European Food Safety Authority

The EFSA Journal (2008) 796, 1-22

SCIENTIFIC OPINION

Animal welfare aspects of husbandry systems for farmed Trout [1]

Scientific Opinion of the Panel on Animal Health and Animal Welfare

(Question No EFSA-Q-2006-147)

Adopted on 11 September 2008

[1] For citation purposes: Scientific Opinion of the Panel on Animal Health and Animal Welfare on a request from the European Commission on the Animal welfare aspects of husbandry systems for farmed trout. *The EFSA Journal* (2008) 796, 1-22.

PANEL MEMBERS*

The Scientific Panel for Animal Health and Welfare (AHAW) of the European Food Safety Authority adopted the current Scientific Opinion on 11 September 2008. The Members of the AHAW Scientific Panel were:

Bo Algers, Harry J. Blokhuis, Donald M. Broom, Patrizia Costa, Mariano Domingo, Mathias Greiner, Daniel Guemene, Jörg Hartung, Frank Koenen, Christine Müller-Graf, David B. Morton, Albert Osterhaus, Dirk U. Pfeiffer, Ron Roberts, Moez Sanaa, Mo Salman, J. Michael Sharp, Philippe Vannier and Martin Wierup.

* A minority opinion was expressed from Prof. Donald Broom based on the view that the accepted Report and adopted Opinion are incomplete and that in order to answer the mandate from the European Commission, the introductory chapters on the welfare, biological functioning and farming of fish should be included (Annex II).

SUMMARY

Council Directive 98/58/EC concerning the protection of animals kept for farming purposes lays down minimum standards for the protection of animals bred or kept for farming purposes, including fish. Following a request from the European Commission, the AHAW Panel was asked to deliver a Scientific Opinion on the animal welfare aspects of husbandry systems for farmed trout. The Scientific Opinion was adopted on 11th September 2008.

The scientific opinion focus on the two main species farmed as ''trout' in Europe, these are the rainbow trout, *Oncorhynchus mykiss* (Walbaum 1792); and the brown trout; *Salmo trutta* L 1758. Trout are produced across Europe for various purposes and in a wide variety of production systems however there has been no systematic survey of production systems and husbandry procedures at a European level.

From the data presented in the scientific report, several factors affecting trout welfare were identified: abiotic and biotic factors, feed and feeding, husbandry, genetic, disease and disease control measures. A risk assessment approach was carried out to obtain a risk ranking for these groups of factors, estimate which hazards are more important for each life stage and enable a comparison of the different production systems. Due to the limited amount of quantitative data related to production systems and effects of potential hazards on trout welfare, the risk assessment was mainly based on expert opinion.

Water quality is an important factor when considering trout welfare; however water quality is the result of a complex of interacting factors. Also the absolute level or the rate of change at which any particular abiotic factor exceeds the adaptive capacity of the fish is not easily predicted since it is co-dependent on: size of the fish, previous experience, health status and other abiotic factors. Trout have the capacity to adapt to a range of external environmental factors. Once their adaptive capacity is exceeded they may suffer from physiological or pathological disturbances. Where available tolerance levels for the various abiotic factors were indicated.

Potential welfare effects caused by interaction of individuals of the same or different species were also considered. Predation is a significant welfare issue for farmed trout in many systems however there is no systematic data available on the scale of the problem. Effective and legal alternative predator control strategies are required. Intra-specific aggression can cause poor welfare, causing for example fin damage and reduced access to food. Stocking density is relevant to welfare but its effects are mediated through other variables such as water quality and fish behaviour. Consequently it is difficult to set clear guidelines for both maximum and minimum stocking densities that would safeguard welfare. Instead the monitoring of the fish condition should be regarded as a preferred option.

Farmed trout are almost exclusively fed on commercial feed and problems may occur through changes in formulations or poor storage. While there are advantages and disadvantages of various feeding methods related to growth there is no clear indication of the relative benefits or disadvantages for fish welfare. Trout as poikilotherms do not have an energy demanding fixed temperature to maintain and will naturally undergo periods of inappetance. The length of time that food can reasonably be withheld for husbandry reasons without affecting welfare is related to size, lipid reserves, life stage and temperature and it is not possible to specify a simple maximum acceptable duration for food deprivation.

Husbandry and management are central to maintaining the health, welfare and productivity of farmed fish. Fish are handled for a variety of purposes during the production cycle, however, much of this is standard husbandry practice and has not been the subject of scientific publications. It was recommended that the frequency and duration of handling events should be

minimised and fish should be exposed to air for a short time as possible. A substantial proportion of the welfare experience of farmed fish is related to the systems in which they are grown. As the sophistication of the infrastructure and loading of the system increases (biomass per unit volume) so the system becomes more susceptible to acute failures. Thus effective backup systems, a higher level of contingency planning and staff training are required.

It was concluded that genetic selection for resistance to endemic diseases constitutes a benefit in the context of welfare though selective breeding may modify other desirable traits unless carefully managed. Poorly structured breeding programmes have the risk of inbreeding with associated poor reproductive performance and egg survival, loss of genetic variation and development of undesirable physiological side effects such as deformities. No evidence was found to suggest that, when rainbow trout are reared in good environmental conditions triploids do not have similar survival and growth, (and in some cases better growth), than diploids. The reduction in aggression associated with all-female production coupled with triploidy can constitute a benefit in the context of welfare.

As with any form of intensive livestock production, health and diseases are a major welfare issue for the trout industry in Europe. There have, however, been very few attempts to collect systematic data across the whole industry except for notifiable diseases. Endemic diseases related to management practices are often of greater welfare significance than the currently notifiable diseases.

A major welfare issue is the lack of available veterinary medicines. Vaccines have produced major welfare benefits for the industry; however, they are currently only available for a small number of diseases. Furthermore the administration methods and inherent toxicity of some therapeutants and vaccines can in itself lead to adverse effects on welfare.

In conclusion of the risk assessment no major differences concerning overall welfare risk between the different production systems used for each life stage were found. However, different production systems of the same life stage can differ for specific risks, as a result of the different conditions. Measures to improve welfare should be adapted to different production systems and take into consideration the specific requirements of each life stage.

A minority opinion was received based on the view that the accepted Report and adopted Opinion are incomplete and that in order to answer the mandate from the European Commission, the general chapters on the welfare, biological functioning and farming of fish should be included.

Key words: **Trout, welfare, risk assessment, fish farming, stocking density, water quality, feeding, disease.**

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Council Directive 98/58/EC concerning the protection of animals kept for farming purposes lays down minimum standards for the protection of animals bred or kept for farming purposes, including fish.

In recent years growing scientific evidence on the sentience of fish has accumulated and the Council of Europe has in 2005 issued a recommendation on the welfare of farmed fish[2]. Upon requests from the Commission, EFSA has already issued scientific opinions which consider the transport[3] and stunning-killing[4] of farmed fish.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In view of this and in order to receive an overview of the latest scientific developments in this area the Commission requests EFSA to issue a scientific opinion on the animal welfare aspects of husbandry systems for farmed fish. Where relevant, animal health and food safety aspects[5] should also be taken into account. This scientific opinion should consider the main fish species farmed in the EU, including Atlantic salmon, gilthead sea bream, sea bass, rainbow trout, carp and European eel and aspects of husbandry systems such as water quality, stocking density, feeding, environmental structure and social behaviour.

Due to the great diversity of species it was proposed that separate reports and scientific opinions on species or sets of similar species would be more adequate and effective.

It was agreed to subdivide the initial mandate into 5 different questions.

Question 1

- In relation to Atlantic salmon

Question 2

- In relation to trout species

Question 3

- In relation to carp species.

Question 4

- In relation to sea bass and gilthead sea bream

Question 5

- In relation to European eel

This opinion will refer only to question 2 as referenced above.

[2] Recommendation concerning farmed fish adopted by the Standing Committee of the European Convention for the protection of animals kept for farming purposes on 5 December 2005.

[3] Opinion adopted by the AHAW Panel related to the welfare of animals during transport -30 March 2004.

[4] Opinion of the AHAW Panel related to welfare aspects of the main systems of stunning and killing the main commercial species of animals- 15 June 2004.

[5] Food Safety aspects are addressed by a Scientific Opinion of the BIOHAZ Panel (Food Safety aspects of Animal welfare aspects of husbandry systems for farmed fish, Question N° EFSA-Q-2008-297).

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group, chaired by the panel member Ron Roberts, for the preparation of the Scientific Report, which has been used as the basis of this Scientific Opinion: Donald M. Broom, Christine Müller-Graf, David Morton, Børge Damsgård, Rod Wootten, Jimmy Turnbull, Gilles Lemarie and Patrick Prunet.

In addition the Scientific Panel on Animal Health and Welfare wishes to thank Dr Sunil Kadri and Dr Craig MacIntyre for their contribution to this report.

CONCLUSIONS AND RECOMMENDATIONS

1. OUTCOMES FROM THE DATA PRESENTED IN THE SCIENTIFIC REPORT

1.1. Welfare in relation to the production cycle and production systems

CONCLUSION

- Trout are produced for various purposes in a wide diversity of systems. There has been no systematic survey of production systems and husbandry procedures at a European level.

1.2. Factors affecting farmed trout welfare

1.2.1. Environmental conditions - Abiotic factors

1.2.1.1. Water quality

CONCLUSIONS

- Water quality is influenced by a complex of interacting factors. The absolute level or the rate of change at which any particular abiotic factor exceeds the adaptive capacity of the fish is not easily predicted since it is co-dependent on: size of the fish, previous experience, health status and other abiotic factors.
- Trout have the capacity to adapt to a range of abiotic, external environmental factors. However, once their adaptive capacity is exceeded they may suffer from physiological or pathological disturbances.

RECOMMENDATION FOR FUTURE RESEARCH

- Research into the interaction of water quality and fish welfare should be encouraged particularly under commercial farming conditions.

1.2.1.2. Water temperature

CONCLUSIONS

- Tolerance to temperature depends on fish strain, degree of acclimatisation, and interaction with other water quality factors such as oxygen pH and ammonia levels.
- Trout seem to be able to adapt to temperatures in the range of 0-22 °C provided the fish are supplied with well oxygenated water. The lower lethal limit is considered to be around -1°C and the higher lethal limit is above 24 °C. During the egg and alevin stages, temperatures

exceeding (above 15 °C) or sudden temperature variations may cause tissue damage and developmental disorders at later life stages.

- Temperature optimum for growth of rainbow trout appears to be in the range of 16-18 °C.

1.2.1.3. pH

CONCLUSIONS

- Tolerance of trout to acid pH depends on degree of acclimatisation, and interaction with other water quality factors such as aluminium, water hardness, and ammonia levels.
- Rainbow trout and brown trout show significant mortality when exposed to a water pH equal to or below pH 4.
- pH values between 4.5 and 5.5 induce sublethal but significant effects on trout physiology, mainly on acid-base state, osmoregulation, oxygen transport and cardiovascular responses
- Trout can cope with acute high pH (> 9.0) exposure for short-term periods. A pH level of 9.2 is generally considered to be a critical maximum.
- Sudden changes in pH may cause severe negative welfare effects

RECOMMENDATION

- Extreme pH (below pH 5 and above 9) and sudden pH variations should be avoided at all life stages.

1.2.1.4. Ammonia

CONCLUSION

- High levels of un-ionised ammonia can be detrimental to fish welfare.

RECOMMENDATION

- In spite of the difficulty of specifying the exact level at which adverse effects will occur due to the complex inter-relationship with other parameters, a maximum level of 0.012 mg/l of un-ionised ammonia in the water is recommended.

1.2.1.5. Nitrite

CONCLUSIONS

- In flow-through systems the main sources of nitrite are anthropogenic and external to the fish farm. In recirculation systems a malfunctioning biological filter can be responsible for elevated nitrite levels.
- Nitrite levels above 0.1 mg/l NO_2^- in water can be toxic though effects of other ions in the water will affect its toxicity.

RECOMMENDATION

- Maximum concentration for nitrite in water should not exceed 0.1 mg/l NO2-.

1.2.1.6. Aluminium

CONCLUSION

- Aluminium toxicity can have a severely detrimental effect on welfare but is generally the result of rapid reduction in pH due to external factors. Such circumstances are limited to specific locations and are not a general risk.

RECOMMENDATION

- Methods to increase pH can be used to ameliorate the effects of aluminium toxicity; however, these are limited to relatively low flow systems and are unlikely to be effective in severe or very acute incidents.

1.2.1.7. Other metals

CONCLUSIONS

- Metals, such as copper, iron, zinc and cadmium, are toxic to trout and have profound negative physiological effects causing stress and at high concentrations mortality.
- Fish susceptibility to heavy metals toxicity is dependent on the degree of acclimatisation of the fish and other water characteristics such as pH, oxygen concentration, temperature, hardness, salinity and other metals.

1.2.1.8. Water exchange rate and water velocity

CONCLUSION

- Adequate water exchange rate (l/min/kg biomass) is important for oxygen supply and removal of metabolites. However, there is no agreement in the scientific data available as to the ideal flow rate or speed for farmed trout.
- Available space as well as water speed and life stage can affect swimming speed and swimming behaviour
- A moderate increase in swimming speed may reduce agonistic behaviour and stress responses.

1.2.1.9. Suspended solids

CONCLUSIONS

- The physical characteristics and total amounts of suspended solids in water are relevant in determining the extent of possible negative effects in trout gills and skin.
- It is difficult to recommend a maximum concentration of suspended solids since this parameter is greatly affected by shape and size of the particles involved.

1.2.1.10. Oxygen

CONCLUSION

- Dissolved oxygen is a crucial variable affecting the welfare and survival of farmed trout. The amount of oxygen dissolved in the water at saturation is dependent on atmospheric pressure, temperature and salinity. The amount of oxygen required by the trout is dependent on temperature, activity, feeding rate, size of the fish and acclimation.

RECOMMENDATION

- In order to prevent negative hypoxia effects levels of oxygen in the outflow water should not be below 5 mg/l.

1.2.1.11. Carbon dioxide

CONCLUSION

- Dissolved carbon dioxide levels can be higher in recirculation systems and those with high loading and oxygen supplementation compared with more traditional flow through systems. While there are reports of adverse effects on health from high levels of carbon dioxide there is wide disparity in the range of recommended safe levels.

1.2.1.12. Supersaturation

CONCLUSION

- Super saturation especially with nitrogen can lead to gas bubble disease that can have serious welfare implications.
- Recommendations for maximum acceptable levels of supersaturation pose difficulties because safe exposure limits vary with fish size and environmental conditions (hydrostatic pressure).

1.2.1.13. Salinity

CONCLUSION

- Transfer of larger trout to salt water appears to be more successful and less damaging than transfer of smaller fish. However, it is difficult to make specific recommendations since the temperature, ionic strength of the source and recipient water, all affect the influence on the fish.

1.2.1.14. Light

CONCLUSIONS

- Photoperiod is a important factor affecting growth and development in rainbow trout and photoperiod treatments can be employed to advance or delay the spawning time.
- Exposure to light at the egg and alevin stage results in poorer yolk sac conversion and may cause mortality.
- Although light is an effective factor on various physiological functions, its relevance to welfare is not established.

RECOMMENDATION

- Eggs and alevins should be kept at low light intensities.

1.3. Environmental conditions - Biotic factors

1.3.1.1. Predation

CONCLUSIONS

- Predation activities are a significant welfare issue for farmed trout in many systems however there is no systematic data available on the scale of the problem.
- The efficacy of the methods developed to prevent or minimise predation are very variable. There is also a lack of any rigorous scientific investigation or clear practical advice for farmers on the methods to be used to control the predation.

RECOMMENDATION

- Trout should be managed in such a way that predation is minimised, for example by excluding or deterring predators.
- Since in outdoor rearing systems predation control can be in conflict with legislation protecting predators effective and legal alternative predator control strategies are required.

1.3.1.2. Invasive species

CONCLUSION

- Invasive species such as toxic algae or jelly fish in open water systems can have very severe effects on fish welfare though they are rare and unpredictable.

RECOMMENDATION

- Consideration should be given to the development of contingency plans to protect the trout from episodic exposure to invasive species such as jellyfish and toxic algae.

1.3.1.3. Intraspecific interaction (Aggression)

CONCLUSIONS

- Intra-specific aggression is a cause of poor welfare, causing for example fin damage and reduced access to food and others.
- Stocking density, water flow and food distribution all affect aggression but there are no published practical strategies to minimise the problem.

1.3.1.4. Stocking density

CONCLUSION

- Where stocking density is relevant to welfare it is mediated through other variables such as water quality and fish behaviour, meaning that stocking density per se (biomass/volume) is seldom a good way to predict welfare. Consequently it is difficult to set clear guidelines for both maximum and minimum stocking densities that would safeguard welfare.

RECOMMENDATION

- Stocking density per se should not be used as an indicator for good welfare as it is difficult to set appropriate levels of stocking densities, the monitoring of the conditions of the fish should be regarded as a preferred option.

1.4. Feed and feeding

CONCLUSIONS

- Poor welfare may occur through deficiency of macro- or micro-nutrients, excesses of some nutrients, presence of toxins or imbalance of nutrients. Trout are almost exclusively fed on commercial feed and problems may occur through changes in formulations or poor storage.
- While there appear to be advantages and disadvantages of various feeding methods related to growth there is no clear indication of the relative benefits or disadvantages for fish welfare.
- Feed composition and feeding frequency is dependent on fish size. Small larval fish and fry need to be fed frequently with a high protein diet. Frequency and protein content are reduced as the fish grow

- Trout as poikilotherms do not have an energy demanding fixed temperature to maintain and will naturally undergo periods of inappetance.
- There are positive welfare advantages associated with food deprivation prior to handling and other husbandry practices.
- The length of time that food may be with held without impacting on welfare is related to size, lipid reserves, life stage and temperature and it is not possible to specify a simple maximum acceptable duration for food deprivation.
- Over feeding can lead to poor welfare related to deterioration of water quality and lipid overload in organs such as the liver.
- Experimental studies have demonstrated some negative effects on the health of fish through substitution of fish oil and protein in the diet.
- Much of the data related to fish nutrition is not in the public domain and formulation is usually conditioned by economic factors. The partial replacement of fish oil and protein in the diet is common practice in the European trout industry on economic grounds but there has been no independent systematic study of the welfare or health implications of these changes under commercial conditions.

RECOMMENDATION FOR FUTURE RESEARCH

- Further investigation and publications of the risks and benefits of substitution of feed constituents is required.

1.5. Husbandry and Management

CONCLUSIONS

- Fish are manipulated and handled for a variety of purposes during the production cycle; however, much of this is standard husbandry practice and has not been the subject of scientific publications.
- While handling may have a negative effect on the health and welfare of farmed trout, these effects can be limited by correct protocols. Failure to monitor, grade or vaccinate, all of which require handling of the fish, may have an even greater negative effect.
- A substantial proportion of the welfare experience of farmed fish is related to the systems in which they are grown. As the sophistication of the infrastructure and loading of the system increases (biomass per unit volume) so the system becomes more susceptible to acute failures and backup systems, a higher level of contingency planning and staff training are required.

RECOMMENDATIONS

- The frequency and duration of handling events should be minimised and fish should be exposed to air for a short time as possible.
- Numbers of fish involved and duration of crowding events and the duration of crowding should be kept to a minimum. Dissolved oxygen should be monitored during crowding and corrective measures applied if necessary.

- All staff should be adequately trained and planning should ensure that all the necessary labour and infrastructure is available to minimize poor welfare. An assessment of training needs should be carried out periodically to ensure that all staff are competent for the tasks they perform. Appropriate records of individual staff training should be maintained.
- Monitoring of fish and the production environment should be conducted on a regular basis. Accurate farm records should be maintained in order to monitor health, welfare and productivity and to allow for early detection of any abnormality.

1.6. Genetic

CONCLUSIONS

- Poorly structured breeding programmes have the risk of inbreeding with associated poor reproductive performance and egg survival, loss of genetic variation and development of undesirable physiological side effects such as deformities.
- Increasing the genetic resistance to endemic diseases of reared trout greatly improves welfare, together with the reduction of disease load in the environment though selective breeding may modify other desirable traits unless carefully managed.
- There are few publications quantifying the benefits of genetic selection in rainbow trout.
- The reduction in aggression inherent in all-female production systems can constitute a benefit in the context of welfare.
- There is no evidence to suggest that, when rainbow trout are reared in good environmental conditions triploids do not have similar survival and growth, (and in some cases better growth), than diploids.
- The reduction in aggression associated with all-female production coupled with triploidy can constitute a benefit in the context of welfare.
- Induction of triploidy by temperature treatment induces higher juvenile mortality at the eyed stage or at hatching, and higher rates of deformities at hatching than induction of triploidy by pressure treatment.

1.7. Impact of disease and disease control measures on trout welfare

CONCLUSIONS

- As with any form of intensive livestock production, health and diseases are a major welfare issue for the trout industry in Europe. There have, however, been very few attempts to collect systematic data across the whole industry and so the scale of the problem is unknown.
- The diseases affecting farmed trout and the health management options for their control vary in the different production systems that are used. For example, the options for bio-security are limited on open systems such as cage sites, where contact with wild fish and contaminated water cannot be controlled. In re-circulation systems it can be difficult to eliminate persistent infections.

- Diseases result in poor welfare through various clinical and sub-clinical effects. Endemic and management related diseases are often of greater welfare significance than the currently listed notifiable diseases which are the only conditions for which data is compiled at a European level.
- Much of the research on fish diseases in Europe currently concentrates on agents and pathogenesis. There is little emphasis on effective practical health control strategies.
- Outbreaks of disease and their treatment in poikilotherms are generally closely related to the environmental conditions, of which temperature is particularly significant.
- There is a serious lack of available veterinary medicines licensed for use in trout. This is a major welfare issue.
- Vaccines have produced major welfare benefits for the trout farming industry; however, they are currently only available for a small number of diseases. Research into new vaccines is continuing.
- The administration method or the inherent toxic effects of some therapeutants and vaccines can in themselves lead to adverse effects on welfare.

RECOMMENDATION

- In order to promote a better understanding of the relative roles of different diseases in trout welfare, an EU health surveillance programme should be put in place, with collection of data on all diseases and not just the relatively uncommon notifiable ones.
- A more effective licensing and approval system should be established to resolve the current lack of availability in EU of medicines and vaccines, many of which are already widely used elsewhere, often on fish for consumption in the EU.
- Efforts should be made to resolve the current discontinuity that exists between fish disease research and the development of practical health management strategies.

RECOMMENDATION FOR FUTURE RESEARCH

- Research programmes on vaccines should be particularly focussed on technologies which can alleviate the negative welfare effects that currently accompany many otherwise effective fish vaccines.

2. RISK ASSESSMENT

2.1.1. Discussion risk assessment

The risk scores based on expert advice were used to compile a risk ranking by category such as abiotic or biotic to indicate which hazards are the more important for each life stage in the various production systems considered, and also to enable the comparison of the different production systems within life stages. Comparison across life stages is difficult, because of the different length and condition for each life stage.

Genetics was not considered in the overall risk score for indidual life stages since separately the "hazard" therefore appears only once and consequences are life long. Genetic selection can lead to a loss of traits which was assessed as being a risk for welfare however the uncertainty was high.

Interactions could not be directly considered in the risk assessment, although some of the hazards can be closely linked to other factors and it is difficulte to disentangle the importance of each of them when assessing their effect on welfare.

2.1.1.1. Welfare risks associated with eggs incubation

Only hazards belonging to the abiotic factors and husbandry categories were assessed because other categories of factors are not relevant to this life stage. Only welfare impact on subsequent life stages was considered. The ranking by order of the highest risk scores on abiotic factors is summarized in Table 1. The combined uncertainty scores were high for all abiotic factors. All factors assessed for husbandry (rough handling, insufficient sorting and monitoring and lack of staff competence) had the same risk score however the risk was lower than from abiotic factors since the frequency of these hazards is usually low because industry practices are carefully controlled to avoid serious consequences.

No considerable differences between the 2 production systems were found with regards to potential risks to welfare.

Table 1. Welfare risks ranking – eggs incubation

	Trays	**Vertical screen incubators**
Abiotic	High temperature	High temperature
	High light intensity	High light intensity
	Rapid changes of temperature	Rapid changes of temperature
Husbandry	Inappropriate handling / insufficient sorting and monitoring/lack of staff competence	Inappropriate handling / insufficient sorting and monitoring/lack of staff competence

2.1.1.2. Welfare risks associated with farming of alevins

Only hazards belonging to diseases, husbandry and abiotic factors categories were assessed in a single system, trays and the other hazards were not relevant for this life stage. The ranking by order of the highest risk scores is summarized in Table 2. Abiotic factors together showed an overall high risk socre. Rapid changes of temperature, environmental complexity (lack of adequate substrate), too low oxygen content, high CO_2 level and total gas pressure constituted the highest ranks. The diseases considered for this life stage constituted a high risk for welfare. All factors assessed for husbandry (inappropriate handling, insufficient sorting and monitoring and lack of staff competence) had the same risk score however the risk was lower than from abiotic factors for the reasons already mentioned in the previous paragraph on eggs. The combined uncertainty scores were high for all abiotic factors moderate for husbandry and low for diseases.

Table 2. Welfare risks ranking – alevins

	Trays
Abiotic	High temperature/ Rapid changes of temperature / Lack of adequate substrate/ Water oxygen content too low / Water carbon dioxide too high / Total gas pressure
Husbandry	Inappropriate handling, insufficient sorting and monitoring, lack of staff competence
Diseases	Infectious Pancreatic Necrosis Fin and skin damage

2.1.1.3. Welfare risks associated with farming of fry

The highest scores for abiotic factors for both systems were too low water oxygen content and too high water temperature. The most important biotic hazards were intra-specific interaction (aggression), followed by stocking density both too low and too high. High stocking density is closely linked with deterioration of water quality and furthermore has the potential to have fatal consequences. For hazards connected with feeding, the ration was considered the most important hazard. Excess of feed impact on welfare was high due to effects on water quality or due to excessive weight gain.

Management hazards such as inappropriate handling and grading insufficient monitoring and lack of staff competency were ranked equally across all production systems. For the fry life stage diseases also scored highly with regards to the other factors. No difference between production systems was found for the disease risks: Infectious pancreatic necrosis and Rainbow trout fry syndrome were the top hazards.

The ranking by order of the highest risk scores is summarized in Table 3.

Table 3. Welfare risks ranking – fry

	Tanks flow-through without oxygenation	Tanks flow-through with oxygenation
Abiotic	Water oxygen content too low	Water temperature too high
	Water temperature too high	Suspended solids and turbidity
	Heavy metals too high, pH dependent	Water velocity
Biotic	Aggression	Aggression
	Low stocking density	Low stocking density
	High stocking density	High stocking density
Feed	Lack of feed (long term)	Lack of feed (long term)
	Excess of feed (environmental deterioration)	Excess of feed (environmental deterioration)
	Excess of feed (excessive weight gain)	Excess of feed (excessive weight gain)
Husbandry	Inappropriate handling, insufficient sorting and monitoring, lack of staff competence	Inappropriate handling, insufficient sorting and monitoring, lack of staff competence
Diseases	Infectious pancreatic necrosis	Infectious pancreatic necrosis
	Rainbow trout fry syndrome	Rainbow trout fry syndrome
	Eye lesions	Fin and skin damage
	Fin and skin damage	Eye lesions

2.1.1.4. Welfare risks associated with farming of trout on growers

Production of trout on fresh water is done in Europe on various systems according to geography and climatic conditions. In some countries rainbow trout is also produced in sea water. The production systems considered for the risk assessment were, tanks flow through, raceways, freshwater cages, ponds and sea water cages. Additional oxygenation is often used both for tanks and raceways and some hazards were assessed separately with or without oxygenation.

For ongrowers Abiotic factor did not constitute major hazards in comparison with other categories. Low oxygen content and high water temperature were top ranked hazards. For oxygenated tanks, race ways and ponds high carbon dioxide level is among the highest scores. High stocking density was the highest biotic hazard and subsequently intra-specific interaction (aggression) and low stocking density. In freshwater, seawater cages and ponds mixing fish from different origins and predators constitute risks not present in the other systems. Over all production systems the amount of feed played the most important role with excess of feed appearing to be slightly more important than lack of feed which could however lead to mortality. Inappropriate handling and inadequate sorting were ranked as top risks, followed by lack of staff competence and insufficient monitoring. Abiotic factor did not constitute major hazards in comparison with other categories. The welfare risks associated with diseases are higher for conditions such as eye lesions and skin and fin damage connected with secondary infections. For the diseases considered, sea cages scored better.

Overall production systems did not considerably differ, but fresh water cages and sea cages had less risks associated with abiotic hazards. Sea cages scored less for the studied diseases. Management and feeding hazards did not differ in their scores and biotic factors only marginally. For abiotic factors the uncertainty varied and could be high, for feeding and management the uncertainty was low, whereas for biotic and disease a medium uncertainty was found.

Table 4. Welfare risks ranking – ongrowers

	Tanks	Raceways	Ponds	Freshwater cages	Seawater cages
Abiotic	Water oxygen content too low	Water oxygen content too low	Water oxygen content too low	Water oxygen content too low	Water oxygen content too low
	Water carbon dioxide too high(*)	Water carbon dioxide too high(*)	Water carbon dioxide too high(*)	Water temperature too high	Water temperature too high
	Water temperature too high	Water temperature too high / Toxic un-ionised ammonia content	Water temperature too high	Water carbon dioxide too high	Water carbon dioxide too high
Biotic	High stocking density	High stocking density	High stocking density	High stocking density	High stocking density
	Aggression	Aggression	Aggression/ Low stocking density	Aggression	Aggression
	Low stocking density	Low stocking density		Mixing fish	Predators/ Low stocking density
Feed	Lack of feed (long term)	Lack of feed (long term)	Lack of feed (long term)	Lack of feed (long term)	Lack of feed (long term)
	Excess of feed	Excess of feed	Excess of feed	Excess of feed	Excess of feed
Husbandry	Inappropriate handling	Inappropriate handling	Inappropriate handling	Inappropriate handling	Inappropriate handling
	Grading	Grading	Grading	Grading	Grading
	Lack of staff competence	Lack of staff competence	Lack of staff competence	Lack of staff competence	Lack of staff competence
Diseases	Eye lesions	Eye lesions	Eye lesions	Eye lesions	Eye lesions
	Fin and skin damages Proliferative Kidney Disease	Fin and skin damages Proliferative Kidney Disease	Fin and skin damages Proliferative Kidney Disease	Fin and skin damages Proliferative Kidney Disease	Fin and skin damages Infectious pancreatic necrosis

* systems with oxygenation

2.1.1.5. Welfare risks associated with farming of broodstock

Combined risk scores show low water oxygen content, too high water temperature and carbon dioxide level as the most important abiotic factors affecting broodstock welfare. Intra-specific interactions showed a very high risk score, followed by high stocking density, predators and low stocking density. For the broodstock the biotic factors constituted the highest risks. For broodstock the amount of feed was the most important risk with excess of feed appearing to be slightly more important than lack of feed which could however lead to mortality. Inappropriate handling and inadequate sorting were ranked as top risks, followed by lack of staff competence. For the diseases: eye lesion and fin and skin damage ranked highest. The risks were the same across all production systems. The ranking by order of the highest risk scores is summarized in Table 5.

Table 5. Welfare risks ranking – broodstock

	Ponds
Abiotic	Water oxygen content too low
	Water temperature too high
	Water carbon dioxide too high
Biotic	Agression
	Stocking density too high
	Predators
	Stocking desity to low
Feeding	Excess of feed
	Lack of feed (long term)
Management	Inappropriate handling \ Inadequate sorting
	Lack of staff competence
Disease	Eye lesions
	Fin and skin damages

2.1.1.6. Risk associated with production systems

Overall, production systems did not seem to differ much in their risk scores within life stages (biotic, management, feeding).

The risk scores for diseases were in general the highest amongst all different categories considered.

2.1.2. Conclusions and recommendations - risk assessment

CONCLUSIONS

- No major differences concerning overall welfare risk between the different production systems used for each life stage were found.
- Production systems can differ in their risk score for different categories of hazards, since they all can have specific risks: for example tanks can show an overall smaller risk score with regards to disease, but a higher risk score with regards to abiotic factors than freshwater cages.
- Different production systems require different measures to control the welfare risks for farmed trout.
- The uncertainty is still high for certain categories indicating the need for well-documented and peer-reviewed data for some of the hazards.
- Risk ranking is possible for the different life stages but uncertainty can be variable and interaction between factors renders the assessment difficult.

RECOMMENDATIONS

- Measures to improve welfare should be adapted to different production systems and taking in consideration the specific requirements of each life stage.
- More detailed data about current production systems at a European level are necessary since fish farming practices vary considerably.
- Disease was assessed as an important risk factor for the welfare of farmed trout however prevalence data of trout disease in aquaculture in the various production systems need to be collected and published for a thorough assessment.

European Food Safety Authority

Annex I to the EFSA Journal (2008) 796, 1-97

SCIENTIFIC REPORT OF EFSA

ANIMAL WELFARE ASPECTS OF HUSBANDRY SYSTEMS FOR FARMED TROUT [1]

Prepared by Working Group on Trout Welfare

(Question No EFSA-Q-2006-147)

Issued on 11 September 2008

[1] For citation purposes: Scientific report of EFSA prepared by Working Group on Trout welfare on Animal Welfare Aspects of Husbandry Systems for Farmed Trout. *Annex I to The EFSA Journal (2008) 796, 1-97*

SUMMARY

The scientific report on the animal welfare aspects of husbandry systems for farmed trout constitutes the background document to the opinion adopted by the Animal Health and Welfare panel on the 11 September 2008. The scientific report focuses on the two main species of trout farmed in Europe, rainbow trout and brown trout. A description of their life cycle both wild and in production and a description of the main production systems was done. The Working group of experts identified a list of production factors affecting the welfare of farmed trout. These factors were classified in seven categories: abiotic, biotic, feed and feeding, husbandry and management, genetic, disease and disease control measures. The scientific literature but also industrial knowledge was taken into account on the description of the various factors identified and their potential welfare impact. A risk assessment approach was developed to assess the welfare risks for farmed trout of each of the identified hazards for the various life stages of farmed trout in the various production systems considered.

Key words: Trout, welfare, production systems, production factors, abiotic, biotic, feed, husbandry and management, genetic, disease, disease control measures, risk assessment.

ACKNOWLEDGEMENTS

This scientific / technical report was prepared by the working group on Trout Welfare. The European Food Safety Authority wishes to thank the (following) members of, the WG on Trout Welfare for their contribution to the preparation of this statement: Ronald Roberts (chairman), Donald M. Broom, Christine Müller-Graf, David Morton, Børge Damsgård, Rod Wootten, Jimmy Turnbull, Gilles Lemarie and Patrick Prunet.

In addition the Scientific Panel on Animal Health and Welfare wishes to thank Dr Sunil Kadri and Dr Craig MacIntyre for their contribution to this report.

PANEL MEMBERS

This scientific report was peer reviewed by the Members of the Scientific Panel for Animal Health and Welfare (AHAW) of the European Food Safety Authority. The scientific report was used as the basis for a scientific opinion adopted on 11 of September 2008. The members of the AHAW Scientific Panel were:

Bo Algers, Harry J. Blokhuis, Donald M. Broom, Patrizia Costa, Mariano Domingo, Mathias Greiner, Daniel Guemene, Jörg Hartung, Frank Koenen, Christine Müller-Graf, David B. Morton, Albert Osterhaus, Dirk U. Pfeiffer, Ron Roberts, Moez Sanaa, Mo Salman, J. Michael Sharp, Philippe Vannier and Martin Wierup.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Council Directive 98/58/EC concerning the protection of animals kept for farming purposes lays down minimum standards for the protection of animals bred or kept for farming purposes, including fish.

In recent years growing scientific evidence on the sentience of fish has accumulated and the Council of Europe has in 2005 issued a recommendation on the welfare of farmed fish[2]. Upon requests from the Commission, EFSA has already issued scientific opinions which consider the transport[3] and stunning-killing[4] of farmed fish.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In view of this and in order to receive an overview of the latest scientific developments in this area the Commission requests EFSA to issue a scientific opinion on the animal welfare aspects of husbandry systems for farmed fish. Where relevant, animal health and food safety aspects[5] should also be taken into account. This scientific opinion should consider the main fish species farmed in the EU, including Atlantic salmon, gilthead sea bream, sea bass, rainbow trout, carp and European eel and aspects of husbandry systems such as water quality, stocking density, feeding, environmental structure and social behaviour.

Due to the great diversity of species it was proposed that separate reports and scientific opinions on species or sets of similar species would be more adequate and effective.

It was agreed to subdivide the initial mandate into 5 different questions.

Question 1

- In relation to Atlantic salmon

Question 2

- In relation to trout species

Question 3

- In relation to carp species.

Question 4

- In relation to sea bass and gilthead sea bream

Question 5

- In relation to European eel

This report will refer only to question 2 as referenced above.

2 Recommendation concerning farmed fish adopted by the Standing Committee of the European Convention for the protection of animals kept for farming purposes on 5 December 2005

3 Opinion adopted by the AHAW Panel related to the welfare of animals during transport -30 March 2004

4 Opinion of the AHAW Panel related to welfare aspects of the main systems of stunning and killing the main commercial species of animals- 15 June 2004

5 Food Safety aspects are addressed by a Scientific Opinion of the BIOHAZ Panel (Food Safety aspects of Animal welfare aspects of husbandry systems for farmed fish, Question N° EFSA-Q-2008-297).

1. Scope and objectives

This report was prepared on the basis that although rainbow trout is the principle farmed trout species in Europe in the 1880's, there is no single husbandry method which predominates, and there is little in the way of peer reviewed literature on the welfare aspects of the wide range of systems used. Thus while wherever possible information used to inform the report is taken from the available scientific literature, recourse has often been necessary to expert opinion or to the experience of producers. Even less information is available on farming systems used for the native brown trout, which is less widely farmed. Where use of such information was necessary, it is indicated in the text.

The various areas where specific welfare risks have been considered to exist have been defined and analysed in relation to the different stages in the life history of the species and the type of production system.

The welfare risks identified as a result of the review were then subjected to a qualitative risk assessment, with the objective of defining those aspects of current trout husbandry systems which may increase the possibility of negative welfare effects on the farmed fish.

Although it is recognised that transportation of live trout and their slaughter may involve welfare issues, these were excluded from the terms of reference and are not considered.

The objective of the report is to highlight those aspects of current husbandry systems for trout in Europe which may increase the likelihood of negative welfare effects on the fish and to recommend areas where modification of the system as currently used may assist in reducing this risk.

2. Taxonomy of farmed Trout species

All trout are members of the Family Salmonidae, Order Salmoniformes. In Europe two species from two different genera of the Salmoniformes are farmed as ''trout''. These are the rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792) (formerly *Salmo gairdneri* (Richardson), which are called steelhead trout when they it grow in sea water, and the brown trout, *Salmo trutta* Linnaeus, 1758, sometimes called sea trout when in sea water.

Colour variants of both brown trout and rainbow trout, such as the golden trout and the blue trout and albino trout are aberrant colour morphs of the two species. These colour variants do occur naturally but they are selected against in the wild and rarely survive to maturity, except under farm conditions where they are protected from predators (M D Powell *pers.com*.).

Rainbow trout originated from western North America, but they have been used for aquaculture in most temperate areas of the world. Brown trout are native to northwest Europe and wild populations, including anadromous strains, are widely distributed (Berra, 2001).

3. Life Cycle

The life cycle of all trout species is similar. Larvae hatch from eggs deposited in a stream bed 'redd' and survive for a limited period on their yolk sac. As gills and internal organs develop so they move on to start feeding on benthic larvae gradually moving downstream and eating larger prey species before residing in a river or lake, or in the case of sea-going populations, in the sea. Growth is mainly a function of the feed availability and marine migrants are generally much larger than their riverine peers. Adults migrate upstream and form redds where the eggs are laid, usually in late autumn or winter in many wild rainbow trout, but from winter into spring in some wild rainbow trout in their native habitat.

Farmed fish have a similar life cycle, although growth is generally faster and maturity occurs earlier. Adult fish are stripped of gametes and fertilised eggs laid down in trays or cylinders for on-growing. Trout often recover after spawning and may spawn several times in their lifetime.

Most farmed rainbow trout populations in Europe are either sterile or all-female populations. Even when non-sterile populations are released into the natural environment, they rarely establish long-term breeding populations.

4. Overview of trout production systems in Europe

Rainbow trout production in Europe in 2006 was 332361 tonnes (FEAP website) which represents approximaltely 20% of the EEA finfish production. The largest producers were Denmark, France, Germany, Italy, Norway and Turkey (Table 1). Up to ~70% of production is of fish of c. 250g, which are produced in fresh water tanks, ponds, raceways or cages. Also, large trout (> 1kg) are produced (Table 1), particularly in Scandinavia, by sea cage culture.

Brown trout are produced mainly for restocking and data on production figures at European and national level are absent.

Table 1. **Rainbow trout production in Europe 2006**

	Portion size fish (a) (250g)	Large fish (>1 kg) (a)
Austria	1600	230
Belgium	400	-
Croatia	800	-
Czech Republic	600	-
Denmark	29000	7000
Finland	-	14000
France	25000	9000
Germany	25000	9000
Greece (2005)	3000	-
Hungary	25	-

Iceland	-	20
Ireland	1500	1100
Italy	2200	600
Norway (2005)	-	65000
Poland	14000	-
Portugal	15500	-
Spain	2500	1500
Sweden	-	6000 (Sea trout 212)
Turkey	38250	2000
UK	12500	2000 (Restocking 3100)

Source: FEAP (http://www.feap.info/feap/aquaculturedata/default_en.asp)

(a): Quantities in tonnes

Some trout producers are vertically integrated, carrying out all stages of the production cycle, but many farms specialise in different life-cycle stages such as egg production, fry and fingerling production, or ongrowing. Hatcheries produce ova from broodstock and sell on to fingerling producers who grow fingerlings and fry. Fingerling producers supply re-stockers and table producers. Table producers in turn provide fish to processors, while re-stockers will supply fisheries. Almost all trout are produced in intensive systems although trout reared for restocking purposes are usually grown at lower stocking densities. Usually brown trout production is on a much smaller scale, supplying the restocking markets.

Information regarding the number of farms or production systems used in the various Member States is currently not available at European level. The data presented in Table 2 was collected from the Community Reference Laboratory of Fish Diseases - 2006 survey however it is not clear if the number of farms reported represent the number of aquaculture licences/ farms or number of sites. Table 3 on production systems is based on expert opinion.

Table 2. **Number of farms in EEA countries, 2006**

Country	Number of farm	Country	Number of farm
Austria	170	Liechtenstein	
Belgium	98	Lithuania	1
Bulgaria	51	Luxembourg	
Cyprus	7	Malta	
Czech Republic	22	Netherland	45
Denmark	344	Norway	
Estonia	12	Poland	204
Finland	225	Portugal	19
France	634	Romania	62
Germany	3801	Slovakia	55
Greece	93	Slovenia	142
Hungary	3	Spain	128
Iceland	2	Sweden	100
Ireland	11	England and Wales	124
Italy	355	Scotland	65
Latvia	13	N. Ireland	30

Source: Fish Diseases Community Reference Laboratory - 2006 survey

Table 3. **Production systems for rainbow and brown trout**

Production Stage	Most frequent production system in EEA	Other production systems
Eggs	Vertical screen incubators	Trays Hatching jars
Alevins	Trays	Low water depth tanks
Fry	Tanks flowthrough	Tanks recirculated Raceways Ponds
Fresh water ongrowing	Ponds	Tanks recirculated (rainbow trout) Cages (rainbow trout) Raceways Tanks flowthrough
Sea water ongrowing	Sea cages	
Broodstock (pre-spawning)	Ponds	Tanks flowthrough Tanks recirculated (rainbow trout)

4.1. Production Systems

The ongrowing stage of trout production is done in different systems across Europe, because of climatic, geomorphological, and hydrologic differences, and biological constraints on the rainbow trout life cycle.

1. Earth ponds in fresh water

In temperate European countries trout farming has traditionally relied on the use of surface water, or spring or borehole water to feed earth ponds. Water temperatures generally range from 5-6 ºC in winter and up to 22-24 ºC in summer. Water input into such systems is at 130 l/sec per 100 tonnes of production. After passing through the ponds water is returned to surface water, often after removal of suspended solids. The flow-through system ensures removal of faeces and nitrogenous waste. Earth ponds have a low energy input and low investment, and stocking densities are limited to 25-45 kg/m^3. Brown trout are grown at lower densities (<20 kg/m^3), which maintains better heatlh and morphology and condition for the restocking market. The size of ponds varies considerably, often depending on the size of fish. Animals may be moved to larger ponds as they grow. Ponds are usually lined and may be arranged in series, so that water flows from one to the other, or preferably in parallel so that water is not re-used. Depending on water flow, ponds in series may give rise to problems of hygiene and water quality. Water re-use may be necessary if surface water flows are restricted (e.g. in summer). Aeration or oxygenation may be used to maintain stocking densities and productivity, especially at higher temperatures. At higher densities growth rates are lower and outbreaks of disease are more frequent because ponds cannot be easily cleaned. As growth is relatively slow production is mostly limited to fish of 250-400 g, which might be pink or white fleshed. There has been increasing use of all-female stocks from year round egg production which produces better flesh quality and prevents early maturation of males.

2. Concrete raceways in fresh water.

In the mid-1970s improved technology (e.g. oxygenation systems, automatic feeders and graders), together with advances in breeding and genetic manipulation (e.g. all year round egg production using photoperiod modification, triploidy, monosex populations) led to the development of more intensive systems using concrete raceways. These are mostly gravity fed from surface waters, but depending on water flow, temperature and oxygenation, stocking densities of 150 kg/m^3 are routinely achieved. Water use is estimated at 60 l/sec per 100 tonnes of production. Fry are stocked at 0.5 g and grown to either 250-400 g portion size, or to 0.4-4 kg. Fish may be white or pink fleshed. Genotypes used are mostly either all female diploid stock for portion-size production or all female triploids for larger fish.

3. Tanks in freshwater

Rainbow trout may be grown to portion or fillet size (250-500 g) in 9-12 months in, usually circular, freshwater tanks either outside or in a suitable building. Tanks may be up to 10 m in diameter and 1.5 m in depth and constructed of plastic, fibreglass or concrete. Trout are stocked at ca 5 gm and stocking densities range from 20-80 kg/m^3. Above 40 kg/m^3 additional oxygenation is required. Fish are fed by hand and/or by automatic feeders. Tanks are self-

cleaning and normally fed by surface water the flow of which should be sufficient to remove waste products. When located outdoors the tanks are usually covered with nets to prevent predation.

4. Cages in marine or brackish water

In northern European countries (Scandinavia, Scotland) fjordic coastlines have allowed the development of cage production of rainbow trout in marine or brackish water. Most fish produced in this system are >1 kg. Salinity permits a high growth rate but the low rate of water exchange limits the fish density to 15-40 kg/m^3. Fish are introduced directly into seawater at 70-100 g, at which size survival is good. Time to harvest is approximately 12-18 months. In the Baltic, fish are introduced to sea water in spring at c. 1kg and harvested in the autmn at 4-5 kg before cages ice-over. In areas where winters are very severe juvenile trout for marine culture are first grown in indoor tanks or recirculation systems with heated water, with water disinfection and injection of liquid oxygen to improve growth and productivity. These are only economically feasible because the numbers of juveniles needed per kg of final product (4-10kg) are small. Water temperatures in winter may be so low that in brackish water areas (Baltic) cages are ice-covered for 2-4 months of the year, preventing feeding and observation of fish, or requiring an autumn harvest. Fish genotypes used for this production are normal diploid or all-female diploid, or triploid. Production is pink fleshed by the use of artificial colourants. Marine farming is limited to northern areas because if water temperatures rise above 19 ºC there is a very high mortality rate. Degraded water quality may occur in sea water cages due to deposition of excess feed and faeces under the cages and poor water circulation. Cage nets may become fouled with marine growth and must be changed and cleaned as necessary to maintain adequate water circulation. Predator nets are usually fitted above and below water.

5. Cages in freshwater

Rainbow trout may also be grown in floating cages in freshwater lakes. Fish are mostly grown to portion size at 250-300 g or to 500 g for fillet production. There is some limited production of fish up to 3.5kg for smoking. For smaller sizes production takes 9-12 months. Fish are mostly stocked at 5-10g but in some cases up to 30g in size. Stocking densities maybe limited by water exchange rate through the cages but are typically 15-40 kg/m^3. Feeding is by hand or automatic feeders. The water depth under the cages is important in order to avoid mixing of water layers in summer and consequent deoxygenation. In highland lakes cages are not situated near to inflowing rivers because of potential high levels of suspended solids and low pH.

6. Highly recirculating system in fresh water

Technologically advanced recirculation systems have been developed in recent years, especially in Denmark. Such systems have high construction and maintenance costs, but limit environmental impact. Production is based on concrete raceways with a high (95 %) recirculation rate, air-lift aeration, in-tank faecal collection using sludge cones, microsieves, microfiltration and biofiltration with fixed and moving bed filters, and automated feed delivery. Water requirements are estimated at 15 l/sec per 100 tonnes production and stocking densities between 50-150 kg/m^3 are reported with a food conversion of 0.8-0.9. Such systems use relatively low quantities of borehole or surface water.

4.2. Production Cycle

Trout eggs and milt are obtained by manual stripping of brood fish, or by surgical removal. When sperm is removed from testis by surgical operation, it can be diluted and stored in adapted extenders. Trout broodstock are 2-5 years old at maturity, but females are often not used as brood fish until they reach 3-4 years to ensure good quality eggs. Approximately 2000 eggs are produced per kg of fish. At least 2 males are used to fertilise eggs from one female. Maturation of broodstock may be manipulated by changes in photoperiod to produce eggs outside the normal autumn spawning season. This, together with the importation of eggs from other geographic regions e.g. South Africa, make eggs are available in Europe throughout most of the year.

Eggs and milt are mixed without water, hydrated in fresh water and then incubated until the eyed embryo stage is reached in running water in hatchery troughs, in vertical screen incubators or hatching jars. Fertilisation extender may also be used to improve and ensure spermatozoa mobility before adding fresh water. Frozen sperm is only used to preserve genetic resources through cryobanks. In troughs, eggs are placed in baskets or screened trays through which the alevins can drop when they hatch. Hatchery troughs are ca 40-50 cm wide, 20 cm deep and up to 4 m in length. Eggs may also be incubated in vertical screen incubators containing a number of trays and stacked on top of each other. Hatchery jars contain a mass of eggs that are suspended in a water flow introduced from below. After hatching alevins from vertical incubators or jars are normally transferred to troughs (Fig 1a) where usually a substrate such as bio mats is provided. The length of time to hatching depends on water temperature, for rainbow trout the requirement is 300-330 degree days and for brown trout 390-420 degree days (Piper et al., 1982). Eggs and nearly hatched alevins are kept under low light intensity until first feeding. When the alevins have started active food searching they are transferred to freshwater tanks and weaned onto formulated dry feed. At this stage food is provided on a more or less continuous basis using belt feeders. Time as alevins varies but is of the order of 250-300 degree days for all species, dependent especially on temperature.

Average survival rates during incubation and the early fresh water phase range from 48% to 98%, being lowest in the period before the eyed-egg stage and during first feeding. Tanks are usually self cleaning fibreglass or concrete and ideally circular in shape to maintain a regular water current and uniform distribution of fry (Fig 1 b). Square tanks with rounded corners are also commonly used. Tanks are usually self-cleaning with a central drain and water introduced at the side. Various tank sizes (1 – 5 m diameter) are used with low water depth (0.3 - 0.1 m) and flowthrough water renewal.

Within the hatchery water supply may be from surface water for from borehole or spring water. The latter may be advantageous from the point of view of temperature and disease control, but may require aeration or, if supersaturated with nitrogen, degassing by exposure to air.

At a size of ca 5 g fry are normally transferred to grow-out systems. These may be circular tanks (fig 1 b), earth ponds, raceways or cages. As fish grow they will be moved to larger holding systems to maintain suitable stocking densities. These will vary greatly depending on the system, including water quality, and oxygen levels. Fish are frequently graded during the production cycle in order to maintain populations of uniform size. Failure to grade may result in feeding competition and aggressive behaviour. Fish near to market size are frequently graded

to harvest fish of the correct size for processing. Grading may be manual but is usually carried out using mechanical graders where fish pass through bars set at required distances apart.

Fish are fed on formulated dry particulate diet throughout their life according to manufacturer's recommended feeding rates and pellet sizes. Trout are normally fed at 1-1.5 % body weight per day. Feed pellets contain fish meal, oil, grains and other ingredients. Many diets are now high energy with high dietary fat levels (ca 20 %) which are very efficiently converted by rainbow trout to limit protein used and their negative impact on the environmental nitrogen. Fish may be fed manually, or by using automatic feeders which deliver feed at set intervals, or by self feeders. Artificial colourants may be added to the diet to colour flesh pink. Portion sized fish (ca 250 - 300 g) are produced in 9-12 months (ca 720 degree days) in most systems. Brown trout grow more slowly reaching 300-500 g in two years, although larger fish may be produced.

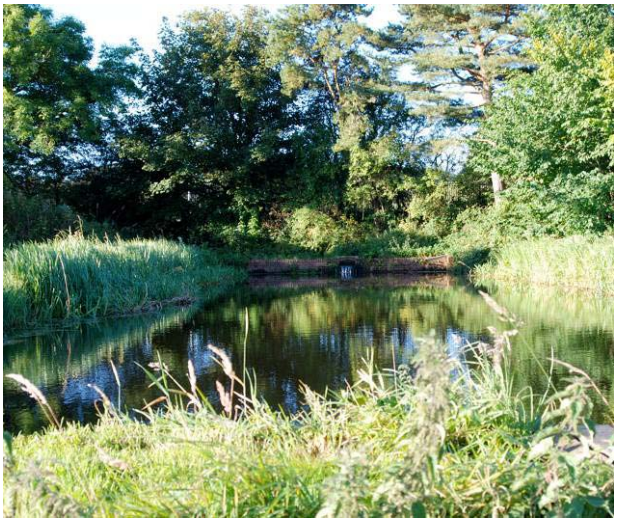
A large proportion of rainbow trout produced are now all-female and triploid i.e. sterile. Such fish avoid the undesirable characteristics and poor growth associated with early maturation of male fish. All female populations are produced by fertilising normal xx chromosome female eggs with milt from sex-reversed masculinised females (xxx chromosomes) which have been treated with methyl testosterone as fry, according to the Council Directive 96/22/CE. Thus, the offspring of these fish which are used for production have not been hormone treated. Eggs are shocked by heat or pressure in early incubation leading to triploid chromosomes and non-development of the ovary i.e. the fish are functionally sterile. Triploids are not considered as GMO according to the Directive 2001/18/EC.

Broodstock are selected for different traits such as growth, external morphology, gutted yield, or flesh colour. The main rainbow trout breeding companies in France, Norway and Finland use technology based modern breeding programmes with sophisticated selection algorithms and genetic parameters (Gjedrem, 2000) in order to avoid the effects of inbreeding, such as loss of genetic variation, poor reproductive performance and loss of variability in disease resistance.

a) D-ended tank for fry or fingerlings

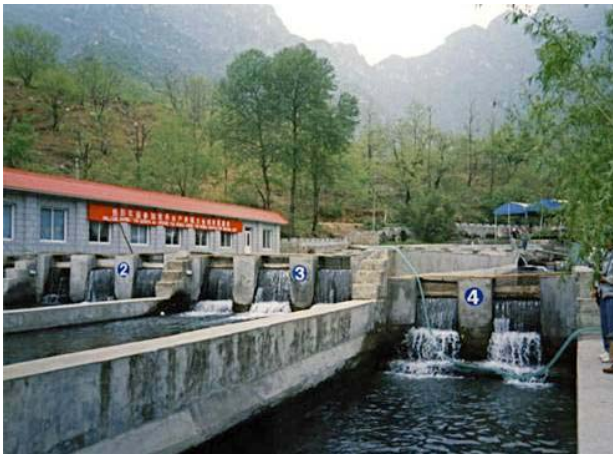
b) Circular tanks with automatic

c) Circular tanks with automatic



d) Earth pond for brown trout growers

e) Freshwater cages for growers



f) Large scale raceway system for growers.

Figure 1. **Trout production systems**

(Photograph courtesy of Dr Rod Wooten)

4.3. Welfare in relation to the production cycle and production systems

While it is possible to discuss the welfare of trout at length there is very little published scientific data to support the many opinions on the subject. There are problems when using controlled laboratory experiments to examine trout welfare in ongrowing systems, as they do not replicate the real farming environment. They may improve our understanding of underlying mechanisms, but do not permit quantification of welfare impacts on real farms. Much of the data from farms has used production or mortality figures, rather than measures of welfare, and deduced tolerance limits do not necessarily coincide with those required for good welfare. Welfare on farms is mostly related to acute challenges such as grading or treatments, but the welfare impacts of such acute events cannot be assessed, and few data are available. One successful approach has been to collect epidemiological data from rainbow trout farms (North et al., 2006a; St-Hilaire et al., 2006) Atlantic salmon farms (Turnbull et al., 2005) and from other species such as poultry (Jones et al., 2005). Even these studies are difficult due the highly confounded nature of the data and its hierarchical nature (i.e. fish in units, in farms categorised by production type). Such complex data requires multi-level modelling approaches which have only recently been applied to aquatic systems and much of this work has yet to be published. Therefore, the following sections attempt to indicate those areas in trout production where welfare issues may arise.

5. Factors affecting Farmed Trout welfare

A list of potential welfare issues for trout farmed in Europe was identified. The description of the factors and its potential welfare impact constitute the first step of the risk assessment methodology described in section 6.

In any environment, trout and other salmonids show habitat preferences (Armstrong et al., 2003). They respond to local temperature, adverse chemical conditions, pollutants, food availability, water flow, the possibility to hide under cover, predator presence and local population density (Bjornn, 1977; Berejikian et al., 2003). The preferences are affected by early experience (Johnsson and Abrahams, 1991) but trout reared in farm conditions still have a wide range of preferences related to biological functioning (Huntingford, 2004).

5.1. Environmental conditions - Abiotic factors

Trout are exposed to a range of abiotic, external environmental factors during the farm life-cycle. Impaired welfare vary with the production systems, and different life-stages can display different optimal and tolerance limits to the various abiotic factors such as water quality (e.g. salinity, temperature, waste products, gases; metals; and particulate matter) light conditions (e.g. photoperiod, intensity, spectral composition, variability and sunburn), sound, hydrostatic pressure, water currents and waves.

Trout can adapt to a range of abiotic factors, but once their capacity to adapt is exceeded they may suffer from a stress response and physiological or pathological disturbances. The level at which abiotic factors exceed the adaptive capacity of the fish are not easily predicted since they

depend on the size and species of the fish, the life stage, previous experience, concurrent diseases and interactions between abiotic factors. Aquatic organisms have tolerance limits within which they can maintain homeostasis. Abiotic factors modulate the physiological, behavioural and endocrine responses of trout, and therefore welfare impacts must be considered in relation to, and interaction with, the environment where the trout is reared. Although it is possible to simply list the major abiotic factors which impact on trout biology, the interactions between these factors are probably more important than the impact of each independent factor. However, much of the literature is on the effects of independent abiotic factors on trout, without consideration of the interactions of abiotic factors, or understanding of the systems involved.

Brown trout and rainbow trout differ in their sensitivity to some abiotic environmental factors. There is also variation in sensitivity within species and between populations, which may be related to genetic differences or previous experience. The widespread distribution of trout farms in Europe, from Finland to Spain and Italy, further complicates interpretation of data on acceptable limits for abiotic factors.

5.1.1. Water quality

There is no doubt that water quality is important for the health and welfare of farmed trout. There is also evidence that effects of management practices such as stocking density are mediated through water quality (North et al., 2006b). However, farmers generally only have a limited capacity to monitor and manipulate many water quality parameters. A recently published review (MacIntyre et al., 2008) describes, in detail, the literature relating to water quality and farmed trout welfare. This review concluded:

"*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 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.*"

In spite of the complexity of the interaction between abiotic factors, in subsequent sections indication of possible welfare impact and ranges will be addressed.

5.1.2. Water temperature

Temperature tolerance is highly dependent on acclimation. In general trout seem to be able to adapt to temperatures in the range of 0-22°C provided the fish are supplied with well oxygen-saturated water (Ihssen, 1986). The lower lethal limit is considered to be around -1° C, and at temperatures approaching that level permanent eye damage is a characteristic feature (Ferguson

et al., 2004). Temperature optimum for growth of rainbow trout appears to be in the range of 16-18°C depending on stage and size (Jobling, 1994).

Optimal range is genetically determined and differs between strains, and previous temperature acclimation history is also important (Neill and Bryan, 1991). Inappropirate rearing temperatures have been associated deformities in salmonids hard and soft tissues.

Aquatic temperatures normally change slowly due to the high specific heat capacity of water. Higher temperatures lead to increased metabolic demand for oxygen but reduce oxygen solubility, often causing life threatening oxygen deficit (Colt and Orwicz, 1991). This may be exacerbated by enhanced oxygen demand due to feeding or to any reduction in respiratory efficiency due to parasitism or hyperplasia of the gill secondary lamellae (Roberts and Shepherd, 1997). Increase in temperature from 9 to 15° C reduces the capacity of water to hold oxygen by almost 13% but increases the metabolic rate by 67.5 % in 100 g rainbow trout. Furthermore ammonia excretion increases by almost 100 % leading to almost 60 % increase in environmental un-ionised ammonia (Klontz, 1993). A rapid drop of the temperature from 14 °C to 9°C induces a stress response (plasma cortisol increase), but an increase from 14 °C to 19 °C does not change cortisol levels (Wagner et al., 1997). However, as the effects of temperature change depend on the rapidity of change and trout population genetics, it is not possible to give a precise threshold which would lead to stress responses.

Environmental conditions, particularly temperature and dissolved oxygen concentrations, are of paramount importance in the successful development of trout eggs and alevins. Poor environmental conditions at these life stages can lead to long lasting skeletal and tissue damage (Finn, 2007). High water temperatures or sudden temperature variations will adversely affect the development of eggs and alevins. Minimum and maximum temperature limits for constant temperature incubation of steelhead and freshwater rainbow trout are 3-14.6 °C (Pennell and Barton, 1996). Problem of fragile bone structure have been reported at high temperature in trout but it is not clear whether mutlifactorial effects may not be the cause of such malformations (Finn, 2007). Alevins are able to withstand higher temperatures up to ca 12 °C. The lower temperature limit is not well defined but eggs and alevins will not be affected at temperatures down to 0º C.

Water temperature modulates the timing of spawning for broodstock, optimal spawning occurring at 10-13 °C in rainbow trout. Ovulation and spawning can occur at higher temperatures (Bromage et al., 2001) , but a sudden drop of 3-5 °C can also induce spawning, although it is inhibited by low temperatures (Elliott, 1981).

5.1.3. pH

Several factors may cause water pH to drop or rise, but generally in seawater the pH is more stable due to a higher buffer capacity. In fresh water pH can be affected by improper filtration systems and increased carbon dioxide due to respiration. Changes in pH can also occur during snow melt in forested areas in higher latitudes leading to sudden drop in pH below 5.5 (Bruslé and Quignard, 2004).

Fish maintain a constant internal pH and an acid/base balance in the blood by altering their pH using bicarbonate ions or acidic carbon dioxide, controlled by carbonic anhydrase in the blood and gills. Acidic pH causes release of bicarbonate ions to buffer the pH back up to normal

values, and carbon dioxide or the removal of bicarbonate ions to lower the blood pH. Salmonids vary in their pH tolerance and generally adults cope better than fry or eggs.

Acidic water elevates blood flow branchial resistance (Sundin and Nilsson, 2000). Acid and alkaline water induced a higher increase in blood lactate and earlier fatigue to swimming fish (Ye and Randall, 1991). Salmonids vary in their pH tolerance and generally adults cope better than fry or eggs. When exposed to pH equal or below 4, rainbow trout and brown trout show significant mortality and it is considered that between 4.5 and 5.5 are pH values which induced sublethal but significant effects on trout physiology, mainly on acid-base state, osmoregulation, oxygen transport and cardiovascular responses (Packer, 1979; McDonald et al., 1980; McDonald and Wood, 1981; Giles et al., 1984). Exposure of trout to pH 4 in soft water, leads to impairment of oxygen transport and although it does not limit resting oxygen consumption it reduces the scope for activity (Ye et al., 1991). At pH 4 there is a blood acidification associated with a decrease of plasma HCO_3^- (Holeton et al., 1983). Chronic exposure to acid pH leads to stabilization of homeostasis parameters but fish are still physiologically affected as indicated by impairment of reproduction (Mount et al., 1988).

Toxicity of acid pH can be modulated by the presence of other ions. Thus, presence of calcium has significant effects on the resistance to acid pH, an observation which confirm that osmoregulatory failure is the sole toxic mechanism of low pH (McDonald et al., 1980). Trout reared in acid pH are more resistant to aluminium toxicity effects and prior exposure to low aluminium levels provides increased resistance to toxicity by acid pH and aluminium (McDonald and Wood, 1981; Orr et al., 1986; Wood and McDonald, 1987).

Alkalosis usually occurs above pH 8 to 9, however, trout can cope with acute high pH (>9.0) exposure periods up to 3-8 days through their ability to counteract high-pH-induced disturbances to ammonia excretion, acid-base homeostasis, and electrolyte balance (Wilkie et al., 1996). Critical limit for acceptable alkalinity in brown trout has been shown to be around pH 9.2 (Bruslé and Quignard, 2004).

Low pH and high levels of aluminium and other metals can be very deleterious to eggs and alevins of trout (Finn, 2007). Eggs are rather more resistant than alevins because of the thick egg shell. Hatching and the growth of larvae are reduced at ca pH 5.5 (Finn, 2007). In fry, the pH level varies on a number of other factors, but the optimal range is 5.5-8.5. The toxicity of acid pH can be modulated by other ions e.g. calcium, aluminium.

5.1.4. Ammonia

Ammonia is not only produced as a waste product by the fish, other animals and plants, and microbial decomposition, but anthropogenically from fertilizers and industrial emissions. Ammonia is excreted from fish gills in 2 forms; unionized (NH_3) and ionized (NH_4^+). Unionized ammonia (NH_3) is the most toxic form and its level is dependent on total ammonia (TAN = $[NH_3] + [NH_4^+]$), pH level, temperature and salinity, ammonia toxicity being higher at high pH, (Fivelstad et al., 1991; Eddy, 2005; Ackerman et al., 2006). Therefore, pH, temperature, salinity and Ca concentrations (Wicks et al., 2002) must be known to estimate ammonia toxicity (Ackerman et al., 2006) (Tables 4 and 5).

Table 4. **Fresh water: Percentage of unionised ammonia (non-ionised form, which is toxic for fish) compared to total ammonia, according to pH level and temperature**

	Water temperature (°C)			
pH	**5**	**10**	**15**	**20**
6	0.01	0.02	0.03	0.04
6.4	0.03	0.05	0.07	0.10
6.8	0.08	0.12	0.17	0.25
7.2	0.20	0.29	0.43	0.63
7.6	0.50	0.74	1.08	1.60
8	1.24	1.83	2.68	3.83
8.4	3.07	4.47	6.47	9.09
8.6	4.78	6.90	9.88	13.68

adapted from (Wedemeyer, 1996)

Table 5. **Salt water: Percentage of unionised ammonia (non-ionised form, which is toxic for fish) compared to total ammonia, according to pH level and temperature**

	Water temperature (°C)			
pH	**5**	**10**	**15**	**20**
7.2	0.17	0.24	0.35	0.51
7.4	0.26	0.38	0.56	0.81
7.6	0.42	0.60	0.88	1.27
7.8	0.66	0.95	1.39	2.00
8	1.04	1.49	2.19	3.13
8.2	1.63	2.34	3.43	4.88
8.4	2.56	3.66	5.32	7.52
8.6	4.00	5.68	8.18	11.41

adapted from (Wedemeyer, 1996)

Most biological membranes are permeable to un-ionised ammonia (Randall and Tsui, 2002). Therefore, 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). Under laboratory conditions effects of acute exposures to high concentrations of ammonia on trout include: increase ventilation rate, hyperexcitability, erratic swimming, lose of equilibrium, convulsion and death. Chronic exposures to ammonia results in alterations of the central nervous system function (Hillaby and Randall, 1979), energy metabolism (Arillo et al., 1981), ionic balance (Soderberg and Meade, 1992) and morphological changes such as fusion of the gill lamellae (Burrows, 1964).

Several factors affect ammonia toxicity. Thurston et al (Thurston et al., 1981) found that tolerance to ammonia decreased with decreasing dissolved oxygen. There is some evidence that

prior exposure of rainbow trout to sublethal levels of ammonia increases their tolerance to environmental ammonia (Meade, 1985).

Exercise increases ammonia toxicity (Shingles et al., 2001). A correlation between plasma ammonia levels and decreased swimming performance has been reported due to the effect of ammonia decreasing muscle membrane potential or by affecting muscle metabolism (Beaumont et al., 1995). Wicks et al (Wicks et al., 2002) shown that 0.04 mg/l of unionised ammonia reduced the critical swimming velocity in rainbow trout. Feed fish are less susceptible to environmental ammonia than unfed fish (Randall and Tsui, 2002). Larvae growth is reduced when chronically (20 days) exposed at unionised ammonia concentrations higher than 0.006 mg/l (Vosyliene and Kazlauskiene, 2004). Tolerance to ammonia increased as fish developed from the larval stage. Maximum tolerance is around 1-4 g body weight, after that tolerance decreases (Thurston and Russo, 1983). From the literature, there is a widespread disagreement regarding safe levels of ammonia in culture systems for trout. However, a maximum level of 0.012 to 0.02mg/l NH3-N is generally considered as a recommended limit (MacIntyre et al., 2008).

5.1.5. Nitrite

Oxygen converts ammonia into nitrite which is slightly less toxic than unionised ammonia. Nitrite destroys erythrocytes and oxidizes the iron in haemoglobin causing reduced oxygen carrying capacity and listlessness.

Although nitrite is not usually a problem in trout farms using flow-through systems, nitrite can be produced in recirculation systems with a malfunctioning biological filter. In flow-through system the main sources of nitrite are anthropogenic.

Toxicity of nitrite depends on water chemistry. Nitrite levels above 0.1 mg/l NO_2^- in water can be toxic (Wedemeyer, 1996) although effects of other ions in the water will affect its toxicity Chloride ions decrease nitrite toxicity by competing with nitrite for transport (Lewis and Morris, 1986) and bicarbonate also inhibits the uptake of chloride from water (Lewis and Morris, 1986). Sulphate, phosphate, nitrate and calcium also affect nitrite toxicity (Russo and Thurston, 1991; Tomasso, 1994). Low dissolved oxygen exacerbates the effect of nitrite toxicity because nitrite decreases the ability of the blood to transport oxygen (Lewis and Morris, 1986).

There are relatively few data on long term effects of nitrite. Fish were able to acclimate to sublethal concentrations of nitrite (Wedemeyer and Yasutake, 1978). However it is important to notice that nitrite concentrations showing minimal or negligible effects only differ by a few-fold (Lewis and Morris, 1986) and only cause respiratory epithelial hyperplasia (Kroupova et al., 2008).

5.1.6. Aluminium

Aluminium (Al) is an important factor in the toxicity of acidified waters to freshwater fish (Poléo et al., 1997) with toxicity being observed in estuaries when heavy rain fall flushes large volumes of acidified freshwater into the sea (Bjerknes et al., 2003) or associated with acid rain.

In fresh waters, Al may occurr in different physico-chemical forms such as simple cations and hydrolysis products, complexes and polymers, to colloids and particles (Salbu and Oughton, 1995; Sposito, 1996). Al toxicity depends on the species of Al present (cationic, neutral or anionic) and hence is affected by pH and the presence of complexing ligands, such as fluorids, and organic material, such as humic acid (Birchall et al., 1989). The predominant toxic forms are known to be the monomeric ones (i.e. Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^{+}$ and $Al(OH)_4^{-}$) (Dickson, 1978; Guibaud and Gauthier, 2003). Fish death is caused from damage to gill epithelia and loss of osmoregulatory capacity (Birchall et al., 1989). Al tends to accumulate in fish gills (Muniz and Leivestad, 1980; Exley et al., 1991), causing ion regulatory and/or respiratory failure (Neville, 1985; Wood and McDonald, 1987; Rosseland and Staurnes, 1994; Gensemer and Playle, 1999). Gill lesions consist of focal to diffuse hypertrophy and hyperplasia of chloride cells and adhesion of lamellae (Fivelstad et al., 2003b).

Al toxicity is related to both Al ion concentration and low pH, and it is difficult to separate the two (Roberts and Shepherd, 1997). Sublethal, acidic aluminium exposure (80 µg/L, pH 5.2) affects rainbow trout blood ions and metabolites, cardiac output, heart rate, and stroke volume (Dussault et al., 2001) and chronic aluminium exposure affects swimming and cardiac performance (Dussault et al., 2004). Low pH and high levels of Al and other metals can be very deleterious to eggs and alevins of trout (Finn, 2007) but eggs are rather more resistant than alevins because of the thick egg shell.

5.1.7. Other metals

Copper, iron, zinc and cadmium, are toxic to trout and have profound negative physiological effects causing stress and at high concentrations mortality (Dubé et al., 2005). Environmental conditions such as pH, oxygen concentration, temperature, hardness, salinity and presence of other metals may modify metal toxicity to fish and hypoxic conditions, temperature increase, and acidification usually render the fish more susceptible to intoxication. Alternatively, an increase in mineral content (hardness and salinity) reduces metal toxicity. Interactions among various metals present in the water may modify their toxicity and synergistic, additive or antagonistic effects may occur (Witeska and Jezierska, 2003).

Lead causes ionoregulatory disruption, rather than respiratory or acid/base dysfunction (Rogers *et al.* 2003). The stress response caused by copper and cyanide increases susceptibility to infection (Carballo *et al.* 1995).

In salmonids cadmium adversely effects growth and reproduction and causes osmoregulatory stress, and it was shown to alter the structure and function of various organs, including the liver (Lemaire-Gony and Lemaire, 1992; Soengas et al., 1996). Rainbow trout can gradually acclimatise to high cadmium levels (Chowdhury et al., 2004), and wild trout can be found in rivers with high levels of cadmium. High levels of toxic metals may derive from the water source and very often, trout are exposed to a mixture of trace metal (Hickie et al., 1993).

5.1.8. Water exchange rate and water velocity

Water exchange rate (i.e. water renewal, l/min/kg biomass) determines oxygen supply and removal of metabolites whether the trout are held in raceways tanks ponds or cages. It is also important for self cleaning (removal of faeces and excess feed) and for setting up water current that affects the swimming behaviour and distribution of the fish in the rearing unit. In self-cleaning raceways, a water velocity > 3 cm/s is used to prevent solids such as uneaten food from settling (Wedemeyer, 1996). If exchange rates are too low, waste products are not removed efficiently and oxygen saturation can become critically low, whereas too high water velocity can result in high energy expenditure and stress leading to poor growth. Water exchange of 1-4 kg/l/min is recommended for trout, depending on temperature and fish size (MacIntyre et al., 2008).

In addition to its effects on water quality, a moderate current speed provides exercise, improves physiological performances and growth and reduces physical damage to the fins through behavioural changes (Jobling et al., 1993). Kawabe (Kawabe et al., 2003) recommended a current speed of 0.48-0.58 body length (BL)/s. Jobling et al. (Jobling et al., 1993) suggested a current speed of 0.75-1.5 BL/s.

5.1.9. Suspended solids

Suspended matter (organic and inorganic) is defined as particulate matter when the diameter is > 1 µm, and they may cause mortalities, but solids >100 µm settle and are not a problem (Chen et al., 1994). Spates increase considerably the quantity of suspended solids. Wastes from industries such as quarrying and sand and gravel extraction are particularly liable to generate spasmodic suspended solid levels with sharp particles. Uneaten food, faecal solids, micro fauna contribute to suspended solids (Chen et al., 1994; Wedemeyer, 1996).

Suspended solids in culture systems should be below 100 mg/l but the effects on fish also depend on the physical characteristics of the particles involved (Wedemeyer, 1996).

Suspended solids abrade and clogg fish gills, causing gill epithelial hyperplasia and excessive mucus generation, they abrade skin and smother eggs during incubation (Wedemeyer, 1996). Steelhead trout exposed to suspended solids over 400 mg/l suffer classical stress responses although no gill damage was observed (Redding et al., 1987) but rainbow trout can survive for 10 months in suspended solid concentrations of 200 mg/l (Alabaster and Lloyd, 1982). Due to the variability in size and shape of suspended solids, recommendations for maximum suspended solids vary: Wendemeyer (Wedemeyer, 1996) suggested 80-100 mg/l while Alabaster and Llyod (Alabaster and Lloyd, 1982) state no evidence of effects at concentration under 25 mg/l. In addition to direct effects, suspended organic solids can reduce oxygen availability and then have effects on fish health (MacIntyre et al., 2008).

5.1.10. Dissolved Gasses

The effects of gas saturation differ among production systems and with life-stages, with fish being very vulnerable to supersaturation of N_2 and O_2, during the early life stages (Geist et al., 2006). Oxygen is often the first limiting factor in most productions systems, but in recirculated systems where O_2 is added artificially carbon dioxide (CO_2) can be the primary limiting factor (Forsberg, 1997; Helland et al., 2005). The concentration of carbon dioxide and oxygen dissolved in a water body has a major effect upon aquatic organisms (Helland et al., 2005; Johansson et al., 2006).

5.1.11. Oxygen

The concentration of dissolved oxygen in water differs with temperature, salinity and the partial pressure of oxygen in the air. The amount of dissolved oxygen (expressed as mg/l) held in a water at 100 % O_2 saturation (in equilibrium with atmospheric oxygen, also called normoxia) decreases with increasing water temperature and salinity (Colt, 1985; Geist et al., 2006). Hypoxia and hyperoxia occur are when oxygen saturation is lower and higher respectively than 100 %. The relative oxygen saturation in water (% saturation) is regarded as the most important parameter for the fish physiology as it is the relative difference in partial pressure of oxygen that drives the diffusion of oxygen over the gills and into the blood stream (Helland et al., 2005). The oxygen consumption (mg O_2/kg fish/min) of rainbow trout increases with water temperature activity, feed consumption and stress level, while it decreases with increasing body size (Colt and Tomasso, 2001). In normoxia, the oxygen consumption in trout ranges from 250 to 500 mg O_2 when expressed in kg of fish biomass per hour and ranges from 200 to 220 g O_2 when expressed in kg of feed ingested (fish fed with commercial pellets following a feeding table) To produce 1 kg of trout, the oxygen requirement ranges from 0.5 to 1 kg of oxygen (Belaud, 1995). Under hypoxia conditions (below 5 mg O_2/l) swimming behaviour, feed intake, growth rate and physiological status are impaired. In extreme hypoxia (below 2 mg O_2/l) metabolic acidosis and mortality occur. Hypoxia may affect egg development and cause early hatching with mortalities and subsequent abnormal development and deformities Metabolic rate may vary according to species and population, a general recommendation for minimum dissolved oxygen levels can be calculated from the equation ($4.0 + 0.5 \times J0_{2max}$) where $J0_{2max}$ is the maximum rate of oxygen consumption for the developmental stage and species (Finn, 2007). Experimentally, the maximum feed intake and growth rate is observed in slightly hyperoxic waters (Dabrowski et al., 2004). Growth, food conversion and sensitivity to confinement stress did not differ in rainbow trout acclimated to hyperoxic (130 %) normoxic (100 %) or hypoxic (65 %) dissolved oxygen (Caldwell and Hinshaw, 1994). In farming conditions dissolved oxygen concentration is usually measured in mg/l. An oxygen level above 5 mg/l in tanks outlet waters is considered not to impair growth performances and is recommended for the health of ongrowing rainbow trout (Smart, 1981; Colt and Tomasso, 2001). Wedemeyer (Wedemeyer, 1996) suggested than 5-6 mg/l is too low to have a safety margin if fish need more oxygen due to increased activity (digestion, swimming, stress, etc). This value in the outlet water is accepted within the industry and considered as economically acceptable by limiting the quantity of gaseous oxygen to inject in waters (inlet water O_2 concentration: 15 mg/l) and accounts for 46,4 % saturation in a fresh

water at 12 °C and 54.9 % at 20 °C (MacIntyre et al., 2008). Lethal oxygen concentration in trout is from 2 to 2.5 mg/l, depending on temperature and fish weight, health and physiological status (Belaud, 1995).

Hyper oxygenation is commonly used in flow-through tanks in the freshwater stage, in order to reduce water renewal rates. Hyperoxia can be induced by inadequate oxygenation Values of 150 % O_2 saturation are commonly used in inlet waters after re-oxygenation in trout farms (MacIntyre et al., 2008). Although hyperoxia may induce a haemoglobin and hematocrit decrease, recovery of blood parameters is rapid when normal conditions are re-established (Jewett et al., 1991).

5.1.12. Carbon dioxide

Carbon dioxide (CO_2) is in equilibrium with the non-toxic carbonic acid (H_2CO_3) bicarbonate ion (CO_3^-) and carbonate (CO_3^{2-}), and its concentration depends on pH, temperature and salinity of the water as well as the respiration of the fish and other aquatic organisms. Monitoring CO_2 can be difficult under commercial conditions, especially in systems with low alkalinity. Increases in carbon dioxide can have a detrimental effect on rainbow trout physiology as it affects gill function, plasma chloride levels and elicits a stress response. Chronic exposure causes poor growth and very high CO_2 levels cause impaired immune function and mortality (Wedemeyer, 1996).

In freshwater salmonids high concentrations of CO_2 leads to significantly slower growth and subsequently smaller fish when exposed for 84 days (Danley et al., 2005) and flared operculae and bright red gill lamella (Summerfelt, 2002). CO_2-specific changes in hematocrit, plasma cortisol, and plasma chloride responses cause physiological stress (Fivelstad et al., 1998; Fivelstad et al., 2003a).

Carbon dioxide levels are an important issue when water renewal is reduced in systems using oxygenation or during fish transportation. Increase CO_2 in the water prevents fish to excrete endogenous CO_2 inducing hypercapnia and a blood pH decrease (acidosis) and reducing oxygen carrying capacity of the blood (Bohr effect). Such effects have been observed at water concentrations of CO_2 aroung 25 mg/l (Westers, 2001). However no mortality was observed with CO_2 concentrations up to 45 mg/l after 90 days exposure (Danley et al., 2001). In hard water, >12mg/l of CO_2 causes nephrocalcinosis (calcareous deposit in ureters) (Harrison, 1979a; Harrison, 1979b).

In freshwater irrespective of the rearing systems, it is generally suggested to have CO_2 levels lower than 10 mg/l (MacIntyre et al., 2008) although this level can be higher depending of other water quality factors like dissolved oxygen, pH, alkalinity (Noble and Summerfelt, 1996).

5.1.13. Super-saturation

Surpersaturation ocurs when gas partial pressure dissolved in water becomes greater than the atmospheric pressure. It can be caused by oxygenation, entrapment of air in piped supplies (leaks in pump or valve systems), sudden decrease in pressure or sudden increase in temperature but also by high waterfalls (hydro-electric flows) and at altitude gradients in fish transported by air (Hauck, 1986).

Super-saturation with N_2, or less frequently O_2, causes a condition known as Gas bubble disease (see section 5.6.7). The degree of supersaturation defines the eventual outcome however that level depends on temperature, size of fish, pressure on incoming water, flow rate making it impossible to establish a general threshold. Super-saturation causes bubbles of super-saturated gas in the bloodstream of affected fish as it comes out of solution.Small vessels rupture and haemorrhage, and in larger vessels, the bubbles can obstruct blood flow. Fish may die without obvious signs, but those that survive may be blind, or suffer cerebral, renal or hepatic vascular rupture and haemorrhage and often clear gas bubbles can be seen as bubbles below the cornea and epidermis (Wedemeyer, 1996). Affected fish do not thrive (Roberts and Shepherd, 1997), and may have increased susceptibility to infection (Huchzermeyer, 2003).

Most commercial farms are designed to avoid it, as it is primarily an engineering problem but when it occurs it has serious welfare implications as well as lower productiviy (Harvey and Cooper, 1962).

5.1.14. Salinity

Rainbow trout is a fresh water species but it is usually possible to acclimatise rainbow trout to seawater. Euryhalinity is only possible when farmed juvenile fish are usually > 50g. The quality of the fresh water may also affect the success of direct transfer to seawater, rainbow trout acclimated to low Ca^{++} level freshwater, are less able to adapt to seawater, than trout of the same size sourced from systems where harder water is used. However, feeding a salty diet helps adaptation to saline conditions (Perry et al., 2006). Fish < 50g reared ion-poor freshwater experience stress and subsequent mortalities (Boeuf, 1993). As rainbow trout do not smoltify in the true sense, considering that there is no preadaptation, and are not fully tolerant of high salinity, they may suffer stress at certain times of year when marine salinity is high and particularly when temperatures are high or very low (Gordon, 1959; Sigholt and Finstad, 1990).

Plasticity of euryhalinity is different in brown trout. While sedentary populations occur in freshwater, migratory population are observed in some coastal rivers which present a life cycle very similar to Atlantic salmon. From eggs up to pre-smolts, the trout parr is kept in fresh water, but full osmoregulatory capacity in seawater (> 30ppt) is achieved after a proper smoltification (parr-smolt transformation).

5.1.15. Light

Light is a complex ecological factor whose components include color spectrum (quality), intensity (quantity) and photoperiod (periodicity). Trout have large optic lobes and vision plays an important part in their foraging escape and social behaviour. The issue of how much light

should be provided for trout and how complex a visual environment they need is important in relation to their welfare. Turbidity is particularly important in relation to light availability in trout ponds (Piper et al., 1982). The aquatic environment has peculiar and extremely variable characteristics moreover, "receptivity" of fish to light changes profoundly from one species to another and, within the same species, from one developmental stage to another (Boeuf and Falcon, 2001).

Photoperiod is a key factor affecting growth and development in rainbow trout (Bromage et al., 1982). Prior to first feeding, the eggs and alevins are adapted to a life in darkness or very low light intensities as they are living in gravel in their natural environment in rivers. Exposure to light at the egg and alevin stage is considered negative as light stimulates physical activity which results in poorer yolk sac conversion efficiency into somatic growth (Boeuf and Falcon, 2001). UV light and visible light exposure during the egg stage may cause mortality (Flamarique and Harrower, 1999).

Long photoperiod (constant long day or constant light) can stimulate growth in fry (Taylor et al., 2005) but its impact on welfare are not clear (Leonardi and Klempau, 2003). Photoperiod is also a key factor in control of age at puberty and timing of spawning in the season for rainbow trout (Bromage et al., 1984; Skarphedinsson et al., 1985).

Photoperiod treatments can advance or delay the seasonal spawning time to allow out-of season egg production (Bromage et al., 1984) but temperature also has a modulating effect on spawning time (Taranger and Hansen, 1993), and combined photoperiod and temperature treatments are used for out-of-season egg production with high egg survival (Bromage, 1995). The welfare consequences of artificial photoperiod treatments are not fully known.

5.2. Environmental conditions - Biotic factors

In nature, rainbow trout and brown trout perform a range of social behaviours, including complex agonistic behaviour, feeding and mating behaviour. Rainbow trout do not normally mate in culture, but agonistic behaviour such as aggression plays an important role for the welfare of the fish throughout the lifespan.

5.2.1. Predation

Potential predators of both trout species vary during the life cycle depending upon the farming system. In freshwater the principal predators are fish-eating birds, such as heron and cormorant, and mammals, such as mink and otter. In the marine environment seals and some birds are important predators and there may be some predation by piscivorous fish.

Eggs and alevins are kept in conditions isolated from the wild so that predation is not a risk, and while fry in indoor tanks are protected, predation may occur in outdoor systems, including tanks, ponds, raceways and cages

Anti-predator behaviour or avoidance response have a behavioural cost, either directly in energy expenditure, or as lost opportunities to feed due to the necessity to hide, and may be frightened when predators such as cormorants have been attacking them, and may not feed for

several days. Farmed fish usually habituate to their farming situation, and respond less as they become acclimatised.

Attacked fish may be injured, killed, suffer stress, or escape through damaged nets. Severe traumatic wounds may become infected (Beveridge, 1987; EIFAC, 1988).

Farmers expend much money on predator control, suggesting that large numbers of fish are lost to or harmed by predators, causing a major welfare problem, but few data are available. Various methods have been developed by farmers to prevent or minimise damage caused by predators, but they have not been investigated scientifically to establish their efficacy, or identify welfare issues. Repeated or prolonged attack, or traumatic wounding, must cause suffering, and cultured fish should be protected against such attack, and from associated stress. Fish that escape to the wild may not be able to adapt to their new environment, but while the survival of escaped fish may be regarded as a welfare issue, nothing practical can be done about it.

Nets or wires over holding facilities, or by bird scarers, may be used to deter bird predators. Anti-predator nets are used to completely surround freshwater and marine cages to prevent predation by birds, and around marine cages to prevent predation by seals. Acoustic scarers may also be used to deter seals. In freshwater otters and mink may be trapped. Predators may be shot, but this requires licences, and does not apply to protected species. Producer's codes of practice recommend anti-predator measures, but non-target species may be inadvertently killed.

Bird predators, particularly gulls, are the final hosts for eye flukes of trout (*Diplostomum* and *Tylodelphys*) which cause blindness in rainbow trout on a significant scale in some farms in Europe (Chappell et al., 1994).

5.2.2. Invasive Species

Invasive species can affect the welfare of farmed trout. The most important of these are various algal species which, under certain environmental conditions, may "bloom" to produce enormous concentrations. Algal blooms may occur in both fresh and marine waters and cause heavy mortalities, especially incaged fish in open water systems (Bruno et al., 1989; Bruslé, 1995). Affected fish may show significant sub-lethal effects such as reduced appetite and respiratory distress (Roberts and Shepherd, 1997; Treasurer et al., 2003). When blooms collapse they cause deoxygenation, production of toxins, and physical damage to the gills (Yang and Albright, 1992). Although their effects are severe, algal blooms are not common. In open waters the effects of the blooms may be ameliorated by additional oxygenation, but this is difficult to apply effectively. Early harvest or slaughter on welfare grounds may be necessary.

In sea cages fish are at risk from jellyfish. Occassionally, dense swarms of jellyfish may be washed into cage sites. Smaller species will pass into the cages whilst larger species will be broken up against the sides of the cages and their tentacles will enter the cages. Mortalities and other severe adverse effects are due to anoxia or obstruction of respiration by the sheer volume of jellyfish, or to sever external injuries caused by the nematocysts on the tentacles. Prevention is extremely difficult, if possible at all, and slaughter on welfare grounds may be the only option.

Nets of sea cages are also colonised by numerous fouling organisms (e.g. algae, molluscs, sea squirts), which, if present is sufficient numbers, may impede water circulation and lead to reduced oxygen levels.

5.2.3. Intraspecific interaction (aggression)

Trout show complex social interactions which depend upon their early rearing conditions and the availability of space (Berejikian et al., 2001; Berejikian and Tezak, 2005). In many circumstances they may defend an area and show aggressive behaviour (Overli et al., 2004a; Overli et al., 2004b). Aggression may depend on stocking density, water flows and adequate feeding, leading to fil-biting and intra-specific aggression, and may be an important welfare issue in trout fry. In brown trout, and possibly rainbow trout, lack of habitat complexity may affect behaviour and growth (Höjesjö et al., 2004).

Aggression occurs among rainbow trout (Abbot and Dill, 1985; McCarthy, 2001), and to a lesser extent in brown trout (Johnsson et al., 2000; Lahti et al., 2002) is often caused by competition for sites close to feeders (Alanara and Brannas, 1997; Adams et al., 1998). In laboratory studies subordinate trout have a lower feed intake and growth compared with dominant fish (McCarthy et al., 1992). The importance of aggression as a decisive factor in social hierarchies has been demonstrated by consistently superior performance of the most aggressive fish. Dominant fish can signal their social status by changing colour, distending their fins, operculae and mouth, and defend a territory in the tank or the cage. Social interactions in trout result in endocrinological changes such as the increase in aggression in growth hormone treated rainbow trout (Johnsson and Björnsson, 1994; Johnsson et al., 1996). Plasma cortisol level may be both a consequence (e.g. (Winberg and Lepage, 1998)) or a cause of aggression, and high cortisol level in both trout species before an aggressive encounter decreases the likelihood of becoming dominant (Pottinger and Carrick, 1999; Sloman et al., 2002). The serotoninergic systems in the brain have been shown to have an inhibitory effect of aggression, and for example diets with supplementary tryptophan, from which serotonin is synthesised, suppress aggression in rainbow trout (Winberg et al., 2001). In maturing fish, there are also higher plasma levels of androgens, such as 11-keto-testosterone, in dominant rainbow trout (Liley and Kroon, 1995; Cardwell et al., 1996).

5.2.4. Stocking density

Stocking density is usually calculated as the total biomass of fish divided by the available space in m^3 of volume. The potential welfare effects of stocking density are related to the indirect effects of density on fish behaviour and water quality traits (Turnbull et al., 2005), but there is disagreement whether behaviour or water quality are the welfare issues.

In rainbow trout, fish density affects fish growth at different fish densities ranging from 14 - 450 kg/m^3 (Holm et al., 1990; North et al., 2006a) although it is not known whether density *per se* is responsible (Ellis et al., 2002; North et al., 2006a). Rainbow trout stocked at densities of 80 kg/m^3 does not produce consistent effects on feeding activity, mean growth rate

or physiological indicators of welfare (North et al., 2006a). Reduced food intake by rainbow trout stocked at 100 kg/m³ may be due to altered appetite, and not due to impaired food access or feed utilization (Boujard et al., 2002) .

Levels of aggression in rainbow trout vary with stocking density with the lowest levels occurring at intermediate densities (Pickering, 1992; North et al., 2006a). Densities Lower, than the optimum level, may result in increased aggression while higher densities may cause increased physical damage to the fish and reduction in the water quality (North et al., 2006b), with accumulation of metabolites such as CO_2.

Crowding to very high densities, associated with pre-slaughter or handling for other reasons, for example to more than 200 kg/m³, also caused an increase in stress indicators such as plasma cortisol concentration, as shown in Atlantic salmon (Veiseth et al., 2006).

Swimming activity also seems to be affected by stocking density. In general, swimming activity of rainbow trout is low under low stocking density (10 to 20 kg/m^3) in cages or tanks (Phillips, 1985; Bégout Anras and Lagardcre, 2004) without organised schooling behaviour, with slow swimming speeds and a decrease in activity during the night (Sutterlin et al., 1979; Phillips, 1985), although this may partly be due to other factors such as water velocity. At high densities from 100 to 136 kg/m³ the swimming activity increases in the night-time almost meeting the diurnal activity level reflecting an increasing constraint on on activity which departed from the natural standard seen under less crowded conditions (Bégout Anras and Lagardcre, 2004).

Male and female broodstock are commonly held together in the same tank/system since there may be pheromonal communication (Vermierssen et al., 2005) between the sexes that may affect the reproductive performance and behaviour of the brood stock. There are no scientific reports on optimal stocking density and sex ratio of trout broodstock, in practice the numbers and size of tanks vary considerably.

5.3. Feed and feeding

5.3.1. Feeding behaviour

The demand-feeding rhythm in rainbow trout is under endogenous control synchronised by two factors, photoperiod and food supply (Bolliet et al., 2001). Under light-dark cycle and free food access, most trout displayed a diurnal pattern of demand-feeding activity, whereas a few fish or groups of fish switched from diurnalism to nocturnalism or vice versa. Under constant lighting conditions and a restricted feeding cycle, the demand-feeding rhythm rapidly synchronised to food availability (Bolliet et al., 2001). The synchronisation of single fish to light-dark cycle or feed availability is slower than that of groups of fish, as social organisation affects the circadian activity in fish (Bolliet et al., 2001). Group feeding pattern may be correlated with genetic selection for growth or for stress responsiveness in brown trout (Overli et al., 2002; Boujard et al., 2007).

The rearing conditions modify feeding behaviour in trout, for example food distribution may differ from the wild situation in that it does not follows biological rhythm and large amounts of food are available (Bégout Anras and Lagardcre, 2004). In juvenile rainbow trout, the

regulation of voluntary feed intake is based on the energy level of the feed, with higher feed intake of a low energy diet than with high energy diet, which has no effect on specific growth (Boujard and Médale, 1994). After short periods of food deprivation, hyperphagia may occur but it has not been demonstrated (Boujard et al., 2000).

On farms the quality of the diet, time of the day, season, water temperature, dissolved oxygen levels, and other water quality variables may affect fish feeding. Reduced water quality rapidly impairs rapidly feeding activity. Feed acceptability, palatability and digestibility vary with the ingredients and feed quality.

5.3.2. Feeding strategy

Feeding rates is determined by fish size, to optimize growth and feed conversion. Small larval fish and fry are fed a high protein diet frequently and usually in excess to ensure adequate feeding, but as fish grow, feeding rate and frequency are lowered, and protein content reduced. Feeding frequency depends on the availability of labour, farm size, and optimal fish growth. Fry and juveniles may be fed continuously by automatic devices or several times a day, and on-growers may be fed once per day in large farms with insufficient labour and many ponds, or twice per day in smaller farms. Generally, growth and feed conversion increase with feeding frequency.

In indoor, intensive trout culture systems, fish may be fed as many as 5 times per day in order to maximize growth at optimum temperatures Fish farmers pay careful attention to feeding activity in order to help determine feed acceptance, calculate feed conversion ratios and feed efficiencies, monitor feed costs, and track feed demand throughout the year. Trout farmers can calculate optimum feeding rates based on the average size in length or weight and the number of fish in the tank, raceway, or pond, using published feeding rate tables. Farmed fish typically are fed 1-5 % of their body weight per day (Craig and Helfrich, 2002; Houlihan et al., 2001). The feeding tables are calculated to optimize the feed efficiency and growth rate, but should be adapted to the farming conditions to avoid food spillage. The feeding tables indicate one ration size for each temperature and each fish mean size, but experimentally, the ration size that optimizes feed efficiency is lower than the ration that results in the highest growth rate (Gélineau et al., 1998).

Trout can be fed manually, by automatic feeders, and by demand feeders, but many fish farmers like to hand feed their fish each day, even if an automatic device is also used, to assure that the fish are healthy, feeding vigorously, and exhibiting no problems. There are a variety of automatic (timed) feeders ranging in design from belt feeders to timed feeders that can be programmed to feed hourly and for extended periods. Demand feeders usually are suspended above fish tanks and raceways and work by allowing the fish to trigger feed release by striking a moving rod that extends into the water. Whenever a fish strikes the trigger, a small amount of feed is released into the tank. Whereas feeding a restricted ration by hand reduced variability in growth among individuals feeding a restricted amount of food by automatic-feeders increases individual variation in growth rate (Gélineau et al., 1998; Houlihan et al., 2001). Increase of the individual variation in growth rate measured by the increase in the variation index of weight or length (σ/m, %) versus time in a sample of the same fish population can be an indicator of a poor feeding strategy.

At early stages (Marcalo et al., 2006) this continual feed delivery is achieved by means of automatic feeders. As the fish grow feeding rate can be reduced. In fry, farmers use dry diets and manufacturers feeding tables to calculate optimal feeding rates for their particular situation. Adverse water quality conditions or temperature extremes and low oxygen will reduce feeding activity and growth.

In ongrowers, encouraging very fast growth through overfeeding (pushing on), or restricting growth by underfeeding (holding back), can affect the health and welfare of the fish (Unpub. Obs).

5.3.3. Food deprivation in Trout

In the wild food availability is variable, and when deprived of food, animals employ various behavioral, physiological, and structural responses to reduce metabolism, to conserve energy reserves. Wild brown trout can restore lost lipid reserves when feeding conditions improve, however, prolonged food deprivation affects development, and in the long term compensation may not be possible (Bureau et al., 2002).

In salmonids, periods of food deprivation may occur in routine hatchery and on-growing procedures prior to sorting, handling, transport, stocking or slaughter to empty the digestive tract, to improve sanitary conditions, maintain ambient water quality and limit bacterial contaminations of flesh after slaughtering.

Before transport it is current practice to deprive fish of food for 48 hours, or longer under cold conditions when gut clearance rates are slow. This reduces faecal contamination of water in the transport tank and minimises the metabolic load such as dissolved CO_2 and ammonia when the fish are crowded together.

Food deprivation may affect fish behavior in rainbow trout. After 3 days, fasted fish were dominant over fed fish, whereas after 6 and 9 days, the competitive ability of fed and fasted fish was similar. After 12 days, there was a tendency for fed fish to be dominant over their fasted competitors (Johnsson et al., 1996).

When food deprivation is severe and has used up all the metabolic reserves of the fish, it leads to the death after a period depending on the initial physiological status, the mean weight of the fish and the water temperature. For example, the 50 % mortality for newly hatched food-deprived fry of trout occurred after 52 days at 7 °C and 24 days at 12°C (Edsall et al., 2003). In larger trout, the initial response some hours after food deprivation was a decrease in oxygen uptake, CO_2 and ammonia release while fish maintained a lower basal heart rate (Yang and Somero, 1993; Blake et al., 2006).

In trout deprived of food for 7 days, there is preferential mobilization of perivisceral lipids, increasing flesh moisture level and decreasing fat conten (Blake et al., 2006). In small rainbow trout after 7 to 14 days of food deprivation at a temperature ranging 12-15 °C, the levels of muscle glycogen decreased by approximately 50 % relative to control value while the plasma cortisol and plasma growth hormone levels increased significantly (Blom et al., 2000).

Under normal (fed) conditions, rainbow trout brain oxidized glucose and lactate at rates higher than in mammals; but after 14 days food deprivation, oxidation rates decrease suggesting brain metabolic depression (Soengas et al., 1998). In rainbow trout after 3-week starvation the

expression of genes involved in lipid metabolism/transport, aerobic respiration, blood functions and immune response were decreased when compared to fed fish (Salem et al., 2007).

Broodstock trout reduce feeding around the time of spawning but digestive tract changes are less than in salmon, and therefore feed is offered throughout spawning and the mending phase. As co-habiting broodstock fish undergo final maturation at different times, feeding is maintained throughout spawning. Micro-nutrients such as vitamin C, thiamine, astaxanthine, and essential fatty acids are important in broodstock performance or gamete quality (Bromage et al., 1992; Sargent et al., 1999; Izquierdo et al., 2001; Palace and Werner, 2006).

5.3.4. Over feeding

Over-feeding should be avoided as it leads to satiation which does not increase growth rates significantly and does not optimize feed conversion ratios in routine conditions. Excess feed after a restricted feeding period, causes increased their gastric capacity and ingestion in trout to build up energy reserves and compensate for retarded growth (Jobling and Koskela, 1996; Pirhonen and Forsman, 1998) but it is unclear whether increased gastric capacity is a welfare issue. Over-feeding may also result in deterioration of water quality due to decomposition of uneaten feed increases the load of dissolved and particulate organic matter in water, which may promote fish diseases (Hinshaw, 1990). Overfeeding may make trout fat, possibly increasing their susceptibility to disease, but no data are available.

5.3.5. Partial protein substitution and alternative sources protein

Currently salmonid farmers feed high-energy diets containing up to 35 % fish oils and protein for muscle growth, increasing feed efficiency and decreasing pollution (Watanabe et al., 1987; Cho et al., 1994; Torstensen, 2000). Because the lipids are the primary non protein energy source in salmonids, the ratio lipids/protein should be carefully controlled as they can lead to an increase in whole-body fatness and they may reduce fish fillet quality (Roselund et al., 2001; Bell et al., 2002).

The optimum protein level for growth is about 40 % in triploid and diploid rainbow trout (Oliva- Teles and Kaushik, 1990; Kim et al., 1991). The best production results are obtained when using high quality fish meals produced at low temperature with minor additions of other protein sources which secure the protein requirements in most of the carnivorous fish, included salmonids. Traditional fishmeal is prepared from dried, ground tissues of whole marine fish, usually menhaden (*Brevoortia* sp.), anchovy (*Engraulis* sp.), and capelin (*Mallotus villosus*). Fish meal varies in amino acid profile, apparent digestibility and palatability to most farmed fish (Hevrøy et al., 2004).

Different protein sources alternative to fishmeal, have been assessed in various studies: krill (Yoshitomi et al., 2007), plants and even bacteria (Aas et al., 2006). Recently, a large number of studies have been carried out on the partial or total replacement of fish meal and fish oil by some plant lipid and protein sources which are generally less expensive and more readily available. In many carnivorous fish and specially salmonids, the most used sources of protein have been soybean meal for both juveniles (Escaffre et al., 1997) and adults (Storebakken et

al., 1998; Mambrini et al., 1999); pea meal, lupin meal (Glencross et al., 2004) and other plant meals as corn, wheat, rapeseed and rice (Davies et al., 1997; Palmegiano et al., 2006). From the experiments, recommendations for soy product incorporation in salmonid diets vary among the studies (Kaushik, 2007). For example, the weight gain and feed efficiency ratio data showed that soybean meal and pea protein concentrate had the best potential for replacing at least 33 % of the fish meal protein in extruded salmon feeds. However several plant protein sources could cause problems such as feed palatability, feed intake, growth, feed conversion, apparent digestibility and utilisation of macronutrients and energy, liver fat deposition, intestinal integrity through patho-histological response of the distal intestine (DI), activities of digestive enzymes in the mid and distal intestinal mucosa, faecal trypsin and plasma insulin concentrations, plasma choslesterol, plasma protein levels, respiratory burst of head kidney leucocytes and plasma myeloperoxidase values can be affected (Carter and Hauler, 2000; Escaffre et al., 2007).

Studies suggest that caution should be exercised in the use of even low levels of extracted soya bean meal in salmonid feeds. These effects can be linked to many specific anti-nutritional factors (Francis et al., 2001) and an amino acid imbalance. The importance of manufacturing process was emphasized (Escaffre et al., 2007). Since most plant protein sources do not meet the essential amino acid (EAA) requirements of fish, plant-based diets need to be supplemented with amino acids (AA) to restore the dietary AA profile to match their IAA requirement (NRC, 1993). More works are needed to improve plant protein meal in IAA and DAA to obtain adequate feed for fish and human consumer.

5.3.6. Feed additive in farmed fish

Commercial feeds contain the nutrients needed by fish, but not always at sufficient levels to meet their total dietary requirements so additional ingredients (nutritive and nonnutritive additives) arer added. The vitamin premixes are concentrates in stable form mixed or not with a carrier, usually a wheat by-product. Prior to mixing with feed, they are ground fine and are added at levels ranging from 0.5 to 4 % of the diet. The main vitamins are A, D3, E, K, B12, C and some others as choline, riboflavin and thiamin. The mineral premixes are also concentrates (zinc, magnesium, copper, iodine, iron, salt) which complete the diet but also which can overcome antagonistic interactions among feed ingredients such as rerduction of the detrimental effects of the phytic acid on the bioavailability of divalent cations when proteins of plant origin are used. The nonnutritive additives have no nutritional value and are included in diets for stability, flavor or influencing fish performances, health status and quality of the final product. They include feed binders, carotenoid supplements, drugs and antibiotics, hormones, antifungal, antioxydants, fiber, flavourings and water.

Binders are used to stabilize pellets when handling or in water. They can be by-products of plants (pregelatinized potato starch, lignin sulfonate, cellulose extract, alginates, agar, …) or originated from minerals with excellent colloidal properties (sodium or calcium bentonite from clay). They are added from 0.5 to 4 % of the diet.

In salmonids which are not able to synthesize pigments, carotenoid pigments are added in broodstock diet to improve egg quality or in on-growers diet to color flesh before slaughtering. Pigments are found in natural materials: herring gull eggs, paprika, zooplankton, krill products and processing wastes from shrimps, crabs and crayfish. The natural material is added to the

diet at a 20 % level or more. Synthetic pigments (canthaxanthin and astaxanthin) are added to commercial feed at a level of about 50 mg/kg diet.

Therapeutants are added to fish feeds to treat, cure, mitigate or prevent diseases. Antibiotics for therapy and prophylaxis are prescription medicines sold through the pharmacy depending on national legislations. The introduction of vaccines against furunculosis in fish farming has eliminated a very large proportion of the use of these drugs. Unregulated feed additive, as B-glucans, which are fragments of cell walls of yeast and mycelia fungi, are able to stimulate in vitro the nonspecific immune response of fish (respiratory burst activity of head kidney macrophages). However, dietary incorporation of the b-glucans was unable to stimulate the macrophage activity in vivo (Bridle et al., 2005).

Probiotics are live single species of microorganisms or a mixture of species added to feeds after pelleting. By colonizing the fish gut, they are supposed to outcompete detrimental microorganisms allowing the fish to spare metabolic energy and to stimulate growth.

Enzyme supplements are either single, purified or crude enzyme preparations added to feeds by spraying after extrusion (they are denatured at temperatures above 65 °C) to enhance the digestion of complex carbohydrates and collagen.

Anabolic steroids and thyroid hormones (testosterone, pituitary growth hormone) have been shown to promote fish growth by increasing feed intake and metabolic efficiency. Their use in fish feeds inducing hormone residues in fish flesh is no longer permitted due to legal restrictions and consumer opposition in many parts of the world. In the current practice of fish culture, the use of hormones is mainly limited to the field of sexual reproduction, and more precisely to induce or synchronize ovulation and stimulate spermiation. Sex steroids are now commonly used to reverse the sex of some species in salmonids, cyprinids, tilapias.

Antimicrobial agents (benzoates, parabens, propionates, benzoic or formic acid) are used at a rate of from 0.01 to 2.5 % to inhibit fungal, microbial or yeast growth in feeds containing more than 12 % moisture. Antioxydants compounds are added to control oxidation of lipids causing off-flavors and odours. They are chemicals compounds used at low level (0.015 - 0.1 %) as phenolics (BHT, butylated hydroxytoluene, BHA, butylated hydroxyanisole), reacting with free radicals, amines (ethoxyquin) and chelating metallic pro-oxidants (acid ascorbic, phytic and tartaric acid, EDTA) or natural compounds as carotenoid pigment and tocotherols.

Fiber is indigestible plant matter such as cellulose, hemicellulose, lignin, pentosans, and other complex carbohydrates that is added to facilitate binding in processing feed. In fish, small amounts fiber in diets (up to 8 %) improves gastric evacuation time and increases the efficiency of protein utilization and fish growth. Higher concentrations may increase fecal waste production, damage the gastro-intestinal epithelium by abrasion, reduce the nutrient intake and may depress growth (NRC, 1993).

Palatability enhancers or flavorings or feed stimulants are mainly used to enhance the feed acceptance in fish larvae when weaning onto artificial feeds or in on-growers fish when fish dietary proteins and lipids are replaced by plant products or in medicated feed. Natural feed stimulants in salmonids are hydrolyzed fish protein concentrates or shrimp meals in which proteins and amino acids play a major part. In rainbow trout, it has been demonstrated that of the 20 amino acids, only glutamic acid, leucine, phenylalanine, proline, and tryptophan at 10^{-2} M were perceived by the trout to be palatable. Mixtures of amino acids at 2 - 4 % diet (L-alanine, L-serine, inosin and betaine) would be more effective than individual compounds (Papatryphon and Soare, 2000).

5.4. Husbandry and Management

Husbandry and management are central to maintaining the health, welfare and productivity of farmed fish. Husbandry can be considered to be taking care of the fish otherwise known as stockmanship and management the strategic decisions regarding the production system.

Here husbandry is restricted to the physical handling of fish and staff competence since feeding and environmental control are described above, and management is restricted to general planning and monitoring. There are many scientific papers on the effect of standard husbandry procedures on the stress response of trout (Pickering, 1992), but little on best practice in husbandry, although best practice guidelines exist in industry codes of practice and other industry standards.

5.4.1. Handling

Farmed trout may be handled when moving between production systems, to adjust biomass, to grade by size or sexual maturity, during vaccination or other treatments and prior to transportation or harvest. Some procedures (i.e. vaccination and monitoring stock) are necessary for the health and welfare of the fish, grading to avoid excessive size discrepancy, and splitting or mixing populations to ensure appropriate biomass to water flow.

Trout eggs are not sensitive to handling and physical disturbances before the eyed stage of development, and consequently they may be easily handled and transported. Alevins are very fragile and susceptible to physical handling, especially when the yolk sac is large, but are less susceptible as the yolk sac is resorbed. Damage at these stages may cause skeletal or other tissue deformities or impaired function later in life (Speare, 2003). Fry are handled during grading and transport. Movement between rearing units can cause physical damage and stress if fry are handled roughly (Pickering, 1992; Ashley, 2007). Grading is normally done by mechanical means using a grid system but can lead to physical damage and stress, especially if fish are left without water. Grading frequency depends on production objectives, growth rate and size heterogeneity, so the number of times individual fish are handled will vary. Water quality such as temperature and dissolved oxygen also contribute to the impact of these procedures on trout. Brown trout show greater increases in cortisol levels after handling than rainbow trout

Ongrower fish for food consumption are usually graded and harvest by mechanical means where fish are crowded and pumped through (Figure 2.). Handling is also associated with vaccination and in order to monitor the stock.

Figure 2**. Ongrowers grading**

(Photograph courtesy of Dr Craig MacIntyre)

Broodstock are normally inspected on a weekly basis for ovulation or the presence of running milt during the spawning period. Such frequent handling stresses the broodstock and may also cause skin damage leading to osmotic losses, bacterial and fungal infections. Staff training and use of adequate anaesthetics are essential to reduce physical damage of the broodstock (Wagner et al., 2002). Prior to handling there is usually a period of crowding or confinement which is also a cause of stress in the fish. Air exposure severely stresses fish. (Barton and Iwama, 1991) which is particularly severe in fish which have recently exercised (Ferguson et al., 1993). Handling can result in damage to the fish followed by bacterial and fungal infection. After showing substantial increases in cortisol there is usually some degree of immunosuppression (Pickering, 1992). If fish are handled out of water, adequate support is given to the body to compensate for gravitational effects and loss of buoyancy support. Live fish must never be held by the gills or tail only. When nets are used they should be in good repair, adequately disinfected and of a design to minimise the potential for damage to the fish. Nets should never be overfilled or fish will be subject to excessive pressure and damage be necessary as part of standard husbandry practice, it is important that the frequency and duration of such events is minimised, and that the handling process is designed and implemented in order to avoid adverse effects.

5.4.2. Staff competence

To ensure the good welfare of farmed fish all staff should be adequately trained to an appropriate level and there should be sufficient staff available not only to undertake routine husbandry but also to deal with predictable episodic events. It is important to recognise that the first signs of poor health or welfare may become apparent to the person feeding the fish and these are often the newest and least experienced members of staff. An assessment of training needs should be carried out periodically to ensure that all staff are competent for the tasks they perform. Appropriate records of individual staff training should be maintained.

5.4.3. Management

Management includes the strategic planning of all aspects of the farming system. Decisions on when to stock, grade, vaccinate or treat trout in production are as important to the fish well being as the procedures themselves. For example grading during high temperatures is avoided by the industry to prevent unnecessary stress and secondary infections.

A substantial proportion of the welfare experience of farmed fish is related to the systems in which they are grown. Common systems include ponds, tanks, raceways and cages with varying levels of intensification and infrastructure. Many of these systems are flow-through but there are variable amounts of water re-use with some pure re-circulation. Correlations between farming system and welfare have to be interpreted with caution (North et al., 2006a). The appearance of fins on fish from farms supplying the restocking market are often said to be better than those on farms producing for the table market (St-Hilaire et al., 2006) but since the value of trout for restocking is directly related to their appearance, fish with poor fins or other signs of damage are often graded out and sold for other purposes or killed. Therefore conclusions based on the remaining fish are not necessarily applicable to the original population. Work currently underway (UK Defra project AW 1205) is examining the welfare implications of various husbandry practices using multi-level modelling on epidemiological data but this is not yet published.

Some general statements can be made regarding the welfare implications of the farming system. As the complexicity and loading of the system increases (biomass per unit volume) so the system becomes more susceptible to acute failures, i.e. an interruption of the water flow into a pond with low stocking density may have little impact, but interruption of the water flow into a highly stocked tank or raceway can rapidly result in respiratory distress and death. Therefore more complex systems require alarm and backup systems, with a higher level of contingency planning and staff training.

5.4.4. Planning and records

An essential part of management is planning and record keeping. Good practice guidelines can be developed for the individual farm, business or be part of a national industry scheme. While such guidelines can provide the basis of good practice it is also necessary to have individual

farm strategies. In some areas these take the form of a veterinary health and welfare plan, developed with the farm veterinarian. In other cases they plans are developed by persons responsible for fish health and welfare within the company. Such plans must not only be available as a source of reference but the staff must also receive training to ensure compliance. The plan should be subject to regular review and retrospectively following any problem of emergency on the farm.

Farm records are essential in order to monitor health, welfare and productivity and to analyse any changes in performance. There are statutory requirements for the keeping of certain records. Despite these requirements records will only be kept effectively if those responsible understand the need for the records and if the process is not too demanding. Records should be easy to keep, and easy to access and understand. They should at a minimum record all fish movements into, off and within the farm, allowing all batches of fish to be traced. They should include records of health or welfare problems, mortalities and treatments. The average weight of the fish, growth, feed used and production performance are essential for effective control of productivity and may ensure that the fish are fed appropriately. Environmental and water quality records can be useful but are frequently restricted to dissolved oxygen, temperature and less frequently pH.

5.4.5. Monitoring

Fish and the production environment are usually monitored and any abnormality investigated using the resources available to deal with problems. Water quality and common diseases may be dealt with by staff, but the farm may require assistance depending on the severity of the problem and how unusual it is. Systematic changes may require the involvement of farm management or external experts, unusual diseases may require veterinarians or other fish health experts, and novel or emerging diseases the help of specialists or researchers.

Monitoring of water quality, such as dissolved oxygen and temperature, is often done by the industry, but may also regularly be carried out by environmental regulatory agencies. Measurements are done manually or by automatic systems with emergency alarms, particularly in intensive systems.

Fish health is monitored through health plans developed between the fish farmer and the veterinarian or other fish health expert, to provide practical procedures to maintain health, enable early disease diagnosis, and specify the actions to be followed by farm staff. Veterinary health plans usually cover six key areas: 1) the responsibilities of nominated staff and the named veterinarian, fish health expert or government agency, 2) the history of disease and disease treatment on the farm, 3) vaccination procedures, 4) monitoring of fish for disease and general health, 5) biosecurity procedures, and, 6) a farm improvement strategy. When a disease problem is identified on the farm, a course of action must be quickly decided upon because of the rapid progression of epidemic fish diseases. The decision to treat or not is based on cost-benefit analysis, environmental legislation (e.g. discharge consents), and the welfare of the fish. Few drugs are available to trout farmers, and their use is further restricted by controls on discharges from the farm. For example, since the banning of malachite green to treat mycoses in food fish, there have been widespread and severe fungal problems in farmed trout.

5.5. Genetic

5.5.1. Domestication

Trout have been domesticated and cultured for restocking of rivers and lakes for sport fisheries (brown trout) or intensive freshwater or seawater aquaculture (rainbow trout), requiring different breeding protocols. For brown trout, systematic breeding has thus been mainly confined to mass selection of the largest and fittest from the locally available populations where as for rainbow trout, breeding protocols have been developed generally for the production of table fish.

Domestication is a process by which a population of animals becomes adapted to man and to the captive environment by some combination of genetic changes occurring over generations and environmentally induced developmental events recurring during each generation (Price, 1984; Price, 2002). There have been changes in farmed trout since the earliest stages of farming, some of which (e.g. body size) are not part of domestication but genetic changes have helped the fish to adapt to the farming situation. However, there seems to have been a relatively small amount of genetic adaptation to husbandry systems and developmental events during the lifetime of individual fish play a significant part in any adaptation.

Domestication is mainly concerned with acquired genetic modification related to the ability under farming conditions to reproduce, to cope with environmental changes, and to enhance defences against diseases which are less significant in the natural environment.

5.5.2. Selection

Rainbow trout have been reared in Europe for over 100 years initially from a relatively small number of individuals imported from North America. A regular importation of eggs from US is still performed from a limited number of US companies. Behavioural traits in culture are therefore a combination of both natural behaviour and changes due to intentional and unintentional breeding. Brown trout occurs naturally in large parts of Europe, but there have been no major breeding programmes, and farmed brown trout are probably based on natural populations.

Poorly structured breeding programmes run the risk of inbreeding (Falconer and Mackay, 1996) with associated poor reproductive performance and egg survival, loss of genetic variation and development of undesirable physiological side effects such as deformities. Until very recently it has been particularly difficult to identify individual trout to their families, so to avoid within family mating (the source of inbreeding) breeding programs have used importations of stock from outside the program (Purdom, 1993). Mass selection (selection from individuals showing the best performances) has been used in rainbow trout but it is only efficient for high heritability traits. Selection based on individual and related performances (family selection) was more widely used in North of Europe but require large rearing facilities. A new selection procedure more adapted to a SME's (Small and Medium Enterprises) has been developed in France (Chevassus et al., 2004). This procedure of selection uses external indirect quality traits or technologies (morphology, fat in the muscle with micro-waves, body thickness and processing yields using ultrasound) and genealogical management of the genetic resources

using genetic fingerprinting (Haffray et al., 2004). Consequently separate strains have developed related to national origin and a considerable international trade in eggs has developed. Development of new improved strains by modern breeding techniques using wide genetic variation is rapidly developing in US, Chile and in Europe (mainly Norway, France, Finland and Denmark).

Traits initially selected using mass selection were growth, early maturation and spawning season, to produce eggs throughout the year. However, the latter trait can be achieved by photoperiodic management of the broodstocks, and is no longer selected for. Most of the commercial strains are autum to winter spawners.

Main traits selected today are growth, body morphology to improve gutted yield and lipid muscle content. These traits have moderate heritability (Gjedrem, 2000; Chevassus et al., 2004; Quillet et al., 2004) and should produce significant genetic gain. More that 70 % of the EU production benefits from juveniles genetically improved according to selection programmes based on scientific basis. Roberge et al. (Roberge et al., 2006) using last relevant genomic micro-array tools have concluded that 5 generations of multitraits commercial selection in Atlantic salmon have little change in gene frequency (genotypes) but mainly modify gene expression. It illustrate for the first time that selection mainly modifies relative gene expression.

Increasing the genetic resistance of artificially reared salmonids to endemic diseases (most of which result in mortality) greatly improves welfare and has the effect of reducing the disease load in the environment and reducing dependence on chemical treatments (Henryon et al., 2005; Guy et al., 2006). Genetic variability for resistance does exists in rainbow trout (Henryon et al., 2002; Henryon et al., 2005; Quillet et al., 2007) candidate traits to implement indirect selection for resistance to viruses have been identified (Quillet et al., 2001; Quillet et al., 2007). Moreover, genetic variability for resistance to bacteria has also been evidenced (Hollebecq et al., 1995). Modern breeding methods tend to give high consideration to improving disease resistance.

Concerns over the sustainability of the culture of trout and other carnivorous species as far as the availability of fish meal and oils for their diet have led to new non- GM molecular approaches to the selection and breeding of rainbow trout specifically for ability to utilize alternative feed sources of vegetable origin (Overturf et al., 2004).

Skeletal deformities reduce the value of harvested fish, and the sustained production of deformed fish challenges to the credibility of the industry and is an ethical issue of increasing importance. Malformations include malformed jaws, fused vertebrae, tail, ribs and softness of the skeleton. Identified causes include water temperature during incubation, currents or phosphorus (P) and zinc (Zn) nutrition. There was no genetic correlation between growth and the rate of vertebral malformations in 2 pedegree populations followed during 3 generations of selection for growth(Kause et al., 2005). These results underline the important role of environmental stressors, a situation also observed in Atlantic salmon (McKay and Gjerde, 1986; Gjerde et al., 2005).

Selection to improve adaptation to one rearing environement may limit mortalities and improve production. Rainbow trout show moderate to high genetic correlations of 0.58–0.86 between body weights in fresh, brackish and salt water (Sylvén et al., 1991; Kause et al., 2003), showing that genotype-by-environment interaction occurs allowing parallel genetic changes in two environments where rainbow trout are farmed for long term breeding programmes.

There are very few publications quantifying genetic progress over the last decades at experimentally or commercially and their impact on welfare in rainbow trout. There is a lack of data on the positive results of selection on productivity, growth improvement, economical benefits, and correlated responses (domestication decreasing stress, adaptation to farming increased gutted and/or fillet yields, increase of disease resistance). This also applies to negative effects on functional systems (respiratory, cardiac, locomotion, reproduction), disease susceptibility, muscle fattening and flesh quality, or genotype/environement interactions. There are no data on genetics and the development of functional systems (respiratory, cardiac, bone) or correlation between the development of these organs and growth.

Experience on other terrestrial animals need to be kept in mind, and a huge effort had been done recently by the animal breeders to introduce such traits in selective breeding programs, the fish specificities and their interactions with environmental factors and farming practices need to be investigated.

5.5.3. GMOs

There are currently legislative and consumer barriers to the development and marketing of transgenic salmonids in Europe. Transgenic salmon have been developed in the US but there is little or no research activity in trout due to low commercial returns from the industry compared to salmon production.

5.5.4. All female stocks

For more than 25 years rainbow trout farmers have been utilizing the techniques of all-female production coupled with triploidy by chromosome set manipulation to produce commercial stocks of table fish and for restocking. The technique is not considered as genetic modification (Directive 2001/18/EC) as triploidy and XX males can also occur naturally in salmonids. It has been used effectively in commercial rainbow trout production for human consumption for a quarter of a century. Such fish are uniform in size, do not develop male precocity and its associated aggressive interactions, and do not develop a gonad, which is of little economic value. The reduction in aggression can constitute a benefit in the context of welfare. Diploid all-female production mainly occurs in fresh water (Spain, Italy, France, UK, Poland, Germany, repersenting about 50% of the EU production) and triploid all-female production mainly occurs in UK, France and Spain (~30,000 t).

The parent stocks are sex reversed female fish which become neo-males with two X chromosomes. All their offspring are therefore female. The gender conversion is done by testosterone hormone inclusion in the diet on the first few days of first feeding, before gonadal differentiation has occurred. All residues of exogenous hormone are dissipated from the reversed fish, which do not anyway enter the food chain, within 72 hours (McIntosh, 1985). The all female eggs from the female fish are subjected to pressure shock in early incubation, leading to triploid chromosomy and non-development of the ovary. Such fish are functionally sterile.

Triploid rainbow trout have been reported to exhibit exactly the same range of physiological coping responses (Benfey and Biron, 2000). Yet, it has also been evidenced that they may exhibit higher mortality in 'extreme' conditions such as transfer to seawater, increased temperature (Quillet et al., 1987; Quillet and Gaignon, 1990). Triploids do however have reduced anaerobic exercise capacity but more rapid recovery from the acidosis associated with physical exhaustion or transportation stresses than diploid fish (Hyndman et al., 2003). All the scientific results confirm that when rainbow trout is reared in good environmental conditions (Sheehan et al., 1999; Wagner et al., 2006), triploids have similar survival and growth, and in same case better growth (few percent) than diploids. Ojolick et al. (Ojolick et al., 1995) reports lower survival than diploids when environmental condition (water temperature at 21°C) are also detrimental for diploids.

The performance of the triploids is dramatically linked with the method to obtain triploids. Triploidisation by temperature treatment induces higher mortality at the eyed stage (89.5 % vs. 68.0 % [controls]) or at hatching (96.8 % vs. 92.3 % [controls]).and higher rates of deformities at hatching (11.7 %) than pressure treatment, which does not differ from controls for survival or deformities (2.8 % vs. 1.9 %) (Haffray et al., 2007), it was also reported a maternal effect on the type of malformation at this stage, possibly associated with egg quality. They concluded that pressure treatment induced a final yield of fry similar to diploid control, but significantly higher than the thermal treatment (81.7 % vs. 49.6 %). No differences were found in performances and morphological anomalies rates at hatchery stages, between triploid fry from pressure treatment and control diploids.

In production, only the indirect method of triploid production has been assessed using temperature and mainly pressure treatments on haploid eggs produced from diploid females. Preliminary research in trout have demonstrated that triploids produced by the direct method by crossing tetraploid male with diploid female have the same performance than diploids (Chourrout et al., 1986).

5.6. Impact of disease on welfare in Trout

Disease in farmed fish is generally closely linked with the husbandry and environmental conditions under which the fish are being reared and many pathogens are ubiquitous in the environment or in the fish's tissues but only manifest themselves in a clinical fashion if husbandry or environmental parameters facilitate their establishment. Thus although clinical disease can usually be considered as a welfare issue in its own right, it is also generally an indicator of an underlying husbandry or environmental deficiency.

Infectious and non-infectious diseases have the capacity to impact on the welfare of trout at various stages of development in freshwater as well as seawater. Farmed trout are susceptible to a range of viral, bacterial, fungal, parasitic, nutritional and other non infectious diseases. A number of serious infectious diseases have been listed in the OIE Aquatic Animal Health Code (OIE; 2008), Council Directive 2006/88/EC but agents of diseases of importance in welfare terms are often ubiquitous and may not be on any such lists.

The Animal Disease Notification System (ADNS) system established by Council Directive 82/894/EC on the notification of animal diseases within the Community requires that notifications are sent to ADNS in accordance with Commission Decision 2005/176/EC. Before

the implementation of the new Fish Health directive Member States were legally obliged to report outbreaks of the following diseases: Infectious Haematopoietic Necrosis (IHN), Infectious Salmon Anaemia (ISA) and Viral Haemorrhagic septicaemia (VHS). The ADNS annual report of 2006 lists 9 outbreaks of IHN in 2005 and 10 in 2006, and 29 outbreaks of VHS in 2005 and 30 in 2006, without details of the species affected. There are no data concerning prevalence of non notifiable diseases.

The present report does not attempt to cover all diseases of rainbow and brown trout but instead will consider several infectious and non-infectious diseases that may have important implications in terms of welfare at some stages of the production cycle in order to serve as examples of the ways in which disease can impact welfare. The following diseases which are considered to be of particular significance to fish welfare because of their: i) severity of effect on physiological integrity of fish, ii) known frequency of occurrence in farming systems and iii) impact of preventive and/or curative measures.

Table 6. **Examples of diseases of farmed trout with potential welfare significance**

Disease	Life stage affected	Environment	Husbandry systems
Viral Haemorrhagic Septicaemia (VHS) A serious and often chronic virus disease causing severe anaemia and nervous signs.	Alevins, fry and fingerlings, freshwater ongrowers, seawater ongrowers, brood stock Brown trout less susceptible than rainbow trout, and not to the same viral serotypes	Freshwater, seawater	Freshwater: tanks, ponds, raceways, cages Seawater: cages
Infectious pancreatic necrosis (IPN) A very significant virus disease causing severe acute and chronic tissue damage	Fry and fingerlings, freshwater ongrowers, seawater ongrowers	Freshwater, seawater	Freshwater: tanks, ponds, raceways, cages Seawater: cages
Enteric Red Mouth Disease (ERM) Bacterial disease causing severe damage at all ages and also requiring vaccination	Fry and fingerlings, freshwater ongrowers,	Freshwater,	Freshwater: tanks, ponds, raceways, cages
Rainbow trout fry syndrome (Infection with *Flavobacterium psychrophila*) Severe bacterial infection of intensive production	Alevins, fry and fingerlings, freshwater ongrowers	Freshwater	Freshwater: tanks, ponds, raceways, cages
Proliferative kidney disease (PKD) Chronic parasitic disease	Fingerlings, freshwater ongrowers	Freshwater	Freshwater: tanks, ponds, raceways, cages
Eye lesions These are of varied aetiology but always a significant welfare concern	Fry and fingerlings, freshwater ongrowers, seawater ongrowers, brood stock	Freshwater, seawater	Freshwater: tanks, ponds, raceways, cages Seawater: cages
Gas bubble disease Common and very serious physical disease (gas embolism)	All stages	Freshwater or pumped seawater	Generally only in pumped systems
Fin and skin damage and associated infections Secondary infection of physical damge during husbandry actions	All stages	Freah and seawater	Freshwater tanks ponds raceways cages. Seawater tanks and cages.

5.6.1. Viral haemorrhagic septicaemia

Viral haemorrhagic septicaemia (VHS) is a serious viral disease, caused by a rhabdovirus and mainly affecting farmed rainbow trout, although outbreaks also have been described in pike fry, brown trout, turbot, whitefish as well as Pacific herring and Pacific cod (Meyers et al., 1994; Meyers and Winton, 1995; Traxler et al., 1999; Hedrick et al., 2003).

VHS has been demonstrated both in freshwater and sea water reared rainbow trout. Infection is generally due to contact with infected individuals or consumption of infected wet fish feed. Mortality due to the disease varies considerably depending on size of fish, water temperature, strain of fish and virus serotype, as well as the history of the disease in a given country. The mortality rates have been reported to be 15 – 50 % in some countries, while in others 50 - 100 % (Håstein et al., 1968; OIE, 2006a).

Several genotypes of VHS virus have been described (OIE, 2006b, Gagne et al., 2007). In July 2007 a first case of pathogenic genotype III VHS virus was diagnosed in Norway in sea farmed rainbow trout (http://www.vetinst.no/nor/Forskning/Aktuelle-tema/Fiskesykdommer/Viral-hemoragisk-septikemi-VHS). Since VHS virus is detected in a number of freshwater and marine fish in the wild, such fish may act as source of disease for farmed fish. VHS virus is only transferred horizontally and VHS virus contaminated eggs may be disinfected by use of iodophors (Ahne and Held, 1980).

VHS has in the literature been described to manifest itself in either an acute, chronic or nervous form, but the different stages may overlap each other. In the acute form the affected fish go off feed and the key changes are lethargy, dark pigmentation, exophthalmos, swollen abdomen (ascites), pale gills, and erratic swimming behaviour. Internally, haemorrhages can be observed in the all internal organs as well as in the adipose tissue and musculature. The kidneys are swollen and the liver greyish-yellowish in colour which may be misdiagnosed as lipoic liver degeneration. Mortalities may be high under certain circunstances.

In the more chronic form of VHS, the pathological changes are less obvious. In the nervous form affected rainbow trout shows signs of motor disorders such as leaping and spiral swimming (Roberts, 2001). No other changes are usually present.

As VHS is a notifiable disease in EU, approved VHS free zones have been established. The consequences of an outbreak of VHS in such zones require eradication procedures if the zone status is to be maintained. Although experimental vaccines have been proven effective against VHS, no commercial vaccines are available. In order to prevent VHS, several biosecurity methods such as use of VHS free brood stock, disinfection of eggs, disinfection of premises, wastes, effluent water etc. may be introduced. Selection of VHS resistant strains of rainbow trout may also be of value in heavily infected areas in which eradication is impossible (Roberts and Rodger, 2001).

5.6.2. Infectious pancreatic necrosis

Infectious pancreatic necrosis (IPN) is one of the most widespread and serious viral diseases affecting salmonids, in both fresh water and seawater (Roberts and Pearson, 2005). The disease is most common in first feeding fry, causing extensive mortalities, but has occasionally been recorded in ongrowing fish and broodstock, where it inhibits egg development. The virus is a member of the Birnaviridae and pathogenic strains are, as with other birnaviruses, immunosuppressive and capable of remaining in infected carrier fish for long periods. In fry IPN is acquired via the digestive tract where it establishes and extends to the pancreas and renal haemopoietic tissue.and subsequent acute pancreatic necrosis and necrotic enteritis are frequently fatal (McKnight and Roberts, 1976).

IPN virus can be vertically transmitted both on the surface of the trout egg and inside it (Bullock et al., 1976). Although testing for the presence of detectable virus by tissue culture or quantitative PCR are helpful in relation to detecting carrier parents, it cannot be relied on entirely and so fry infected with IPN can readily infect a clean site. Affected fry are generally darker, with swollen abdomen, and vent and pop-eyes, and they have behavioural aberrations which allow very early clinical identification of an outbreak and institution of sanitary precautions which can help prevent the typical epizootic losses of up to 90%.

In rainbow trout transferred to sea, carriers may readily develop into clinically infected fish immediately post transfer (stress mediated IPN) or else the disease may occur 2-3 months after transfer to sea. Outbreaks are always worse in populations where there have been poor husbandry events during transfer or thereafter, and although outbreaks can occur in even the best operated farms, attention to fallowing of sites, biosecurity, low stress transport, sea louse control and proper feeding in the first few months all help minimise the level of losses (Smail et al., 1992). Careful management of the clinical outbreak with rapid and careful removal of dead fish, into disinfectant, and biosecurity between nets, cages etc also help limit losses.

IPN outbreaks constitute a serious welfare problem at all production stages. If fish survive acute pancreatitis and enteritis chronic damage may lead to starvation in the worst cases. Slaughter of the entire population may be the most welfare friendly course of action particularly with very small fish. Gading with removal, killing and biosecure disposal of all small, discoloured and deformed fish is often practised, but can exacerbate mortalities because of consequent stress.

Commercial vaccines are now licensed for use in EU and are becoming routine to protect against IPN in salmon but the cost and inability to vaccinate small fish makes it unlikely that they will be used for rainbow trout at present and their effectiveness is as yet unproven in field conditions. In salmon genetic resistant strains are becoming available and are reducing incidence in fry. One such strain has been reported in rainbow trout (Okamoto et al., 1993) but is not available in Europe. Recent improvements in production of SPF eggs, and biosecurity coupled with water sanitization have transformed loss levels in trout fry where they have been applied quickly and assiduously.

5.6.3. Enteric Red Mouth

Enteric red mouth disease (ERM) or yersiniosis is a significant cause of mortality in salmonids worldwide, especially in rainbow trout with losses of 10-15 % over a growth cycle (Horne and Barnes, 1999; Tobback et al., 2007). The disease is caused by the Gram-negative bacterium *Yersinia ruckeri*, a member of the family Enterobacteriaceae. A number of strains of the bacterium have been identified which vary in their pathogenicity to fish (Davies, 1991; Sousa et al., 2001).

The clinical signs of enteric redmouth at early stages of the disease are rather non-specific and include anorexia, darkening of the skin and lethargy. In small fry asymptomatic deaths may occur (Kawula et al., 1996). The disease takes its name from a typical reddening of the throat and mouth, caused by subcutaneous haemorrhaging, but this is not always seen. Subsequently, erosion of the jaw and palate may occur (Horne and Barnes, 1999). There may be haemorrhages on the body surface, at the base of the fins and in the gills.

Exopthalmia may occur and there can be cerebral haemorrhages (Rucker, 1966). Internally there can be congestion of blood vessels and petachial haemorrhages affecting many of the internal organs (Wobeser, 1973). The kidney and spleen may be swollen and there may be yellowish mucoid fluid in the intestine (Busch, 1982).

The disease is usually most acute in fry and fingerlings, but there can be significant losses in older fish. However, in the latter the disease is usually chronic. Fish which have been exposed to the bacterium and which have survived an outbreak may become “carriers” with small numbers of bacteria present in the intestine and lymphoid tissues (Horne and Barnes, 1999) which are difficult to detect. If such fish are exposed to stresses, such as high temperature, high suspended solids, or to handling or high stocking densities, then clinical disease may occur, probably due to suppression of the immune system. The disease is most common at summer temperatures in Europe with a peak at 15-18 °C (Horne and Barnes, 1999). Disease is unusual below 10 °C.

The major source of infection in farmed fish is believed to be the shedding of large numbers of bacteria with faeces of carrier or infected fish (Busch and Lingg, 1975). *Y.ruckeri* is also associated with sediments and surfaces within the aquatic environment and is known to form biofilms on solid surfaces, such as tanks (Coquet et al., 2002). Such biofilms may be a source of recurrent infection in trout farms. Other animals such as aquatic invertebrates and birds have been suggested as vectors (Willumsen, 1989).

Yersinia ruckeri has been reported from broodstock but it is not certain whether true vertical transmission from brood fish to offspring occurs. Sauter et al. (Sauter et al., 1985) suggested that vertical transmission could occur in Chinook salmon, but it seems most likely that proper disinfection of eggs will prevent transmission from broodstock (Dulin et al., 1976).

Prevention of the disease can be achieved by avoiding the introduction of infected stock and using disinfected eggs. However, the bacterium is so widespread that infection is a constant threat in many farms. Maintenance of good husbandry conditions, including reduced densities and minimal handling, especially in situations where high temperatures and low water flows occur, will be important in reducing the risk of clinical disease.

Antimicrobial compounds are often used in the treatment of enteric redmouth. (Bullock et al., 1983; Rodgers and Austin, 1983) and may be successful. However, widespread resistance of

Y.ruckeri has been reported to many of the most commonly used compounds (Post, 1987; De Grandis et al., 1988). Effective vaccines against Enteric Redmouth are available commercially. They are made of formalin-killed whole bacterial cells and offer good levels of protection. Administration is by immersion, spray, injection or oral routes (Tobback et al., 2007). Disease outbreaks may occur in vaccinated fish under high stress conditions where the immune system is compromised. Immunostimulants added to the diet may improve resistance to the pathogen, especially in conjunction with vaccination.

5.6.4. Rainbow trout fry syndrome

Rainbow trout fry syndrome (RTFS) has since the 1980s been the cause of high mortality in rainbow trout fry in European countries (Bernadet et al., 1988; Lehmann et al., 1988; Lorenzen et al., 1991; Toranzo and Barja, 1993; Dalsgaard et al., 2007; Holt et al., 2007; LaPatra, 2007). The disease is also reported from USA and Japan. The RTFS is caused by *Flavobacterium psychrophilum* (formerly *Cytophaga psychrophila*, *Flexibacter psychrophilum*) which was first described as the cause of peduncle disease in rainbow trout fingerlings (Davis, 1947). It affects very large number of fry and young fish and they may suffer a prolonged period of clinical disease before dying, so its welfare significance is high.

RTFS affected fry exhibit by lethargy, loss of appetite, ascites, anaemia including pallor of the gills, liver and kidneys. The spleen is swollen and the vent is often red (Baudin Laurencin et al., 1989; Dalsgaard et al., 2007). In fingerlings and larger fish, the symptoms are less obvious; however, abnormal swimming, moderate anaemia, blindness, skin lesions spinal deformities and visceral haemorrhages may be observed (Dalsgaard and Hørlycke, 1990). Pericarditis may be observed in chronically affected fish.

In larger fish, the disease condition is described as bacterial cold water disease or peduncle disease (Roberts, 2001). The infection usually occur at water temperatures between 8 °C – 14 °C and with a mortality up to 50-60 % (Dalsgaard et al., 2007) but if the temperature raises above 12 °C, mortality decreases drastically. The highest mortality is usually recorded when large number of fry is stocked together or if the organic load of water is high (Roberts, 2001).

As RTFS usually occurs when the fish are young and not fully immunocompetent and also may not have started feeding, it may be difficult to treat affected fish properly (Roberts, 2001). Fingerlings and larger fish may however be treated with antibiotics (Roberts, 2001; Dalsgaard et al., 2007). Antibiotic resistance has been reported (Roberts, 2001; Dalsgaard et al., 2007). Vaccines have also been developed using antigens isolated from the *F. psychrophilum* cell walls (LaFrentz et al., 2004; Aoki et al., 2007). No commercial vaccines are registered at present.

5.6.5. Proliferative kidney disease

Proliferative kidney disease (PKD) has been reported from a number of countries in Europe and North America. PKD is a serious disease causing large economic losses in particular in farmed rainbow trout and brown trout. PKD is caused by the myxozoan *Tetracapsuloides bryosalmonaea* (Canning et al., 1999).

PKD affected fish show abnormal swimming, lethargy, exophthalmus, and the appetite is reduced during the development of the disease. Furthermore, the abdomen is swollen (ascites), the gills are pale and swollen as are the heart, liver and kidneys. The kidneys have a greyish appearance and due to destruction of the haematopoietic tissue in the kidneys as part of the host inflammatory response to the presence of the parasite. Affected fish get a pale appearance due to increasing anaemia (Clifton-Hadley et al., 1987).

Clinical disease increases with increasing temperatures and at temperatures >15 °C develops more rapidly. However, outbreaks of PKD have been reported even at lower temperatures.

It is important that affected fish are handled carefully in order to avoid stress that may result in increased mortality. Minimal handling and movement of the fish and limited feeding during the higher temperatures of the summer period oxygen supplementation and reduction of water temperature may assist if feasible.

5.6.6. Eye lesions

Eye lesions in rainbow trout have several causes, including vitamin deficiency, parasites, viral and bacterial infections, mechanical damage, irritants, neoplasia, genetic factors, light and gas-bubble disease. Eye lesions vary in severity and even severe eye pathologies may not be fatal per se. However, they can result in poor growth and increased susceptibility to a range of infectious diseases (Ersdal et al., 2001). One particular eye condition common in farmed trout in ponds is eye-fluke infection by the intermediate stages of the trematode parasite of fish eating birds, *Diplostomum sp.* (Shariff et al., 1980). This renders the fish blind and easy to predate upon and so maintains the life cycle. Eye pathology is also a key presenting sign in gas bubble disease which is described below. Cataracts associated with the exclusion of blood meal from salmonid diets n the 1990' and consequent marginal nutritional deficiency, were common in rainbow trout and Atlantic salmon. This has largely been corrected but exemplifies the potential that any nutritional modification has for creating an unexpected welfare issue (Wall, 1998).

5.6.7. Gas bubble disease

Super-saturation with N_2, or less frequently O_2, causes gas bubble disease. This is an important cause of loss in farmed fish. Most commercial farms are designed to avoid it, as it is primarily an engineering problem but when it occurs it has serious welfare implications (Harvey and Cooper, 1962). It was originally observed in fish below entrained hydro-electric flows and closely resembles the human condition of 'divers-bends'. In farms or in public aquaria can be due to leaks in pump or valve systems or sudden temperature gradients. It has also been associated with altitude gradients in fish transported by air (Hauck, 1986).

The degree of supersaturation defines the eventual outcome. Supersaturation causes bubbles of super-saturated gas to come in the bloodstream of affected fish as it comes out of solution. In small vessels this can lead to rupture and haemorrhage, and even in larger vessels, the bubbles can obstruct blood flow. Fish may die without obvious signs but those that survive may be blind, or suffer cerebral, renal or hepatic vascular rupture and haemorrhage and often clear gas

bubbles can be seen as bubbles below the cornea and epidermis. They are invariably compromised in one way or another, and do not thrive (Roberts and Shepherd, 1997).

5.6.8. Fin and Skin damage

The skin and fins of farmed fish may be damaged due to various health related problems, such as handling trauma, predator attack, UV lights, bacterial or parasite infections. There may be several causal relationships between fin damage and welfare related risk factors in rainbow trout. The fins may be damaged directly by aggressive interactions, or indirectly by inadvertent contact between the fish and tank walls, nets, or other farming equipment. The causes may be amplified due to rearing technology and rearing methods, such as the degree of intensive rearing, water quality traits, stocking density and feeding regimes, but the strength of these relationships in rainbow trout are only partly known (e.g. (Moutou et al., 1998; Arndt et al., 2001; North et al., 2006b)).

The development of fin damages may in general be affected by health status and the immune competence of the fish, and initial fin damage may turn into severe secondary infection including multiple bacterial and fungal infections. Fin damage have complex potential consequences for the welfare of the fish, ranging from reduction in feed intake and growth, chronically stress responses, impact on immune status, and pain responses (Abbot and Dill, 1985) as described for Atlantic salmon (Turnbull et al., 1996).

The skin of all fish is extremely delicate. The epidermis overlying the scales is not keratinized and is usually very thin (Bullock and Roberts, 1974). In farmed conditions a number of husbandry actions depend on physical crowding, netting or grading fish and unless managed carefully, these lead to loss of epidermis and consequent osmotic effects. Also the loss of the limiting membrane allows invasion of the resultant ulcers by parasites, bacteria and fungi. Many of these are not particularly infectious but all cause delays in healing of the open lesions. Some however can be serious invasive pathogens and such external lesions are often the portal of entry.

5.7. Impact of Disease Control Measures on trout welfare

Infectious diseases of farmed fish are controlled by baths for external parasites, antibiotics and chemotherapeuants generally via the diet but occasionally via the parenteral route, and vaccines, which may be applied by bath, feed or injection. Also neutraceutical compounds such as immuno-enhancers or feed components which may reduce stress are incorporated into feed for health management during specific husbandry events.

In fish for consumption, it is important that these therapeutic or neutraceutical components whether provided via the water or the feed are not only efficacious, but equally importantly, do not have negative effects on the environment or leave residues for the human consumer. In order to ensure such safety, there are regulatory requirements in place, which require any efficacy, environment or residue issues to be resolved before they can be used. For this reason that malachite green previously used extensively in trout farming as a efficient fungicide is now

unavailable since its breakdown product leuco-malachite is an extremely long lasting residue in fish tissues and claimed to be carcinogenic.

The technical requirements relating to the marketing authorisation, production, labelling, classification, distribution and advertising of veterinary medicinal products have been laid down by Directive 2001/82/EC. The approval system follows various procedures: i) national ii) decentralized to be used for products that have not yet received authorization in an EU country iii) mutual recognition mening that EU countries may approve the decision made about a medicinal product by another EU country and iv) centralized when in accordance to Regulation (EC) No 726/2004 the application is submited to the Committee for Veterinary Medicinal Products - EMEA and on approval the marketing authorisation is valid throughout the Community. Veterinary medicinal products intended for use in aquatic species have to satisfy all the usual requirements for approval prior to marketing authorization. The main criteria for authorization are quality, efficacy and safety. Safety for humans both operators and consumers is crucial for the approval decision. The impact of aquaculture on the prevalence of human antimicrobial resistant pathogens is mostly unknown (MacMillan, 2001). Issues such as the environmemtal fate and impact, the probability of resistance transfer and the probability of human exposure should be considered.

The high cost associated with the authorization processs deters interest from the pharmaceutical industry to the aquaculture market. As trout husbandry tries to ensure minimal requirement for such therepeutants, and the individual worth of the single animal is low, often the market size for such products does not justify the manufacturer's investment on the cost of the licensing. National regulatory agencies and European Medicines Evaluation Agency (EMEA) have simplified procedures for minor species or the application of the "cascade" principle to addresss this problem. However salmonids are considered as major species and the cascade mechanism has been used in a limited way because of the difficulties to extrapolate between different fish species and from terrestrial animals to fish. Consequently the number of authorized veterinary medicinal products (VMP) is at the moment extremely low (Table 7).

The lack of availability of useful veterinary medical products is considered within the industry as a major welfare constraint. The absence of authorized VMP's might favour the illegal usage with severe consequences for human health, animal health and the environment.

Table 7. **Veterinary Medicinal Products authorized for use for farmed trout in Members States countries in 2008**

Country	Antibacterial	Antiparasitic	Antifungal	Anesthetics
Czech Republic	Flumequine			
Denmark	Oxolinic acid, Trimethoprim+sulfadiazine			

Finland	Bentsalkolium chloride; Formaldehyde; Chloramine; Orimycin; Potassium chloride	Formaldehyde; emamectin benzoate	Formaldehyde	Benzocain
France	Flumequine Oxolinic acid, Oxytetracycline Sulphadiazine+Trimethoprim	None	None	None
Greece	Oxytetracycline Flumequine Oxolinic acid Trimethoprim+sulfadiazine	-	-	-
Ireland	Oxytetracycline	Teflubenzuron		MS222
Italy	Amoxicillin, Flumequine, Sulfadiazine+Trimethoprim, Chlortetracycline, Oxytetracycline		Bronopol	
UK	Oxytetracycline	Emamectin benzoate		MS222

Source: Data collected by questionnaire at the consultation meeting (Members states and stakeholders representative) on Animal Welfare aspects of Husbandry Systems for Farmed Fish held on 4 March 2008 in Parma.

5.7.1. Biosecurity

Health biosecurity is the state of having applied appropriate measures to prevent or limit the possibility of pathogens entering populations from an extraneous source. It may be applied at the regional national level, area farm level or between particular holding facilities. These measures include disease surveillance, border controls, as well as national and international controls and stock and equipment movement controls, disinfection (Danner and Merrill, 2006), husbandry disciplines and good record keeping. A particular requirement, often recognised solely in the breach, is disinfection of transportation equipment both before and after transportation of fish. Although these are increasingly being applied and by preventing disease outbreaks have a significant welfare benefit, currently, in the trout industry, they are hindered

by lack of understanding of the principles, lack of enforcement, and failure to understand the risk basis upon which they are applied. The degree of discipline that proper biosecurity programmes require, they require in their application are somehow inimical to many farmers and invariably enhancement of biosecurity can only be induced following direct example of benefit after a serious disease outbreak. Increasing awareness of the risks associated with the movement of live fish, internationally, including aquarium fish, has led to concern for improvements in biosecurity at international and national level. At farm level however, there is still great need for training and establishment of robust biosecurity arrangements that will be both applied and monitored. Currently, however, there is a lack of adequate information on the efficacy of the various disinfectants used in terms of the fish pathogens, and in particular the toxicity of many of the disinfectants used both in relation to the fish and the environment. A start has been made to resolving these issues, (Scarfe et al., 2006; Graham et al., 2007), but inadequate knowledge and application of biosecurity is still a major factor in relation to trout welfare.

Although EU Legislation requires reporting of "increased mortality", most farms do not currently have any formal means to differentiate between expected mortalities and unexpected or increased mortalities, although this permits biosecurity monitoring and medicines control as well as mortality awareness. Presence of a named Veterinary Surgeon and regular and detailed Veterinary audit are important to the management of disease in the aquatic environment just as in terrestrial conditions and support welfare. Mortalities of fish during the production cycle can result from a variety of causes, including, disease, damage, predation and adverse environmental conditions. Since any population of animals suffers from mortalities it would be -unreasonable to aim for no mortalities. At present there is no data on what level of mortalities are experienced in the various farming systems or what level of mortalities might be considered acceptable. Very poor welfare (e.g. disease, poor growth and mortalities) is not cost-effective for the farmer, so even the farms that have relatively poor fish welfare have found a balance between welfare and productivity. However, in many cases high or increasing mortalities are an indication of disease problems with serious economic and welfare implications. At present many farms rely on the experience of the farm manager to decide when mortalities require additional action. This is not a simple task since mortalities vary over time depending on a variety of factors such as life stage, temperature, farming system, presence of endemic diseases and others.

5.7.2. Vaccination of trout

Intensification in fish farming has resulted in the emergence of disease problems, in particular of infectious origin, which were unknown, or at least not previously diagnosed, in the wild. Since the establishment of fish farming as an industry, antimicrobials have been used for “fire fighting” of epizootics in order to keep disease problems in aquaculture at an acceptable level. In the longer term, however, such usage is unsustainable option, and while initially, considerable volumes were used, the level of antibiotic consumption for control infectious diseases has been significantly reduced due to better husbandry methods and particularly, due to the introduction of vaccines into the major sectors of farmed fish production (Hastein et al., 2005; Berg et al., 2006b).

In rainbow trout farming killed microorganinsms or else modified bacterial toxins are generally used as vaccine antigens. They are usually based on whole cell proteins. The main vaccines

used in trout culture are against enteric red-mouth (ERM) (Raida and Buchmann, 2008) and *Listonella anguillarum* the cause of vibriosis in marine farmed trout and those are generally given orally. In marine farmed trout oil and water emulsion vaccines are generally given by injection against: vibriosis, furunculosis cold water vibriosis and IPN.

Table 8. **Authorized Veterinary Immunological Products for farmed trout**

Country	Disease – Method of administration
Czech Republic	Enteric redmouth disease - immersion and oral
Denmark	Enteric redmouth disease - immersion and oral
Finland	Furunculosis + Vibriosis - injection
	Furunculosis – immersion and injection.
	Vibriosis – immersion, injection and oral
France	Entericredmouth disease - immersion, injection and oral
	Vibriosis – immersion, injection and oral
Greece	Enteric Red mouth disease - immersion and injection
	Vibriosis - immersion, injection and oral
Italy	Enteric Red mouth disease
	Vibriosis
	some auto- vaccines or experimental vaccines e.g. Lactococcus garviae
UK	Enteric redmouth disease - immersion and oral
	Vibriosis – immersion, injection and oral
Norway	Vibriosis,
	Furunculosis
	Winter ulcers

5.7.3. Methods of Vaccination

Vaccines applied to farmed fish, have generally been given via a parenteral route. With ERM and Vibriosis, bath immersion vaccination, can be used (Raida and Buchmann, 2008), though often this may be followed up when the fish get older, by parenteral injection (Smith, 1988).

Vaccination by injection is usually intraperitoneal, but in potential brood fish, as with salmon, the adhesions, where an adjuvant is used, cause major clinical effects on the developing ovaries and testes and significantly limit fecundity. In such fish, which are not for consumption, vaccination is usually intramuscular into the dorsal median sinus.

Immersion vaccination, when feasible, is carried out by direct immersion, by hyper-osmotic infiltration by immersion in hypertonic saline before or with the vaccination procedure or by spraying the vaccine onto the fish (the "shower" method). The immersion method may induce

an effective immune response in fish and an advantage of the method is that a large number of fish may be vaccinated at the same time with a minimum of handling. It cannot however be used with multivalent vaccines and even where the system lends itself to use of immersion vaccination, the higher dose levels required means that for economic reasons it is only economically feasible with very small fish (Smith, 1988).

5.7.4. Welfare aspects of vaccination

Provided other factors such as husbandry and welfare are adequate, it prevents disease outbreaks, reduces mortality considerably and reduces the use of medicinal products. Thus in general it has a positive welfare effect as where it is effective, few fish will suffer from the disease it protects against, and the environmental loading of such pathogens is also reduced for other fishes, in the absence of outbreaks. In the case of bacterial diseases, where vaccination has had the greatest effect, introduction of vaccination has greatly reduced the use of antimicrobials and also modified the multiple disease resistance patterns of such pathogens, which were emerging.

Although vaccination is proven effective in protection against many serious fish diseases, conferring major welfare advantage, nevertheless, there are also certain disadvantages regarding vaccination by injection, in particular. These are:

- Handling at vaccination;
- Growth retardation;
- Deformities in the vertebral column;
- Adhesions which later organise and contract as scar tissue in the peritoneal cavity.

Handling

Poor welfare may be caused by crowding, catching, anaesthesia and possible pain associated with the vaccination.

Growth

Salmon breeding companies have observed that (Sørum and Damsgård, 2004) that unvaccinated salmon consistently gain up to a kilo more than their vaccinated peers in the same time period to harvest under the same conditions. This considerable growth penalty is also likely to apply in the case of vaccinated trout.

Adhesions in the peritoneal cavity

Oil in water emulsion adjuvant enhances the immune response to most parenteral fish vaccines. The adjuvant act by stimulating a chronic inflammatory response at the site of injection. Inflammatory peritonitis holds the antigen within the peritoneal cavity attracting into the area macrophages and lymphocytes as well as an enhanced blood supply ensuring maximal uptake of the antigen by the host (Mutoloki et al., 2006; Russell et al., 2006). The inflammatory response enhances the efficacy of vaccines against many fish microbes. Endotoxin antigens of the serious aeromonad and vibrio bacteria, among the most dangerous fish pathogens, are themselves highly necrotoxic and aggressive and retention in the peritoneum rather than allowing their dispersion is important.

Progress has been made in developing vaccines to limit the adhesion development, using less aggressive oils not only for welfare reasons but also because the effect on growth and processor resistance to the adhesions resulting in serious financial loss (Midtlyng et al., 1996). The chronic inflammatory responses in fish ultimately lead to the melanisation of the tissue and its contraction as collagenous scarring develops. The melanisation is a serious quality problem but the contraction may also cause constriction of the digestive tube or other organs (Koppang et al., 2005). The degree of inflammatory fibrosis and melanosis caused by a particular vaccine is assessed on a scale known as the Speelberg scale (Midtlyng et al., 1996; FVS, 2003). Vaccines are assessed in terms of three parameters, 1) degree of fibrous adhesions within the peritoneal cavity; 2) degree of melanisation and 3) position and nature of the lesions, on a scale of 1-6.

Peritoneal adhesions represent the most serious welfare issue in relation to the use of injectible vaccines. There is little doubt that the use of such vaccines since their commercial introduction in the US in 1987 has transformed the ability to control serious diseases but there is strong justification for seeking alternative methods of inducing protective immunity against these serious diseases which do not require the production of such severe side effects as part of their efficacy.

Apart from the nature of the vaccine itself, there is now a considerable amount of field evidence from salmon farming, that the husbandry conditions of the fish can affect the degree to which the vaccinal lesions develop**.** These include the size of the fish, the temperature at the time of the vaccination and a variety of husbandry and environmental factors. Vaccination may also influence the development of spinal lesions in salmon and and marine-reared trout may be equally susceptible (Vagsholm and Djupvik, 1998).

Vaccine injection should not be carried out on fish less than 12 g and at a water temperature above 15 °C, the risk for lesions is reduced if the size of the fish is at least 70 g and the water temperature is 10 °C or below. Injection of vaccines without adjutants may, however, to be carried out in fish down to 10 g (Gudding, 2000). Nevertheless, the larger the fish are at the time of injection, the better and also reduction of the water temperature at the time of vaccination will improve the result (Berg et al., 2006a).

In fries biosecurity and management methods may be applicable. Vaccines are available for a very limited range of diseases e.g. enteric redmouth. Vaccination is not normally carried out on fish less than 10 g in weight. Immersion is the normal method of vaccine administration.

6. Risk assessment

6.1. Introduction

Animal welfare problems are generally the consequence of animal environment changes resulting from management or production factors as well as environmental, genetic and disease factors and interactions thereof. Presently there are no standards for animal welfare risk assessment, but previous studies exist where risk assessment for animal welfare has been explored (Anonymous, 2001; EFSA, 2006).

Risk assessment is a systematic, scientific-based process to estimate the magnitude of and exposure to a hazard impact and include 4 steps: hazard identification; hazard characterisation; exposure assessment and risk characterisation.

In food risk assessment terminology (*Codex alimentarius*), a hazard is a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect. The risk is a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food.

Making a parallel to the *Codex alimentarius* risk assessment methodology, a hazard in animal welfare risk assessment is a factor with a potential to cause negative animal welfare effect (adverse effect).

A risk in animal welfare is a function of the probability of a negative animal welfare effect and the severity of that effect, consequential to the exposure to a hazard(s). The probability of a given target population to be exposed to a particular hazard was scored as frequency of exposure. Once exposed, the proportion of the population affected will vary and was assessed as the likelihood of effect. Consequences of exposure have been scored by severity of the effect in the individual and duration of the effect. While hazards and risks usually relate to negative welfare impacts, the risk assessment approach could also be also extended to include positive welfare consequences (resulting in risk-benefit analysis).

The degree of confidence in the final estimation of risk depends on the uncertainty, and variability. Uncertainty comes from the evaluation and extrapolation of information obtained from epidemiological, experimental, and laboratory animal studies and whenever attempts are made to use data concerning the occurrence of certain phenomena obtained under one set of conditions to make estimations or predictions about phenomena likely to occur under other sets of conditions for which data are not available. Uncertainty also comes from having incomplete knowledge. Uncertainty can be evaluated by carrying out further studies to obtain the necessary data or quasi-formally by using expert opinion or by simply making a judgment.

Variability is a biological phenomenon (inherent dispersion) and is not reducible. Reduction in variability is not an improvement in knowledge but instead, it would reflect a loss of information. Variability cannot always be separated from uncertainty, and together they are referred as total uncertainty.

6.2. Steps of risk assessment

For risk assessment of welfare of farmed trout the different production systems, as well as the different life stages were identified. The different life stages were: eggs and alevins, fry, parr, smolt, ongrowing and broodstock (Table 9). The different production systems vary depending on the life stage and are summarized in table 10.

Table 9. **Life stages duration**

	Duration (d/d = degree days)	**Estimated % of the total life cycle**
Broodstock		
Eggs	300 – 330 d/d	10
Alevins	250 - 300 d/d	10
Fry	250 d/d	10
Fresh water ongrowing	9 -12 months	70
Sea water ongrowing	12 – 18 months	

Table 10. **Production systems by life stages**

	Production life stages					
Production systems	Eggs	Alevins	Fry	Fresh water ongrowing	Sea water ongrowing	Broodstock
Trays	X					
Vertical incubators	X	X				
Tanks flow-through with oxygenation			X	X		
Tanks flow-through without oxygenation			X	X		
Fresh water cages				X		
Ponds				X		X
Raceways				X		
Sea water cages					X	

6.2.1. Hazard identification

The aim of this step is to identify causes or factors that have a potential to change the trout welfare. Although negative or positive changes can be accessed only negative impacts were considered. A list of potential categories of hazards to trout welfare was drawn up (Table 11). The identified hazards were grouped in different categories such as abiotic, biotic, genetic, management and disease. The hazard tables referred to the different life stages of trout as well as to the different types of production systems.

Table 11. **Factors or hazards**

HAZARD IDENTIFICATION	HAZARD SPECIFICATION
ABIOTIC	
Water velocity	too low / too high
Light	period / intensity
Water temperature	rapid change/ high /low
Suspended solids	
Salinity	too high
pH	too high or low in combination with Al
Oxygen content	too low
Heavy metals	too high
Environmental complexity	
Carbon dioxide content	too high
Ammonium content	too high, pH dependent
Nitrite	Too high
Total gas pressure	
BIOTIC	
Stocking density	high/low
Intra-specific interaction	
Predators	
Other invasive species (e.g.algae)	
Mixing fish from different origins	
FEEDING	
Excess of feed	
Dietary toxins (fungal toxins)	
Non fish meal based diets	
Nutrients	Surplus/deficiency
Lack of food	short time/long time
Dietary toxins	
MANAGEMENT	

Grading	Frequency /Methods
Lack of staff competence	
Insufficient monitoring	Health /Biomass
Handling	
GENETIC	Growth/Disease
Genetic selection	
Triploids	
DISEASES	
Viral haemorrhagic septicaemia	
Infectious pancreatic necrosis	
Enteric Red Mouth	
Rainbow trout fry syndrome	
Proliferative kidney disease	
Eye lesions	
Gas bubble disease	
Fin and Skin damage	

The tables in Appendix A cover the four essential steps of risk assessment: 1) hazard specification, 2) hazard characterisation 3) exposure assessment and 4) risk characterization.

Production factors (hazards) could have a direct or indirect effect on trout welfare by causing changes in trout s' environment. We addressed single factors without interactions. Since production factors can interact and welfare problems are usually due to multiple exposures to different factors, any positive or negative interactions with other factors need to be reviewed. Interactions and positive effects are described in the text if deemed necessary.

6.2.2. Hazard characterization

The objectives of this step are:

- to examine and describe the consequences of an exposure to one or several hazards; and
- to assess the relationship between the level of the hazard in terms of frequency and duration and the likelihood and magnitude of the adverse effect occurring at population level.

The severity of the adverse effect was scored according to scientific evidence of the level of physiological and behavioural responses (Table 12).

Table 12. **Severity of adverse effect**

Evaluation	Score	Explanation

Negligible	**0**	No pain, malaise, frustration, fear or anxiety as evidenced by measures of the normal range of behavioural observations, physiological measures and clinical signs for >95 % of the species or strain/breed
Mild	**1**	Minor changes from normality and indicative of pain, malaise, fear or anxiety
Moderate	**2**	Moderate changes from normality and indicative of pain, malaise, fear or anxiety
Substantial	**3**	Substantial changes from normality and indicative of pain, malaise, fear or anxiety.
Severe	**4**	Extreme changes from normality and indicative of pain, malaise, fear or anxiety, that if persist would be incompatible with life.

The duration of the adverse effects, i.e. the consequences of the hazard, were scored on a 0 to 100 % scale considering the whole life of the fish and not just the particular life stage.

If the adverse effect is fatal then the duration before death would be the key welfare issue, even though death itself might indicate a prior welfare problem. The life time can be judged as the "potential life time" or the "real life time". If the adverse effect is death the duration of the effect over the potential life time is very short but 100 %.over the real life time. In this assessment, it was decided to score the duration of the effect over the "potential life time" of the animal, but indicating if a hazard was so severe that it could lead to immediate death.

A hazard is not only described by the the severity of its adverse effect, but also by the likelihood of the adverse effect occurring which equates to the proportion of the population affected (Table 13).

Table 13. **Likelihood of effect (proportion of population affected)**

Evaluation	Score	Explanation
Negligible	**0**	The event would almost certainly not occur
Extremely low	**1**	The event would be extremely unlikely to occur
Very low	**2**	The event would be very unlikely to occur
Low	**3**	The event would be unlikely to occur
Moderate	**4**	The event would occur with an even probability
High	**5**	The event would be very likely to occur

The uncertainty value (low, medium, high) depends on the type of information available, whether there are different studies with differing conclusions, but also whether scientific information exists, and how certain the information is likely to be. The qualitative assessment of uncertainty for each assessment of any scientific evidence is also scored (Table 14).

Table 14. **Uncertainty**

Evaluation	Score	
low	**1**	Solid and complete data available; strong evidence in multiple references with most authors coming to the same conclusions
medium	**2**	Some or only incomplete data available; evidence provided in small number of references; authors' conclusions vary from one to the other; Solid and complete data available from other species which can be extrapolated to the species considered
high	**3**	Scarce or no data available; evidence provided in unpublished reports, or based on observation or personal communications; authors' conclusions vary considerably between them

6.2.3. Exposure Assessment

Exposure assessment is the qualitative, semi-quantitative or quantitative evaluation of the probability of the specific scenario of exposure. It takes into account the frequency and duration of exposure to one or several hazards during the life stage of the trout. The frequency of exposure (Table 15), is, how often a particular hazard would be encountered.

Table 15. **Frequency of exposure**

Evaluation	Score	Explanation
Negligible	**0**	The exposure would almost certainly not occur
Extremely low	**1**	The exposure would be extremely unlikely to occur
Very low	**2**	The exposure would be very unlikely to occur
Low	**3**	The exposure would be unlikely to occur
Moderate	**4**	The exposure would occur with an even probability
High	**5**	The exposure would be very likely to occur

The duration of the hazard for a given life stage was also described using a value from 0 % to 100 %. For instance a predator attack could only last a short period of time while a temperature change could last for much longer. The uncertainty of the information was judged as well using the same criteria as above.

Experts were asked individually to fill in the tables, based on current scientific knowledge and published data. Due to the low number of experts in relation to the large number of tables on average two experts filled in each table. Their scores were then compared and discussed in the working group and referred to the literature. In numerous cases differences of scoring appeared to be a problem of scaling of the risk scores or interpretation of the risk factor. The scores served as a basis for the overall risk scoring

6.2.4. Risk characterization

Risk characterisation integrates hazard characterisation and exposure assessment into a risk score. This step aims to estimate the likelihood of occurrence of the adverse effect in a specific production system at a specific life stage of the trout.

Exact quantitative figures were not possible due to the limited amount of quantitative data, a semi quantitative risk assessment has been used. The methodology used does not give a precise numerical estimate of the risk attributed to certain hazards; however, the output can be used to rank the problems and designate areas of concern, as well as provide guidance for future research.

Risk score = (Magnitude)*(likelihood of adverse effect)*(Exposure)

Magnitude = (severity of adverse effect)*(duration of the adverse effects)

Exposure = (frequency of hazard)*(duration of hazard)

The scores of frequency of hazard, severity and likelihood of effect were standardized to give even weighting to the scores (frequency of hazard /5; severity / 4 and likelihood of hazard / 5) Duration of hazard and duration of effect were divided by 100. Eventually, the risk score was multiplied by 100 to make it easier to read.

Interactions of the hazards cannot easily be considered with this approach and each hazard is looked at individually.

Uncertainty scores were not used in the risk estimate directly but are included for transparency to indicate the uncertainty of the data and areas requiring future research. To simplify the presentation the two uncertainty scores were summed up in a single figure according to an uncertainty classification matrix (Table17).

Table 16. **Combined Uncertainty Scores**

		Uncertainty (exposure assessment)		
Uncertainty (Hazard characterization)		High	Medium	Low
	High	High	High	High

	Medium	High	Medium	Medium
	Low	High	Medium	Low

The risk score gives a ranking indicating the importance of the risks and allowing comparison of hazards in different production systems in the same life stage. Risk scores have been added up, to to compare the different production systems.

The Tables in the **Appendix A** include all values agreed by the experts of the working group to assess the various factors identified in different production systems for the various life stages of farmed trout.

6.3. Discussion risk assessment

The risk scores based on expert advice were used to compile a risk ranking by category such as abiotic or biotic to indicate which hazards are the more important for each life stage in the various production systems considered, and also to enable the comparison of the different production systems within life stages. Comparison across life stages is difficult, because of the different length and condition for each life stage.

Genetics was not considered in the overall risk score for indidual life stages since separately the "hazard" therefore appears only once and consequences are life long. Genetic selection can lead to a loss of traits which was assessed as being a risk for welfare however the uncertainty was high.

Interactions could not be directly considered in the risk assessment, although some of the hazards can be closely linked to other factors and it is difficulte to disentangle the importance of each of them when assessing their effect on welfare.

6.3.1. Welfare risks associated with eggs incubation

Only hazards in the abiotic factors and husbandry categories were assessed because other categories of factors are not relevant to this life stage. Only welfare impact on subsequent life stages was considered. The ranking by order of the highest risk scores on abiotic factors is summarized in Table 17. The combined uncertainty scores were high for all abiotic factors. All factors assessed for husbandry (rough handling, insufficient sorting and monitoring and lack of staff competence) had the same risk score but the risk is lower than for abiotic factors as hazard frequency is usually low because of avoidance of serious consequences by industry.

No considerable differences between the two production systems were found with regards to potential risks to welfare.

Table 17. **Welfare risks ranking – eggs incubation.**

	Trays	Vertical screen incubators
Abiotic	High temperature	High temperature
	High light intensity	High light intensity
	Rapid changes of temperature	Rapid changes of temperature
Husbandry	Inappropriate handling / insufficient sorting and monitoring/lack of staff competence	Inappropriate handling / insufficient sorting and monitoring/lack of staff competence

6.3.2. Welfare risks associated with farming of alevins

Only hazards belonging to diseases, husbandry and abiotic factors categories were assessed in a single system, trays and the other hazards were not relevant for this life stage. The ranking by order of the highest risk scores is summarized in Table 18. Abiotic factors together showed an overall high risk socre. Rapid changes of temperature, environmental complexity (lack of adequate substrate), too low oxygen content, high CO_2 level and total gas pressure constituted the highest ranks. The diseases considered for this life stage constituted a high risk for welfare. All factors assessed for husbandry (inappropriate handling, insufficient sorting and monitoring and lack of staff competence) had the same risk score however the risk was lower than from abiotic factors for the reasons already mentioned in the previous paragraph on eggs. The combined uncertainty scores were high for all abiotic factors moderate for husbandry and low for diseases.

Table 18. **Welfare risks ranking – alevins**

	Trays
Abiotic	High temperature/ Rapid changes of temperature / Lack of adequate substrate/ Water oxygen content too low / Water carbon dioxide too high / Total gas pressure
Husbandry	Inappropriate handling, insufficient sorting and monitoring, lack of staff competence
Diseases	Infectious Pancreatic Necrosis
	Fin and skin damage

6.3.3. Welfare risks associated with farming of fry

The highest scores for abiotic factors for both systems were too low water oxygen content and too high water temperature. The most important biotic hazards were intra-specific interaction (aggression), and stocking density both too low and too high. High stocking density is closely linked with deterioration of water quality with possible fatal consequences. For hazards connected with feeding, the ration was considered the most important hazard for welfare risk.

Excess of feed impact on welfare was high due to effects on water quality or due to excessive weight gain.

Management hazards such as inappropriate handling and grading insufficient monitoring and lack of staff competency were ranked equally across all production systems. For the fry life stage diseases also scored highly with regards to the other factors. No difference between production systems was found for the disease risks: Infectious pancreatic necrosis and Rainbow trout fry syndrome were the top hazards (Table 19).

Table 19. **Welfare risks ranking – fry**

	Tanks flow-through without oxygenation	**Tanks flow-through with oxygenation**
Abiotic	Water oxygen content too low	Water temperature too high
	Water temperature too high	Suspended solids and turbidity
	Heavy metals too high, pH dependent	Water velocity
Biotic	Aggression	Aggression
	Low stocking density	Low stocking density
	High stocking density	High stocking density
Feed	Lack of feed (long term)	Lack of feed (long term)
	Excess of feed (environmental deterioration)	Excess of feed (environmental deterioration)
	Excess of feed (excessive weight gain)	Excess of feed (excessive weight gain)
Husbandry	Inappropriate handling, insufficient sorting and monitoring, lack of staff competence	Inappropriate handling, insufficient sorting and monitoring, lack of staff competence
Diseases	Infectious pancreatic necrosis	Infectious pancreatic necrosis
	Rainbow trout fry syndrome	Rainbow trout fry syndrome
	Eye lesions	Fin and skin damage
	Fin and skin damage	Eye lesions

6.3.4. Welfare risks associated with farming of trout on growers

Production of trout on fresh water is done in Europe on various systems according to geography and climatic conditions. In some countries rainbow trout is also produced in sea water. The production systems considered for the risk assessment were, tanks flow through, raceways, freshwater cages, ponds and sea water cages. Additional oxygenation is often used both for tanks and raceways and some hazards were assessed separately with or without oxygenation.

For ongrowers abiotic factor did not constitute major hazards in comparison with other categories. Low oxygen content and high water temperature were top ranked hazards. For oxygenated tanks, race ways and ponds high carbon dioxide level is among the highest scores. High stocking density was the highest biotic hazard followed by intra-specific interaction (aggression) and low stocking density. In freshwater, seawater cages and ponds mixing fish from different origins and predators constitute risks not present in the other systems. Over all production systems the amount of feed played the most important role with excess of feed appearing to be slightly more important than lack of feed which could however lead to mortality. Inappropriate handling and inadequate sorting were ranked as top risks, followed by lack of staff competence and insufficient monitoring. Abiotic factor did not constitute major hazards in comparison with other categories. The welfare risks associated with diseases are higher for conditions such as eye lesions.and skin and fin damage connected with secondary infections. For the diseases considered, sea cages scored better.

Overall production systems did not considerably differ, but fresh water cages and sea cages had less risks associated with abiotic hazards. Sea cages scored less for the studied diseases. Management and feeding hazards did not differ in their scores and biotic factors only marginally. For abiotic factors the uncertainty varied and could be high, for feeding and management the uncertainty was low, whereas for biotic and disease a medium uncertainty was found (Table 20).

Table 20. **Welfare risks ranking – ongrowers**

	Tanks	**Raceways**	**Ponds**	**Freshwater cages**	**Seawater cages**
Abiotic	Water oxygen content too low	Water oxygen content too low	Water oxygen content too low	Water oxygen content too low	Water oxygen content too low
	Water carbon dioxide too high (*)	Water carbon dioxide too high (*)	Water carbon dioxide too high (*)	Water temperature too high	Water temperature too high
	Water temperature too high	Water temperature too high / toxic un-ionised ammonia content	Water temperature too high	Water carbon dioxide too high	Water carbon dioxide too high
Biotic	High stocking density	High stocking density	High stocking density	High stocking density	High stocking density
	Aggression	Aggression	Aggression/ low stocking density	Aggression	Aggression
	Low stocking density	Low stocking density		Mixing fish	Predators/ Low stocking density

Feed	Lack of feed (long term) Excess of feed	Lack of feed (long term) Excess of feed	Lack of feed (long term) Excess of feed	Lack of feed (long term) Excess of feed	Lack of feed (long term) Excess of feed
Husbandry	Inappropriate handling Grading Lack of staff competence	Inappropriate handling Grading Lack of staff competence	Inappropriate handling Grading Lack of staff competence	Inappropriate handling Grading Lack of staff competence	Inappropriate handling Grading Lack of staff competence
Diseases	Eye lesions Fin and skin damages Proliferative Kidney Disease	Eye lesions Fin and skin damages Proliferative Kidney Disease	Eye lesions Fin and skin damages Proliferative Kidney Disease	Eye lesions Fin and skin damages Proliferative Kidney Disease	Eye lesions Fin and skin damages Infectious pancreatic necrosis

* systems with oxygenation

6.3.5. Welfare risks associated with farming of broodstock

Low water oxygen content, too high water temperature and carbon dioxide level are the most important abiotic factors affecting broodstock welfare. Intra-specific interactions showed a very high risk score, followed by high stocking density, predators and low stocking density. For the broodstock the biotic factors constituted the highest risks. The amount of feed was the most important risk with excess of feed appearing to be slightly more important than lack of feed which could however lead to mortality. Inappropriate handling and inadequate sorting were ranked as top risks, followed by lack of staff competence. For the diseases: eye lesion and fin and skin damage ranked highest. The risks were the same across all production systems (Table 21).

Table 21. **Welfare risks ranking – broodstock**

	Ponds
Abiotic	Water oxygen content too low Water temperature too high
	Water carbon dioxide too high

Biotic	Agression
	Stocking density too high
	Predators
	Stocking desity to low
Feeding	Excess of feed
	Lack of feed (long term)
Management	Inappropriate handling \ Inadequate sorting
	Lack of staff competence
Disease	Eye lesions
	Fin and skin damages

6.3.6. Risk associated with production systems

Overall, production systems did not seem to differ much in their risk scores within life stages (Biotic, management, feeding). Risk scores for diseases were in general the highest amongst all different categories considered.

6.4. Uncertainty

The combined uncertainty score indicates how certain the experts were of the knowledge for a particular field and whether this could be backed up with published references.

The uncertainty for certain areas tended to be high, also reflected in the fact that expert opinion had to be used, since not a lot of data were available. Information on management issues varied between fairly certain (low uncertainty) to medium uncertainty.(for alevins and fry). For feeding experts gave a low uncertainty score. For biotic factors the whole range of uncertainty scores was covered depending on each particular hazard. The uncertainty score for the impact of genetic selection on trout welfare was high, as very little information for this species is available.

6.5. Data gaps

- There is insufficient information about the production systems for trout
- Key operational data on water quality parameters under commercial conditions is mostly not available.
- Prevalence data on trout disease is not available

- Effects of many of the environmental hazards on welfare are mostly unknown although tolerance levels have been determined under experimental conditions.

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Abbreviations

ADNS	Animal Disease Notification System
BKD	Bacterial Kidney disease
DO	Dissolved Oxygen
EEA	European Economic Area
EMEA	The European Medicines Agency
EU-15	The first 15 members of the European Union
EU-25	The first 25 members of the European Union
FEAP	Federation of European Aquaculture Producers
GnRH	Gonadotrophin releasing hormone
HSMI	Heart Skeletal Muscle Inflammation
IPN	Infectious pancreatic necrosis
IPNV	Infectious pancreatic necrosis virus
OIE	World Organization for Animal Health
PKD	Proliferative kidney disease
ppt	parts per thousand
RTF	Rainbow trout fry syndrome
VMP	Veterinary Medicinal Product
VHS	Viral Haemorrhagic Septicaemia

Glossary

Alevin	First stage of the trout life-cycle following hatch: the alevin has a very limited swimming ability and is provided with nutrition by an attached yolk sac. In the wild alevins remain within the redd in which they are hatched, and in aquaculture within the container in which they are hatched.
Broodstock	A population of fish selected to provide genetic material for the next generation. In a modern breeding programme, broodstock populations are selected and isolated at the egg stage and grown through all the life stages separately from production stocks. Broodstock are maintained beyond the end of the ongrowing stage in order to reach sexual maturity.
Closed systems	A rearing system with control of inlet and outlet water, e.g. tanks, raceways or closed bags.
Crowding	The situation in which the movements or other activities of individuals in a group are restricted by the physical presence of others
Degree days	Average temperature in degree centigrades multiplied by the number of days.

Eyed Stage	The stage of development of eggs during which the eye is visible.
Fry	Early life stage of trout beginning from independence of yolk sac as primary source of nutrition and ending when fish begin move from their hatching site: the redd in the wild and hatching container in aquaculture. In aquaculture the term First Feeding Fry is often used to describe the stage at which fish have entered the water column and begun to feed.
Hypercapnia	A condition with elevated carbon dioxide concentration in the water.
Hyperoxia	A condition with oxygen saturation above 100% of the normal atmospheric equilibrium for a given temperature and salinity.
Hypoxia	A condition with oxygen saturation below 100% of the normal atmospheric equilibrium for a given temperature and salinity r.
Oxygenation	In aquaculture: the mixing of pure oxygen and water; this generally refers to a process by which oxygen pressurized in a gas cylinder is diffused into the water mass to be oxygenated, for example for fish transport.
Redds	Spawning areas, often with a gravel substratum.
Restricted feeding	A reduced ration usually bellow fish appetite
Sea Trout.	sea going form *Salmo trutta* L
Starvation	A period of food deprivation such that the animal metabolises tissues that are not food reserves but are functional tissues.
Stocking density	The number of fish per unit volume of water. This term is the reciprocal of the space allowance (the volume of water occupied per fish).
Supersaturation	A condition in which a medium, such as a solvent, contains concentration of a substance higher than it can normally hold at a given temperature and pressure, e.g. oxygen supersaturation in water.
Water quality	The extent of presence in water of any substance that may have an effect on fish in that water.

Risk Analysis Terminology

Exposure Assessment	The quantitative and qualitative evaluation of the likelihood of hazards to welfare occurring in a given fish population.
Hazard Identification	The identification of any factor, from birth to end of life, capable of causing adverse effects on fish health/ welfare.
Hazard characterisation	The qualitative and quantitative evaluation of the nature of the adverse effects associated with the hazard. Considering the scope of the exercise of the working group the concerns relate exclusively to fish/trout welfare.
Risk	A risk in the context of this report is a function of the exposure to an adverse effect, the magnitude and the likelihood, consequent to a hazard for trout health/ welfare.
Risk	The process of determining the qualitative or quantitative estimation,

Characterisation	including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse effects on welfare in a given fish/ trout population based on hazard identification, hazard characterisation and exposure assessment.
Risk Assessment	A scientifically based process consisting of the following steps: i) hazard identification, ii) hazard characterisation, iii) exposure assessment and iv) risk characterisation.
Quantitative Risk Assessment	A risk assessment that provides numerical expressions of risk and an indication of the attendant uncertainties
Qualitative Risk Assessment	A risk assessment based on data which, while forming an inadequate basis for numerical risk estimations, nevertheless, when conditioned by prior expert knowledge and identification of attendant uncertainties, permits risk ranking or separation into descriptive categories of risk.
Risk Analysis	A process consisting of three components: risk assessment, risk management and risk communication.
Uncertainty Analysis	A method used to estimate the uncertainty associated with model inputs, assumptions and structure/form. This includes also uncertainty, due to the lack of reliable publications, uncertainty in the scientific results etc.

European Food Safety Authority

The EFSA Journal (2008) 796- Annex II , 1-19

Animal welfare aspects of husbandry systems for Farmed Trout[1]

MINORITY OPINION

This minority opinion from Prof. Donald M. Broom is based on the view that the accepted Report and adopted Opinion are incomplete and that in order to answer the mandate from the European Commission, the introductory chapters on the welfare, biological functioning and farming of fish should be included.

[1] For citation purposes: Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission on Animal welfare aspects of husbandry systems for farmed trout. *The EFSA Journal* (2008)796-Annex II, 1-31

SUMMARY

Fish are very diverse in their body form and have a wide range of sensory systems, some of which, such as electroreceptors and the lateral line system, are not shared by birds and mammals. As vertebrates, fish, birds and mammals share a similar general brain structure. Over and above this, however, comparative neuroanatomy highlights many differences among vertebrate groups; it also highlights differences in brain structure among species of fish. On the other hand, studies of brain function suggest a number of parallels between fish and other groups. Fish have nociceptors and these look like and have a similar response profile to those of birds and mammals. The question of whether fish experience the input of these receptors as pain remains controversial but experiments have shown the brain is active during such stimulation and that painkillers reduce prolonged behavioural and physiological responses. It is clear that the responses given by fish to nociceptive stimulation are more complex than simple reflexes, including significant shifts in behavioural priorities and the performance of anomalous behaviour. In this context, our working position is that juvenile and adult fish have the capacity to perceive painful stimuli and experience at least some of the adverse affective states that we associate with pain in mammals. Data suggest that the affective state of fear sometimes motivates behaviour in fish. The systems in mammals and birds that result in the production of adrenaline and cortisol have close anatomical and functional parallels in fish. Fish show physiologically and behaviourally similar freeze and flight responses and prolonged cortisol production is associated with immunosuppression.

WELFARE CONCEPTS

Attitudes to animal welfare encompass three aspects: what animals feel or experience; how animals are functioning; and how the subject animals compare with their 'natural' wild counterparts (Fraser, 1999) and these influence how animal welfare is understood. Feelings and experiences are part of animal functioning and have effects that may be assessed. However, observations on animals in the wild are not involved in welfare assessment, but give a guide as to their likely functioning when removed from the environment in which they have evolved.

Welfare is a characteristic of an individual animal and is concerned with the effects of all aspects of its genotype and environment on the individual (Duncan 1981). Broom (1986) defines welfare as follows: "the welfare of an animal is its state as regards its attempts to cope with its environment". According to this definition, an animal's welfare depends on the ease or difficulty of coping and also the extent of any failure to cope, which may lead to disease and injury. Furthermore, welfare also includes pleasurable mental states and unpleasant states such as pain, fear and frustration (Duncan 1996, Fraser and Duncan 1998). Such feelings cannot be measured directly but may be inferred from measurements of physiology and behaviour and are a component of coping systems (Cabanac 1979, Broom 1998, Panksepp 1998).

Whenever animals are overtaxed by environmental impacts, welfare is poor to some degree and aspect of animal welfare (MacIntyre et al., 2008).

When considering fish, application of these welfare concepts appear more difficult to develop and require specific consideration. There are several reasons for this. First, there is less knowledge of basic biology particularly of brain functioning in relation to awareness of pain and fear then for mammals or birds (Rose, 2002).._Fish are poikilothermic animals which live in an aquatic environment. Environmental factors have a major impact on fish biology and coping with environmental changes is a major task for fish. There are many publications, on the impact of external factors on fish physiology and behaviour: Such biological knowledge is a valuable source of information when assessing fish welfare (Iwama, 2007). In this context, the concept of 'needs' is central to discussions of animal welfareThe needs can be fulfilled by

physiological changes and by carrying out certain behaviours. Such behavioural requirements are more difficult to evaluate as sophisticated experimental evidence is required to determine their strength (Hughes & Duncan 1988, Jensen & Toates 1993, Broom and Johnson 1993, Vestergaard 1996). Such experiments have rarely been conducted in fish even though a failure to meet such needs in some way may contribute to poor welfare.

Where welfare or health are referred to as good in this report, these words imply a state that is positive for an individual and by implication for the population as a whole, Where welfare or health are referred to as poor, a negative state is implied. The following sections will deal with the major recognisable adverse states in the fish species being studied with a review of the available scientific data and its interpretation.

CONCLUSION

The concept of welfare is relevant to all farmed animals, including farmed fish, and some aspects of fish welfare can be scientifically assessed. However, although the same methodology is relevant in studying the welfare of fish, birds and mammals, much less research has been carried out on fish.

WELFARE ASSESSMENT IN FISH

The scientific assessment of welfare is discussed by Huntingford *et al.*(2006) and and FSBI (2002).. Welfare assessment may be based upon a list of needs, for example measuring the hazards associated with the non-fulfilment of these needs. It may be assessed in various ways. Poor welfare can be assessed by how far an individual animal has deviated from what is normal for animals in a good environment (Morton and Griffiths, 1985), i.e. one that meets all of their needs. Normality is not necessarily that which is natural for wild fish and an assessment of deviation from normality must be based upon baseline studies of farmed fish in a satisfactory environment., taking into account their previous experiences e.g. (specific) rearing environment. To understand, compare and develop actions to improve fish welfare, defined protocols of welfare measures or indicators are needed.

Some welfare research involves measuring direct indicators of poor or good welfare while other research evaluates what is important to animals by studies demonstrating positive preferences and motivation (Dawkins 1990) and also aversion i.e. negative preferences and how hard an animal will work to avoid, as opposed to access, an environmental variable. Some such work on preferences and motivation has been conducted with fish, but there is not a large amount of data on these issues. Measures of physiological functioning, productivity, health and pathology and behaviour all form the basis of welfare assessment. As an example, measuring disease resistance or the functioning of the immune system offers one way of estimating the welfare "cost" of certain aquaculture conditions. Compromised immune performance can lead to disease outbreaks with associated direct negative welfare consequences. Moreover, lowered disease resistance is generally believed to be a consequence of maladaptive physiological stress, and disease challenge testing may therefore also be an indirect measure of such stress conditions.

Due to the complex causal relationships among the various needs of farmed fish and their behavioural and physiological consequences, it is impossible to find one single measurement or welfare indicator that will cover all possible welfare relevant effects of all possible rearing systems, farmed species and potential situations. Some of the methods used and evaluation of the results will be species and system specific. When the welfare of fish or other animals is assessed, sets of measures can be used, which might be physiological (Oliveira *et al.,* 1999,

Ellis *et al.*, 2004), behavioural or pathological (see Huntingford *et al.*, 2006). Whilst a single measure could indicate poor welfare, a range of measures will usually provide a more accurate assessment of welfare because of the variety of coping mechanisms used by the animals (Koolhaas *et al.*, 1999, Huntingford and Adams 2005) and the various effects of the environment on individuals. Useful welfare indicators must to be valid reflections of welfare and repeatable. In addition to measures, which are the outputs of good husbandry, farm practices that help to ensure good welfare provide important indirect welfare indicators, independent of the condition of the fish. Such indicators of welfare through good practice include staff training, good husbandry protocols, monitoring and biosecurity systems, health plans and contingency plans. These complement measures of welfare outcomes by indicating ways by which poor welfare can be avoided.

Indicators that do not necessarily give information about individual fish are commonly used by fish farmers to assess changes at a population level. Indeed, many fish farms have strategies for real-time monitoring of such indicators, which include feed intake, growth rate and mortality. In the case of feed intake, the indicator is not the feed intake per se, but the deviation from an expected feed intake based on biomass and water temperature. Production variables of this kind have a place in welfare assessment and a failure of fish to feed and grow often indicates poor welfare. However, high performance levels (e.g. high feed intake and good growth) do not necessarily indicate good welfare. At a population level, changes in rate of mortality may be a useful indicator of poor welfare.

Indicators at the individual level cover all measurements of individual fish in a system, either by non-invasive monitoring in free-swimming fish, or with targeted sub-sampling of fish. Examples of individual measures are fin condition and parasite load. Representative sub-samplings are difficult in large farm systems, but can work well in smaller systems. The individual indicators commonly relate to the ability of the fish to maintain a normal physiological (and possibly behavioural) state, including the ability to mount effective immune responses.

INTRODUCTION TO THE BIOLOGY AND FUNCTIONING OF FARMED FISH SPECIES

1.1. Diversity of teleost fish forms and environmental adaptations

The three major groups of fish are: Agnatha (hagfish, lampreys), Chondrichthyes (sharks, rays, sturgeons) and Actinopterygii (bony fish with teleosts being the most prevalent). Most aquaculture finfish species are teleostean fish (Evans *et al.*, 2005). There are more than twenty thousand living species of teleosts that have been evolving over 500 million years, representing every aquatic environment and a vast range of physiological and behavioural traits.

Each species has developed a set of tolerance limits for each environmental factor, and within such ranges ecological interactions are further limiting the natural distribution and habitat selection (Randall *et al.*, 2002, Helfman *et al.*, 1997). The tolerance ranges are species specific and may be wide or narrow, developing the species into opportunistic generalists or specialists designed for long-lasting natural ecological niches. Individual fish have abilities to to cope with a changing environment, including large annual changes in e.g. water temperature and food availability. As a result of such plasticity, fish have been able to inhabit every conceivable aquatic environment, from a Tibetan lake at an altitude of 5,250m to the pacific depth at –8,370m. They are also extraordinary diverse in terms of numbers of species, body forms, life-styles and physiologies. Fish genomes are more varied and plastic in comparison with other vertebrates, owing to frequent genomic changes (Cossins and Crawford, 2005).

Teleost fish share many common morphological and physiological adaptations with other vertebrates, including many components of the neural and endocrine systems, immune system and the physiological stress cascade. However, some key systems such as the respiratory- and osmoregulatory systems differ markedly form land-living vertebrates due to the particular challenges imposed by living in water. Respiration (i.e. exchange of gases such as oxygen and carbon dioxide) takes mainly place over the gills (except in the early larval stages). The gills are also involved in uptake and excretion of ions and maintenance of osmoregulatory balance. The intimate physiological contact of all body fluid compartments and tissues through gills, skin and gastro-intestinal system with the external environment is a situation that can lead to major physiological challenges. Variations in water conditions (including oxygen levels, temperature, pathogen, salinity and water-borne pollutants) can have a direct and unavoidable impact on susceptible cells, tissues and organs. This close physiological contact is more easily defined and its impact more readily studied than in terrestrial species. Fish are sensitive sentinels of environmental challenge particularly pollution (Cossins & Crawford 2005).

Some fish species go through marked metamorphosis or habitat changes such as transfer from freshwater to sea-water that often represent critical periods with reduced capacity to withstand stressors or infectious diseases. The intimate contact with the water, including pathogens, represents a challenge in terms of barrier functions as a part of the disease defence. Breakdown of the integrity of these barriers, e.g. due to various forms of stress, may lead to increased susceptibility to infectious diseases. The development of acquired immune function often takes place after the metamorphosis from larval to juvenile form which represents a challenge on vaccination, in particular in marine farmed fish species that are exposed to a suite of pathogens from early life stages.

Fish in a natural habitat display complex swimming, feeding, anti-predator and reproductive behaviours, and such behavioural traits are linked to genotypical differences between species and individual animals, and are modified by phenotypical development and learning. In addition, several fish species undergo ontogenetic niche shifts during their lifespan, and consequent changes in behaviour, e.g. change in salmon from a territorial parr in the river to a schooling fish which migrates from freshwater to sea-water, and years later to a mature fish which migrates back to the river prior to spawning **(**McCormick *et al.*, 1998).

1.2. Environmental factors and fish physiology.

The main environmental factors which control spatio-temporal distribution of fish are temperature, salinity, light, oxygen, food, pollutants, hydrodynamics and substratum. Moreover, the physiological processes of fish are carried out under environmental conditions harsher and more restrictive in many ways than those experienced by terrrestrial animals (Wedemeyer, 1997). For example, the concentrations of the gases in the aquatic environment are highly variable compared with those in air. Oxygen depletion in water is not unusual and at times respiration can be difficult. All these reasons explain why coping with changes in environmental factors is a major ability for fish species that is relevant when considering fish welfare.

During the last 40 years, considerable research effort has been devoted to the effects of environmental factors on fish physiology (Somero and Surarez, 2005).

Scientific information on the effects of environmental factors on physiological functions in fish, including development, growth, reproduction, excretion, osmoregulation, respiration and immunity are summarised in several text books on fish ecophysiology (Evans 1993, Rankin 1994, Bruslé and Guignard 2004). Teleost fish share with other vertebrates many common developmental pathways, physiological mechanisms and organ systems. The challenge

imposed by aquatic life leads to major physiological roles for exchanging epithelia such as gills. This is not only related to the major physiological functions (i.e. respiration, osmoregulation, excretion, acid-base balance regulation) carried by the gill which then play a central role in a suite of physiological responses to environmental and internal changes but also to the huge surface exchange built up by the gill which are a major entry for many biotic or abiotic water compounds (Evans, 2005). An example of fish ecophysiologyis the study of the effect of xenoestrogen on sex differentiation on trout reared in cages (Jobling et al., 1998) which led to literature on the effect of endocrine disruptors (Sumpter and Johnson, 2005).

Literature on fish behaviour and analysis of behavioural responses exhibited by fish exposed to stressors are mostly devoted to fish in their natural environment (Schreck, Olla and davis, 1997). Fewer studies have looked at fish behaviour in production systems. Feeding behaviour (Volkoff and Peter, 2006), social interaction and hierarchies (Gilmour et al., 2005) are important in fish aquaculture.

CONCLUSION

Fish live in the aquatic environment and respond to harmful chemicals and many other stressors at intensity levels frequently far below those that can be perceived by terrestrial animals

1.3. Sensory systems in fish

Both conservation and innovation in the organisation of sensory systems occur across vertebrates. Fish perceive optical, positional, chemical, tactile, mechanosensory and electrosensory (lateral line), acoustic, and magnetic stimuli by receptors innervated by particular brain regions (Hodos & Butler 1997). Some basic patterns of sensory innervation are common to all vertebrates for the relay of sensory inputs from putative stressors in the environment to the brain, directly impacting on the fish's welfare.

The optical characteristics of water affect illumination intensity and spectral quality. This has led to evolution of the fish eye to cope with these challenges. Fish eye adaptations allow the efficient collection of light (Warrant & Lockett 2004) and other specialisations (Siebeck & Marshall 2001). They do not have eyelids or nictitating membranes and the large choroidal complexes are subject to pressure changes and to gaseous embolism. Thus the fish eye is particularly vulnerable to a variety of husbandry effects leading to poor welfare (Roberts 2001).

Sound and vibrations travel well in water and fish are highly responsive to and potentially easily disturbed by exposure to such systems. However, it is not clear whether or not salmonid fish are disturbed by such stimuli (Wysocki *et al*. 2007).

The ear of bony fish comprises three semi-circular canals, a utricle and a sacculae and lagena. The auditory receptors comprise a very variable set of sensory organs that perceive sound from the environment. The ascending auditory pathways in mammals and fish are similar. The vestibular system of vertebrates detects position and motion of the head and is important for equilibrium or balance and coordination of head, eye and body movements.

Fish have highly elaborate chemosensory detection of information from the environment including other fish. Chemicals detected by the fish and conveyed to the brain via cranial nerve I are involved in olfaction. Structural organisation of the peripheral olfactory organ is variable

throughout fish species, although the ultrastructural organisation of the olfactory sensory epithelium is extremely consistent (Hara 1994). Olfactory signals such as those involved in reproduction and feeding may be processed independently through two distinct subsystems (Laberge & Hara 2001, Nikonov *et al.,* 2005). The neuronal components are similar to the olfactory systems of mammals except that there is no connection between respiratory structures and the olfactory system in fish. Chemical pollution and chemical signals such as alarm pheromones may often cause poor welfare in fish so consideration of the impact of olfactorily important chemicals in the fish environment can improve welfare.

The taste buds of vertebrates are the receptors of the gustatory or taste organ that may occur in the oropharyngeal cavity and elsewhere on the body surface (Hara 1994).

The lateral line system detects mechanosensory information and is found in all fishes and some amphibians but has been lost in reptiles, birds and mammals. The sensory organ consists of hair cells called neuromasts located in the lateral line canals or on the head and body. The lateral line system allows fishes to respond to water movements and other movements relatively close to the fish. This system alerts fish to prey, predators, school neighbours, water flow from environmental obstacles, and in salmon reproductive vibrations (Satou *et al.,* 1994) that facilitates orientation behaviour (Montgomery *et al.,* 1997).

Magnetoreceptors have not been identified with certainty in any animal, and the mode of transduction for the magnetic sense remains unknown. However, magnetite particles embedded in specific cells in the basal lamina within the olfactory lamellae of rainbow trout, *Oncorhynchus mykiss*, have been identified (Walker *et al.,* 1997). All fish can use their lateral line to detect local movement and electroreception is widespread in fish, including farmed species. The implications for welfare are starting to be considered (Spiess *et al.*, pers. comm.).

CONCLUSION:

Fish have a wide range of sensory systems, some of which, such as electroreceptors and the lateral line system are not shared by birds and mammals.

1.4. Comparative Brain Structure

As in all vertebrate brains, the fish brain consists of forebrain (i.e. telencephalon and diencephalon), midbrain (mesencephalon), and hindbrain (rhombencephalon). The pallium constitutes the exterior surface of the telencephalon, in mammals the neocortex is a greatly expanded part of the pallium. Thus, the general anatomy of the teleost (bony fish) brain is similar to that of other vertebrate brain, however, the fish brain is smaller relative to body size and less complex in structure than that of higher vertebrates (Kotrschal *et al.*, 1998). Moreover, among fish there is a marked inter species variation in brain anatomy, often reflecting sensory specialization, fundamental differences in embryonic development, and the degree of cell migration and proliferation and intraspecific variation in brain structure is evident (Butler 2000).

The fish brain grows continuously throughout life and appears to be highly responsive to the environmental conditions that the fish experiences as it develops (Ramage-Healey & Bass 2007, Dunlap *et al.*, 2006, Kihslinger & Nevitt 2006, Kihslinger *et al.,* 2006, Lema 2006).

In vertebrates specific brain structures have been associated with emotions and motivated behaviour. It is now indicated that the same function can be served by different structures in different groups of animals (e.g. cognitive functions in birds and mammals, Jarvis *et al.,* 2005) and structures that seem to be different may be more homologous than had previously been

thought. Comparative anatomical studies have shed some light on the potential functional role of fish brain structures in relation to motivational and affective states. The issues are complex and there is considerable disagreement among specialists about the extent of commonality of brain function within the vertebrates. Fish do not have the extensive analytical cortex that mammals have and sensory processing is carried out in different regions of the brain according to the adaptations of the particular group of fishes. Fish do not have the extensive cerebral cortex that mammals have, this being smaller relative to body size and without the characteristic folded and layered appearance of the mammalian cortex. Additionally, sensory processing is carried out in different regions of the brain according to adaptations of the particular group of fishes (Rose 2002, Vogt 2003).

The possibility cannot be excluded that parts of the brain other than the cerebral cortex have evolved the capacity for generating negative emotional states in fish (Huntingford *et al.*, 2006). The concept of pain in vertebrates revolves around the perceived noxiousness of certain stimuli, and may have been conserved through evolution as a protective strategy.

At the level of the telencephalon, fish lack the higher cortical centres that have been demonstrated as necessary for full processing and experience of pain in mammals (Rose 2002). Extensive interconnections exist between the telencephalon, diencephalon and mesencephalon in fish (Rink & Wullimann 2004). Neural pathways that connect to various forebrain structures are of fundamental importance to consciousness and the perception of pain and fear in mammals (Willis & Westlund 1997). The pallium (the grey matter that covers the telencephalon) has thickened to various extents in different classes of vertebrates, and in mammals it consists of a laminated structure, the cerebral cortex (Striedter 1997). Unlike mammals, in the majority of modern fish species, the pallium is unlaminated (Vogt 2003), however there is evidence to suggest it has developed into a highly differentiated structure with respect to the processing of sensory information (Bradford 1995, Butler 2000). The telencephalon in fish contains several brain structures that are thought to be functionally homologous to those associated with pain and fear in higher vertebrates (Bradford 1995, Chandroo *et al.*, 2004, Portavella *et al.*, 2004), and this is known to be active during a potentially painful event (Dunlop and Laming 2004). Therefore, information about noxious stimuli, such as those resulting from tissue damage, in fish may be processed in a functionally homologous way, not yet fully characterised, to that involved in processing noxious stimuli in mammals. In mammals, the hippocampus, a telencephalic structure, is involved in memory and learning of spatial relationships whereas the amygdala, a structure which is also telencephalic, has long been known to be important in arousal and emotions, particularly fear responses (Carter 1996, Maren 2001). Recent studies have identified structures in the teleost telencephalon that appear to be homologous to the mammalian amygdala and hippocampus with alterations in fear, spatial learning and memory retrieval when these areas are lesioned (Portavella *et al.*, 2002). Another important structure in the fish brain, the hypothalamus, is thought to perform functions similar to those of the hypothalamus in other vertebrates. The hypothalamus is involved in various functions, including sexual and other social behavior, and is also responsible for the integration of both internal and external signals including those originating from those telencephalic areas that have been implicated in fear responses (Fox *et al.*, 1997, Portavella *et al.*, 2002, Chandroo *et al.*, 2004).

CONCLUSION

Our understanding of the extent to which brain structure and function in fish are comparable with other vertebrate groups is limited. As vertebrates, fish, birds and mammals share a similar general brain structure. Over and above this, however, comparative neuroanatomy highlights many differences among vertebrate groups; it also highlights differences in brain structure

among species of fish. On the other hand, studies of brain function suggest a number of parallels between fish and other groups.

1.5. Sentience

Sentience refers, among other properties, to the ability to experience pleasurable and adverse states, a key issue when considering the welfare of any animal and a focus of public concern and there are discussions of this matter in relation to fish (Broom 2006, 2007, Yue *et al.* 2008).

Animals that have some cognitive ability at a certain stage of their development, start development without such ability. Hence it is relevant to consider at what time, during the life of a fish, their perceptual and cognitive abilities develop. It is likely that fish develop some cognitive ability only when they are able to perceive external stimuli. While little is known about the development of cognitive ability, we have some evidence concerning the stage of life at which the development of responsiveness to external stimuli starts (EFSA, 2005).

1.6. Pain

Pain is defined as an aversive sensation associated with tissue damage. As non-human animals are unable to communicate the experience of pain directly, a number of criteria have been defined to provide a guide as to whether an animal might be capable of experiencing pain (Bateson 1991, Broom 2001a, b, Sneddon 2004). These criteria include: (i) the existence of functional nociceptors (ii) the presence and action of endogenous opioids and opioid receptors (iii) the activation of brain structures involved in pain processing (iv) the existence of pathways leading to higher brain structures (v) the action of analgesics in reducing nociceptive responses vi) the occurrence of avoidance learning vii) the suspension of normal behaviour associated with a noxious stimulus.

Each of these areas will be considered in turn to assess how well fish fulfil these criteria and how their functioning compares to the nociception and pain systems of higher vertebrates.

Nociception is the detection of a noxious stimulus and is usually accompanied by a reflex withdrawal response away from that stimulus immediately upon detection. Noxious stimuli are those that can or potentially could cause tissue damage so stimuli such as high mechanical pressure, extremes of temperature and chemicals, such as acids, venoms, prostaglandins and so on, excite nociceptive nerve fibres. Martin & Wickelgren (1971) and Mathews & Wickelgren (1978) identified sensory neurones in the skin and mouth of a lamprey (*Petromyzon marinus*) during heavy pressure, puncture, pinching or burning, and found that the output was like that which would be recorded in a mammalian nociceptor when responding to a painful stimuli. Studies of the rainbow trout (*Oncoryhnchus mykiss*) have shown that nociceptors are present on the trout face and are innervated by the trigeminal nerve (Sneddon 2002, 2003a). These studies on nociceptor anatomy and physiology strongly support the hypothesis that the rainbow trout has the sensory equipment for detecting potentially painful stimuli. Studies of nerve responses, nerve and other tissue regeneration, behavioural responses and effects of analgesics indicate nociceptive function in the fins of salmonid and other fish (Becerra et al 1983, Geraudie and Singer 1985, Turnbull et al 1996, Chervova 1997).

Fish have the necessary brain areas for nociceptive processing to occur (e.g. pons, medulla, thalamus; Sneddon 2004).The functional possibility for high level processing, such as that carried out in the cortex in humans, is crucial in terms of pain perception. In term of anatomy the fish brain is far smaller relative to body size and simpler in structure than of a human. Moerover, fish lack cortical structure such the neocortex, which plays a key role in the

subjective experience of pain in humans (FSBI 2002; Rose 2002). However, it is not impossible that parts of the brain other than the cerebral cortex have evolved the capacity of generating negative emotional states in fish (Huntingford et al. 2006).

In fish as in other vertebrates, nociceptive information is relayed to the brain from the periphery via two major tracts. The trigeminal tract conveys information from the head while the spinothalamic tract conveys information from the rest of the body. In fish the trigeminal has been shown to project to the thalamus as it does in other vertebrates (Goehler & Finger 1996, Finger 2000). The elasmobranch (Ebbesson & Hodde 1981) and teleost (Goehler & Finger 1996, Finger 2000) groups both have the same basic components of ascending spinal projections as higher vertebrates.

The possession of opioid receptors, endogenous opioids and enkephalins is one of the requirements to determine whether nociception can occur in an animal (Bateson 1991, Broom 2001a, b). These substances are involved in analgesia in the mammalian central nervous system and are produced in order to reduce pain internally. Met-enkephalin and leu-enkephalin are present in all vertebrates which have been tested and there are at least six opioid receptors described for teleost fish (Dores and Joss 1988, Dores *et al.*, 1989, Dores and Gorbman 1990, McDonald and Dores, 1991). Opioids elicit antinociception or analgesia through three distinct types of receptors in mammals (Newman *et al.*, 2000) and these have been identified in the zebrafish, *Danio rerio* (Stevens 2004). When goldfish are subjected to stressful conditions, there is an elevation of pro-opiomelanocortin, the precursor of the enkephalins and endorphins, just as there would be in humans (Denzer and Laudien, 1987). Goldfish which are given electric shock show agitated swimming but the threshold for this response is increased if morphine is injected and naloxone blocks the morphine effect (Jansen and Greene 1970). Work by Ehrensing *et al.*, (1982) showed that the endogenous opioid antagonist MIFI down-regulates sensitivity to opioids in both goldfish and rats. Opiate receptors and enkephalin like substances have also been found in various brain areas of goldfish, *Carassius auratus* (Finger 1981, Schulman *et al.*, 1981) and rainbow trout, *O. mykiss* (Vecino *et al.*, 1991). The distribution of enkephalins in the fish brain shows a similar pattern to that seen in higher vertebrates (Simantov *et al.*, 1977, Vecino *et al.*, 1992). In general it is clear that there are very many similarities amongst all vertebrates in their opioid systems.

A simple reflex response to a noxious stimulus can indicate nociceptive function, however, adverse affects on an animal's normal behaviour beyond a simple reflex may indicate a psychological component that is indicative of suffering, and suggests that the animal may be perceiving pain. Reflex responses occur instantaneously and within a few minutes but some of the responses of fish may be prolonged.(Sneddon 2006). A recent study investigated the behavioural response of rainbow trout that had been given subcutaneous injections of acetic acid and bee venom (algesics) to the lips (Sneddon *et al.*, 2003a). These fish showed an enhanced respiration rate for approximately 3 hours, did not feed within this period, and showed anomalous behaviours such as rubbing of the affected area on the aquarium substratum and glass and rocking from side to side on either pectoral fin (Sneddon 2003b, Sneddon *et al.*, 2003a). These, therefore, appear to represent changes in behaviour over a prolonged period as a result of nociceptive stimulation.

The ability of analgesics to modulate nociceptive responses is also indicative of pain perception since the selectively act on this system. The adverse behavioural responses seen in the rainbow trout, *O mykiss*, were quantified and when morphine was administered to fish injected with acid, there was a dramatic reduction in this rubbing behaviour as well as rocking behaviour and the enhanced respiration rate was also ameliorated (Sneddon 2003b, Sneddon *et al.*, 2003a). Further to this, acid injected fish did not show an appropriate fear response to a novel challenge supporting the idea that this painful stimulus dominates the fish attention (Sneddon *et al.*,

2003b). Studies have shown that goldfish are able to learn to avoid noxious, potentially painful stimuli such as electric shock (Portavella *et al.*, 2002, 2004). Learned avoidance of a stimulus associated with a noxious experience has also been observed in other fish species (Overmier & Hollis 1983, 1990) including common carp, and pike, avoiding hooks in angling trials (Beukema 1970a, b).

There are strong debates on the question of pain in fish with opposing views (Rose 2002, Derbyshire *et al.*, 2007, Sneddon 2004, 2006). For example, Derbyshire *et al.*, (2007) argue that the results from Sneddon's studies presented above can be interpreted as showing a remarkable capacity of trout to withstand oral trauma which would be expected as trout normally feed on potentially injurious prey such as crayfish, crabs and spiny fish. They also suggest that there is an important difference between knowledge about sensation and sentience (Derbyshire *et al.*, 2007). Rose (2002) argues that there are major neurobehavioral differences between fish and humans, particularly at the level of brain regions responsible for pain awareness in humans. In fish, in which the cerebral hemispheres were removed, leaving the brainstem and spinal cord intact, ome behaviour was still possible(Overmier and Hollis, 1983). Because the experience of fear and pain depends on cerebral cortical structures in mammals and these are absent in fish brains, Rose (2002) concluded that awareness of fear and pain is impossible in fish. However, evidence of an active nociceptor system in fish associated with effects of administration of noxious substances on normal behavioural repertoire has led to the inference that fish potentially have the capacity for long-term suffering (Chandroo et al. 2004, Sneddon 2006, Braithwaite and Boulcott 2007).

CONCLUSION

It has been convincingly demonstrated that fish have nociceptors and that these look like and have a similar response profile to those of birds and mammals. The question of whether fish experience the input of these receptors as pain remains controversial but experiments have shown the brain is active during this stimulation and that painkillers reduce prolonged behavioural and physiological responses. It is clear that the responses given by fish to nociceptive stimulation are more complex than simple reflexes, including significant shifts in behavioural priorities and the performance of anomalous behaviour. In this context, our working position is that juvenile and adult fish have the capacity to perceive painful stimuli and experience at least some of the adverse affective states that we associate with pain in mammals.

1.7. Fear

Fear serves a function that is fundamental to survival and is the activation of a defensive behavioural system that protects animals against actual or potentially dangerous environmental threats. In higher vertebrates, fear involves mainly the amygdaloid and hippocampal regions of the brain although other areas are also implicated. Studies in fish have shown that these responses also appear to be dependent upon cognitive mechanisms and homologous limbic brain regions in the telencephalon. The dorsomedial (Dm) telecephalon in fish has been implicated in emotional learning and is thought to be homologous to the amygdala in mammals (Bradford 1995, Butler 2000, Portavella *et al.*, 2004). In mammals the hippocampus is involved in memory and learning of spatial relationships and it is the dorsolateral (Dl) telencephalon in fish that is thought to be functionally homologous to the hippocampus. Dm lesions impaired acquisition of an avoidance response but had no effect on performance in a spatial learning task, while Dl lesions affected spatial learning but did not impair the acquisition of the

avoidance response (Portavella *et al.,* 2002). Therefore Dm and Dl areas of the fish telencephalon share functional similarities with the amygdala and hippocampus, respectively, in mammals.

Studies on fear conditioning in mammals measure levels of freezing and startle behaviour (Fendt & Fanselow 1999). In fish, a number of different behavioural responses to potentially threatening stimuli have been described and include escape responses such as fast starts (Chandroo *et al.,* 2004, Domenici & Blake 1997, Yue *et al.,* 2004) or erratic movement (Cantalupo *et al.,* 1995, Bisazza *et al.,* 1998), as well as freezing and sinking in the water (Berejikian *et al.,* 1999, 2003). Such behaviours may serve to protect the individual from the threat and a number of studies have illustrated that these behaviours can be shown in response to conditioning. Many fish species also release chemical alarm substances when injured. These are thought to act as warning signals, as conspecifics show a behavioural fright response to these chemicals (Smith 1992, Lebedeva *et al.,* 1994, Brown & Smith 1997, Berejikian *et al.,* 1999). These alarm behaviours include dashing movements, vigorous movements in the aquarium substratum, and fast swimming towards hiding places, remaining there for an extended period. These behaviours are thought to be associated with predator evasion (Hamdani *et al.,* 2000).

Learned avoidance studies not only show that a consistent suite of behaviours are produced in response to fearful stimuli in fish but they also provide evidence that the displayed behaviour is not merely a reflex response. Learning to avoid an aversive stimulus in the future implies a cognitive process of recognising that the behavioural response will lead to the desired effect of avoidance (Yue *et al.,* 2004). This may support the suggestion that an affective state such as fear may serve to motivate behaviour in fish.

Learning is thought to be mediated in part by receptors in the brain that are activated by N-methyl-D-aspartic acid (NMDA). Administration of selective antagonists of NMDA receptors impair learning mechanisms such as associative learning and conditioned fear in mammals (Miserendino *et al.,* 1990, Sanger & Joly 1991, Kim *et al.,* 1991, Maren 2001). Experiments with goldfish have shown that intracranial administration of MK-801, an NMDA receptor antagonist, blocks specific aspects of Pavlovian fear conditioning in fish (Xu & Davis 1992, Xu 1997).

CONCLUSION

Fear often depends on cognitive and learning ability and fear responses by fish are described for various situations, suggesting that the affective state of fear sometimes motivates fish.

1.8. Stress responses

Selye (1973) defined stress as “the nonspecific response of the body to any demand made upon it”. Following a period of controversial debates about the definition of stress and stressors, all recent reviews on stress in teleost fish define this term as a condition in which the homeostasis is threatened or disturbed as a result of the actions of intrinsic or extrinsic stimuli commonly defined as stressors (Wendelaar Bonga 1997, Iwama *et al.,* 1997, Barton 2002, Chrousos 1998, Wendemeyer *et al.,* 1990). The problems associated with Selye’s concept of stress are discussed by Broom and Johnson (2000) and there is debate about whether or not the concept should be limited to that which is detrimental to the fish. The response to stressors is often an adaptative mechanism that allows the fish to cope with stressors in order to maintain homeostasis. If the intensity of the stressors is overly severe or long lasting, physiological

response mechanisms can become detrimental to fish welfare or maladaptative (Barton 2002, FSBI, 2002, Wendelaar Bonga 1997).

During the last 20 years, there has been extensive research devoted to the biology of stress in fish. Physiological and behavioural responses to a large variety of physical, chemical and biological stressors including those seen in aquaculture have been measured (for review see Wendelaar-Bonga 1997, Iwama *et al.*, 1997, Barton 2002, FBSI 2002, Conte 2004, Ashley 2007). Hypothalamic-pituitary-interenal (HPI) axis responses are generally considered as an adaptive strategy to cope with a perceived acute threat to homeostasis, for example poor water quality. Although fish are able to tolerate acute adverse water quality conditions, when they become too challenging or prolonged, fish cannot maintain homeostasis and experience chronic stress which in the long term can impair immune function, growth and reproductive function. Furthermore, chemicals may have toxic effects at the level of cell and tissue but, in addition, elicit an integrated stress response which may be specific to the toxicant.

The stress physiology of fish is directly comparable to that of higher vertebrates. Stress physiology is manifested by primary, secondary and eventually tertiary stress responses (see review Wedemeyer *et al.*, 1990, Wendelaar Bonga 1997, FSBI 2002, Ashley 2007). The primary stress response to short term potentially harmful situations involves, amongst other things, the release of catecholamines (adrenaline and noradrenaline) from the chromaffin cells into the circulating system. Simultaneously, activation of the hypothalamic-pituitary-interenal (HPI) axis is observed. The corticotrophin releasing factor (CRF) is released from the hypothalamus and acts on the pituitary resulting in the synthesis and release of adrenocorticotrophic hormone (ACTH) which in turn stimulates the synthesis and mobilisation of glucocorticoid hormones (cortisol) from the interrenal cells. Released catecholamines and cortisol will result in an activation of various physiological and behavioural mechanisms that constitute the secondary and possibly tertiary stress responses. The secondary changes include alteration of secretion of other pituitary hormones and thyroid hormones, changes in turn-over of brain neurotransmitters, mobilisation of energy by breakdown of carbohydrate and lipid reserve and by oxidation of muscle protein, improvement of respiratory capacity via increased heart stroke volume and increase blood flow to gills. As a consequence of this last effect, disruption of the hydromineral or osmoregulatory balance can be observed.

Primary and secondary stress responses are short-term effects of acute, short-lived challenges. When these responses are prolonged or repeated and fish has no way to avoiding or escape the challenge, a series of tertiary effects become apparent, including changes in immune function and disease resistance (Pickering 1992, Balm 1997), in growth (Barton *et al.*, 1987, Pickering *et al.*, 1991) and in reproduction (Pankhust and vander Kraak 1997, McCormick 1998, Schreck *et al.*, 2001).

Behavioural responses are often shown early in defence against adverse environmental changes, often triggered by the same stimuli that initiate the primary physiological stress responses. The exact behavioural response depends on the stressor in action. For example, the response to an approaching potential predator might be escape, whereas the response to an approaching competitor might be attack. The behavioural response to abiotic environmental stressors, such as inappropriate water temperature, oxygen or water current, includes a range of responses in movement pattern, spatial choice and social interactions, but these responses are poorly described in most fish species. In addition, individuals of the same species may differ in the nature and magnitude of their behavioural responses to various stressors. Such behavioural differences, together with the physiological variation with which they are associated, are referred to as coping strategies. Some individuals adopt what is called a proactive coping strategy, showing adrenaline-based fright and flight responses, while others adopt a reactive coping strategy, showing cortisol based “freeze” and hide responses (Korte *et al.*, 2005).

However it is not clear to what extent these are general strategies. These differences are correlated with variation in brain serotoninergic activity (Schjolden and Winberg 2007) and are also affected by the extent of exposure to stressors.

Chronic stress is a major factor in the health of fish (Conte, 2004). As in mammals, there is a clear link between stress and immune status arising mostly through the effects of cortisol which can suppress many aspects of the immune system (Wendelaar Bonga 1997). However, the relationship between stress and immune system goes in two directions since components of the immune system can influence stress responses through modification of the secretion of hormones (Ottaviani *et al.*, 1996, Balm 1997). While disease is not always connected to poor environmental conditions (Huntingford *et al.*, 2006), aquaculture practice presents many situations where stress and physical injury can increase susceptibility to naturally occuring pathogens (Ashley, 2007). For example, diseases associated with low temperatures over winter period have been described in a number of different species (Tort 1998b). Fin erosion is also an important problem in aquaculture which often occurs as results of aggressive interactions. Fin erosion may increase susceptibility to infections (Turnbull *et al.*, 1996). One example of the strong interaction between environmental stress and a serious infectious disease is the case of furunculosis. Many fish may carry the causative pathogen but clinical outbreaks occur normally after stressful events such as grading or transportation of fish. So predictable is the response that a predictive test for identifying carrier populations is the 'furunculosis stress test' where samples of healthy fish are injected with cortisone to identify individuals which might become clinical cases if stressed (Hiney *et al.*, 1994).

An acute stress response does not necessarily imply any harmful consequence as such a response may be important to the maintenance of homeostasis. However, mid- and long-term exposure to stressors generally leads to maladaptative effects and sometimes to chronic stress, which are associated with decreased welfare. Such effects have been described with chronic effects on growth, reproduction or immune function and disease resistance. So, while studies on stress responses do not necessarily give us a complete view of welfare in fish, deleterious effects of several components of the stress response observed after chronic exposure to stressors are indicative of poor welfare (Huntingford *et al.*, 2006, Ashley *et al.* 2007).

Measurements of the levels of both glucose and lactate in the plasma may sometimes be biomarkers of stress in fish (e.g. Arends *et al.*, 1999; Acerete *et al.*, 2004). Measures of the expression of stress related genes might also provide useful markers (e.g. Gornati *et al.*, 2004). Chronic stress has been also studied and exerts a strong effect on haematology (Montero *et al.*, 2001), metabolism (Mommsen *et al.*, 1999), neuroendocrine function (Dibastistta *et al.*, 2005b), and osmoregulation (Wendelaar Bonga, 1997). However, reliable indicators of chronic stress are still under investigation and will probably rely on a range of measurements.

Avoidance of the maladaptative consequences of prolonged stress is a central concern in aquaculture and assessments of potential methods to reduce stress responses is an active area of research (Ashley 2007). Thus, fish have been selectively bred for reduced emergency responses: High responding (HR) and low responding (LR) lines of rainbow trout have been generated by selection for consistently high or low cortisol response to a standard confinement test (Pottinger and Carrick 1999). In addition, these two strains of rainbow trout also show a divergence in sympathetic reactivity as a response to confinement (Schjolden and Winberg 2007). However, all testing was conducted under controlled laboratory conditions and the welfare and productivity of LR strains have not yet been compared under commercial conditions. Manipulation of fish diet has been also shown to play an important role in inter-renal sensitivity: For example, vitamin E added in the diet has been shown in sea bream to slow down elevation of plasma cortisol levels in response to a stressor and to increase survival rate

(Montero *et al.*, 2001). In African catfish (*Clarias gariepinus*), vitamin C fed during early development induced lower inter-renal gland activity (Merchie *et al.*, 1997).

Although much research has been devoted to stress biology in fish, major questions concern the development of new techniques for non-lethal and non-invasive sampling of physiology and behaviour of fishes which would allow measurement of stress outside a controlled laboratory environment (Scott and Ellis 2007), including meat quality measurements (Skjervold *et al* 1999). Cumulative stress responses at different life stages and methods for evaluating stress in relationship to fish performance have not been much studied.

If stressors and failure to cope persist, the final consequence is death. Mortality rate is therefore a useful welfare indicator as mentioned in Chapter 5. In fish species, there is variation amongst species in the mortality rate in the wild. Amongst salmonids, the egg is large so mortality in alevins and fry is lower than in some species with less food reserve available. When considering the mortality rate, that which occurs in the wild is not directly relevant as farmed fish should be cared for and protected from starvation, predation and avoidable disease. Taking into account the biological functioning of the fish species, mortality rate can give information about the extent of stress and poor welfare.

CONCLUSION

In common with all vertebrates, fish possess a suite of adaptative behavioural and physiological strategies that have evolved to cope with stressors. The systems in mammals and birds that result in the production of adrenaline and cortisol have close anatomical and functional parallels in fish except that the adrenaline and cortisol production are from the more diffuse chromaffin and inter-renal tissue rather than from a discrete adrenal gland. Fish show physiologically and behaviourally similar freeze and flight responses and prolonged cortisol production is associated with immunosuppression.

NEEDS OF FISH

A need is a requirement on the part of an animal to obtain a particular resource or to respond to a particular environmental or bodily stimulus. The exact set of needs for any given species is a consequence of its biology. In general needs are associated with all of the major biological functions of the animal. In aquaculture, the fish experience only a part of the range of natural variation in environmental factors. Some factors may be less variable than in the wild, e.g. food availability, while other factors vary more than in nature, e.g. oxygen concentration. In addition, while fish in nature may swim away from adverse or sub-optimal conditions, the farmed fish spatial and temporal environment, gives few options for individual preference. Nevertheless all farmed animals have needs and good welfare depends upon these being met to a greater or lesser degree.

However, there is variation in the importance of the various needs for the welfare of the individual. Needs range from resources whose absence results in rapid death to those whose presence improves welfare for a period, but lack of which would never result in death

The following list of needs is not in order of importance and reflects current knowledge. Some needs require being satisfied only at intervals of some hours or only when fish are at certain life stage, young or adult. The causes of some problems of fish are multifactorial and may be related to more than one need. The welfare risk assessment refers to hazards that are linked to

the known needs of a particular species. Those hazards or factors have been identified for each species.

1 Need for adequate physical and chemical environmental conditions:

1A. To have access to appropriate oxygen concentration

All fish need oxygen of a certain partial pressure, the actual value varying according to species.

1B. To avoid harmful substances or environmental conditions in water

All fish need an appropriate aquatic environment. Inappropriate water conditions, for example too high salinity or carbon dioxide concentration, too much ammonia or other toxic chemicals, or suboptimal pH can harm fish.

1C. To have appropriate visual, olfactory and other environmental conditions

It may be that problems are caused to fish of particular species by inappropriate light, vibration, chemical stimuli, pressure changes, or electrical changes.

1D. To avoid extreme temperatures

Although fish are poikilotherms, adverse temperature conditions can harm fish for various reasons including impact on oxygen availability and demand, so they need to avoid them if possible. Body temperature modification in most fish, where it can occur at all, is behavioural.

1E. To osmoregulate

Fish need to maintain relative stability in the ionic composition and osmotic strength of their body fluids, for example when exposed to inappropriate salinity.

1F. To have space for movement

Fish require space to carry out various functions, such as food searching, social interactions and responses to threats, and crowding can lead to problems. The fish species vary greatly in what space they need.

2 Need to have appropriate social interactions

Some fish species shoal for much of their lives and good welfare may depend upon such behaviour. Other species are social for part of their lives or for none of their lives. Some fish need to avoid attacks by conspecifics.

3 Need to avoid predation

Many fish living in natural conditions are very vulnerable to predation. The biological functioning fish of most species is strongly adapted to maximise the chance of recognition of danger from predators and escape from it.

4 Need to feed for maintenance and growth

A variety of nutrients are needed by fish. Fish also need to avoid feed containing dietary toxins and anti-nutrients.

5 Need to maintain good health condition

Fish use various behaviours, anatomical adaptations, physiological responses and immune responses to combat pathogens. They need to avoid any physical or chemical impact that causes tissue damage.

RECOMMENDATION

Since there is evidence in fish for the range of abilities and functions associated with learning and cognition and with affective states such as pain and fear, the welfare of fish should be considered during all aspects of their husbandry.

Fish farming in Europe

World aquaculture has significantly increased during the last fifty years from a production of less than a million tonnes in the early 1950s to 59.4 million tonnes by 2004. Consumption of farmed fish is about 45.5 million metric tons whereas around 60 million tons are wild caught fish from both fresh and sea-water. The 70% of the total aquaculture production comes from the Chinese aquaculture, 22% from the Asian and the Pacific region whereas Europe contributed to approximately 4% of world farmed fish production (FAO;

http://www.fao.org/newsroom/en/news/2006/1000383/index.html).

Nevertheless Europe has the largest production of some species like Atlantic salmon, European sea bass and gilthead sea bream. Currently, Norway is the top producer in Europe, with an annual salmon production of more than 580,000 tonnes representing a 41% of increase in the production rate from 1998 to 2003. Other major producing countries of farmed fish in EEA are Spain, France and Italy. United Kingdom and Greece are also centres of fish farming activity and smaller quantities are produced in several other European countries (Table 1).

Table 1**. Finfish aquaculture production in EEA countries in 2005**

Country	2005	% growth 1995 - 2005
Norway	652306	135.30
United Kingdom	143012	64.50
Greece	80136	268.36
Spain	57346	100.74
France	50352	-23.11
Italy	47642	-27.49
Denmark	38732	-13.41
Poland	36607	45.78
Germany	35130	-22.02

Czech Republic	20455	9.51
Ireland	15384	15.68
Finland	14355	-17.24
Hungary	13661	45.95
Netherlands	8675	213.63
Iceland	8246	136.61
Romania	7284	-63.27
Sweden	4805	-20.20
Portugal	4115	137.31
Bulgaria	2971	-31.70
Austria	2420	-17.07
Cyprus	2315	419.06
Lithuania	2013	17.44
Slovenia	1335	72.04
Switzerland	1214	4.57
Belgium	1200	41.84
Slovakia	955	-40.94
Malta	736	-18.58
Estonia	553	75.56
Latvia	542	3.24
Total	1253283	63.29

(Source: Eurostat, 2008)

European finfish aquaculture species comprises a range of teleosts including salmonids like Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) and arctic charr (*Salvelinus alpinus*), sea basses (mainly European sea bass *Dicentrarchus labrax*), sea breams (mainly gilthead sea bream *Sparus aurata*), carps (e.g. common carp, crucian carp, grass carp and silver carp), flatfish like turbot (*Psetta maxima*), halibut (*Hippoglossus hippoglossus*) and sole (*Solea vulgaris vulgaris or Solea solea*), European eel (*Anguilla anguilla*), catfish (*Clarius sp.*) and gadoids like Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) (Table 2).

Table 2. *Yearly production of the main farmed fish species in EEA in tonnes*

Species	1995	2000	2005
Atlantic salmon	347 861	589 606	733 332
Rainbow trout	258 168	286 629	261 805
Gilthead seabream	17 487	58 747	71 475

Common carp	75 000	72 178	69 557
European seabass	17 000	40 869	49 202
European eel	6 819	10 658	8 202
Atlantic cod	317	169	8 115
Turbot	2 978	4 785	6 838
Catfish	1 482	3 640	6 674
Silver carp	8 851	4 909	2 568
Atlantic halibut	-	35	1 445
Grass carp	1 334	1 526	1 090
Arctic charr	531	1 028	905
Haddock			72
Sole	30	23	11

*catfish (Clarius Spp. and Silurius spp)

Source: Eurostat, 2007

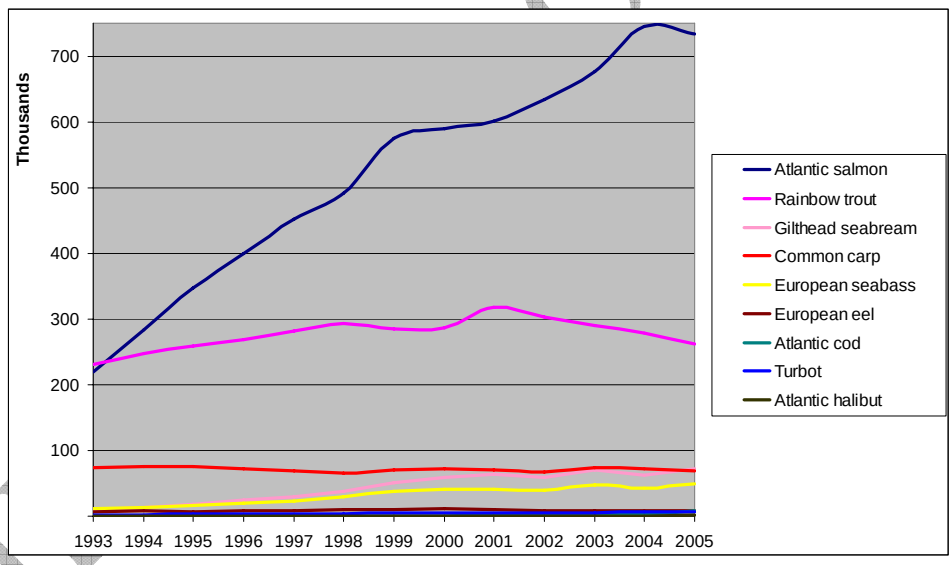

Figure 1: *Yearly production (thousands of tons) of the main farmed finfish species in EEA* (Source: Eurostat, 2007).