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SID 5 Research Project Final Report

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Project identification

1. Defra Project code: AW1205
2. Project title: The interaction between water quality and welfare in farmed rainbow trout
3. Contractor organisation(s): University of Stirling, Stirling, Scotland, UK, FK9 4LA
4. Total Defra project costs (agreed fixed price): £ 295,209.00
5. Project: start date .... 01 April 2004
   end date .... 31st March 2008

6. It is Defra's intention to publish this form.
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Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

Conclusions of the project

This project used a wide range of research approaches to study the relationship between water quality (WQ) and trout welfare. We confirmed that poor WQ can have a negative effect on the health and welfare of farmed trout (Obj 1, 3 & 4). Trout farmers generally had systems and procedures in place to monitor and maintain WQ; these are largely based on guidelines and past experience but are also dependent on the competence of the farm staff (Obj 2, 3, 5 & 6). A large epidemiological study of farmed trout (Obj. 5) indicated that WQ on commercial trout farms rarely deteriorated to levels which would have a negative effect on the welfare of the fish, although episodic events such as droughts or algal blooms may have a detrimental effect (Obj 2). In acidic water conditions very few negative effects were observed even at high loading rates, providing dissolved oxygen was maintained (Obj 4). Under those conditions WQ had to deteriorate to potentially lethal concentrations before there were detectable effects on welfare (Obj 4). Levels of WQ parameters that can negatively influence welfare vary according to the farming system, other aspects of water chemistry and the previous experience of the fish (Obj 1). However, our results indicate that monitoring (*e.g.* DO, ammonia, pH and temperature) and controlling (*e.g.* DO and ammonia) a limited number of WQ parameters may safeguard many aspects of trout welfare (Obj 1, 2, 4 & 5). The British Trout Association Code of Practice recommend a minimum DO concentration of $6 mg.l^{-1}$, our results suggest this is adequate to safeguard many aspects of welfare affected by WQ. There was agreement among stakeholders that fish based indicators combined with practical experience are the best way to monitor the welfare of fish (Obj 3 & 6). There is still the need for schemes to maintain and improve farmer competence along with adequate record keeping and documentation (Obj 3 & 6). There is also the need for more robust guidelines on behavioural indicators of fish welfare (Obj 3 & 6). This project has produced a very wide range of outputs which have already been widely disseminated and discussed with UK and European stakeholders. The research has also produced robust information on the relationship between WQ and farmed trout welfare which could inform policy, legislation and guidelines.

All milestones (some with agreed adjustments) were met in full and on time. The outputs and findings of the specific scientific objectives are summarised below.

01 A literature review of information relating to WQ and welfare (Published paper in Appendix 1)
A review of literature concerning water quality and its role on the welfare of farmed rainbow trout was conducted and completed in March 2005. It concluded that the levels of WQ parameters that can negatively influence welfare vary according to the farming system, other aspects of water chemistry and the previous experience of the fish. However, it is likely that monitoring or controlling a limited number of WQ parameters may safeguard many aspects of trout welfare. A copy of the literature review received approval from Defra and was included in the proceedings of the 1st Fish Welfare Conference of the Fish Veterinary Society which were published in 2008 (*Fish Welfare:* Edited by E. Branson).

02 Description of current status of WQ monitoring and control on farms (Draft manuscript in Appendix 2)
This objective was divided into two phases with initial telephone interviews, followed by farm visits. The telephone interviews of rainbow trout farmers produced an up-to-date list of active farms willing to

participate in Objective 5. The list also proved to be valuable for the selection of suitable sites for AW1204. In addition to collecting data on a wide range of site characteristics and production practices for UK trout farms, this exercise improved communication with farmers and informed them of the project and its aims and objectives. The work demonstrated that the most commonly monitored WQ parameters are temperature and dissolved oxygen with pH being measured less frequently. Most on-farm measurements appear to be reasonably accurate and monitoring is generally only conducted when it is deemed necessary from previous experience. Monitoring by the EA and SEPA may have some value in welfare monitoring and episodic events such as spates or algal blooms must also be considered when assessing the interaction between WQ and fish welfare.

A summary of the work conducted in this objective was appended to the 2005-06 annual report and a manuscript is nearing completion.

03 Focus groups to discuss potential indicators of welfare (Published paper in Appendix 3)
A series of focus groups were conducted with stakeholders involved in the UK trout farming industry. Stakeholders were drawn from fish farmers, fish veterinarians, animal welfare organisations, retailers, academics and consumers. The aims of this exercise were to explore criteria that could be used to evaluate fish welfare, identify stakeholders' concerns regarding the welfare of farmed trout and enhance participation and ownership of the project. It is difficult to summarise briefly the detailed opinions of so many stakeholders but it is possible to say that there was a large amount of agreement between the various stakeholder groups. The process of stakeholder consultation in collaboration with the Fish Veterinary Society was a highly significant improvement over previous dialogue between groups with conflicting agendas.

A detailed report of this activity was provided with the 2005-06 annual report. A more concise version of the report is included as a book chapter in the proceedings of the 1st Fish Welfare Conference of the Fish Veterinary Society meeting (jointly co-ordinated by this project under Objective 03 and published in 2008).

04 A tank based study to examine the effects of deteriorating WQ on various indicators of welfare (Report in Appendix 4)
This study aimed to reproduce the complex deterioration in WQ that occurs under farming conditions in a controllable system which minimised the social effects of high stocking density. It was envisaged that the experiments would provide a realistic means of obtaining robust data on the effects of deteriorating WQ on specific welfare indicators. The main experiment in this objective used 4 replicated cascades each consisting of 5 tanks where water was pumped from one tank to the next. Subsequently through the involvement of an MSc student the scope was expanded to include static water tank experiments to investigate very severe deterioration in WQ.

The systems had low pH, low suspended solids and dissolved oxygen was maintained above critical levels at all times. These experiments demonstrated that despite very high loading rates, exceeding 3kg/l/min, there was very little evidence of a negative effect on welfare. These experiments have shown that the most sensitive and quantifiable indicators of WQ associated with poor WQ are gill pathology, growth and indices of condition with plasma cortisol.

These experiments also played a role allowing the sampling protocols for Objective 5 to be refined. A summary of the work conducted in this objective was appended to the 2005-06 annual report and a manuscript is being prepared for publication.

05 Farm based epidemiological studies of relationship between water quality and indicators of welfare (Draft manuscripts in Appendix 5 and 6)
This study aimed to establish the role of WQ, farming system and management factors in the welfare of farmed rainbow trout. 44 farms were visited at two times of the year with 189 units and 3700+ fish individually assessed. Multilevel modelling techniques were used to investigate the intricate relationships between farm management, rearing system and WQ variables and fish based measures of welfare.

A range of measurements were taken from sample fish, from which 5 were selected as being relevant to welfare. These welfare indicators were the condition of the fins, the blood cortisol concentrations (an indicator of the stress response), condition of the gills, the condition of the spleen (an indication of the disease state of the fish) and the population mortality rate. The 5 welfare indicators were combined into a welfare score and analysed using multilevel modelling techniques.

This study established that WQ, within the limits recorded, is not a major problem for the welfare of farmed rainbow trout in the UK. Generally, the quality of water throughout the farms investigated was considered satisfactory (92% of farming systems measured had dissolved oxygen > $5 mg.l^{-1}$). Trout farmers recognise the importance of WQ to the welfare of fish and the success of their business, therefore will work hard to maintain WQ at a level conducive to good welfare. However, exposure to disease was identified as highly detrimental to the welfare of farmed rainbow trout, and the primary risk factor for poor welfare in this study. The study also identified that changes to certain husbandry and management practices may bring about improvements to the welfare of farmed rainbow trout.

A full description of this study is being prepared as two manuscripts for publication.

06 Stakeholders' workshop / technology transfer (Draft manuscript in Appendix 7)
This objective was intended to disseminate the findings of the project, to discuss the identified risks to welfare from WQ and to agree key auditable welfare measures. The scope was further expanded to identify future priorities for farmed fish welfare.

The workshop was conducted with Defra's support at the Innovation centre, Reading in November 2007. All major stakeholder groups involved in fish welfare attended. The results of AW1205 were disseminated in poster format and discussed by stakeholders as part of the participation exercises that were conducted. All participants were extremely positive after the workshop and consensus between all groups was achieved by the end. The workshop resulted in a prioritised list of actions for farmed fish welfare as well as practical solutions for addressing those priorities. Stakeholders were also asked to take responsibility for addressing the priority areas. These not only included researchable constraints (e.g. centrally collated mortality data and behavioural welfare indictors) but also necessity for training.

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:

- the scientific objectives as set out in the contract;
- the extent to which the objectives set out in the contract have been met;
- details of methods used and the results obtained, including statistical analysis (if appropriate);
- a discussion of the results and their reliability;
- the main implications of the findings;
- possible future work; and
- any action resulting from the research (e.g. IP, Knowledge Transfer).

Introduction

Evidence from a variety of sources including the FAWC report (1996) and previous Defra project AW1203, suggested that water quality (WQ) is a better predictor of welfare than management parameters, which only influence welfare indirectly (*e.g.* stocking density). The main aim of this project was to investigate the interaction between WQ and trout welfare and provide the scientific information necessary to incorporate WQ into a system for monitoring and auditing fish welfare on trout farms.

Additional information

Prior to the start of the project the British Trout Association (BTA) endowed the Niall Bromage Ph.D. studentship which was awarded to Mr Craig MacIntyre. This studentship was conducted in parallel with the Defra project and was effectively additional industry funding for the project. This additional funding, which was discussed with Defra prior to submission of the project proposal allowed the scope of the project to be expanded. Due to the nature of the project it is impossible to define those aspect of the work resulting from BTA funding and those from Defra funding and therefore outputs are considered and reported together.

Scientific objectives and extent to which they have been met.

1 A literature review of information relating to water quality and welfare.

A detailed review of this complex and contradictory body of literature will be conducted and related to current trout farming practice in the UK.

Completion milestone 01/03/05. Completed in full and on time.

2 Description of current status of water quality monitoring and control on farms.

The activities leading to this objective are intended to be a preparatory phase for Objective 05, providing information regarding the current nature of the industry essential to the planning of Objective 05 and developing the necessary links with farm staff. Objective 02 will involve a large number of farms, from which the 60 required for Objective 05 will be recruited.

Completion milestone 01/03/05. Completed in full and on time.

3 Focus groups to discuss potential indicators of welfare.

A series of focus groups will be held to explore the criteria that are used to evaluate welfare by stakeholders in trout farming. The participants will be drawn from farmers, animal welfare organisations, fish veterinarians, retailers, consumers and academics. The output will be a list of widely acceptable and measurable welfare indicators to be used in parallel with existing measures of damage, physiological stress response and production.

Completion milestone 01/04/05. Completed in full and on time.

4 A tank based study to examine the effects of deteriorating water quality on various indicators of welfare.

Replicated cascades (series of experimental tanks), will provide robust data on the effect of deteriorating water quality, on specific welfare indicators independent of other influences on welfare. This approach is suggested as an alternative to examination of individual water quality parameters in isolation, which has little relevance to farm conditions. Such information will allow more insightful analyses of subsequent farm data.

Completion milestone 01/07/05. Completed in full and on time.

5 Farm based epidemiological studies of relationship between water quality and indicators of welfare.

An epidemiological approach will be used to record water quality and welfare indicators on at least 240 systems on 60 farms representing the diversity of the industry throughout the year. Data loggers with multi-probes (pH, temperature, DO, ammonia and turbidity) will be used to monitor water quality over 24-hour periods in each system. A raft of previously identified welfare indicators (Objectives 01, 03 and 04) will also be recorded. Other data will be collected including any farm records of water quality and husbandry procedures prior to the sampling period. In addition 24-hour water samples will be collected on a sub-sample of systems to validate the probe records and measure parameters outwith the capacity of the probes (e.g. CO_2, NO_2, NO_3).

Completion milestone 01/10/07. Completed in full and on time.

6 Stakeholders' workshop / technology transfer.

The project will conclude with a stakeholders' workshop to discuss the identified risks to welfare from water quality and agree key auditable welfare measures. It is envisaged that this may subsequently lead to a Hazard Analysis and Critical Control Point (HACCP) and/or Hazard Analysis and Risk Assessment (HARA) approach to the control of welfare through water quality and other influences. The implementation of such a system is not an

appropriate objective for this research project, but is being implemented in other species by collaborators from Bristol.
Completion milestone 01/11/07. Completed in full and on time.

End of project 31/4/08
Submission of final report 30/6/08

Methods and results

Obj 1 A literature review of information relating to water quality and welfare

"A detailed review of this complex and contradictory body of literature will be conducted and related to current trout farming practice in the UK".

A review of literature concerning WQ and its role on the welfare of farmed rainbow trout was conducted and completed in March 2005. This review received overall approval from Defra and was included in the proceedings of the 1st Fish Welfare Conference of the Fish Veterinary Society which were published in 2008 (*Fish Welfare:* Edited by E. Branson). The abstract of the literature review is shown below and the full review is attached as Appendix 1.

Literature review abstract:
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. 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.

The water quality parameters which have the greatest potential to affect the health of fish are primarily those over which the farmer can exert a degree of control, *i.e.* dissolved oxygen, carbon dioxide, ammonia, nitrite and nitrate, suspended solids and gas supersaturation. Parameters which are largely outwith the control of the farmer (after the location of the farm has been confirmed) include acidity, alkalinity, temperature, hardness and heavy metals.

The concentration of dissolved oxygen in the water is widely recognised as the most important water quality parameter. Existing recommendations for minimum dissolved oxygen levels relate mostly to avoidance of effects on health and productivity. Recommended minima of 5-6 $mg.l^{-1}$ are widely cited, but levels up to 9 mg/L have been suggested for salmonids. This wide range reflects the influence of temperature which controls both the concentration of oxygen water can hold and the metabolic rate of the fish.

Ammonia is a toxic metabolite produced by fish and reported chronic effects include growth suppression, ion imbalances and increased fin erosion. Although gill damage is a commonly cited effect of ammonia, it is unclear if and how ammonia causes gill damage. Despite the large amount of literature available, estimates of lethal concentrations and safe values vary greatly between studies. This is because the effects of ammonia are strongly influenced by other water chemistry factors (*e.g.* pH, temperature, ionic strength and dissolved oxygen), and the size of the fish and history of previous exposure. A maximum safe level of 0.02 $mg.l^{-1}$ is widely quoted, although as other recommendations vary by a factor of 20, some researchers have concluded that it is not possible to recommend a maximum safe concentration.

Ammonia is oxidised to nitrite, which is also a toxic substance. Nitrite is actively taken up by fish, which can result in internal concentrations far greater than the water concentrations. Nitrite affects the ability of fish to transport oxygen in the body, and severe nitrite exposure is characterised by the blood turning brown. The current recommended maximum concentration of nitrite is 0.1 $mg.l^{-1}$, although the toxic effects of nitrite are mediated by the concentration of chloride ions in the water.

Carbon dioxide is produced by fish during respiration, and in high concentrations can be toxic. Recommendations for safe levels vary between 9 and 30 $mg.l^{-1}$, with other water chemistry parameters, such as pH, alkalinity and dissolved oxygen, affecting toxic concentrations.

Suspended solids have the potential to reduce the health status of farmed fish, yet the literature on this subject is sparse. Recommendations for safe levels vary greatly, with a concentration of 100 $mg.l^{-1}$ suggested, although there is evidence that levels as low as 40 $mg.l^{-1}$ can result in damage to fish gills. Variation in the size, shape and material of the particles are thought to explain the differences in safe levels between studies.

Water alkalinity and hardness have been shown to have protective qualities in many studies, primarily through the buffering action against large pH changes. Low pH or acidic water can have deleterious effects on the transport of oxygen around the body. Water temperature affects the toxicity of other substances, the metabolic rate of fish, the physical characteristics of water, and microbes which can cause disease or reduce the oxygen in the water.

In conclusion, there are contradictions in recommended safe levels for all key water quality parameters due to interdependence of toxic levels with a wide variety of other parameters. There is also a lack of reliable data from 'real' aquaculture situations, with many toxicity studies conducted under controlled conditions very different from those on commercial trout farms. Water quality limits could be introduced for some parameters, although the selection of limits would represent pragmatism rather than best scientific practice. Further research may enable more appropriate limits to be prescribed based on categorisation of water type (*e.g.* acid, alkaline or neutral, soft or hard water). Detailed standardised protocols for measurement of these parameters suitable for fish farmers would need to be developed. Additionally, limits for routine exposure and specific events (*e.g.* transport, grading) would need to be differentiated. An alternative or complementary method for safeguarding welfare would be to require farmers to screen for fish health and disease indicators of poor water quality, and use the information and their experience to introduce and adapt farm-based management plans for their local inflow systems and water.

Obj 2 Description of current status of water quality monitoring and control on farms

"The activities leading to this objective are intended to be a preparatory phase for Objective 05, providing information regarding the current nature of the industry essential to the planning of Objective 05 and developing the necessary links with farm staff".

This objective was completed in July 2005 after a Defra approved amendment to the original completion milestone. The milestone amendment was requested to allow the co-ordination of activities undertaken in the parallel fin erosion project (AW1204). A full report of this objective is attached as Appendix 2.

The questions addressed by this objective

1. What (if any) WQ parameters are currently measured under existing farm practice?
2. What is the accuracy of WQ monitoring equipment on farms compared with laboratory calibrated equipment?
3. How often the farms are visited by the EA or SEPA?
4. What are the normal ranges of key WQ parameters for particular sites (EA or SEPA)?
5. What is the occurrence of historical WQ problems *e.g.* failed EA/SEPA inspections, spate, algal blooms *etc*?

Methods

A list of UK trout farmers was compiled from the records of a previous DEFRA project (AW1203), the BTA membership list, www.intrafish.com and the Yellow Pages.

Farmers were contacted by telephone when the aims of the project were described and basic questions posed about the individual farms' operations and WQ recording. Farms were then categorised and screened for suitability for inclusion in the project. It was decided that only rainbow trout (*Oncorhynchus mykiss*) would be investigated and that farms that were solely fisheries would be excluded from the project, given the extensive nature of such operations and lack of fish rearing facilities.

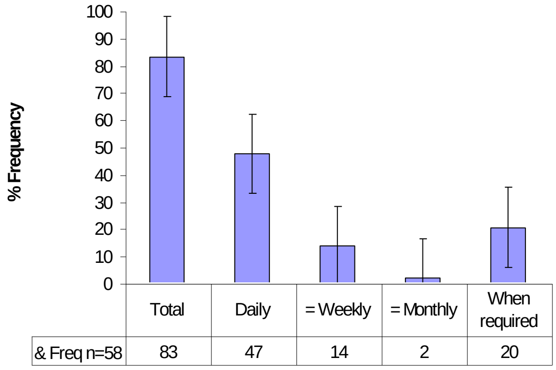
109 farms were considered suitable for the project, of which 107 expressed an interest in further participation. The 109 suitable farms constituted approximately 80% of UK rainbow trout production by weight.
Farm visits were made to 58 farms to collect more detailed information on farming practices and to compare the on-farm WQ monitoring equipment with regularly calibrated laboratory equipment.

Results:

1. What WQ parameters are currently measured under existing farm practice?

Dissolved Oxygen: 56% of farmers contacted by telephone and 83% of farmers visited measured DO. A possible reason this discrepancy is that farm visits were primarily made to large, commercial farms, whilst telephone contact was made with every farmer possible. During farm visits, information was gathered on the frequency that DO measurements were taken (Fig. 1).

Temperature: 62% of farmers contacted by telephone and 90% of farmers visited measured temperature. The frequency with which temperature measurements were taken is shown in Fig. 2. Other Parameters: DO and temperature were the most commonly measured WQ parameters. 11% of farmers visited measured pH and 10% recorded water flow.



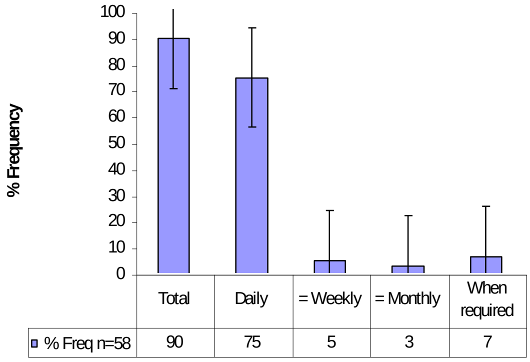


Figure 1. Frequency of DO measurements taken by farmers

Figure 2. Frequency of DO measurements taken by farmers.

2. What is the accuracy of WQ monitoring equipment on farms compared with calibrated laboratory equipment?

During the visits, farm WQ monitoring equipment was compared with regularly calibrated equipment. Unfortunately, many farmers do not take measurements until June/July, and at the time of the visits much of the equipment was out of use and not calibrated. However, measurements were taken from 23 of the 59 farms visited (Table 1).

Table 1 Comparison of farm water quality equipment and regularly calibrated laboratory equipment.

Water quality parameter	Deviation from lab equipment	Number of measurements	% Frequency					
Dissolved oxygen	<0.5mg/l	21	50	Mean	-0.41	Min.	-3.2	n=42
	<1.0mg/l	11	26	Median	-0.002	Max.	1.4	
	>1.0mg/l	10	24	St. Dev	1.14			
Temperature	<1^{o}C	32	91	Mean	0.13	Min.	-1.2	n=35
	<2^{o}C	3	9	Median	0	Max.	1.6	
	>2^{o}C	0		St. Dev	0.49			
pH	Only one farm visited measure pH- farm and laboratory equipment agreed							

3. How often the farms are visited by the EA or SEPA?

Of the farms visited during the project 61% were visited by the EA or SEPA monthly, 22% every 6 weeks to 2 months, 12% every 6 months and 5% annually. Farms visited by the EA or SEPA every 6 months or annually were generally cage sites situated on lochs. Most farmers (93%) received notification of the results of their inspection.

4. What are the normal ranges of key WQ parameters for particular sites (EA or SEPA)?

The WQ information collected by the EA and SEPA was made available but the quality of this information varied considerably and was not considered suitable for analysis. However, EA and SEPA data from 2 farms were used to validate and compare the results collected from our multi-parameter WQ data loggers (sondes) during Obj 5.

Data comparing the WQ values measured from a farm in the south of England (Table 2) and a farm in Scotland (Table 3) are shown below.

Table 2 Comparison between EA/SEPA water quality data and data collected using sondes continuously over 24 hours.

EA Data		**Our Data**
08/10/2004	**Date**	06/10/2005
11.18	**Temperature**	12.39
10.1	**DO (mg/L)**	10.55
7.99	**pH**	7.95
0.141	**Total Ammonia**	0.08
0.00274	**Ammonia (NH3)**	0.0016
9.3	**Susp. Solids**	5

SEPA Data		**Our Data**
27/08/2002	**Date**	03/08/2005
14.4	**Temperature**	14.31
7.9	**DO (mg/L)**	8.28
7.2	**pH**	7.1
102	**Elec Cond**	164
0.49	**Ammonia**	0.26
2	**Susp. Solids**	0

The high level of agreement in the results of the two measurements indicates that the data logging sondes used in Obj 5 were producing results comparable to the WQ measured by SEPA or the EA.

5. What is the occurrence of historical WQ problems e.g. failed EA/SEPA inspections, acid run-off, algal blooms etc?

40% of farmers had failed EA/SEPA inspections. The majority of failures related to increased levels of suspended solids. However, the EA and SEPA pass/fail levels are intended for the health of biota downstream of the fish farm and a fail due to suspended solids may not adversely affect the health of the farmed fish.

23% of farmers had suffered losses due to flooding, while 35% of farms had suffered drought. A further 8% of farms had experienced problems from algal blooms, and 20% from pollution incidents, although most pollution incidents related to upstream pollution such as that from forestry or agriculture practises.

Concluding comments
In addition to the data collected, this component of the project was important in establishing relations with the trout farmers. This was particularly important for the farm based epidemiology study under Scientific Obj 5.

Obj 3 Focus groups to discuss potential indicators of welfare

"A series of focus groups will be held to explore the criteria that are used to evaluate welfare by stakeholders in trout farming. The participants will be drawn from farmers, animal welfare organisations, fish veterinarians, retailers, consumers and academics. The output will be a list of widely acceptable and measurable welfare indicators to be used in parallel with existing measures of damage, physiological stress response and production".

This objective was completed in April 2005 after a Defra approved amendment to the original completion milestone.

In total three series of focus group meetings were held, with representatives from a range of stakeholder groups represented (Table 4).

Table 4. Stakeholders, venues and dates of focus group meetings

Stakeholders	Venue	Date
Trout farmers	British trout farming conference, Sparsholt College, Winchester	Sept. 2004
Fish veterinarians, academics, farmers, governmental and NGO's representatives	Fish Vet Society meeting, Edinburgh	Nov. 2004
Retailers, governmental and NGO's representatives	Novartis House, London	Jan. 2005

The report detailing the discussions and outputs from the focus group meetings was an invited chapter in the proceedings of the 1st Fish Welfare Conference of the Fish Veterinary Society, which were peer reviewed and published in 2008 (*Fish Welfare:* Edited by E. Branson). The abstract of that chapter is shown below. The detailed report is included as Appendix 3.

Focus group meetings report abstract:
This chapter summarises the findings of a series of focus and discussion groups that were conducted with stakeholders in the UK trout farming industry, as part of the Defra funded project investigating the interaction between water quality and welfare in farmed rainbow trout. Stakeholders were drawn from fish farmers, fish veterinarians, animal welfare organisations, retailers, academics, and representatives from governmental and non-governmental; organisations. The aims of this exercise were to explore the various criteria that could be used to evaluate fish welfare, and to identify stakeholders' concerns regarding the welfare of farmed trout. Although the main focus of this summary relates specifically to indicators of trout welfare, in many respects the work was more far-reaching with both discussion topics and stakeholders' experience extending into other areas of commercial aquaculture and other livestock industries.

It was recognised that selection of the most appropriate indicators would largely depend on the context and purpose of the assessment. Possible uses for a system of welfare assessment would include auditory or regulatory purposes, forming the framework for risk assessments (*e.g.* Hazard Analysis and Critical Control Points [HACCP], Hazard Analysis and Risk Assessment [HARA] systems), and for Quality Assurance and accreditation schemes. However, there was a high level of agreement between the various focus groups regarding the most important parameters for assessing welfare, which were grouped under the following headings: operational welfare indicators (OWI), environmental quality, farm records, targeted sampling of fish, demonstration of good stockmanship and harvest-based measures.

Transportation of fish was seen to be in need of regulation and the lack of effective licensed disease treatments was seen to represent a major challenge to fish welfare. The need to communicate any measures that are taken to safeguard or promote fish welfare to the consumer was considered to be important in making improved welfare economically sustainable. Within the remit and constraints of the present project it was not possible to carry out consumer focus groups, but considering the increasing profile of fish welfare and the level of investment in research directed towards its study, research into consumer opinions of fish welfare would be beneficial.

Obj 4 A tank based study to examine the effects of deteriorating water quality on various indicators of welfare

"Replicated cascades (series of experimental tanks), will provide robust data on the effect of deteriorating water quality, on specific welfare indicators independent of other influences on welfare. This approach is suggested as an alternative to examination of individual water quality parameters in isolation, which has little relevance to farm conditions. Such information will allow more insightful analyses of subsequent farm data".

Objective 04 was completed by its target milestone completion date of July 2005. The scope of this objective was expanded by the incorporation of an MSc. project student to include an investigation into the effects static water conditions on fish welfare. The experiments conducted in this objective are outlined below with a more detailed report provided in Appendix 4.

Experiment 1: Cascade experiment to simulate WQ deterioration caused by high loading rates and water reuse.

Methodology

The experiment was conducted between October 2004 and July 2005. Four replicated cascades, where water was pumped from one tank to the next (see Fig. 3) of five 0.3 m^3 tanks were stocked with rainbow trout (n = 120; mean weight = 53.4 g; stocking density = 21.4 $kg.m^{-3}$). Thirty fish from each tank were PIT tagged.

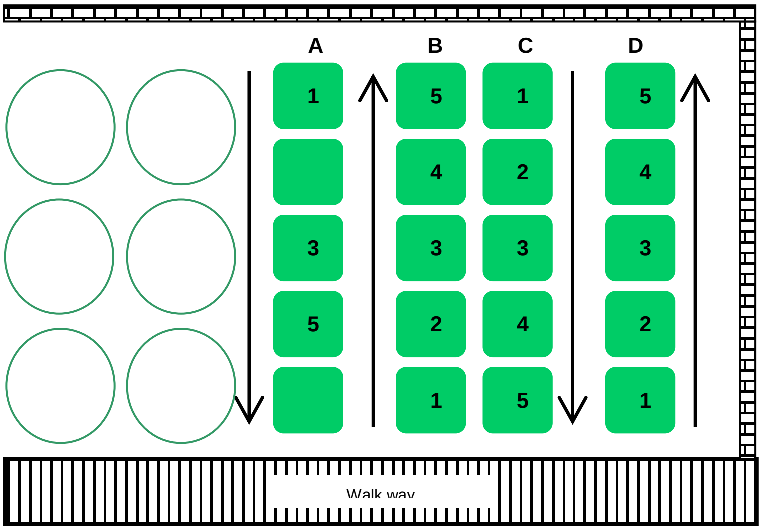


Figure 3. The layout of experimental tanks. Arrows show the direction of water movement.

Submersible pumps were used to move water along the cascade with the flow adjusted to provide an incoming water flow approximately 2 $L.min^{-1}$ greater than water removal to the following tank. The excess waste water was removed via the standpipe. During the last 3 weeks of the experiment the direction of water flow in the cascades was reversed to investigate the potential acclimatisation of fish to the relative water conditions.

The bottom tank (tank 5) in each cascade was fitted with an oxygen monitoring system. Additional aeration was supplied to tank 1 to maintain DO >5 $mg.l^{-1}$ in tank 5, thus ensuring that fish were not inappropriately exposed to low oxygen levels. WQ parameters (*i.e.* pH, temperature, DO, ammonia, and conductivity) were continuously monitored using multi-parameter sondes. One sonde was allocated to each cascade and moved between tanks every 24 hours. Water samples were taken each month to validate the sondes and to measure parameters out with the capacity of the probes (*i.e.* suspended solids, CO_2, NO_2^-, NO_3^-). A 24h parallel monitoring session was also carried out to provide further cross-validation of the sondes' accuracy.

Sampling occurred at monthly intervals. Weight and length was recorded from PIT tagged fish and all rayed fins were measured at the beginning, middle and end of the experiment. A further 10 untagged fish from each tank were sacrificed for welfare assessment with the following parameters measured: length, weight, condition factor, fins lengths of all rayed fins, liver, gut, and spleen weight, blood samples for the analysis of cortisol, lysozyme activity, glucose and haematocrit, and gill samples for assessing pathologies. Feed intake was measured using x-radiography of fish supplied food containing radio-opaque beads.

Results

Water temperature ranged from 1.9°C in February to 16.8°C in July. The incoming water supply was slightly acidic (pH=6.3-6.7) and very soft, with a specific conductivity ranging from 0.04 to 0.05 mS.cm^{-1}. WQ generally deteriorated along each cascade. DO decreased along each cascade and there was a build up in ammonia. An example of the levels observed is provided in Fig. 4.

Oxygen had to be supplemented from October 2004 until mid November and again from mid March until July. During peak water temperatures additional oxygenation and aeration was supplied to tanks 1 and 2 to maintain DO at >5mg.l^{-1} in tank 5. Consequently during June and July tanks 1 and 2 were supersaturated with approximately 150 and 120% DO respectively, above the recommended safe level for salmonids of 110% (Wedemeyer, 1997). Oxygen saturation in tanks 3, 4 and 5 was around 90, 60 and 40% respectively. Towards the end of the experiment DO was frequently <4 mg.l^{-1} in tank 5. The lowest DO concentrations ranged from 2.6 to 3.4 mg.l^{-1} in the cascades, well below the recommended limit of 5 mg.l^{-1}.

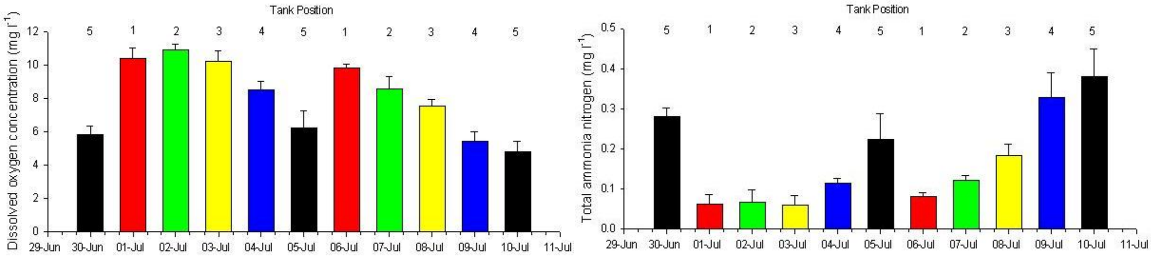


Figure 4. Dissolved oxygen and ammonia concentrations (mean ± S.D., n=100) recorded over a 24h period along a tank cascade.

The maximum total ammonia nitrogen (TAN) was 0.72 mg.l^{-1} (tank 5 in July). However, 0.0053 mg.l^{-1} of NH_3 was present at that time, well below the recommended safe limit of 0.02mg.l^{-1} (Wedemeyer, 1997). There was a slight decrease in pH along each cascade, almost certainly due to a build up of CO_2. However, pH was always within the recommended safe limits for salmonids (pH 6-9; Wedemeyer, 1997). The concentration of suspended solids was generally very low and showed no consistent patterns along the cascades. The highest concentration was 3.54 mg.l^{-1}; well within the recommended safe limit of 80 mg.l^{-1}.

Loading rates and stocking density

Stocking density was initially 21.4 kg.m^{-3} and increased steadily throughout the experiment despite the removal of fish for sampling (Fig. 5). At the end of the experiment the stocking density ranged from 66 to 73 kg m^{-3}. Loading rate increased along each cascade from 0.19 kg.l^{-1}.min^{-1} (tank 1) to 1.2 kg.l^{-1}.min^{-1} (tank 5). Loading rate increased to a maximum of 0.55 and 3.56 kg.l^{-1}.min^{-1} in tanks 1 and 5 respectively.

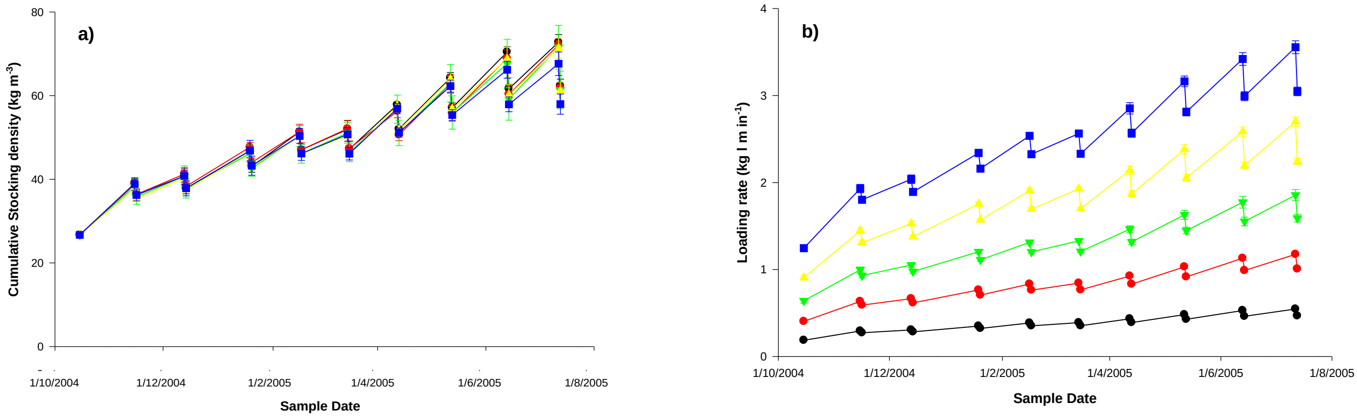


Figure. 5. Stocking density (a) and loading rate (b) (mean ± SEM, n=4) at different positions along the cascade.

Mortality was low (<2%) throughout the experiment and was unrelated to tank position. Fish weight increased from 53g to approximately 300g but there were no differences resulting from tank position (p=0.39). Condition factor (CF) increased in all treatments until February after which it remained steady for the remainder of the experiment. Tank position had no effect on the CF of PIT-tagged fish (p=0.388) although amongst the untagged fish (p<0.001) those from tank 1, 2 and 3 had a higher CF than those from tank 5 (p<0.05). There was a significant effect of tank position on hepatosomatic index (HSI) (p=0.041), with fish in tanks 1 and 2 having a greater HSI than those in tank 5. Tank position had an effect on viscera somatic index (VSI) (p=0.011), with the VSI of fish in tank 3 higher than those in tanks 4 and 5 (p<0.01). Feed intake varied over time (p<0.001), but there were no consistent differences relative to tank position. There were significant changes in relative fin length over the course of the experiment for all fins ($p<0.05$) except the dorsal fin ($p=0.301$). However, no differences related to tank position within the cascade. Eleven gill pathologies were quantified (*i.e.* epithelial lifting, separation and hyperplasia, lamellar fusion and inflammation, interlamellar inflammation, clavate lamellae, and the numbers of thrombi, mucus cells, chloride cell and parasites). Only epithelial hyperplasia and chloride cell numbers were affected by tank position (Fig. 6). There was no effect of tank position on haematocrit ($p=0.899$) although there were changes over time ($p<0.001$). Similar patterns were observed for lysozyme activity and plasma concentrations of glucose and cortisol.

The null hypothesis behind the reversal of the cascade was H_o *Fish are not more severely affected by sudden deterioration in WQ compared with gradual changes*. No evidence was found to reject the null hypotheses and therefore we can conclude that the lack of observable effects in the cascade were not the result of acclimation to gradual changes.

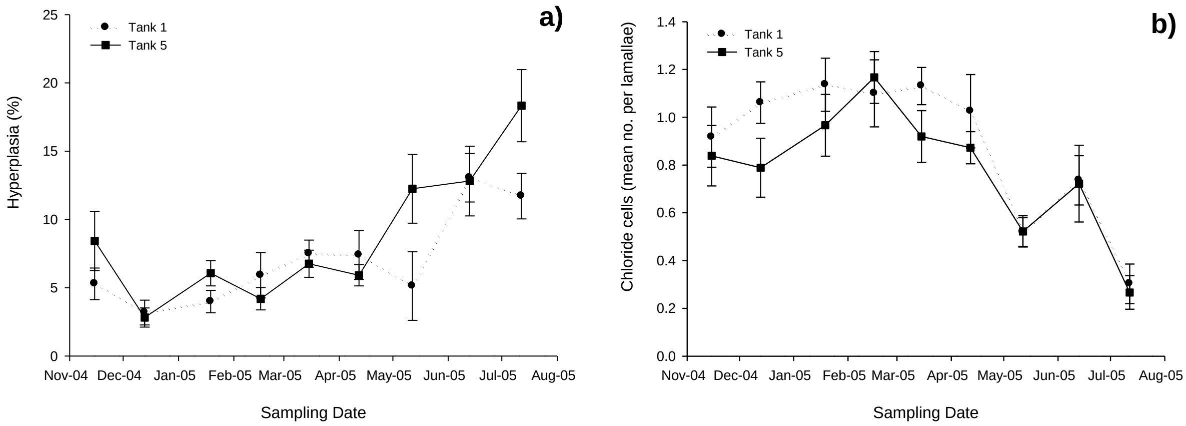


Figure 6. Gill hyperplasia (a) and chloride cell number (b) recorded in fish from tanks 1 and 5 of the cascade.

Experiment 2: The effects of static water culture on indicators of welfare

Methodology

The experiment was conducted for 22 days from 16th May 2005. Fish (250-350g; $n=50$ per tank; stocking density approx. 10 $kg.m^{-3}$) were allocated to 4 tanks and left to acclimatise. At the start of the experiment the inflow of water to two tanks was stopped with the aim of achieving a rapid build up of waste products. A flow rate of 30 $l.min^{-1}$ was maintained in the remaining two tanks. Each of the static tanks was flushed for 5 sec every 3 days in order to prevent excessive build up of uneaten food.

DO was monitored in all tanks with additional aeration supplied if levels fell below 5 $mg.l^{-1}$. At the start of the experiment a system failure left fish in one tank exposed to very low DO levels and there were several mortalities. This tank was removed from the experiment and the water flow to one of the control tanks was stopped converting it to a static tank. Therefore the experiment included two static tanks (original static (OS) and new static (NS)) and one control (C). WQ measurements were taken as described for experiment 1. Fish were sampled on days 3, 8, 11, 16 and 22 of the experiment. At each sample point 10 fish were killed and assessed in a similar way to that described in experiment 1. Gill samples were taken and assessed from five fish at each sample point.

Results

DO levels in the OS and NS tanks remained fairly constant between about 8-10 $mg.l^{-1}$ (Fig. 7). DO levels in the control tank (measured between days 8 and 11) were between 11-12 $mg.l^{-1}$. TAN levels increased within the static tanks reaching 20.9 $mg.l^{-1}$ on day 16 (Fig.7). TAN measured in the control tank (days 1-4 and 8-11) decreased from 0.33 $mg.l^{-1}$ to 0.09 $mg.l^{-1}$. NH_3 increased dramatically after day 6 in the OS tank and day 8 in the NS tank, and by days 8 and 9 respectively, had exceeded the recommended safe level of 0.02 $mg.l^{-1}$ (Wedemeyer, 1997).

Suspended solid levels rose dramatically in the static tanks (Fig. 7), from 0 to 58.9 $mg.l^{-1}$ and 42.4 $mg.l^{-1}$ respectively for the OS and NS tanks. However levels did not exceed those at which sub-lethal effects might be expected (*i.e.* 80 $mg.l^{-1}$ (Wedemeyer (1997)). Levels in the control tank remained low throughout. The specific conductivity of water in the static tanks rose steadily during the experiment. Levels in the control tank (days 8 and 11) remained low. The pH in the static tanks increased during the experiment (from 6.07 to 7.26; Fig. 7) although never exceeded recommended limits (*i.e.* pH 6-9 (Wedemeyer (1997)). pH in the control tank (days 8 to 11) remained low (<6.45). Water temperature in the static tanks ranged from 9.5ºC to 12.3ºC. The temperature in the control tank recorded during days 9 to16 varied very little, ranging between 10.3-11ºC.

In all tanks there was an overall decrease in condition factor and hepatosomatic index, while the splenosomatic index increased in the NS and OS tanks and remained level in tank C.

Feed intake remained fairly constant in the control group, but there was a general decrease in the static tanks. By day 16 many of the fish in the OS treatment showed no ingested feed (Fig. 8). Plasma cortisol concentrations on day 3 were elevated in the OS and NS tanks compared with the control (p<0.001). However, subsequently cortisol levels were low with no significant differences between any of the tanks (Fig. 8). There was no overall difference in lysozyme activity related to the different treatments (p=0.063), but there were highly significant differences between tanks on certain days of the trial (Fig. 8). Plasma glucose concentrations were lower in fish from the static tanks compared with the control (p<0.05; Fig. 9). Haematocrit data was only collected on day 16, when the OS and NS tanks had markedly higher (51.0%) and lower (29.4%) values respectively than the control tank (38.4%) (p<0.05).

Generally, the incidence of gill pathologies (epithelial lifting, necrosis and hyperplasia, fused lamellae, lamellar and interlamellar inflammation, clavate lamellae and chloride cells) were higher in the static tanks compared with the controls. However, levels of epithelial separation and parasites were lower in the static tanks, with the numbers of thrombi and mucus cells unaffected by treatment.

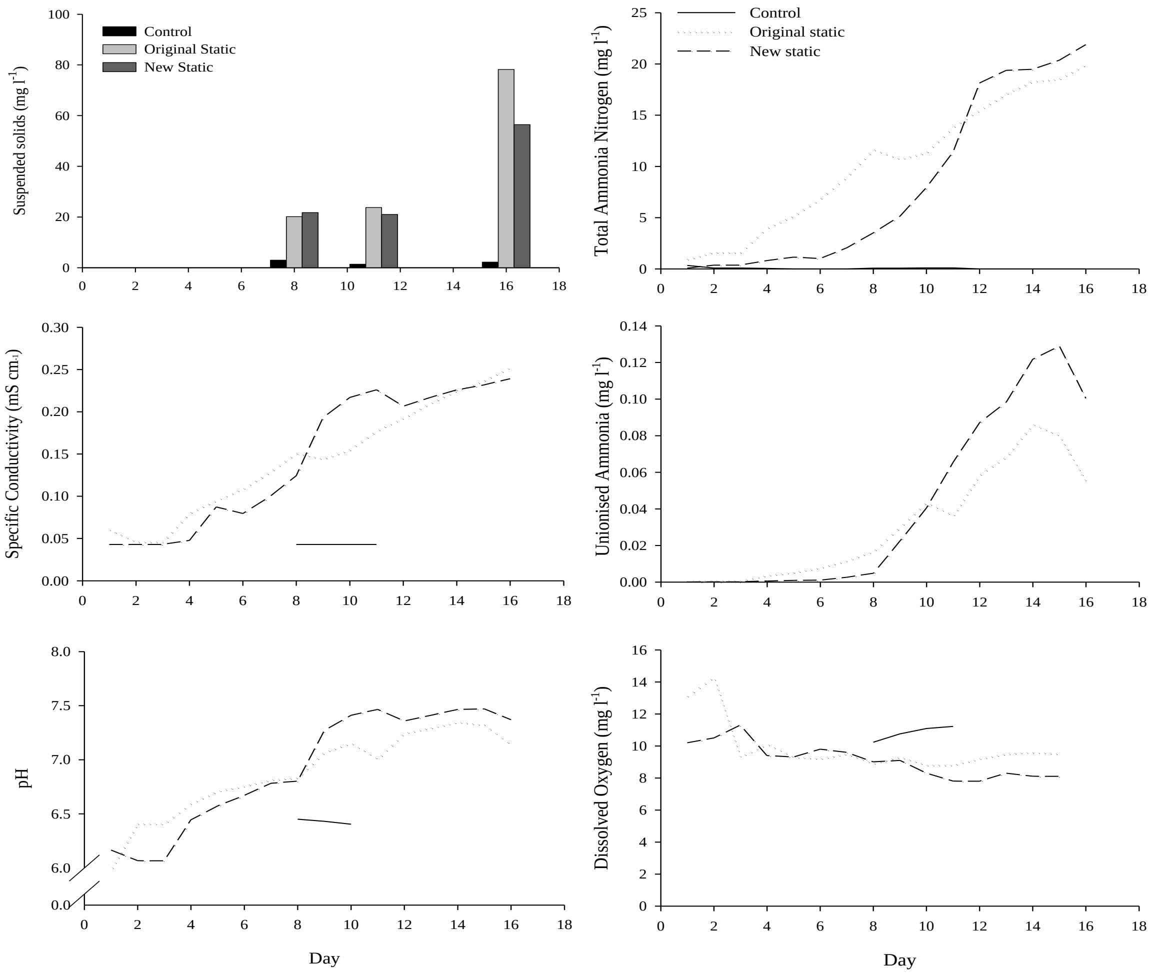


Figure 7 The water quality parameters measured in static water (OS and NS) and control (C) tanks

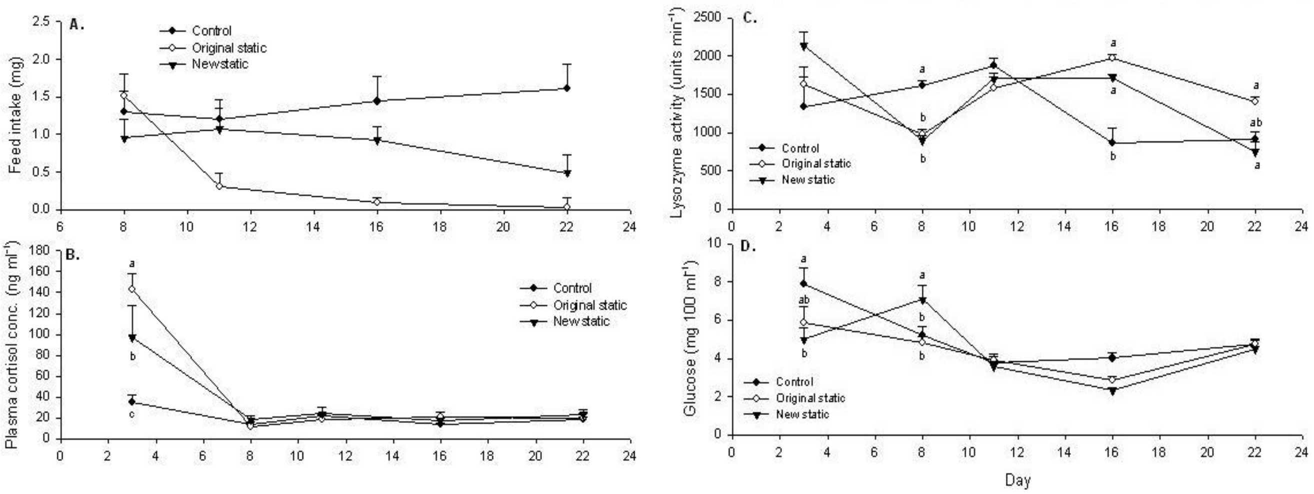


Figure 8. Feed intake (A), plasma cortisol (B), lysozyme activity (C) and glucose (D) (mean ± SEM, n=10) in rainbow trout held in flow-through (control) or static water. Different lettering denotes statistical differences (*p*<0.05).

Summary and Discussion

Two experiments attempted to identify welfare indicators that are sensitive to WQ deterioration in rainbow trout. Experiment 1 attempted to recreate the conditions that might be seen on a commercial fish farm by reusing water along a series of tanks. The loading rates, levels of WQ deterioration and fluctuations in temperature, day length and fish growth represented the ranges found on commercial trout farms. Experiment 2 resulted in rapid and extreme deterioration in WQ in excess of conditions that would normally be encountered on trout farms.

During experiment 1 WQ did not deteriorate sufficiently to result in consistent changes in most of the welfare indicators measured, despite achieving loading rates in excess of recommended levels. DO was the parameter most affected by the cascade and to maintain levels >5 $mg.l^{-1}$ at the bottom of the cascade it was necessary to inject the inflow water with oxygen, and provide additional oxygenation and aeration at the top of the cascades. This resulted in heavily supersaturated water at the top of the cascade. DO at the bottom of the cascade regularly fell below 4 $mg.l^{-1}$, but all other WQ parameters remained well within recommended safe limits.

In experiment 2 WQ decreased dramatically in the static tanks compared with the flow-through control, with levels of TAN exceeding 20 $mg.l^{-1}$. Specific conductivity and pH in the static tanks also increased. By the end of the experiment levels of unionised ammonia (NH_3) approached lethal concentrations in the static tanks.

Experiment 1 has shown that growth and condition indices have potential value as welfare indicators even at WQ levels within recommended safe limits. Plasma cortisol levels in experiment 2 were initially elevated in the static tanks, but this increase was only transient and despite extreme WQ deterioration subsequent samples found cortisol levels in the static tanks to be comparable with the control tank.

In both experiments epithelial hyperplasia was the gill pathology most sensitive to WQ deterioration. Significant changes in the numbers of chloride cells were found in both studies but these were not consistently associated with WQ and accurate identification of chloride cells was problematic.

Identifying welfare indicators that are sensitive to WQ deterioration:

These experiments have shown that the most sensitive and quantifiable indicators of WQ associated with poor WQ are gill pathology, growth and indices of condition with plasma cortisol. Importantly both of these experiments resulted in highly unstable systems that required 24-hour supervision and a constant supply of aeration/oxygenation. Therefore any system with heavy loading will require effective monitoring and contingency planning.

Ref:

Wedemeyer, G.A. (1997) Rearing conditions: effects on fish in intensive culture. In: Iwama, G.K., Pickering, A.D., Sumpter, J.P. and Schrek, C.B., (Eds.) *Fish Stress and Health in Aquaculture*, Society for Experimental Biology, Cambridge University Press.

Obj. 05 Farm based epidemiological studies of relationship between water quality and indicators of welfare

"*An epidemiological approach will be used to record water quality and welfare indicators on at least 240 systems on 60 farms representing the diversity of the industry throughout the year. Data loggers with multi-probes (pH, temperature, DO, ammonia and turbidity) will be used to monitor water quality over 24-hour periods in each system. A raft of previously identified welfare indicators (Obj. 01, 03 and 04) will also be recorded. Other data will be collected including any farm records of water quality and husbandry procedures prior to the sampling period. In addition 24-hour water samples will be collected on a sub-sample of systems to validate the probe records and measure parameters outwith the capacity of the probes (e.g. CO_2, NO_2, NO_3).*"

Objective 04 was completed by its target milestone completion date of October 2007. The experiments conducted in this objective are outlined below and two draft manuscripts are provided in Appendix 5 and 6.

This study examined welfare of rainbow trout (*Oncorhynchus mykiss*) on commercial farms, adopting an epidemiological approach to study 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, including size, condition factor, the condition of the fins, somatic indices of the liver and spleen, the stress hormone cortisol and haematocrit. Information was collected on the provenance of the fish, as well as production data for stocking density, growth, disease, history and mortality rates. Details of the system, including the addition of aeration or oxygenation, were recorded, as were management and husbandry parameters, such as feeding strategies, if the fish were farmed for the table market or restocking. Particular emphasis was placed on water quality. 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.

Fig. 9 and 10 describe the range of dissolved oxygen and unionised ammonia (UIA) recorded from the 189 systems during this study. The values given are the mean values taken over the 24 hour period. From the literature review for Obj 1, a minimum DO concentration of 5-6 mg.l^{-1} was suggested by some, and anecdotal comments made by fish farmers confirm this is the minimum concentration that many farmers aim to maintain. From the systems sampled, 93% had DO concentrations > 5mg.l^{-1}, while 86% of systems had DO concentrations >6 mg.l^{-1}. Although the literature review highlighted controversies over recommended maximum concentrations of UIA, a maximum level of 0.02 UIA mg.l^{-1} is frequently cited. The maximum mean value for UIA recorded for this study was 0.0097mg.l^{-1}, and the maximum UIA value recorded throughout the entire study was 0.016 mg.l^{-1}, both below the recommended maximum of 0.02mg.l^{-1}. Ninety-three percent of systems had carbon dioxide levels under 20 mg.l^{-1}, with 71% less than 10 mg.l^{-1}. For parameters outside the control of farmers, temperature ranged from 2.8°C to 19°C and water hardness ranged from <5 to 290 mg.l^{-1} as $CaCO_3$.

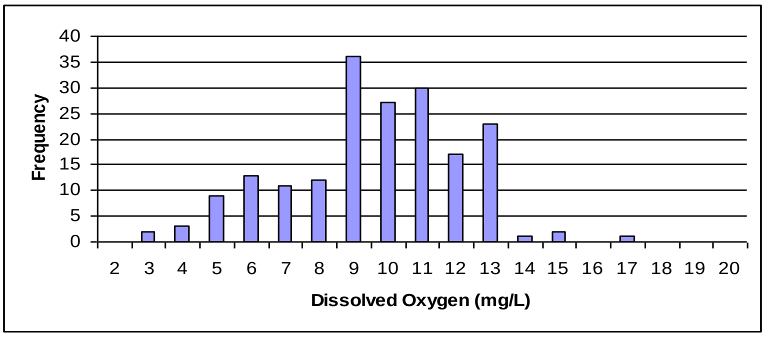
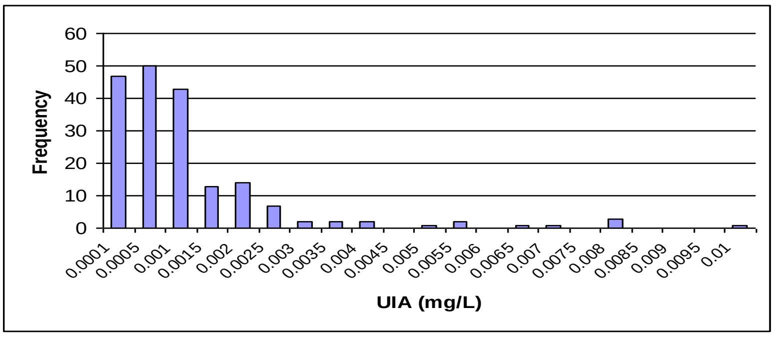


Figure 9. Frequency of dissolved oxygen measurements recorded from all systems.

Figure 10. Frequency of unionised ammonia (UIA) measurements recorded from all systems.



Welfare is a complex and multifactorial subject. To gain as broad a perspective as possible on the welfare of farmed fish, a range of parameters measured from the fish were used to construct a welfare score. The parameters selected for inclusion in the welfare score were the condition of the gills, the condition of the fins, the somatic index for the spleen, the concentration of the stress hormone cortisol in the blood, and the mortality rate for the population in the individual system. These data for these parameters were adjusted to be comparable (see appendix 6 for full explanation) and standardised so that all had a mean = 1, and were then combined into an aggregate welfare score. This welfare score and its components were used as response variables in the subsequent multilevel modelling.

Analysis of the data using multilevel modelling revealed that very few WQ parameters affected either the aggregate welfare score or the components of the score when analysed individually. Fluctuations in DO concentrations were associated with an increase in the stress hormone cortisol, however, DO was not associated with any effects on the aggregate welfare score. The primary factors associated with mortality rates were the number of diseases that the population of fish had been exposed to and the season, with higher mortality rates reported during summer months. Fish farmed for restocking purposes had better fins than those farmed for the table market, restocking fish also had better overall welfare scores than table fish. However, it is not possible to determine whether this is due to improved rearing conditions/husbandry or removal of poorer fish.

Analysis of the aggregate welfare score also revealed that the most significant aspect affecting overall welfare was disease, irrespective of whether the disease was current at the time of sampling. Exposure to any disease was strongly associated with poor welfare using the parameters measured for this study. Fig. 11 illustrates the association between welfare and the condition factor (an indication of the general condition of the fish described as a function of weight and length). Fish with a low condition factor (K) have poor welfare, which improves as K increases until a K of *ca.* 1.3, at which point increasing K is associated with a reduction in welfare. The 4 visible groupings in the graph (‘a’ – ‘d’) relate to the number of diseases the fish had been exposed to, with the highest welfare scores attributed to fish that were disease free.

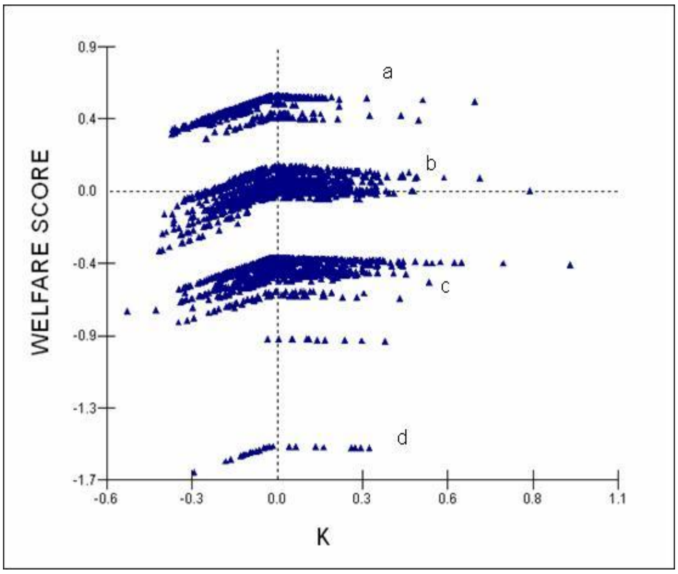


Figure 11. Welfare score modelled against condition factor K. Groupings ‘a, b, c, d’ refer to fish not exposed to any diseases, fish exposed to 1 disease, 2 diseases and 3 or more diseases respectively.

From the results of this study, there is little evidence that poor WQ is a major problem for the welfare of farmed rainbow trout in the UK. The majority of farms had WQ parameters within current recommended limits, and WQ was not strongly associated with poor welfare following analysis of the data.

Obj. 06 Stakeholders’ workshop / technology transfer

“The project will conclude with a stakeholders’ workshop to discuss the identified risks to welfare from water quality and agree key auditable welfare measures. It is envisaged that this may subsequently lead to a Hazard Analysis and Critical Control Point (HACCP) and/or Hazard Analysis and Risk Assessment (HARA) approach to the control of welfare through water quality and other influences. The implementation of such a system is not an appropriate objective for this research project, but is being implemented in other species by collaborators from Bristol.”

This objective was completed in November 2007 after a Defra approved amendment to the original completion milestone. The amendment was requested to allow the results of Obj 5 to be disseminated at the workshop. A draft manuscript for peer review publication is attached as Appendix 7.

The workshop was conducted on 22nd November 2007 at Defra’s Innovation Centre. Individuals were invited from the following stakeholder groups: fish farming representatives, researchers, fish veterinarians, governmental and non-governmental organisations, research and grant funding bodies, consumer groups, retailers and ethicists. In total 31 participants attended, with each stakeholder group represented by at least one individual.

Workshop aims

The aims of the workshop were to disseminate the results of AW1205. However, the workshop coincided with Defra's 5 year review of fish welfare research and consequently the scope was expanded to consider current and future priorities for fish welfare. During informal discussions prior to the workshop it became apparent that many stakeholder groups did not have a clear understanding of each others' stance regarding fish welfare. Therefore the following aims were adopted:

1. To prompt discussion amongst stakeholders aiming to establish:
   - What each stakeholder group can do to improve fish welfare
   - What each stakeholder group thinks others can do to improve fish welfare.
2. To create a prioritised list of actions which stakeholders feel should be addressed.
3. To improve communication and understanding between the stakeholder groups.

Workshop format

Initially, attendees were invited to view and discuss 9 pre-prepared posters, which contained information and statements relating to farmed fish welfare. This encouraged discussion amongst stakeholders and provided some, but not all, information on fish welfare. One poster was used to disseminate the results of AW1205.

Stakeholders were then asked to propose issues relating to farmed fish welfare that they felt should be addressed. These ideas were reduced to a focused list of the most important issues using a number of peer review exercises. Once a focused list had been agreed, strategies to address the issues were developed, again using a peer review to assess proposals that were generated. Finally, each proposal was discussed in plenary and stakeholders were asked if they felt they could take some role in addressing the action points following the meeting.

Critically, each exercise during the day was conducted using small groups of regularly mixed stakeholders. Therefore the outcomes were not strongly influenced by any one stakeholder group, but represented the broad views of the industry stakeholders as a whole.

Results

In total 32 priority areas for farmed fish welfare (including duplicates) were initially identified by stakeholders. These were reduced to 12 ideas and finally a list of the 8 most important issues were agreed using a system of voting (Table 5).

Table 5. The priority areas identified by stakeholders as most important for fish welfare. The relative importance of each priority area (based on votes) is given. The 4 shaded ideas were considered least important and were not considered in further depth.

	Priority area	Relative importance
1	The role of genetic selection in producing fish more suited to the fish farming environment.	13.0
2	Integration and application of behavioural and physiological welfare indices.	10.8
3	We need to know what good welfare is for various farmed species because you can't improve welfare if you don't know what good welfare is.	16.2
4	Address the issues of staff training and new trained workers coming to the industry because well trained, competent staff are important to welfare.	8.1
5	How do we ensure that best practice for welfare in aquaculture is followed by individual businesses?	6.5
6	We need more data on mortalities.	15.1
7	The need for welfare monitoring and documentation systems because we need baseline data about the farming environment, mortalities, fish performance and husbandry practises to identify problems and research issues.	8.1
8	The need for a more liberal regime in Europe for introducing (licensing) new medicines, which requires government commitment.	9.7
9	Risk assessment and contingency planning because welfare problems can be avoided (or corrected).	5.9
10	The development of welfare standards backed by actual scientific research and not just guesswork.	3.2
11	We must understand the costs and benefits of fish welfare because improvements must be economically and environmentally sustainable.	2.2
12	Disseminate more widely fish welfare research so it can be used and applied.	1.1

The need for a “better understanding of what is good welfare” was seen as the most important priority area (16.2% of votes). The need for “more data on mortalities” also had a high number of votes (15.1%) and this priority area was considered sufficiently similar to the need for “welfare monitoring and documentation systems” (8.1%) that the two ideas were merged. The “role of genetic selection in producing fish suited to the farming environment” and a need for “integration and application of behavioural and physiological measures” were also considered highly important. All other priority areas had <10% of the votes. Proposals to address these priority areas and are shown in Table 6.

Table 6. Proposals stakeholders developed to address the most important priority areas for farmed fish welfare.

	Priority area	Proposal to address the priority area
1	The role of genetic selection in producing fish more suited to the fish farming environment.	1. To hold a round table of trade associations on identifying and sharing information on genetic problems and opportunities. 2. Conduct a broader stakeholder meeting to establish if there are problems and to increase awareness.
2	Integration and application of behavioural and physiological welfare indices.	Research and define behavioural welfare indices to add to current knowledge so that a minimum welfare standard can be agreed between stakeholders.
3	We need to know what good welfare is for various farmed species.	Develop and validate a checklist of practical indices of bad welfare to be used on all fish farms.
4	Address the issues of staff training and new trained workers coming to the industry.	Publish an agreed set of stockman competences which farmers have to demonstrate that they actively use and is part of their written animal health and welfare plan.
5	How do we ensure that best practice for welfare in aquaculture is followed by individual businesses?	Develop a pilot scheme to establish best practice from a representative sample of farms for a specific species during a specific phase and event.
6	We need more data on mortalities.	Seek agreement from the main trade associations and other stakeholders’ representatives to use centrally shared data.
7	The need for welfare monitoring and documentation systems.	
8	The need for a more liberal regime in Europe for introducing (licensing) new medicines, which requires government commitment.	Campaign to rationalise and streamline the international approval of veterinary medicines for use in the fish farming industry.

Conclusions

The final workshop was professionally facilitated and based around peer review processes and development of consensus. The anecdotal opinion of the participants at the end of the workshop was overwhelmingly positive. At the conclusion of the meeting, stakeholders were asked if they felt they could take some role in addressing the action point following the meeting. Whilst it would be inappropriate to provide specific details of this, in all but one case stakeholder group(s) took responsibility for addressing the priority areas, however, the funding agencies emphasised that they could not guarantee to fund the research aspects in the immediate future. The only priority area where no stakeholder group took responsibility for an action was “ensuring best welfare practise is followed by individual farmers”.

In summary, this meeting was the second during this project to bring together stakeholders involved in farmed fish welfare and it identified current and future priority areas. The workshop provided an important list of actions for improvements to the welfare of farmed fish. Priorities were established in consultation with all stakeholders and can therefore be viewed as an honest and accurate opinion of the future needs for this area. The outputs are of considerable importance to the future of farmed fish welfare and should serve to focus the actions of all stakeholders.

Discussion of the results and their reliability

This study involved a literature review (Obj 1), to which all the participants contributed and which has been peer reviewed and published. We consider this to be a thorough and considered review. A survey of current practices was undertaken (Obj 2) involving a large proportion of the trout farming industry, again this was thorough and provided a reliable picture of trout farming practices at that time. The focus group discussions (Obj 3) were conducted as planned and the resulting paper has been peer reviewed and published. By the nature of this exercise there is the capacity for the facilitators to bias the direction of the discussion and there is inevitably a degree of subjectivity in the interpretation and synthesis of the information. The cascade experiment and the associated static tank experiments (Obj 4) were carried out as rigorously as possible using continuous monitoring data loggers which were internally calibrated and externally compared with alternative means of measurement. More effort was expended on these experiments than originally intended since we wished to ensure that the apparent lack of effect was a robust result. It is impossible to extrapolate the results to situations with different water chemistry but we feel the results are robust and representative of the effects in acid water where dissolved oxygen is maintained. A very great deal of time and effort also went into the epidemiological study (Obj 5). This study collected the largest and most comprehensive data set on the UK trout farming industry practices, WQ and fish welfare. The experimental design and subsequent adaptations were only undertaken after detailed consultation with statisticians and produced a robust sample framework. All samples were adequately controlled, replicated and validated. Statistical analysis was undertaken using multilevel models, which are currently considered to be the most and possibly only valid way of analysing data with such a hierarchical structure. The final workshop based on peer review and building consensus established priorities in consultation with all stakeholders and can therefore be viewed as an honest and accurate opinion of the future needs for this area.

Major conclusions

This project used a wide range of research approaches to study the relationship between WQ and trout welfare. We confirmed that poor WQ can have a negative effect on the health and welfare of farmed trout (Obj 1, 3 & 4). Trout farmers generally had systems and procedures in place to monitor and maintain WQ; these are largely based on guidelines and past experience but are also dependent on the competence of the farm staff (Obj 2, 3, 5 & 6). A large epidemiological study of farmed trout (Obj. 5) indicated that WQ on commercial trout farms rarely deteriorated to levels which would have a negative effect on the welfare of the fish, although episodic events such as droughts or algal blooms may have a detrimental effect (Obj 2). In acidic water conditions very few negative effects were observed even at high loading rates, providing dissolved oxygen was maintained (Obj 4). Under those conditions WQ had to deteriorate to potentially lethal concentrations before there were detectable effects on welfare (Obj 4). Levels of WQ parameters that can negatively influence welfare vary according to the farming system, other aspects of water chemistry and the previous experience of the fish (Obj 1). However, our results indicate that monitoring (*e.g.* DO, ammonia, pH and temperature) and controlling (*e.g.* DO and ammonia) a limited number of WQ parameters may safeguard many aspects of trout welfare (Obj 1, 2, 4 & 5). The British Trout Association Code of Practice recommend a minimum DO concentration of $6 mg.l^{-1}$, our results suggest this is adequate to safeguard many aspects of welfare affected by WQ. There was agreement among stakeholders that fish based indicators combined with practical experience are the best way to monitor the welfare of fish (Obj 3 & 6). There is still the need for schemes to maintain and improve farmer competence along with adequate record keeping and documentation (Obj 3 & 6). There is also the need for more robust guidelines on behavioural indicators of fish welfare (Obj 3 & 6). This project has produced a very wide range of outputs which have already been widely disseminated and discussed with UK and European stakeholders. The research has also produced robust information on the relationship between WQ and farmed trout welfare which could inform policy, legislation and guidelines.

Future work

This project through the final stakeholders' workshop is in a position not only to recommend future work on the basis of the opinions of the participants but also the priorities of a wide range of stakeholders and therefore we reproduce here the summary table from Objective 6. These priority areas were not ranked but are listed in arbitrary order

Table 7. Proposals stakeholders developed to address the most important priority areas for farmed fish welfare.

	Priority area	Proposal to address the priority area
1	The role of genetic selection in producing fish more suited to the fish farming environment.	1. To hold a round table of trade associations on identifying and sharing information on genetic problems and opportunities. 2. Conduct a broader stakeholder meeting to establish if there are problems and to increase awareness.
2	Integration and application of behavioural and physiological welfare indices.	Research and define behavioural welfare indices to add to current knowledge so that a minimum welfare standard can be agreed between stakeholders.
3	We need to know what good welfare is for various farmed species.	Develop and validate a checklist of practical indices of bad welfare to be used on all fish farms.
4	Address the issues of staff training and new trained workers coming to the industry.	Publish an agreed set of stockman competences which farmers have to demonstrate that they actively use and is part of their written animal health and welfare plan.
5	How do we ensure that best practice for welfare in aquaculture is followed by individual businesses?	Develop a pilot scheme to establish best practice from a representative sample of farms for a specific species during a specific phase and event.
6	We need more data on mortalities.	Seek agreement from the main trade associations and other stakeholders' representatives to use centrally shared data.
7	The need for welfare monitoring and documentation systems.	
8	The need for a more liberal regime in Europe for introducing (licensing) new medicines, which requires government commitment.	Campaign to rationalise and streamline the international approval of veterinary medicines for use in the fish farming industry.

Project outputs and Knowledge transfer

In addition to the formal outputs listed in Section 9 of this report, experience from this project has been incorporated into the latest draft of the Appendix to the Council of Europe Recommendation on fish welfare concerning Atlantic salmon and rainbow trout and the European Food Safety Authority draft report on the welfare of farmed trout. Participants in the project have also made major contributions to the European COST Action 867 – Welfare of fish in European Aquaculture. There have also been numerous informal discussions between the participants in this project and a wide range of stakeholders including for example, trout farmers, retailers, legislators, a range of animal welfare organisations (RSPCA Freedom Foods, Compassion in World farming etc.) and even a national radio interview. Finally as part of the project activities and with advice from Defra a web site was developed and is still actively maintained [fishwelfare.net]. This site has become a major source of information on fish welfare with 2,366 visits to the site by 13th June 2008, the majority of which were from Europe (48%) and North America (35%), with 13% from within the UK.

This project has also led directly to a number of other research projects described below:

1. A COST Action (867) "Welfare of fish in European aquaculture" has been funded and successfully established.

2. A STREP application under the EC 6th Framework Programme has been funded which will extend the scope of the current project to examine the economic consequences of safeguarding fish welfare. The project, ("Evaluation and Modelling of Benefits and Costs of Fish Welfare Interventions in European Aquaculture") aims to incorporate the biological implications of selected welfare indicators with the consequences for the entire value chain to develop bio-economic models for implementation of fish welfare strategies.

3. A SARF/Defra project "Development of a scheme for monitoring sentinel farms in the UK trout industry" has been funded and commenced. This project will provide a web-based application for benchmarking mortality data within the trout farming industry. The project draws on knowledge and contacts gained during AW1205 to assist the development of an industry wide monitoring scheme.

4. A SARF project "Developing practical strategies for reducing the spread of harmful organisms during the transportation of live fish" has been funded and commenced. This project will review current transport procedures and attempt to identify practical methodologies to reduce the spread of signal crayfish and other pathogens. Furthermore it will consider the welfare of fish during transportation.

5. An application has been submitted to the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3R's) to investigate non-invasive methods for monitoring welfare and identifying alternatives to lethal endpoints for fish experiments.

eferences to published material

his section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

Presentations

MacIntyre, C.M. Meeting on trout welfare for review of production systems and husbandry management procedures for trout in capacity as External Expert to Working Group. EFSA, Parma, Italy. 10th June 2008.

MacIntyre, C. M. On-farm operational welfare indicators for rainbow trout. COST Action 867 Meeting, Krakow, Poland. 15-17 April 2008.

MacIntyre, C. M. “Fish welfare and water quality.” Fish Welfare During Transport Forum. Humane Slaughter Association. Inverness, Scotland. 25th October 2006.

Turnbull, J. F. “Research activities at Stirling.” COST Action 867 Meeting, University of Bordeaux, Arcachon, France. 9-11 September 2006

North, B. P. “On-farm assessment of fish welfare: a UK trout farming perspective.” Aquaculture Europe 2005. Trondheim, Norway, 5-12, August 2005

North, B. P. “Trout Welfare update on current activities.” The British Trout Farming Conference” Sparsholt, 2nd & 3rd September 2004.

North, B. P. “Stocking density and fish welfare.” Fish Veterinary Society Welfare Meeting. Edinburgh, 23rd & 24th November 2004.

Publications

MacIntyre, C. (2008) What next for fish welfare. Aquaculture News 34, 8-9.

MacIntyre, C. M., Turnbull, J. F. (2007). Is the trout industry ready for welfare assessment? Fin Fish News 3, 24-26.

MacIntyre, C., North, B. P., Nikolaidis J., Turnbull, J. (2006). Interactions between water quality and trout welfare (Defra project AW1205). Fin Fish News 1, pp. 9-13

MacIntyre, M. C., Ellis, T., North, B. P. & Turnbull, J. F. (2008). *The influences of water quality on the welfare of farmed trout: a review*. In *Fish Welfare*. Ed. E. J. Branson. Blackwell Publishing Ltd. 150-184pp. ***Appendix 1 to this report***

North, B. P., Ellis, T. Bron, J., Knowles, T. G. & Turnbull, J. F. (2008). The use of stakeholder focus groups to identify indicators for the on-farm assessment of trout welfare. In *Fish Welfare*. Ed. E. J. Branson. Blackwell Publishing Ltd. 243-267pp. ***Appendix 3 to this report***

Publications in preparation for peer reviewed journals

Berrill, I. K., MacIntyre, C. M. & Turnbull, J. F. The effects of different blood handling techniques on plasma cortisol and haematocrit determination in rainbow trout (*Oncorhynchus mykiss* Walbaum).

Berrill, I. K., MacIntyre, C. M., Jones, E., Cooper, T., Ellis, T., Knowles, T. G., & Turnbull, J. F. Current and future priorities for farmed fish welfare.

North, B. P., , MacIntyre, C. M., Nikolaidis, J., Mettam, J. & Turnbull, J. F. Tank based studies to examine the effects of deteriorating water quality on various indicators of welfare in rainbow trout, *Oncorhynchus mykiss*.

MacIntyre, C.M., North, B.N., Nikolaidis, J., Turnbull, J.F. Rainbow trout farming in the UK and the potential for monitoring welfare.

MacIntyre, C.M., Berrill, I.K., North, B.N., Knowles, T., Turnbull, J.F. An epidemiological study of welfare in farmed rainbow trout: description of data and selection of welfare indicators.

MacIntyre, C.M., Berrill, I.K., North, B.N., Knowles, T., Turnbull, J.F. Identification of risk factors for the welfare of farmed rainbow trout in the UK.

Other outputs

MacIntyre, C. M. (2008) Water quality and welfare assessment on United Kingdom trout farms. Ph.D. Thesis. University of Stirling.

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