βX is a fixed effect predictor variable which varies at level 1 (*ijk*) or level 2 (*jk*). The subscripts *i, j, k* refer to the 3 levels of the model, level 1, *i*, is the fish within a batch, $i = 1,\dots,3699$, level 2, *j*, is the batch within a farm, $j = 1,\dots,189$, and level 3, *k*, is the farms within the UK trout industry, $k = 1,\dots,44$. The terms v_{0k}, u_{0jk} and e_{0ijk} are the random effect residual variances at the levels of the farm, batch and fish respectively.

All data were centred prior to analysis, a common practice in this type of modelling (Knowles *et al.* 2008). Centring the data aids interpretation of both the constant in the model (β_0) and of parameter estimates for the centred predictor variables (βX). The parameter estimate then shows how the response variable (y) changes for a given off-set from the mean of that predictor variable. For example, in a simple model where the response variable ‘aggregate welfare score’ is predicted only by stocking density, if stocking density were not centred, the constant in the model would be interpreted as the mean welfare score when stocking density = 0, an impossibility. In a model in which stocking density was centred, the constant would represent the mean welfare score at the mean stocking density, which is more meaningful and comprehensible (Knowles *et al.* 2008).

If categorical variables are included within a model, MLwiN uses one of the categories as a reference and compares the effect of the other categories against the reference. For example, if the mean welfare score was modelled by system type, MLwiN would select one category, *e.g.* ‘pond system’ as a reference, calculate the mean welfare score for fish in pond systems, and provide coefficient terms for the other 3 system types, raceways, cages and tanks.

It was impractical to attempt to model every combination of the 57 predictor variables in this study. The approach taken for this analysis was to model all predictor variables individually in a bivariate model, and those variables that were significantly associated with the response variables were retained. Variable were adjudged to be significant if the standard error of the coefficient was ≤ 50% (Rasbash *et al.* 2004). Combinations of the 7 variables calculated for each of the water quality parameters measured over 24 hours (DO, temperature, pH, NH_3) were tested and significant combinations retained. The final model was prepared by combining all retained predictor variables and also any other variables considered to have a potential biological effect. Predictor variables were added and removed until the model of best fit was attained based on the maximum likelihood (Browne *et al.* 2002). Standardised residuals were checked for normality at all levels of the model

With a dataset such as this, a very large amount of analysis could be carried out. The following results represent the hypotheses that were most pertinent to the larger study.

5.4 Results

The individual components of the welfare score were analysed for risk factors separately before the aggregate welfare score was analysed.

5.4.1 Cortisol

Table 5.3 provides a summary of the model for the cortisol welfare score. As the sign of the cortisol values was reversed, a increasing cortisol score represents decreasing blood plasma cortisol concentrations.

Table 5.3 Model summary for risk factors for cortisol welfare score.

	Coefficient *Explanatory variable*	SE of Coefficient
Intercept †	0.0325	0.0225
Water quality		
DO Δ/15min/24 hour	-0.3110	0.1320
System type		
Cages	-0.4260	0.0640
Interaction term		
Cannon suppl. by hand * cages	0.4840	0.1170
Hand feeding * cages	0.2860	0.0950
Fish measures		
Condition factor K	0.1770	0.0400
Deviation of K from 1.3	-0.1190	0.0520
Estimate of random effects	*Partitions of variance*	
Farm	0.009	11.54%
Batch	0.029	37.18%
Fish	0.040	52.28%
Variance explained by model	18%	
† Reference categories are fish in pond systems fed by demand feeder		

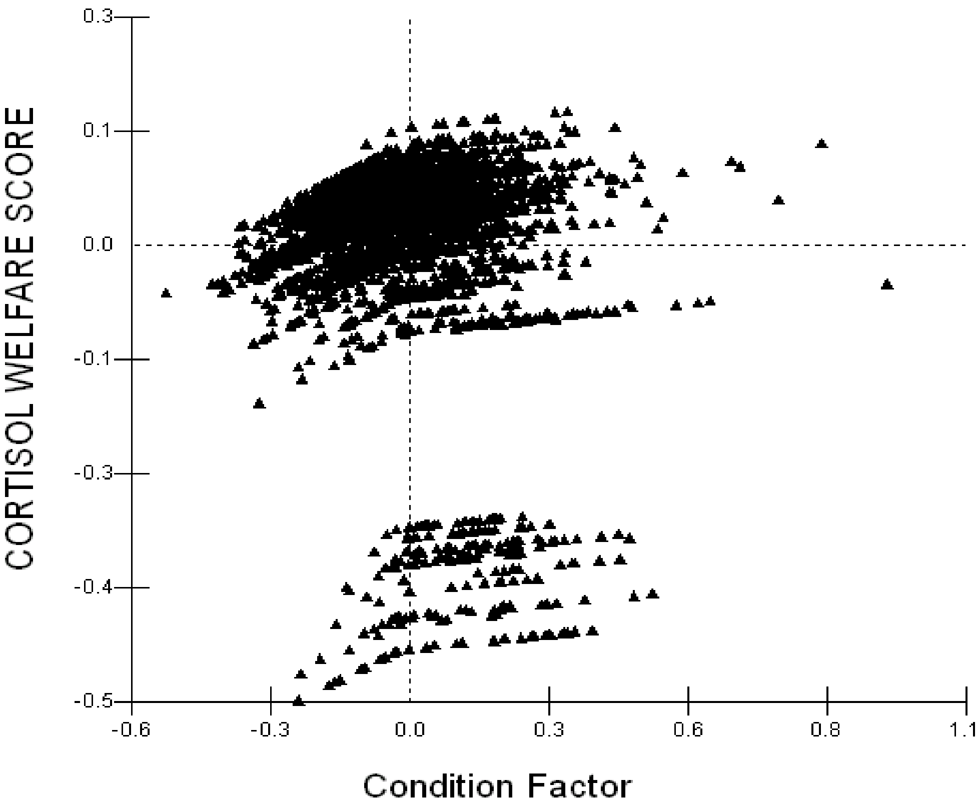

Figure 5.1 Predicted cortisol welfare score against condition factor. The bottom grouping refers to predicted cortisol concentrations for fish found in cage systems.

An increase in the mean change in DO concentrations per 15 minutes for 24 hours was associated with increasing cortisol levels in the blood. No other water quality parameters were significantly associated with cortisol levels. Fish farmed in cage systems had significantly higher cortisol levels than other system types (figure 5.1), however hand fed fish and those fed by feed cannon with supplemental hand feeding in cages had lower cortisol levels than those fed by other methods. The condition factor K and the deviation of K from 1.3 were both associated with cortisol levels. An increase in K was associated with lower blood cortisol levels, however this linear increase in K was moderated by deviation from the K mean, which reduced the coefficient of the slope (figure 5.1). This means that, as the condition factor increased, cortisol levels in fish reduced, however if the condition factor was too large, then an increase in cortisol levels was observed. This model accounted for 18% of the original variance in the dataset, with the majority of the variance explained at the level of the farm and batch.

5.4.2 SSI

Risk factors for the SSI are summarised in table 5.4. An increase in the SSI welfare score indicates a decrease in the weight of a fish' spleen relative to its body weight.

Table 5.4 Model summary for risk factors for SSI welfare score.

	Coefficient *Explanatory variable*	SE of Coefficient
Intercept	0.0514	0.0180
Water quality		
DO mean	-0.1730	0.0050
NH_3 mean	-0.0033	0.0016
NH_3 SD	0.0185	0.0073
Oxygenation added	-0.0789	0.0300
Interaction terms		
Stocking density * raceways	-0.0032	0.0007
NH_3 * raceways	-0.0129	0.0033
Fish		
Length of fish (mm)	0.0007	0.0002
Estimate of random effects	*Partitions of variance*	
Farm	0.0039	5.32%
Batch	0.0143	19.77%
Fish	0.0542	74.91%
Variance explained by model	22%	

The length of fish was included in the model as a potential confounder, with the spleens of larger fish being relatively smaller than those of smaller fish. An increase in mean DO concentrations was associated with an increase in spleen size. Additionally, fish in systems that were oxygenated also exhibited this response. Increasing levels of unionised ammonia NH_3 were linked with larger spleens, although if the standard deviation of the 24 hour sample was large, indicating fluctuating NH_3 concentrations, then the spleen size was reduced. Increasing stocking density was associated with larger spleens in raceways, while higher NH_3 concentrations in raceways were associated with larger spleens than for other system types. This model accounted for 22% of the total variance, with the variance explained almost entirely at the farm level of the model.

5.4.3 Fin Condition Score

Table 5.5 summarises the risk factors for the fin condition score. An increase in the fin condition welfare score indicates less damage to fins and that the fins are in a better condition.

Table 5.5 Model summary for risk factors for fin condition welfare score.

	Coefficient *Explanatory variable*	SE of Coefficient
Intercept †	-0.2050	0.0570
Water quality		
pH mean	-0.1320	0.0670
Temperature mean	0.0139	0.0053
Farming purpose		
Restocking	0.4860	0.0850
Interaction terms		
Stocking density * restocking	-0.0032	0.0007
Fish		
Length of fish (mm)	-0.0009	0.0003
Estimate of random effects	*Partitions of variance*	
Farm	0.0663	24.94%
Batch	0.0667	25.08%
Fish	0.1329	49.98%
Variance explained by model	21%	
† Reference category is table ifsh		

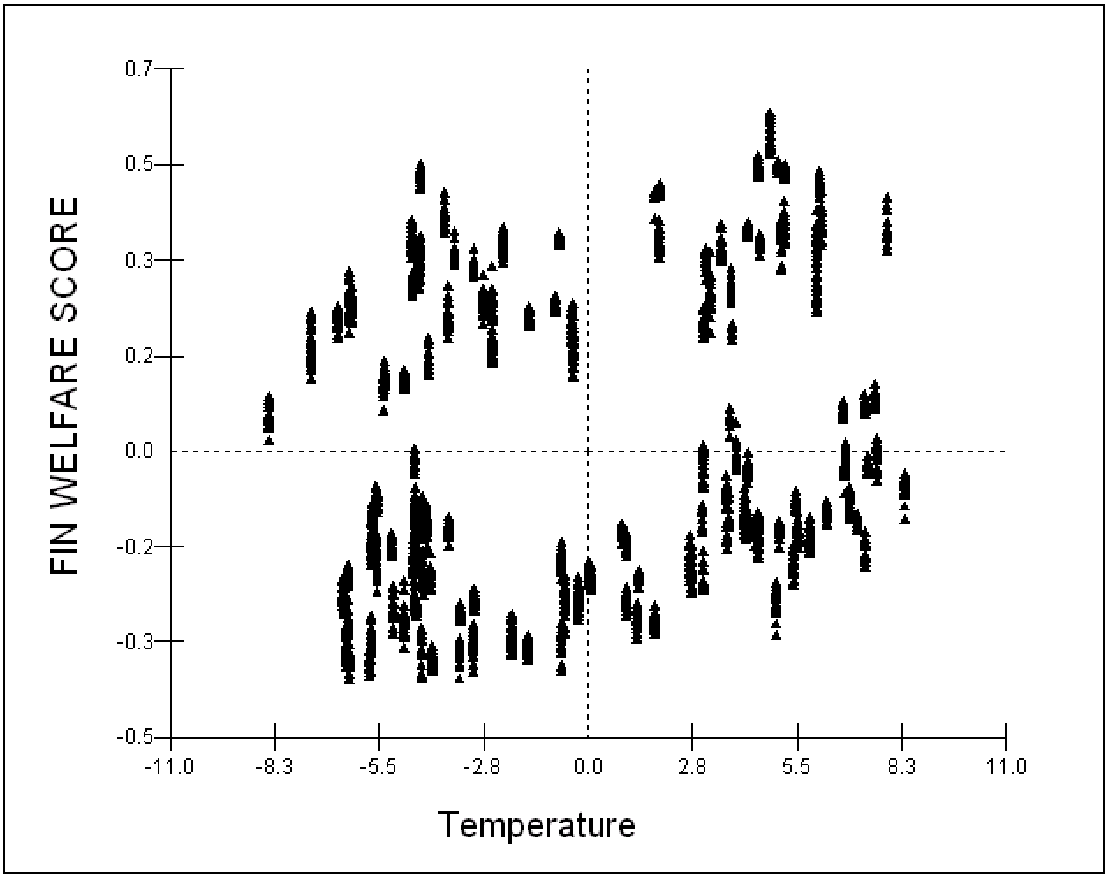

Figure 5.2 Predicted fin welfare score and temperature. The top grouping in the graph refers to fins from restocking fish, and the bottom group, with worse fin condition, refers to table market fish.

The condition of the fins was most strongly associated with the purpose of the farming, fish farmed for restocking had better fins than those farmed for the table (figure 5.2). While stocking density was not associated with fin condition for table fish, higher stocking densities for restocking fish were associated with worse fins, although the association was not strong. Larger fish were observed to have worse fins than smaller fish, however, again the effect of fish length on fin condition was minimal. Water quality was associated with fin condition, with increasing pH values associated with fish with worse fin conditions. Increasing water temperatures were associated with better fin conditions; this effect was not due to seasonal changes in water temperature, as season did not have a significant association with fin condition.

This model accounted for 21% of the total variance, again primarily at the farm level of the model.

5.4.4 Mortality

Table 5.6 summarises the risk factors for mortality levels. As mortality levels apply to an entire batch of fish in a system, there is no variance at the level of the fish, and therefore this is a 2 level model. An increase in the mortality welfare score signifies a reduction in batch mortality levels for the month prior to sampling.

Table 5.6 Model summary of risk factors for mortality welfare score.

	Coefficient *Explanatory variable*	SE of Coefficient
Intercept †	-0.1160	0.0285
Number of diseases		
1 disease	-0.1211	0.0168
2 diseases	-0.1355	0.0161
3 or more diseases	-0.2069	0.0225
Farming purpose		
Restocking	0.0729	0.0270
Season		
Winter	0.2175	0.0095
Ploidy		
Triploid	0.1038	0.0200
Estimate of random effects	*Partitions of variance*	
Farm	0.0236	32.42%
Batch	0.0492	67.58%
Variance explained by model	20%	
† Reference categories are diploid, table fish with no diseases sampled in summer		

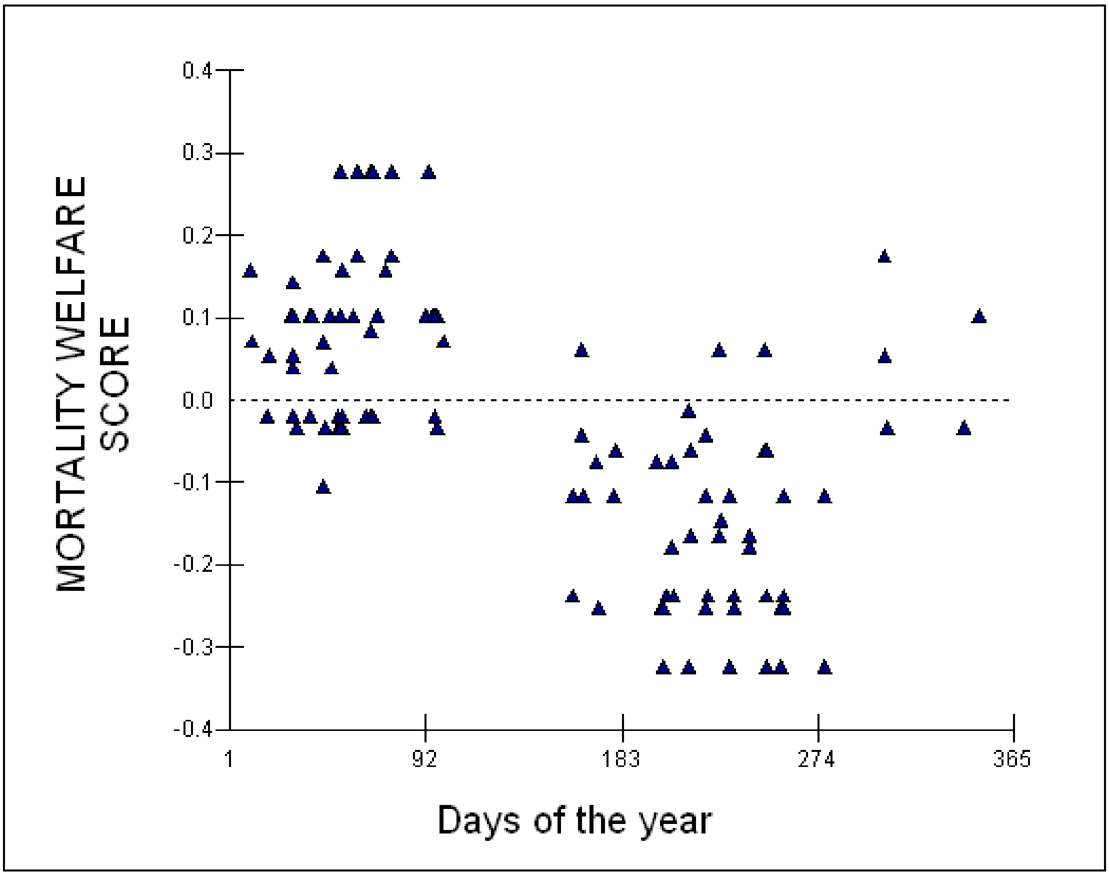

Figure 5.3 Seasonal effect on mortality rate. Highest mortality rates (the lowest mortality welfare scores) are found during summer periods.

Exposure to any disease led to an increase in recorded batch mortality levels for the month prior to sampling, irrespective of which diseases involved. No individual disease accounted for an increase in mortality levels. The season the fish were sampled in was also significant, with lower mortality levels observed during winter months. In the bivariate models, both season and temperature were associated with mortality levels, with lower mortality levels at lower temperatures (figure 5.3), although neither were significant when both terms were included in the model together. Seasonal effects explained more of the variance than temperature and thus remained in the model. Fish farmed for restocking had significantly lower mortality levels than table fish, while triploid fish had lower mortality levels than diploid fish. This model explained 20% of the total variance.

5.4.5 Welfare Score

Table 5.7 summarises the risk factors for the overall welfare score. Increases in the welfare score signify improving fish welfare.

Table 5.7 Model summary of risk factors for total welfare score.

	Coefficient *Explanatory variable*	SE of Coefficient
Intercept †	0.0255	0.1220
Water quality		
NH_3 Δ/15 min/24 hour	-0.0510	0.0210
Farming purpose		
Restocking	0.5344	0.1467
Number of diseases		
1 disease	-0.4660	0.1218
2 diseases	-0.4078	0.1205
3 or more diseases	-0.4270	0.1624
Fish measures		
Condition factor K	0.2890	0.0980
Deviation of K from 1.3	-0.3330	0.1264
Estimate of random effects	*Partitions of variance*	
Farm	0.2390	37.23%
Batch	0.1694	26.32%
Fish	0.2346	36.45%
Variance explained by model	14%	
† Reference categories are table fish not exposed to any diseases		

The risk factor that had the greatest effect on the overall welfare score was the purpose of farming, with fish farmed for restocking having better welfare than table fish. Variables that were recorded and that could potentially account for the difference between table and restocking farming practices were investigated, however none were found to be significantly associated with the welfare score. The only water quality parameter found to be associated with the welfare score was the average change in NH_3 per 15 minutes. Greater changes in NH_3 were associated with worse welfare scores, although the association was not strong. Any diseases that the fish had been exposed to was associated with worse welfare: as with the mortality component of the welfare score, this was irrespective of the diseases involved. Exposure to diseases was only associated with the mortality component of the welfare score; to test if the overall welfare score was unduly influenced by mortality levels, the mortality component was removed from the welfare score and analysed for risk factors. The only risk factors which emerged as significant were exposure to any diseases which was associated with worse welfare and farming fish for restocking purposes which was associated with better welfare. It was therefore concluded that mortality levels did not have an undue influence on the overall welfare score.

An association was observed between increasing K and improving welfare; however as with the cortisol component of the welfare score, fish with a condition factor with a large deviation from the idealised mean of 1.3 had worse welfare. This point is illustrated in figure 5.1, which is the predicted values for the welfare score from table 5.7 graphed against K. Increasing K was associated with improving welfare up to an inflection point, where after increasing K was associated with worse welfare. Piecewise linear regression with breakpoint estimation indicated the inflection point was 0.02, which corresponds to the idealised mean of K of 1.3. In figure 5.4, there are 3 main groupings of data points, ‘a-c’, with a smaller grouping ‘d’: these correspond to groupings of the disease history of batches, with the highest welfare scores for fish that had not been exposed to any diseases (‘a’), then one disease (‘b’), two diseases (‘c’), and three or more diseases (‘d’).

Given the associations observed in this study between welfare and table or restocking production, the dataset was divided by farming purpose and re-analysed. Risk factors for table fish are summarised in table 5.8 and are similar to the risk factors for the overall welfare score for the entire dataset, with fish sampled in winter associated with better welfare scores and any exposure to diseases associated with worse welfare scores. An increasing K was associated with improving welfare scores, however unlike the overall welfare score the deviation from the idealised mean did not have any association with the score.

Risk factors for the welfare of restocking fish are summarised in table 5.9. Feeding restocking fish with a demand feeder was associated with lower welfare scores than using any other method, with the highest welfare scores associated by feeding fish with a feed cannon. Restocking fish kept in tank systems had significantly lower welfare scores than fish kept in other systems, while fish exposed to up to 2 diseases had worse welfare than those exposed to none. There were 3 batches of restocking fish exposed to 3 diseases, however no association was found between those batches and the welfare score.

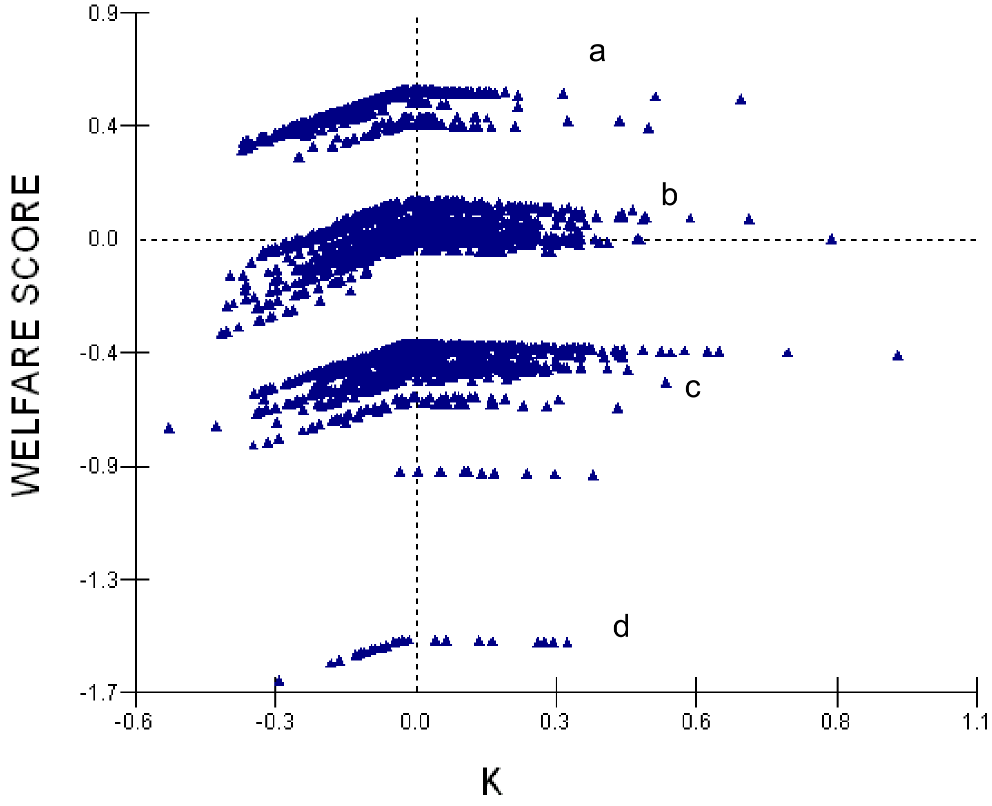

Figure 5.4 Predicted welfare score modelled against condition factor K. Groupings 'a, b, c and d' refer to fish not exposed to any diseases, fish exposed to 1 disease, 2 diseases and 3 or more disease respectively.

Table 5.8 Model summary of risk factors for total welfare score for table fish.

	Coefficient *Explanatory variable*	SE of Coefficient
Intercept †	-0.1713	0.1620
Season		
Winter	0.2219	0.1109
Number of diseases		
1 disease	-0.4048	0.1631
2 diseases	-0.3987	0.1521
3 or more diseases	-0.4736	0.2044
Fish measures		
Condition factor K	0.2941	0.1160
Estimate of random effects	*Partitions of variance*	
Farm	0.2900	39.78%
Batch	0.1991	27.31%
Fish	0.2400	32.91%
† Reference categories are fish sampled in summer not exposed to any diseases		

Table 5.9 Model summary of risk factors for total welfare score for restocking fish.

	Coefficient *Explanatory variable*	SE of Coefficient
Intercept †	0.4600	0.1618
Feeding method		
Demand feeder suppl. by hand	0.6439	0.2114
Hand feeding	0.3252	0.1545
Feed cannon	0.9261	0.3566
System		
Tank	-0.4653	0.1344
Number of diseases		
1 disease	-0.6187	0.1402
2 diseases	-0.3929	0.1537
Fish measures		
Deviation of K from 1.3	-0.5737	0.1838
Estimate of random effects	*Partitions of variance*	
Farm	0.0385	10.25%
Batch	0.1107	29.48%
Fish	0.2263	60.27%
† Reference categories are fish in ponds fed by demand feeder not exposed to any diseases		

5.4.6 Partitioning of Variance

Table 5.10 describes how the variance for the welfare score is partitioned across the whole of the dataset and by system type at the 4 levels of the model, between farms, between systems and within systems. Across the entire dataset, the variance is partitioned evenly, with no single level standing out. The least amount of variance was found in pond systems (total = 0.58), with most of the variance found within systems. For the welfare score in cage systems, very little variance was found between farms, with nearly 60% of the variance occurring between systems. The greatest variance totals were found in raceway and tank systems, where the most amount of variance occurred between farms (56% and 43% respectively).

Table 5.10 Partitioning of variance for welfare score for all systems, and by individual system type.

	All systems	Ponds	Raceways	Cages	Tanks
Variance between farms	0.282	0.18	0.467	0.034	0.354
Variance between systems	0.224	0.157	0.142	0.425	0.244
Variance within systems	0.236	0.243	0.226	0.256	0.221
Total	0.742	0.58	0.835	0.715	0.819

5.5 Discussion

5.5.1 Validity of Welfare Score

PCA was unable to establish any biologically plausible groupings in the data for use as an objective welfare score. The approach taken in place of these groupings was to combine welfare indicators into an aggregate welfare score (Tuyttens *et al.* 2008). Each of the welfare indicators in the aggregate score reflects different functional aspects of welfare, with fin damage an indication of physical injury, large increases in the SSI signalling disease status, plasma cortisol levels indicative of the stress response, the gill condition the ability to take up oxygen, and batch mortality levels the health status of the population in a system. The aggregate welfare score was also a useful tool for the identification of risk factors for welfare in farming operations, given the range of welfare aspects it covered.

5.5.2 Cortisol

The risk factors associated with cortisol levels in fish were fluctuations in DO levels, cage systems and feeding methods. Natural diurnal fluctuations (Boyd, 1990) can result in large changes in DO, where DO concentrations fall throughout the night, due to minimum photosynthesis combined with maximum respiration by plants and algae, and then begin to increase again at dawn. Examination of the output from the water monitoring sondes used in this study showed that there was frequently a dip in DO concentrations at first light, presumably due to an increase in fish activity and therefore oxygen consumption (Boyd, 1990). Fish cannot acclimate to sudden large changes in DO concentrations, leading to activation of the stress response and avoidance behaviour (Wedemeyer, 1996). Other fluctuations in DO could be as a result of increased fish activity, due to, for example, disturbance by predators, feeding and grading. Normal husbandry practices, such as feeding and grading, were not suspended during sampling for this study, however time since last grading was not significantly associated with plasma cortisol concentrations.

There was a strong association between growing fish in freshwater cages and high plasma cortisol levels in rainbow trout. Analysis of the data did not reveal any systematic differences between cages and other system types that could account for the increased plasma cortisol levels. There are certain characteristics common to all the cage systems sampled from, such as slightly acidic water with no alkalinity or hardness, DO concentrations were all well above the current recommended minimum, water quality parameters remained stable over the 24 hour period monitored, with no sudden large fluctuations, and all fish were produced for the table market. Given the consistency of environment, there must be some other aspects of cage systems or husbandry procedures for cage systems, not recorded during this study, that accounted for the observed increased cortisol levels, as the association between cortisol and cage systems was strong. It was hypothesised that sampling bias may account for this observed increase; netting fish from large cage systems (circular 80m^2) can take longer than land-based systems, using drag rather than dip nets. For these larger cage systems, the stress response in fish would be activated at the onset of netting, and therefore it is possible that the observed increase in plasma cortisol concentrations could be as a result of the time from onset of the stressor until immersion in the anaesthetic. However, the majority of cage systems sampled from were square 16m^2 cages and fish were able to be collected using dip nets, the method used for all other system types, and therefore the observed increase in cortisol concentrations is unlikely to be due to netting procedures. The time taken to transport fish from cage to shore to sampling station for blood sampling was estimated at 5 minutes longer on average than for land-based systems to sampling station, another possible source of sampling bias. However, all fish were placed in the same, lethal dose of anaesthetic (2-phenoxyethanol), with time to loss of sensibility estimated at ± 1 minute for all fish. It is unlikely that a significant secretion of cortisol occurred after loss of sensibility (Tort *et al.* 2002) and therefore transport time from system to sampling station was unlikely to be a factor.

Fish in cage systems that were fed by hand and by feed cannon supplemented by hand had significantly lower cortisol levels than fish fed by feed cannon alone or by an automated feed system. This suggests that any form of hand feeding is less stressful for fish than other methods of feeding in cage systems, possibly through ensuring that enough food is provided for each fish and that it is well distributed throughout the system. Feeding methods were not significantly associated with plasma cortisol concentrations in other system types, indicating that there is some dynamic within cage populations of fish that makes feed cannons or automated feed systems inherently stressful to fish.

5.5.3 Fin Condition

The risk factors for fin condition were very different to those for cortisol. Small fish had better fin conditions than larger fish, although the association was weak. The length of fish was included as a proxy for the age of fish, as the hatch date was not available for the majority of batches sampled from. The weak association found in this study supports the conclusion of Barrows and Lellis (1999) that fins get worse throughout the production cycle. Alternatively, it is possible that the association between length and fin condition is due to the behavioural strategy adopted by fish (Adams *et al.* 1998) and that larger, dominant fish in a hierarchy fought aggressively for a food resource, which could result in fin damage, as has been observed in juvenile steelhead trout (Abbott & Dill 1985) and Atlantic salmon (Turnbull *et al.* 1998). In experiments on Atlantic salmon, Adams *et al.* (1998) found that within a single population fish adopted a variety of behavioural strategies including aggressive high food acquisition and non-aggressive low food acquisition, as there were individuals that had damaged fins but grew rapidly and individuals with very little fin damage that grew very slowly, a result that was also found for rainbow trout (North *et al.* 2006a).

High water pH values were associated with poor fin condition. High pH values are usually found in waters with high alkalinity and hardness, however in this study no associations were found between fin condition and alkalinity and hardness. Bosakowski and Wanger (1994) found an association between low alkalinity and fin damage, however were unable to establish a causal link and the mechanisms behind it. High mean water temperatures were associated with better fin condition in fish, however no association was observed between the season the fish were sampled in and fin condition. Turnbull *et al.* (1996) suggested that the rate of healing for fin damage is affected by temperature, which might explain why better fins were found at higher temperatures. Ellis *et al.* (2008) in their review concluded that water quality was unlikely to be a primary cause of fin damage, however it is possible that water quality is a secondary factor, affecting the severity of damage caused.

The strongest association for fin condition existed for the farming purpose, with fish farmed for restocking purposes strongly associated with good fin condition. There are 2 possible hypotheses for this difference; 1) that farming conditions for restocking fish are more conducive to production of fish with better fins, and 2) or that restocking farmers select fish for their fin condition during grading. For the first hypothesis, it is not known what farm conditions could account for the difference, as although restocking farms had on average lower stocking densities than table farms, which appears to be generally true within the industry (North *et al.* 2006b), stocking density was not associated with the fin condition of table fish. Table farmers are often pressurised to produce harvest weight fish in as short a time as possible, and due to market pressures will sometimes ‘push on’ or ‘hold back’ fish by increasing or decreasing feeding accordingly; it is possible that these pressures will affect fin condition, through increased aggression or other social interactions. The second hypothesis concerns restocking farmers selecting fish on the basis of their fin condition, discarding those fish that do not meet the grade, and improving the overall fin quality in a population through selection. Conversely, table farmers will grade fish according to size, frequently using automated graders, and will not select for fin condition. If the second hypothesis is accepted, then there may not be any differences in farming conditions that account for the difference in fin condition. Of course, aspects of both hypotheses may be accepted. Stocking density was found to be associated with fin damage on restocking farms, with an increase in stocking density associated with deteriorating fin condition: this association did not exist for fish farmed for the table market. There is some evidence for increased fin damage at higher stocking densities (Bosakowski & Wagner 1994, Winfree *et al.* 1998, North *et al.* 2006a), however other studies and reviews have failed to establish such a link (Ellis *et al.* 2002, Latremouille 2003, Ellis *et al.* 2008). It has been suggested that any stocking density effect might be mediated through water quality and behavioural interactions (Ellis *et al.* 2008). This study found little evidence for a mediating water quality mechanism and therefore would support the hypothesis that the effect of stocking density on fin damage is mediated through behavioural interactions.

5.5.4 SSI

The spleen is one of the major filtering organs in the body, along with the kidney, and is responsible for removing foreign bodies as well as for the production and storage of erythrocytes (Noga 2006). Many diseases can result in splenomegaly, a marked enlargement of spleen, for example Proliferative Kidney Disease, *Aeromonas* spp., the RTFS agent *Flavobacterium psychrophilum* and haemoparasite infections. The risk factors associated with the SSI were all weak and were dominated by water quality parameters. Increasing DO concentrations and systems provided with oxygenation were associated with larger spleens. Ritola *et al.* (2002) observed an increase in erythrocyte numbers during hyperoxia, and it is possible that this could account for the associated increase in spleen size with higher DO concentrations.

High concentrations of unionised ammonia NH_3 were associated with larger spleens, however the association was ameliorated in systems with large variability in NH_3 concentrations, suggesting that the spleen can tolerate high NH_3 concentrations for short periods. However, there is little evidence that gross changes in spleen size result from high ammonia concentrations (see MacIntyre *et al.* 2008 for a review) although poor water quality has been shown to affect melanomacrophage centres within fish spleens (Agius & Roberts 2003).

A very weak association was observed between fish length and SSI, with larger fish having relatively smaller spleens. This is the opposite of what Wells and Weber (1990) reported, however the association in our study was very weak and may be an artefact. Unionised ammonia and stocking density were associated with larger spleens of fish in raceway systems: it is not known why these associations should occur specifically within raceway systems, although stocking densities in raceways were on average the highest recorded during this study (chapter 4), with high stocking densities possibly leading to higher NH_3 concentrations.

Despite the spleen's intimate involvement in the immune system of fish, and the association between splenomegaly and certain diseases (see chapter 4, Noga 2006), no associations were found between SSI and disease. Splenomegaly is associated with Rainbow Trout Fry Syndrome (RTFS), and the fish in 28% of batches sampled for this study had been exposed to the RTFS at some time (chapter 4), however no association existed between SSI and RTFS. Few batches of fish were exhibiting clinical signs of disease at the time of sampling, and the individuals sampled may not have suffered or have been suffering from the diseases causing splenomegaly

5.5.5 Mortality

Diseases were strongly associated with mortality rates, however, as discussed above, data on diseases recorded presence of the disease in the batch and in many cases this referred to historical previous outbreaks. Only a few batches had clinical signs of disease at the time of sampling. This may indicate that the effect of diseases on a batch of fish extends past the recorded clinical outbreak. The observed persistent effect on mortality may be due other problems arising in populations stressed and weakened by disease outbreaks. (Wendelaar Bonga 1997) combined with unrecorded chronic diseases.

Season was also strongly associated with mortality rates, with higher rates recorded in batches of fish sampled during summer months as previously reported (Roberts 1975, McGurk *et al.* 2006). In addition high feeding and growth rates may combine with lower DO levels to increase mortalities.

In this study triploid fish were associated with lower mortality rates, a finding which disagrees with previous publications (Yamamoto & Iida 1994, Ojolick *et al.* 1995). This was not the purely the result of the higher prevalence of triploid fish farmed for restocking compared with table fish. Neither is there any evidence that triploids are more resistant to diseases (Yamamoto & Iida 1995). The previous studies examined performance under sub-optimal environmental conditions; it is possible that, in this study, conditions were within tolerable ranges for triploid fish and that they outperformed diploid fish in terms of survival.

5.5.6 Welfare Score

The total welfare score, comprised of the welfare scores for plasma cortisol concentrations, fin condition, SSI, monthly mortality rates and the gill condition, was strongly associated with disease and the farming purpose. The number of diseases was associated with poor welfare, as it was with the monthly mortality rate. The strong association between mortality and disease did not mask any other risk factors, as analysis of the welfare score without the mortality component had the same, strong associations. The disease history therefore appears to have a strong association with welfare, or at least the functional welfare indicators measured for this study. Apart from mortality rates, no associations existed between the other components of the welfare score and disease when analysed individually, however when the components of the score were combined, strong associations existed. No trend was observed in the effect of different types of diseases on welfare, whether the causal agent was viral, bacterial or parasitic.

The other significant association for the overall welfare score was the farming purpose, with fish farmed for restocking strongly associated with higher welfare scores. Table fish are generally farmed more intensively than restocking fish (North *et al.* 2006b), however none of the risk factors associated with intensification were significant for the overall score. As with the fin condition score, restocking fish may have better functional welfare because farmers have selected the best fish in a batch and rejected the rest, or alternatively restocking farmers provide better environmental and husbandry conditions and the fish have better welfare. From the predictor variables measured for this study, the data do not provide any other explanation for the association. A weak association was found between large changes in NH_3 concentrations and poor welfare, a finding which agrees with that of Thruston *et al.* (1981a), who reported that rainbow trout tolerated constant concentrations of ammonia better than fluctuating levels

The model and graph in table 5.7 and figure 5.1 illustrate the relationship between the welfare score and condition factor K. It has long been considered that thin fish, with low K, have poor welfare (Goede & Barton, 1990), and that generally welfare improves with increasing K. However, these results indicate that, while fish with larger K have better welfare than those with low K, functional welfare decreases as K increases above the idealised mean of 1.3 used in this study. It is therefore suggested that if condition factor is included as a welfare indicator in future studies that both very high and very low K are both considered to be indications of poor welfare.

Separate analysis of only table market fish did not reveal any other risk factors associated with the welfare score. However, analysis of restocking fish showed that, in addition to the association between disease and welfare, the method of delivering feed was also important. Compared with a demand feeder, all other feeding methods were associated with better welfare. Demand feeders provide a defensible food source, while the other feeding methods distribute food more widely, and it is possible that demand feeders result in the formation of dominance hierarchies, which could have led to increased plasma cortisol levels and fin damage, and thus worse welfare, in restocking fish (McCarthy *et al.* 1992, Kadri *et al.* 1996, Wendelaar Bonga 1997, Adams *et al.* 1998). Dominance hierarchies are more likely to form at lower stocking densities (Bagley *et al.* 1994), as found on restocking farms, and it is suggested that this is the reason that feeding method was identified as a risk factor for restocking and not table fish.

5.6 Conclusions

This study has identified a range of risk factors affecting different functional aspects of welfare in farmed rainbow trout, from associations linked to different system types, feeding methods and environmental conditions. This study has not attempted to prove causal associations between the risk factors and welfare. In an observational on-farm study such as this, where husbandry and environmental conditions were not controlled in any way, causal relationships cannot be established due to the inability to ensure that other factors did not cause the effect on welfare (Martin *et al.* 1987). However, while causation cannot be established, the risk factors identified are linked to welfare and are present when good or bad welfare occurs.

The associations identified between disease, the farming purpose and welfare meet the first of A.B.Hill's tenets of causality, 'strength of association' (Hill, 1965), although further investigation is required to prove causality. For example, disease is unquestionably a cause of poor welfare in a population of fish during an outbreak. What is not clear is if the effects of disease persist in a population after the end of the disease outbreak, causing continuing poor welfare. Disease has been recognised as one of the greatest challenges facing trout farmers in the UK at the current time, with a lack of effective treatments for many diseases (Read 2008, Wall 2008).

The differences between methods of restocking farming and table farming need to be investigated, to determine if welfare on table farms can practically and economically be improved using techniques employed on restocking forms. No association was found in this study between stocking density and welfare, or other risk factors associated with intensification. The length of the production cycle to the table harvest weight of 400g does not appear to be drastically different for restocking and table fish (anon. table and restocking fish farmers, pers. comm.). According to one restocking farmer, the main difference in farming methods is grading, with restocking farmers handling fish with greater care and grading less frequently (anon. pers. comm.).

There is a need for behavioural indicators to be developed that reflect positive experiences in fish (Turnbull *et al.* 2008), and are integrated with functional welfare indicators for clearer picture of how fish perceive their environment. While research has been conducted on dominance hierarchies in fish and the conditions under which the hierarchies form (McCarty *et al.* 1992, Kadri *et al.* 1996, Adams *et al.* 1998, Juell & Fosseidengen 2004), little work has been done on trout behaviour in freshwater systems, aside from Ross *et al.* (1995), with no literature available for trout distribution or behaviour in freshwater cage sites. Information is also lacking on fish behaviour under farm conditions where dominance hierarchies do not form.

The primary focus for this study was to investigate the relationship between water quality and trout welfare. From the results of this study, there is little evidence that water quality is a major welfare problem on UK rainbow trout farms, despite detailed water quality measurements taken. Throughout all the components of the aggregate score there was no systematic evidence that poor water quality was a major problem. Fish farmers work hard to maintain good water quality (Read 2008), with the majority of farms sampled from having water quality within the guidelines set out by Wedemeyer (1996, see table 2.7 chapter 2). The BTA Code of Practice recommends low ammonia concentrations and a minimum DO concentration of 6mg/L (Anon 2002), and there is some evidence that maintaining DO concentrations at this level provides fish with a level of protection against toxic metabolites, such as ammonia (Thruston *et al.* 1981b, North, unpublished data). Poor water certainly has the potential to cause poor welfare (see MacIntyre *et al.* 2008 for a review), however farmers are aware of this (North *et al.* 2008, Read 2008), as it is in their own economic interests to ensure that water quality does not have an impact on production, and farmers use their experience to avoid adverse impacts of water quality deterioration.

5.7 References

Abbott, J.C., Dill, L.M. 1985. Patterns of aggressive attack in juvenile steelhead trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences..* **42**: 1702-1706.

Adams, C.E., Huntingford, F.A., Turnbull, J.F., Beattie, C. 1998. Alternative competitive strategies and the cost of food acquisition in juvenile Atlantic salmon (*Salmo salar*). *Aquaculture*. **167**: 17-26.

Adams, C.E.; Turnbull, J.F.; Bell, A.; Bron, J.E.; Huntingford, F.A. 2007. Multiple determinants of welfare in farmed fish: stocking density, disturbance, and aggression in Atlantic salmon (*Salmo salar*). *Canadian journal of fisheries and aquatic sciences*. **64(2)**: 336-344.

Agius, C., Roberts, R.J. 2003. Melano-macrophage centres and their role in fish pathology. *Journal of Fish Diseases*. **26(9):** 499–509.

Anon. 2002. *British Trout Association Code of Practice*. British Trout Association. pp11.

Appleby, M.C., Hughes, B.O. 1997. *Introduction*. In: Appleby, M.C., Hughes, B.O. (Eds). Animal Welfare. CABI, Oxfordshire. ppxi-xiii.

Bagley, M.J., Bentley, B., Gall, G.A.E. 1994. A genetic evaluation of the influence of stocking density on the early growth of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. **121**: 313-326.

Barrows, F.T., Lellis, W.A. 1999. The effect of dietary protein and lipid source on dorsal fin erosion in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture.* **180**: 167-175.

Bosakowski, T., Wagner, E.J. 1994. A survey of trout fin erosion, water quality, and rearing conditions at state fish hatcheries in Utah. *Journal of the World Aquaculture Society.* **25**: 308-316.

Boyd, C.E. 1990. *Water quality in ponds for aquaculture*. Auburn University, Alabama, USA. pp 482.

Braithwaite, V.A., Boulcott, P. 2007. Pain perception, aversion and fear in fish. *Diseases of Aquatic Organisms*. **75**: 131-138.

Browne, W.J., Draper, D., Goldstein, H., Rasbash, J. 2002. Bayesian and likelihood methods for fitting multilevel models with complex level-1 variation. *Computational Statistics & Data Analysis.* **39**: 203-225.

Chandroo, K.P., Duncan, I.J.H., Moccia, R.D. 2004. Can fish suffer?: perspectives on sentience, pain, fear and stress. *Applied Animal Behaviour Science*. **86**: 225-250.

Dawkins, M.S. 2004. Using behaviour to assess animal welfare. *Animal Welfare.* **13**: S3-7.

Dawkins, M.S. 2006. A user's guide to animal welfare science. *Trends in Ecology & Evolution.* **21(2)**: 77-82.

Duncan, I.J.H., Fraser, D. 1997. *Understanding animal welfare*. In: Appleby, M.C., Hughes, B.O. (Eds). Animal Welfare. CABI, Oxfordshire. pp19-31.

Ellis, T., North, B., Scott, A. P., Bromage, N. R., Porter, M. & Gadd, D. 2002. The relationships between stocking density and welfare in farmed rainbow trout. *Journal of Fish Biology*. **61**: 493-531.

Ellis, T, James, J.D., Stewart, C., Scott, A.P. 2004. A non-invasive stress assay based upon measurement of free cortisol released into the water by rainbow trout. *Journal of Fish Biology*. **65(5)**: 1233-1252.

Ellis, T., Oidtmann, B., St-Hilaire, S., Turnbull, J.F., North, B.P., MacIntyre, C.M., Nikolaidis, J., Kestin, S.C., Knowles, T., Hoyle, I. 2008. *Fin erosion in farmed fish*. In: Branson, E.J. (ed). Fish welfare. Blackwell Publishing, Oxford. pp121-149.

FAWC. 1996. Report on the welfare of farmed fish. Farm Animal Welfare Council, Surbiton, Surrey.

Fraser, D. 1999. Animal eithics and animal welfare science: bridging the two cultures. *Applied Animal Behaviour Science*. **65**: 171-189.

Goede, R.W., Barton, B.A. 1990. Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. *American Fisheries Society Symposium*. **8**: 93-108.

Hill, A.B. 1965. The environment and disease: Association or causation. *Proceedings of the Royal Society of Medicine*. **58**: 295-300.

Hoyle, I., Oidtmann, B., Ellis, T., Turnbull, J., North, B., Nikolaidis, J., Knowles, T.G. 2007. A validated macroscopic key to assess fin damage in farmed rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. **270**: 142-148.

Huntingford, F., Adams, C. 2005. Behavioural syndromes in farmed fish: implications for production and welfare. *Behaviour.* **142(9-10)**: 1207-1221.

Huntingford, F.A., Adams, C., Braithwaite, V.A., Kadri, S., Pottinger, T.G., Sandøe, P., Turnbull, J.F. 2006. Review Paper: Current issues in fish welfare. *Journal of Fish Biology*. **68(2)**: 332-372.

Iwama, G.K. 2007. The welfare of fish. *Diseases of Aquatic Organisms*. **75**: 155-158.

Jones, T.A., Donnelly, C.A., Dawkins, M.S. 2005. Environmental and management factors affecting the welfare of chickens on commerical farms in the United Kingdom and Denmark stocked at 5 densities. *Poultry Science*. **84**: 1155-1165.

Juell, J., Fosseidengen, J.E. 2004. Use of artificial light to control swimming depths of Atlantic salmon (*Salmo salar*) in production cages. *Aquaculture*. **233**: 269-282.

Kadri, S., Huntingford, F.A., Metcalfe, N.B., Thorpe, J.E. 1996. Social interactions and the distribution of food among one sea-winter Atlantic salmon (*Salmo salar*) in a sea cage. *Aquaculture*. **139**: 1-10.

Knowles, T.G., Kestin, S.C., Haslam, S.M., Brown, S.N., Green, L.E., Butterworh, A., Pope, S.J., Pfeiffer, D., Nicol, C.J. 2008. Leg Disorders in Broiler Chickens: Prevalence, Risk Factors and Prevention. *PLoS ONE*. **3(2)**: e1545. doi: 10.1371/journal.pone.0001545

Latremouille, D.N. 2003. Fin erosion in aquaculture and natural environments. *Reviews in Fisheries Science*. **11**: 315-335.

MacIntyre, C.M., Ellis, T., North, B.P., Turnbull, J.F. 2008. *The influences of water quality on the welfare of farmed rainbow trout: a review*. In: Branson, E.J. (ed) Fish welfare. Blackwell Publishing, Oxford. pp150-184.

Martin, S.W., Meek, A.H., Willeberg, P. 1987. *Veterinary epidemiology: Principles and methods.* Iowa State University Press, USA. pp 343.

McCarthy, I.D., Carter, C.G., Houlihan, D.F. 1992. The effect of feeding hierarchy on individual variability in daily feeding of rainbow tout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Biology*. **41**: 239-256.

McGurk, C., Morris, D.J., Auchinachie, N.A., Adams, A. 2006. Development of *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea) in bryozoan hosts (as examined by light microscopy) and quantitation of infective dose to rainbow trout (*Oncorhynchus mykiss*). *Veterinary Parasitology*. **135(3-4)**: 249-257.

Noga, E.J. 2006. *Spleen, thymus, reticulo-endothelial system, blood*. In: Ferguson, H.W. (Ed). Systematic Pathology of Fish, 2nd Ed. Scotian Press.

North, B.P., Turnbull, J.F., Ellis, T., Porter, M.J., Migaud, H., Bron, J., Bromage, N.R. 2006a. The impact of stocking density on the welfare of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. **255**: 466-479.

North, B.P., Ellis, T., Turnbull, J.F., Davis, J., Bromage, N.R. 2006b. Stocking density practices of commercial UK rainbow trout farms. *Aquaculture*. **259**: 260-267.

North, B.P., Ellis, T., Knowles, T., Bron, J.E., Turnbull, J.F. 2008. *The use of stakeholder focus groups to identify indicators for the on-farm assessment of trout welfare.* In: Branson, E.J. (ed) Fish welfare. Blackwell Publishing, Oxford. pp243-267.

Ojolick, E.J., Cusack, R., Benfey, T.J., Kerr, S.R. 1995. Survival and growth of all-female diploid and triploid rainbow trout (*Oncorhynchus mykiss*) reared at chronic high temperature. *Aquaculture*. **131**: 177-187.

Rasbash, J., Steele, F., Browne, W., Prosser, B. 2004. *A user's guide to* MlwiN. University of Bristol. pp256.

Read, N. 2008. *Fish farmer's perspective of welfare*. In: Branson, E.J. (ed) Fish welfare. Blackwell Publishing, Oxford. pp101-110.

Ritola, O., Tossavainen, K., Kiuru, T., Lindström-Seppä, P., Mölsä, H. 2002. Effects of continuous and episodic hyperoxia on stress and hepatic glutathione levels in one-summer-old rainbow trout (*Oncorhynchus mykiss*). *Journal of Applied Ichthyology*. **18**:159-164.

Roberts, R.J. 1975. *The effects of temperature on diseases and their histopathological manifestations in fish.* In: The Pathology of Fishes. Ribelin, W.E., Migaki, G. (Eds). The University of Wisconsin Press, USA. pp 477-496.

Ross, R.M., Watten, B.J., Krise, W.F., DiLauro, M.N., Soderberg, R.W. 1995. Influence of tank design and hydraulic loading on the behavior, growth, and metabolism of rainbow trout (*Oncorhynchus mykiss*). *Aquacultural Engineering.* **14(1)**: 29-47.

Thurston, R.V., Chakoumakos, C., Russo, R.C. 1981a. Effect of fluctuating exposures on the acute toxicity of ammonia to rainbow trout (*Salmo gairdneri*) and cutthroat trout (*S. clarki*). *Water Research*. **15**: 911-917.

Thurston, R.V., Phillips, G.R., Russo, R.C. 1981b. Increased toxicity of ammonia to rainbow trout (*Salmo gairdneri*) resulting from reduced concentrations of dissolved oxygen. *Canadian Journal of Fisheries and Aquatic Sciences.* **38**: 983-988.

Tort, L., Puigcerver, M., Crespo, S., Padrós, F. 2002. Cortisol and haematological response in sea bream and trout subjected to the anaesthetics clove oil and 2-phenoxyethanol. *Aquaculture Research*. **33(11)**: 907-910.

Turnbull, J.F, Richards, R.H., Robertson, D.A. 1996. Gross, histological and scanning electron microscopic appearance of dorsal fin rot in farmed Atlantic salmon, *Salmo salar* L., parr. *Journal of Fish Diseases*. **19**: 415-427.

Turnbull, J.F., Adams, C.E., Robertson, D.A. 1998. Attack site and resultant damage during aggressive encounters in Atlantic salmon (*Salmo salar*) parr. *Aquaculture*. **159**: 345-353.

Turnbull, J.F., Bell, A., Adams, C., Bron, J., Huntingford, F.A. 2005. Stocking density and welfare of cage farmed Atlantic salmon: application of a multivariate analysis. *Aquaculture*. **243**: 121-132.

Turnbull, J.F., Kadri, S. 2007. Safeguarding the many guises of farmed fish welfare. *Diseases of Aquatic Organisms*. **75**: 173-182.

Turnbull, J.F., North, B.P., Ellis, T., Adams, C.E., Bron, J., MacIntyre, C., Huntingford, F.A. 2008. *Stocking density*. In: Branson, E.J. (ed). Fish welfare. Blackwell Publishing, Oxford. pp111-120.

Tuyttens, F., Heyndrickx, M., De Boeck, M., Moreels, A., Van Nuffel, A., Van Poucke, E., Van Coillie, E., Van Dongen, S., Lens, L. 2008. Broiler chicken health, welfare and fluctuating asymmetry in organic versus conventional production systems. *Livestock Science*. **113**: 123-132.

Wall, T. 2008. *Disease control, vaccination and medicine availability related to welfare*. In: Branson, E.J. (ed). Fish welfare. Blackwell Publishing, Oxford. pp195-201.

Wedemeyer, G.A. 1996. *Physiology of fish in intensive culture systems*. Chapman & Hall. pp 232.

Wells, R.M.G., Weber, R.E. 1990. The spleen in hypoxic and exercised rainbow trout. *Journal of Experimental Biology*. **150**: 461-466.

Wendelaar Bonga, S.E. 1997. The stress response in fish. *Physiological Reviews*. **77**: 591-625.

Winfree, R.A., Kindschi, G.A., Shaw, H.T. 1998. Elevated water temperature, crowding, and food deprivation accelerate fin erosion in juvenile steelhead. *Progressive Fish-Culturist*. **60(3)**: 192-199.

Yamamoto, A., Iida, T. 1994. Oxygen consumption and hypoxic tolerance of triploid rainbow trout. *Fish Pathology*. **29**: 245-251.

Yamamoto, A., Iida, T. 1995. Non-specific defense activities of triploid rainbow trout. *Fish Pathology*. **30(2)**: 107-110.

6 General Discussion

6.1 Water Quality

The principal aim of this thesis was to investigate the relationship between water quality and the welfare of farmed rainbow trout. Despite there being calls from non-governmental organisations for the setting of prescriptive limits for certain water quality parameters, *e.g.* Compassion in World Farming (Lymbery 2002), at the conclusion of this study there is no evidence that setting such limits would be practical or would benefit the welfare of farmed trout. In chapter 2, we discussed the setting of prescriptive water quality limits, with 2 main issues highlighted; the standardisation of measurements and the setting of appropriate limits. Standardisation of measurements is a problem that could be overcome if water quality monitoring was taken up industry wide, however there is no scientific basis for the setting of prescriptive water quality limits. As discussed in chapter 2, toxicological studies often give disparate or conflicting recommendations for safe levels, the test conditions bear little resemblance to conditions found on commercial trout farms, and the duration of exposure affects how fish respond to water quality.

The current UK trout industry guidelines for dissolved oxygen (DO) suggest a minimum of 6mg/L at the outflow of a system (Anon 2002). In a study carried out for the Defra AW1205 project (not a part of this thesis), the effects of deteriorating water quality, with high suspended solids and toxic metabolites, were ameliorated by maintaining DO above 5mg/L under experimental conditions (North, unpublished data). This suggests that the current industry guidelines are adequate. While there is no scientific basis for the setting of prescriptive water quality limits, there may in the future be a political basis for such limits, in which case it is suggested that DO should be maintained above 6mg/L and unionised ammonia below 0.02mg/L. At the present time, there is insifficient evidence to set a limit for carbon dioxide. From the results of the epidemiological study (chapters 4 & 5), it does not appear as if poor water quality is a major problem on UK trout farms, as there was no consistent effect of water quality on welfare. Trout farmers are aware that poor water quality can lead to poor welfare, which is against their economic interests, and therefore it appears that generally farmers are maintaining water quality at a level that does not result in poor welfare. However, this finding should not encourage farmers to abdicate from their responsibilities for monitoring the main water quality parameters, arguably DO and temperature. Results from the telephone survey of chapter 3 showed that only 54% of trout farmers measured DO. There is a growing need for farmers to be able to demonstrate that fish are provided with suitable environmental conditions, and with the availability and relatively low cost of DO probes (with thermometers) (chapter 3), it is suggested that all trout farmers should have DO probes and be capable of measuring DO and temperature.

6.2 Disease

It is well recognised that within the UK trout industry, disease is one of the primary factors that can affect fish welfare (North *et al.* 2008, Read 2008, Wall 2008). A disease outbreak within a population is often accompanied by an increase in mortality levels, and while death itself is not a welfare issue, the process of dying is (Wall 2008). The epidemiological study found that poor welfare was associated with disease, irrespective of which disease was involved and how many diseases the population had been exposed to. The association between disease and welfare was not restricted to increased mortality levels in this study. Analysis of the aggregate welfare score without the mortality component was still strongly associated with disease, despite disease not being a risk factor for any of the other components of the welfare score when analysed individually, however when combined a strong association was evident.

6.3 *Farming Purpose*

In the epidemiological study, restocking fish generally had better welfare than table fish. This might have been because restocking fish were provided with better environmental and husbandry conditions more conducive to good welfare, or that restocking farmers selected fish during grading based on the general condition of their body and fins, which would have resulted in a better welfare score in this study. The data were unable to provide any explanations for the association between better welfare and restocking practices. Table fish are generally farmed more intensively than restocking fish (North *et al.* 2006b), however none of the risk factors associated with intensification were significant in this study for the overall welfare score, such as poor water quality, stocking densities, numbers of fish in a unit, biomass in a unit or oxygenation. Other aspects of farming that may differ between table and restocking production and were not recorded for this study are, *inter alia*, feeding rate, specific growth rates over the production cycle, frequency of grading, method of grading (hand versus automated) and if fish are ‘pushed on’ or ‘held back’ to meet market demands. Several fish farmers commented that having to ‘push on’ or ‘hold back’ a batch for retailers resulted in poor welfare (anon. fish farmers, pers.comms). One restocking farmer felt that the main difference between table and restocking fish lay in grading, with restocking fish graded by hand, rather than pumped through an automated system, and being graded less frequently than table fish. Table farmers are often under pressure to produce fish of a specific size, which leads to greater frequency of grading, while restocking farmers often have greater latitude with size of the fish at point of sale.

6.4 Behavioural Strategies

The formation of dominance hierarchies is frequently cited in studies on salmonids (McCarthy *et al.* 1992, Alanärä & Brännäs 1996, Kadri *et al.* 1996, Adams *et al.* 1998, North *et al.* 2006a), where a defensible food source can lead to monopolisation of food by a few individuals. While dominance hierarchies are not desirable for farmers, due to the increased size heterogeneity and reduced growth within a population (Jobling 1995), the relationship between dominance hierarchies, or rather the individual competitive strategies and welfare has yet to be established. Three behavioural strategies have been observed in salmonids within hierarchies; dominant individuals, subdominants and subordinate individuals, categorised by high aggression/high food intake, high aggression/low food intake and low aggression/low food intake respectively (Adams *et al.* 1998). Welfare is concerned with the physical and mental state of an animal: if an animal adopts a dominant competitive strategy, with the attendant aggression, both administered and received, can this be considered to be poor welfare? Functional welfare measures may provide mixed results, with damaged fins and elevated cortisol levels indicating poor welfare, but with good growth, indicating good welfare. However, considering the mental state of the fish, is the animal suffering? Does aggressive dominance behaviour promote positive experiences in fish? After all, it has won the battle for the food resource, albeit at a cost. It is arguable that adopting a subordinate strategy results in poor welfare under functional and feelings-based welfare definitions. Functional welfare will be good with respect to little or no damage to fins from aggressive interactions, but poor with respect to elevated cortisol levels (Wendelaar Bonga 1997) and very poor growth. Under feelings-based definitions, welfare may be poor due to limited accessibility to food, although this would be balanced by limited aggressive interactions. Purely from a welfare perspective, if dominance hierarchies are deemed to result in poor welfare for any of the fish in a population, then husbandry conditions that promote hierarchical formation should be avoided, possibly through wider distribution of food and/or increasing stocking densities.

6.5 On-Farm Welfare Assessment

There are currently no on-farm welfare assessment schemes for rainbow trout in the UK, although it is understood that the RSPCA, through its Freedom Foods scheme, are preparing welfare standards for the UK trout industry (J. Avinezious, RSCPA, pers.comm). In order to improve the welfare of farmed rainbow trout, it will be necessary for farmers to participate in a welfare assessment scheme that seeks to safeguard or improve welfare standards.

On-farm animal welfare can be measured using animal-based (the responses to the environment) parameters and/or resource-based (*i.e.* requirements for good welfare) parameters (Main *et al.* 2003). There is currently no ‘gold standard’ for assessing animal welfare (Spoolder *et al.* 2003), partly due to the various ways that welfare can be defined (Turnbull & Kadri 2007), and therefore different research groups have adopted different assessment methods in terrestrial animals (See Johnsen *et al.* 2001 for a review). Assessment using resource-based parameters only is easier and less time consuming than assessment using animal-based parameters (Spoolder *et al.* 2003), however this approach is only appropriate for welfare assessment when the effects of environmental conditions and husbandry practices on animal welfare are well understood. As has been demonstrated in this and previous studies on fish welfare (e.g. Ellis *et al.* 2002, North *et al.* 2006a), it is difficult to predict fish welfare using resource-based measures. This may be due to the ability of fish to adjust to a variety of environmental challenges (Turnbull & Kadri 2007) through behavioural and physiological adaptations. Animal-based parameters provide the most direct insight into how the animal is coping with its environment. It is therefore suggested that any on-farm welfare assessment scheme contains both animal- and resource-based parameters to ensure that the welfare of fish is safeguarded and important environmental effects on fish are not overlooked.

6.6 Future Work

6.6.1 Welfare assessment

In the epidemiological study for this thesis, a welfare score was developed using functional welfare indicators. While functional welfare is certainly an important aspect of welfare, it has been argued that it is the subjective experiences of the animal that is most important (Dawkins 1997, 2006, Duncan & Fraser 1997, Fraser 1999, Duncan 2006). Assessment of welfare using functional indicators is frequently limited to identifying poor rather than good welfare (Turnbull *et al.* 2008), however the assumption that if the animal is functioning well, then it has good welfare, is not always true, for example if a social animal is denied companionship (Huntingford & Kadri 2008). Assessment of behaviour is necessary to understand if an animal has good welfare, or positive subjective experiences, (Dawkins 2004, 2006).

Dawkins (2004) proposed 2 questions for good welfare, ‘are the animals healthy?’, and ‘do they have what they want?’. Functional welfare indicators can answer the first question, however for the second question; preference testing and behavioural indicators are required. While the assessment of fish behaviour under commercial farm conditions is inherently problematic (Turnbull & Kadri 2007, Turnbull *et al.* 2008), if we want understand fish welfare as fully as possible, then behavioural indicators of welfare are required.

Therefore it is suggested that we should develop behavioural welfare indicators, that reflect both good and bad aspects of welfare, and can be used for assessing the welfare of fish on commercial farms.

6.6.2 Interventions

Whay (2007) identified 3 stages in the process of improving the welfare of farmed animals; 1) assessment of welfare, 2) identification of risk factors, 3) interventions in response to the risk factors. This thesis has contributed to the first 2 stages, providing a method for assessing functional aspects of welfare and identifying risk factors that contribute to poor welfare. Fulfilling the criteria for assessment of welfare has not yet been accomplished, as welfare assessment is lacking behavioural welfare indicators, however, the method employed in this thesis represents the best available knowledge. This thesis identified the primary risk factors relating to welfare of farmed rainbow trout, and in order to drive improvements in fish welfare, interventions are required.

Interventions have been defined as “a systematic attempt to change peoples’ behaviours” (Rutter & Quine 2002 cited in Whay 2007). Improvements in fish welfare can only be brought about if stakeholders in the industry are engaged and motivated to make changes. Awareness of welfare has grown considerably in the past decade with UK trout farmers (Read 2008), who have been active participants in fish welfare research (*e.g.* North *et al.* 2006a, b, Hoyle *et al.* 2007, Ellis *et al.* 2008, this thesis). Within the UK trout industry, a successful intervention affecting the welfare of farmed trout concerned humane slaughter (Read 2008). The FAWC report (1996) highlighted humane slaughter as an area that needed attention, and many stakeholders, including farmers, a government department, retailers, a non-governmental organisation and welfare scientists collaborated to develop a solution that humanely slaughters fish economically and practically (Lines *et al.* 2003, Lines & Kestin 2004, 2005).

The likelihood of an intervention being successful depends on: 1) a person's perception of the severity of the issue, 2) the perceived benefits derived from implementation of the intervention, and 3) the barriers preventing implementation, such as effort, cost, social pressures, likelihood of success, complexity, and sustainability (Whay 2007). Taking the risk factor of disease as an example; for farmers disease is a serious problem (Read 2008), and the benefits of preventing or reducing disease on their farms are numerous and not restricted to improvements in welfare. However, there are barriers to success, not least the current lack of available treatments for many diseases (Wall 2008). In order for any interventions to be successful for the reduction of disease on rainbow trout farms, new treatments would need to be made available to farmers, which would involve scientists and legislators working in collaboration with farmers. The cost and complexity of licensing new treatments is seen as prohibiting (Read 2008) and this is an area where relevant stakeholders could consider making the behavioural or procedural changes necessary to bring about real improvements in fish welfare.

6.7 References

Adams, C.E., Huntingford, F.A., Turnbull, J.F., Beattie, C. 1998. Alternative competitive strategies and the cost of food acquisition in juvenile Atlantic salmon (*Salmo salar*). *Aquaculture*. **167**: 17-26.

Alanärä, A., Brännäs, E. 1996. Dominance in demand-feeding behaviour in Arctic charr and rainbow trout: The effect of stocking density. *Journal of Fish Biology*. **48**: 242-254.

Anon. 2002. *British Trout Association Code of Practice*. British Trout Association. pp11.

Dawkins, M.S. 1997. Why has there not been more progress in animal welfare research? *Applied Animal Behaviour Science*. **53**: 59-73.

Dawkins, M.S. 2004. Using behaviour to assess animal welfare. *Animal Welfare.* **13**: S3-7.

Dawkins, M.S. 2006. Through animal eyes: What behaviour tells us. *Applied Animal Behaviour Science*. **100**: 4-10.

Duncan, I.J.H. 2006. The changing concept of animal sentience. *Applied Animal Behaviour Science*. **100**: 11-19.

Duncan, I.J.H., Fraser, D. 1997. *Understanding animal welfare*. In: Appleby, M.C., Hughes, B.O. (Eds). Animal Welfare. CABI, Oxfordshire. pp19-32.

Ellis, T.E., North, B.P., Scott, A.P., Bromage, N.R., Porter, M., Gadd, D. 2002. The relationships between stocking density and welfare in farmed rainbow trout. *Journal of Fish Biology*. **61**: 493-531.

FAWC. 1996. *Report on the welfare of farmed fish*. Farm Animal Welfare Council, Surbiton, Surrey.

Fraser, D. 1999. Animal ethics and animal welfare science: bridging the two cultures. *Applied Animal Behaviour Science*. **65**: 171-189.

Hoyle, I., Oidtmann, B., Ellis, T., Turnbull, J., North, B., Nikolaidis, J., Knowles, T.G. 2007. A validated macroscopic key to assess fin damage in farmed rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. **270**: 142-148.

Huntingford, F.A., Kadri, S. 2008. *Welfare and fish*. In: Branson, E.J. (ed) Fish welfare. Blackwell Publishing, Oxford. pp19-31.

Jobling, M. 1995. Simple indices for the assessment of the influences of social environment on growth performance, exemplified by studies on Arctic charr (*Salvelinus alpinus*). *Aquaculture International*. **3(1)**: 60-65.

Johnsen, P.F., Johannesson, T., Sandøe, P. 2001. Assessment of farm animal welfare at herd level: Many goals, many methods. *Acta Agriculturæ Scandinavica Section A-Animal Science*. **Suppl. 30**: 26-33.

Kadri, S., Huntingford, F.A., Metcalfe, N.B., Thorpe, J.E. 1996. Social interactions and the distribution of food among one sea-winter Atlantic salmon (*Salmo salar*) in a sea cage. *Aquaculture*. **139**: 1-10.

Lines, J.A., Robb, D.H., Kestin, S.C., Crook, S.C., Benson, T. 2003. Electric stunning: a humane slaughter method for trout. *Aquacultural Engineering*. **28(3-4)**:141-154.

Lines, J., Kestin, S. 2004. Electrical stunning of fish: the relationship between the electric field strength and water conductivity. *Aquaculture*. **241**: 219-234.

Lines, J., Kestin, S. 2005. Electric stunning of trout: power reduction using a two-stage stun. *Aquacultural Engineering*. **32**: 483-491.

Lymbery, P. 2002. *In too deep – the welfare of intensively farmed fish*. A Compassion in World Farming report, Hampshire, UK.

Main, D.C.J., Kent, J.P., Wemelsfelder, F., Ofner, E., Tuyttens, F.A.M. 2003. Applications for methods of on-farm welfare assessment. *Animal Welfare*. **12**: 523-528.

McCarthy, I.D., Carter, C.G., Houlihan, D.F. 1992. The effect of feeding hierarchy on individual variability in daily feeding of rainbow tout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Biology*. **41**: 239-256.

North, B.P., Turnbull, J.F., Ellis, T., Porter, M.J., Migaud, H., Bron, J., Bromage, N.R. 2006a. The impact of stocking density on the welfare of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. **255**: 466-479.

North, B.P., Ellis, T., Turnbull, J.F., Davis, J., Bromage, N.R. 2006b. Stocking density practices of commercial UK rainbow trout farms. *Aquaculture*. **259**: 260-267.

North, B.P., Ellis, T., Knowles, T., Bron, J.E., Turnbull, J.F. 2008. *The use of stakeholder focus groups to identify indicators for the on-farm assessment of trout welfare.* In: Branson, E.J. (ed) Fish welfare. Blackwell Publishing, Oxford.

Read, N. 2008. *Fish farmer's perspective of welfare*. In: Branson, E.J. (ed). Fish welfare. Blackwell Publishing, Oxford. pp101-110.

Rutter, D., Quine, L. 2992. *Social cognition models and changing health behaviours*. In: Rutter, D, Quine, L. (eds). Changing health behaviour. Open University Press, UK.

Spoolder, H., De Rosa, G., Hörning, B., Waiblinger, S., Wemelsfelder, F. 2003. Integrating parameters to assess on-farm welfare. Animal Welfare. 12: 529-534.

Turnbull, J.F., Kadri, S. 2007. Safeguarding the many guises of farmed fish welfare. *Diseases of Aquatic Organisms*. **75**: 173-182.

Turnbull, J.F., North, B.P., Ellis, T., Adams, C.E., Bron, J., MacIntyre, C., Huntingford, F.A. 2008. *Stocking density*. In: Branson, E.J. (ed). Fish welfare. Blackwell Publishing, Oxford. pp111-120.

Wall, T. 2008. *Disease control, vaccination and medicine availability related to welfare*. In: Branson, E.J. (ed). Fish welfare. Blackwell Publishing, Oxford. pp195-201.

Wendelaar Bonga, S.E. 1997. The stress response in fish. Physiological Reviews. **77**: 591-625.

Whay, H.R. 2007. The journey to animal welfare improvement. *Animal Welfare*. **16**: 117-122.