Harmonisation of the Care and Use of Fish in Research

Report from an international consensus meeting

23rd – 26th May 2005

Gardermoen, Norway

Introduction:

The purpose of this meeting was to bring together fish researchers and others interested in the care and use of fish in research. A total of 99 delegates from 10 countries participated.

Background for the meeting:
The European organisation FELASA (Federation of European Laboratory Animal Science Associations, http://www.felasa.org) exists to aid collaboration between national and regional organisations promoting the welfare of animals used in research. The theme of the 9th FELASA symposium, held in Nantes in June 2004 was "Internationalisation and harmonisation in laboratory animal care and use issues". There were a number of lectures on the subject of fish as research animals, but it was clear that there is an international need both to exchange existing knowledge on the care and use of these species and to explore areas where our present knowledge is inadequate.

The Laboratory Animal Unit at the Norwegian School of Veterinary Science arranged this meeting to further these aims. A comprehensive website containing this report, all the presentations from the meeting and a list of guidelines and other resources within the use of fish in research, is under development (http://oslovet.veths.no/fish).

A preliminary report from the meeting was presented at the World Congress on the Use of Animals in the Biosciences held in Berlin in August 2005 (http://www.ctw-congress.de/act2005). This full report is available on the website of the Norwegian School of Veterinary Science (http://oslovet.veths.no/fish) and has been circulated to all participants for prior approval.

Acknowledgements:

This report was largely written by Home Office inspectors Kathy Ryder and Ngaire Dennison, U.K., who have also added their own comments to many for the lecture abstracts.

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Summary of the meeting:

- **There is a need for species-specific guidelines that take into account the differences between research disciplines**
  - "Fish" constitute a group of over 30,000 species in a wide diversity of habitats, showing a range of physiological and behavioural traits. Guidelines for the care and use of fish in research must reflect this diversity. Species-specific guidelines must be developed for many aspects of fish welfare.
  - The construction of an overview of existing guidelines is a priority. A website addressing this need has been established since the meeting: http://oslovet.veths.no/dokument.aspx?dokument=148
- **There is a need for easy access to the latest knowledge**
  - The participants of the meeting expressed their gratitude for the opportunity to come together and exchange the latest knowledge on, among other topics:
    - Best practice for the care and use of fish in research
      - Comparisons to other laboratory animals
    - Health and welfare monitoring
      - Definitions of fish welfare
    - Good Clinical Practice (GCP)
    - The research possibilities and limitations for different fish species and strains
    - The ability of fish to feel pain
    - Knowledge of the environmental needs
      - Enrichment possibilities and limitations
      - Optimal temperature, photoperiod and other factors
    - How to apply the 3R's in fish research
      - Better design of fish models to reduce the number of fish and their suffering
      - Refinement of endpoints
      - Anaesthesia and humane killing
      - Alternative methods to the use of live fish
      - Relevance of fish research
  - Annual meetings of this kind combined with a website could provide a means of updating researchers more effectively.
    - There are many laws, regulations and guidelines for the care and use of fish in research. These are constantly changing, and this creates a need for an updated overview.
    - A closed discussion group on the Internet, especially for those working directly with research fish, would provide an opportunity to exchange practical experience and help to solve acute problems.
- **There is a need for more standardised models for fish research**
  - Variation in, among other things, the genotype and health status of fish used in research is one of the main reasons for difficulties in providing fish models

with repeatable results. This is also one of the main causes of the large numbers of fish used in research.

- Standardisation of health and welfare monitoring
  - Currently, the monitoring of health and welfare is very dependent upon visual inspections, growth rates and mortality rates.
  - Knowledge of the microbiological status of the fish is often limited.
  - Barriers and procedures to prevent infectious agents gaining entry to the system, such as water treatment, quarantine of fish and the avoidance of live feed are needed.

- Standardisation of the care and housing of fish
  - Sedation, anaesthesia, analgesia, surgery and slaughter methods require improvement in order to minimise their impact on fish. Pre-anaesthesia sedation and the use of local anaesthetic at the site of surgery appear to be successful in reducing adverse physiological effects. For example, the use of eugenol post-vaccination has been shown to improve appetite.

- Standardisation of water quality, light regimes, temperature and other environmental factors.

- Standardisation of sampling techniques and procedures.
  - Variations in blood sampling procedures, in particular, are known to affect research results drastically
  - Identification of the best methods for individual identification of fish in a range of research situations is needed, both for field studies and laboratory trials

- Laboratory experiments must provide results that can be extrapolated to farm conditions, when this is the aim of the study
  - Fish vaccine trials may show good results in laboratory studies, but may cause side-effects in field studies

➢ **There is a need for more knowledge and debate on the ability of fish to feel pain**

- All those involved in fish research, from technicians to scientific staff, should be updated on the latest knowledge within this field

- Research will never be able to provide exact data on how a fish feels, and the debate on this issue will therefore always exist. A forum for debate, in the form of scientific meetings or via the Internet, is therefore needed.

- The precautionary principle that it is likely that fish have the ability to feel pain should be applied and studies should be designed accordingly. While some understanding of the response of fish to noxious stimuli exists, little is known about the effects of, for example, chronic disease or poor husbandry in fish.

➢ **There is a need for harmonisation of reporting systems**

- The ways in which the numbers of experimental fish are reported vary greatly between countries, despite the attempts to harmonise this under the European Convention ETS 123. The presentation of such statistics is politically sensitive. Consistency in the definition of what constitutes an experimental animal is required. This will make it easier to compare the use of fish between countries, and it will provide a better platform for developing appropriate welfare standards.

- **There is a need for implementation of the 3R's in regulations that make it mandatory to use live fish**
  - Regulations for the testing of, for example, fish vaccines and toxins do not in many cases allow any refinement of the method or reduction of the number of fish.

Summary of presentations

Monday 23rd May:

The challenges of refining methods used in fish research

Magnor Nerheim, Norwegian Ministry of Fisheries and Coastal Affairs

Broad discussion of the position/ need for research within Norway from a member of the Fisheries Ministry. Norwegian Government supports this meeting and is very interested in knowing the outcomes.

Fish amount to 94% of all research animals in Norway. It is seen as an obligation in Norway to increase food production via aquaculture and therefore that relevant research is required. Acknowledge this will cause some animal suffering, but that this should be minimised.

There is a very large aquaculture industry in the country, mainly Atlantic salmon and rainbow trout. The rising global demand for seafood is beyond the capacity of fisheries, so that number of species kept for aquaculture is increasing and includes cod, halibut and catfish: reflected in research being performed.

Research is therefore required in the areas of health and welfare (essential for successful farming), environmental influences on fish and optimisation of cost-effective production.

There are difficulties with research on health in fish because of the huge range of species such that results from one species may not be relevant (or may even be totally misleading) for other species. The importance of vaccination to maintain health within aquaculture was stressed.

Refinement of endpoints is required, away from current use of death for the endpoint, so that animal suffering may be reduced.

High ethical standards should be applied, with the importance of attitudes and awareness and the need for alternatives stressed.

Reporting fish experiments: Are national statistics adequate?

Bjørn Groven, Norwegian Animal Research Authority (NARA)

Brief description given of the Norwegian system of regulation via NARA and the fact that recent changes in Government structure mean that the system should be refined to be more effective. The reporting of animal numbers used needs to be improved and particular attention is needed in improving the definition of what constitutes an experimental animal.

NARA regulates work at approved facilities and also “field” studies. Speaker considers that the recent reorganisation of the various Ministries has decreased the effectiveness of the functioning of NARA and that a better system is required, with emphasis on alternatives to animal use and reduction in animal numbers, which requires the collaboration of all parties involved within fish research. The evaluation of whether there is an alternative to the use of animals needs to be more rigorous and it is essential to have competence within care and use for the fish that are used.

National statistics for the use of animals in research vary between EC states because of the differences in the National laws. The requirements of Appendix B of ETS123 have harmonised the basic reporting of the 16 countries that have ratified the treaty, and the harmonisation of reporting will be further improved with 4 more countries also signed up to ETS123.

Norway use this reporting system, applied to all free-living/ mature stage fish, but in addition have 2 additional tables, one for “non-experimental” animal use and one for “painful procedures” on animals.

Animals do not need to be reported as experimental where any of the following exemptions apply:

1. recognised clinical procedures
2. simple identification methods, blood sampling, etc. not affecting the animals normal way of life or causing only temporary pain/ distress
3. breeding and rearing (for a scientific purpose)
4. experiments that do not produce an abnormal physiological state (most feeding and environmental studies)
5. fertilised eggs

Animals are reported in the “non-experimental” category if they fall into categories 2 and 4 above. Where the animals are killed without any prior treatment (for research or education and training purposes) and where they have been bred for scientific use but have been surplus or used for certain other purposes, e.g. health screening, these animals are also counted as "non-experimental". Fish make up 98% of all non-experimental animals counted (1.7 million). Of these numbers, 122,556 were used in category 2 (minor procedures) and 1.14 million in category 4 (no abnormal physiological state induced).

In terms of experimental animals in Norway, fish make up 94.5% of total numbers (i.e. 800,000 fish compared to a total of 27,000 for all other species). By comparison, the annual losses in aquaculture run at 34.77 million fish, and over a third of these (just over 15 million) of these die from disease – i.e. with significant potential suffering to the animal.

A number of problems are seen with reporting of animal numbers, the most significant of which are reporting animals at the wrong time (the year the experiment ended rather than when it began), not counting control animals, reporting the numbers based on the results (numbers completing an experiment) rather than the numbers actually used and counting animals from what should be non-experimental categories. To improve the reporting of numbers used, it is essential to improve the definitions of experimental and non-experimental animals.

Discussion/ questions: It was identified that the Swedish system has a simpler definition of an experimental animal – any animal used for a study where the **aim** is research is counted (even where the work is observation) – this caused a massive increase in experimental animal number the year it came in, as numbers include e.g. experimental trawling, with thousands of fish involved per trawl. Some Norwegian members of the audience expressed concern about tagging/ blood sampling/ anaesthesia (non-experimental procedures), as there is not the same requirement for training of personnel for non-experimental compared with experimental work. They felt this raised ethical concerns.

Note added by UK Home Office delegates: The UK system counts any fish from free feeding stage or vertebrate from halfway through gestation, which has commenced a procedure which may cause pain, suffering, distress or lasting harm. Many feed trials are not counted (or regulated) and observational studies are also not counted. Controls are not counted if they do not suffer. Blood sampling does cross the threshold of pain and therefore need to be counted. Vertebrates that are marked using instantaneous methods are also not counted, but this rarely occurs in fish since anaesthesia is very often used. All fish marked for scientific purposes are counted, but those marked for husbandry are not. It is sometimes difficult to distinguish between these categories. UK statistics for use of animals in scientific procedures can be found at: http://www.homeoffice.gov.uk/docs/animalstats.html

A summary of the reporting system in Norway revealing many weak points has been published after the meeting http://oslovet.veths.no/analysisfish2003.pdf.

Experiences from inspection of fish research facilities in the UK

Kathy Ryder, Home Office Inspectorate

Brief discussion of the annual numbers and the purpose of use of experimental fish in the UK. The range of types of experimental facility and issues relating to assessment of such facility as "fit for purpose" were highlighted. Improvements in environments will be needed in the future, with an emphasis on the physiological and behavioural needs and requirements of the fish.

Slow but increasing upward trend in the numbers of fish used in research in the UK. There is a noticeable bias in the use of fish from some types of work – fish make up 12% of all animals used in the UK in regulatory toxicology work, but only 6% of the overall numbers. Improvements in environmental complexity are now considered the norm in other species, but progress in this area in fish facilities has been slow. The range of facilities means that it may be difficult to apply improvements directly from one to another, but consideration in how enrichment can be provided should be tackled, using evaluation systems locally. It is recognised that there may be problems in the use of enrichment for certain types of study and tank systems – but this should not prevent attempts in addressing the issue of environmental complexity for improving welfare.

What can we learn from established health monitoring practice for other species?

Jeffrey Needham, The Microbiology Laboratories

The principles of mammalian health monitoring can be applied to fish in order to raise health standards, but there are specific factors associated with fish that need to be considered.

Speaker considers that, in terms of health monitoring practices, the fish research community is where the rodent research community was 40 years ago. Health control in its simplest form is the type of situation seen in farm animals where there are border controls enforced by Governments that allow freedom from certain named diseases. Health monitoring/ surveillance by comparison is more complex. It was stressed that health surveillance is not "quality control". Performed correctly health monitoring ensures:

- standardisation of animal supply
- standardisation of experiments and therefore results
- decreased animal suffering caused by disease
- decreased animal numbers used, decreased experimental cost (fewer failed studies)
- saves time (fewer failed studies)
- protects animals from human infections
- protects people from zoonoses
- decreases cross-infection between animals

FELASA guidelines for health monitoring for other species have standardised frequency of testing (every 3 months), number of animals examined, what microorganisms are sought and how results are presented. The issues of sample size, which animals to test, the methods available (including their accuracy, sensitivity and shortcomings) and frequency of testing, as they pertain to the fish situation, now need to be considered to move health monitoring forward for fish species. The "microbiological entity" (the population of fish in a system which have the same exposure to pathogens e.g. water system) needs to be clearly thought out in order that sampling and isolation systems can be devised. The aquatic medium, with an increased ease of pathogen spread between fish compared to land-organisms, the large number of potential pathogens that may need to be sought, the (currently unregulated) supply of fish and housing to minimise the risk of cross-infection are all need to be specifically addressed. The range of species and huge size variation may raise particular challenges.

Note from UK: Ian Bricknell has agreed to create a list of pathogens for some species (probably salmonids and Danios) which could feasibly be screened for.

Health monitoring of fish used in research

Renate Johansen, Norwegian School of Veterinary Science

The speaker indicated a number of concerns with the current methods of selection of fish for experimental use. She indicated that there is a prevailing opinion that, once the desired results are obtained, the experiment shouldn't be repeated or it will come up with a different answer. This tends to lead to very large trials with a lack of comparability between trials. The importance of the standardisation of fish models was stressed. The choice of fish for studies needs to be better informed and the experimenters need to be "fussier" in their selection. The effects of subclinical disease on results are largely unknown and research and guidelines in this area are desperately required.

There is a big difference between "are you healthy?" and "are you sick?" – these are not the same question and a fish that looks OK is not necessarily healthy. Current practice is to check that fish aren't sick prior to commencing research, but often little/ nothing is known about health status/ previous history. The importance of the interaction between the fish and its environment means that it is not possible to concentrate purely on simply which pathogens are present. Water quality, immune status, nutritional state, virulence of the pathogen, "stressors" and intercurrent disease (infectious or non-infectious) are just some of the factors that may influence the expression and incidence of disease when pathogens are present. Knowledge of all these factors is essential. There are problems with gaps in our knowledge; e.g. source and life cycle of pathogens and how environmental factors influence pathogenesis.

Currently used monitoring methods tend to use gross clinical signs (e.g. behavioural changes, loss of appetite), mortality rate, age, size and growth rate/ efficiency. Gross post mortem, with tests for pathogen presence may be carried out on some fish during or at the end of studies – but a large number of types of examination used in other species (faecal examination, haematology, etc.) are often not used. One of the reasons for this may be that it is not known in many cases what is normal, so that it is hard/ impossible to know what is abnormal.
For selection of fish, specific information is required such as, the relevance of the species to the question under investigation. The presence of any pathogens may also be important but - it can be difficult to detect infection in subclinically infected fish (limitation of detection methods), prevalence of disease can be low (large numbers are required to determine prevalence) and it may not be known (and may not be predictable) how the disease may affect results. Serious consideration therefore should be given as to whether specific pathogen free (SPF) fish should be used on some, or even in all, studies. In addition, the relevance of the choice of the individual fish is vital (is the best animal the smallest, the largest, an average size?). An example of this cited was the incidence of epicarditis in Halibut, where the highest growth rate fish had the highest presence of the disease (45-90%) compared with only 10% of small fish showing evidence of the disease. Thus, taking just one size out of a group may not be representative of the group as a whole. Another example is the heart in farmed fish is more variable in shape compared to wild fish and therefore is likely to be poorer in terms of mechanical performance – but what is normal for the farmed fish - are they "good performers" or are they abnormally overweight? The criteria of selection, why these were chosen and, if possible, how this may effect the result, should be identified prior to a study commencing. Guidelines are needed for each species for health monitoring and probably also for each type of research. Speaker is working towards 2 papers on health monitoring that may help inform such guidelines.

Note Added by Home Office Delegates UK: The lifetime history of criteria such as disease, growth, food-conversion rate may be more important than a snapshot.

Environmental monitoring in fish experiments

Trond Rosten, Norwegian Institute for Water Research

Keeping fish in tanks is equivalent to keeping people in a respirometer – the experimenter controls all the variables (inputs and outputs). A better understanding of the water environment and its effects on the fish is therefore required by scientists, with improvements in how and what is monitored needed.

The field of monitoring of the water environment is a very large one. Consideration should be given to the basic needs of fish – they need to be able to breathe, swim, eat, "escape"/ hide and maintain homeostasis. By comparison, the needs of the researchers are for valid experimental conditions, large (statistically relevant) numbers per group, the ability to monitor/ measure the parameters of an individual compared to the "herd", to be able to manipulate the environment and to keep within budget! Often insufficient money is allocated to environmental monitoring – the importance of this aspect of fish studies needs to be realised and budgeted for.
A particular issue relates to work that is trying to mimic the conditions seen within the aquaculture industry. There is a lack of scientific data on a relevant scale: these are large ($100m^3$ +), dynamic, environments compared to the controlled experimental environment of small units.
Many of the commonly used fish have "environmental timing" (born with limited hypo-osmotic regulation ability for seawater and develop seasonally good osmotic regulation to allow move to a sea environment), therefore effects of aspects of the environment such as salinity depend on time in life cycle. This is one of the factors that makes toxicity of compounds in combination and individually and their relevance in water extremely complex.
The speaker believes that all labs should monitor at least to the level/ parameters set as standard in commercial farming (16 parameters in raw water, with additional monitoring of operational and fish tank water). Aluminium was given as an example of a toxic solute, which may appear in raw water under certain environmental conditions (heavy rain). Knowledge is needed on how water quality influences biological effects – for example whether the parameter gives an "optimum" with deficiency and toxicity states (as for oxygen for level of swimming performance compared to oxygen saturation) or whether performance is stable until a toxic state is reached (e.g. effects on health of CO_2 and pH). The picture may be complicated by interactions between parameters, for example if pH drops due to an increase in CO_2, tolerance of ammonia is increased because it is shifted to a less toxic ionic form, ammonium. The density and therefore "joint metabolic load" needs to be considered. A number of systems are available to provide water-monitoring packages relevant for different situations. The most

commonly used standard for water quality evaluation in Norway is the VK-programme described by Rosseland *et al*,. (2004). More information about monitoring water quality can be found at http://www.niva.no/vk2005.

Reference:

Rosseland, B., Rosten, T., Salbu, B., Kristensen, T., Maroni, K., Baeverfjord,. G. Water quality and its effect on aquaculture operations. Abstract and presentation at the PROFET (Q5AM-2002-0256) conference in Dublin, 16th-17th April 2004.

Note from Home Office UK: In the UK, the FAWC Report on the Welfare of Farmed Fish (September 1996) sets commercial "standards", but it gives no parameters other than oxygen and no levels are described. It is a very general document.

Neurophysiology in fish in relation to pain

Anne Sverdrup, ILAB, Bergen

The speaker stated that current literature is contradictory as to whether fish feel pain or not. Fish possess a spinothalamic tract, although the pathways from this to the telencephalon differ from that of humans. Transmitter peptides found in the pain system in humans, such as glutamate and substance P, exist in fish and recent work by *Sneddon et al* shows that morphine acts on such receptors and can alter the behaviour to noxious stimuli. The structures present in fish species studied to date would appear to indicate the potential for perception of pain, but, if fish do feel pain, it is not in the same way as mammals and more behavioural research is needed.

Fish have a developed nocioception system that transmits information from the periphery to the thalamus and onwards to the telencephalon. However, the presence of a functioning nocioception system does not itself infer an ability to detect pain, as this system functions to identify damaging stimuli, but does not interpret the stimulus as painful. Therefore the question is one of whether the telencephalon is sufficiently developed to interpret the information as painful. Pain is a subjective experience and is complicated in human subjects by the possibility of the presence of painful stimuli without pain and the presence of pain with no apparent painful stimulus. The study of pain is therefore very difficult in animals and even more so in fish where there is no understanding of the possible emotional aspects of painful stimuli.

There is a split in the literature as to whether fish can feel pain and no work has been done to date that looks at both sides of the nocioceptive response in fish, i.e. the path from the nocioceptor to dorsal horn has been studied, but not from the dorsal horn up the spine. The neural system of fish is not constant between species. Species specific adaptations include an increased taste nucleus in goldfish and special electric sensors and an electromotor nucleus to allow sensing of movement at great distances in sharks/ rays. Species specific adaptations may be additional variables that have to be considered when considering the response to nocioception.

Human pain responses are under segmental control to allow inhibition of spinal responses ("gate" control) – this system has not been documented in fish and is difficult to study in species that are not able to express themselves.

How should we conduct fish research in the light of our current knowledge of pain perception?

Victoria Braithwaite, University of Edinburgh

Current knowledge would appear to indicate that fish possess the structures required for nocioception and that at least some species possess reasonable cognitive function, which suggests the potential capability for pain perception. Handling and other stressful procedures adversely affect behaviour and consideration should be given to the use of analgesia for invasive procedures. Many fish possess unique sensory systems that we don't understand and we have no idea how what we do in the lab impacts on these impressive additional senses. It is important that we develop methods to identify how aversive or important different factors are to fish and devise ways to measure cognitive state, but the huge diversity of the taxonomic group may hamper applicability of the results.

This presentation was broken down into 3 questions:

1. What do we know?

Nocioception:

-there is a nocioceptive system in fish, at least round the mouth, with equivalent response to noxious stimuli as is seen in mammals.

-Aδ and C fibres are present

-responses to stress are seen with classic primary and secondary phases

Cognitive processing:

The attentional state of trout treated with a noxious stimulus has been investigated and animals subjected to this had significantly decreased neophobia compared to controls, which was eliminated with morphine treatment. This is proposed to be an "attention deficit" that is caused by pain, which is reversed by analgesia and is similar to responses seen in mammals. This suggests reasonable cognitive function.

2. What are the implications of what we know?

-*effects of handling* are clearly stressful to fish, based on measurement of physical parameters and the observation from aquaculture that fish do less well if they are handled frequently, which has lead to recognised good practice for handling being adopted in industry. Frequent handling in the lab could therefore affect the background behaviour of fish if, for example, transfers between tanks are performed poorly. Work done in the speakers lab suggests an improved performance with total "in water" transfer (fish encouraged into a submerged cup that is then moved to the receiving tank) compared to the use of a net and that fish will "choose" the former transfer method.

-*marking/ tagging*; these involve handling and are typically performed out of water, as well as being invasive as techniques in themselves. Should we therefore routinely consider the use of analgesia for such procedures, especially when rodent work shows that animals recover quicker from equivalent methods when they receive analgesia.

-*social interactions;* these need to be considered on a species by species basis – for example behavioural work in guppies held on their own is likely to be meaningless as the biggest drive to their behaviour in this situation is the search for conspecifics. There is an impact on behaviour where animals are wild caught as opposed to captive bred in relation to adaptation to new environments.

-*enrichment*: there is evidence in cod that they learn faster, are less timid and have fewer aggressive interactions with tank-mates in enriched rather than barren environments. The tank enrichment should be considered relative to each study – a bare tank may be appropriate for ecotoxicology work, but if the fish are to be released for restocking there may be a need to raise them in an enriched environment to improve their chances in the wild.

3. What do we need to find out?

-better understanding of the unique *sensory systems* of fish is needed to understand the impact of the laboratory situation.

-a better understanding of *anaesthesia* is needed; what is best for each species depending on the purpose of the study.

-knowledge is needed as to *what parts of the body* (in addition to the mouth) *are sensitive to noxious stimuli*; for example, what are the consequences of fin erosion and do fish suffer from pain to chronic damage?

-*behavioural syndromes/ personality traits* need further investigation – fish can be screened for a number of traits (e.g. bold versus timid) that tend to be consistent across tests.

-quantification on how aversive or important different factors are is needed, e.g. by demand studies and work on cognitive states.

Questions/ discussion: General agreement of those present that the presence of nocioception in fish has been proved. Noxious stimuli give a response via adrenaline that should be minimised wherever possible, since this has physiological consequences even if suffering is not present. The consensus was that fish should be treated as if they suffer pain, even without definite evidence that they do.

<u>Tuesday 24th May:</u>

How do we run an accredited laboratory animal facility for mammals? What can we learn from each other?

Adrian Smith, Norwegian School of Veterinary Science

AAALAC accreditation is a recognition that quality control methods are in place in a facility. The accreditation process provides a framework that considers the policies for the care and use of animals, their housing and environment, management and veterinary care and the establishment's physical facilities. A Master plan allows organisational planning and project management. It requires risk assessments to have been performed and disaster planning to be in place. Proof of competence of staff by CV and training records is required. Reappraisal of procedures is ongoing which helps ensure the evolution of practices over time to ensure they remain current. Sound solutions to potential problems are a key part of the system. Such ideas are highly relevant to fish research as part of an integrated approach to improving working practices and ensuring the consideration of the use of alternative and improved methods.

An inflatable cushion which could be used for supporting fish during surgery out of water was described: Brattelid T. & Smith A.J. (2000): Methods of positioning fish for surgery or other procedures out of water. Laboratory Animals 34: 430-433.

Canadian guidelines for the care and use of fish in research, teaching and testing

Gilly Griffin, Canadian Council on Animal Care

The Canadian system for regulating the care and use of animals in research is highly devolved and therefore comprehensive guidelines are required by the local animal care and use committees. The guidelines developed are evidence based whenever possible, involve a high level of expert and peer input and include sections on facilities, capture, transport, husbandry, procedures, health, euthanasia and disposal and also the current societal concerns. These guidelines are aimed at scientists, vets, animal carers and the animal care committees that are involved in fish research in the Canadian setting, although it is hoped that they will have a broader relevance.

Note added by Home Office delegates UK: these are excellent guidelines and will be of more practical help to those setting up and running systems than those likely to come from the European revision of Appendix A. However, the two different roles of the documents should be clear. The Canadian Guidelines lay out best practice, whereas the European Appendix A lays down minimum standards. The latter are required for enforcement, the former are better for encouragement.

The American Fisheries Society Guidelines for the Use of Fishes in Research was referenced:

http://www.fisheries.org/html/Public_Affairs/Sound_Science/Guidelines2004.shtml

The CCAC guidelines for fish have been published after the meeting: http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GDLINES/Fish/Fish%20Guidelines%20English.pdf

Good Clinical Practice (GCP): a tool to refine fish research

Hanne Bergendahl, Norwegian Medicines Agency

GCP is not as well incorporated into veterinary clinical trials compared to those done in humans, although all clinical field trails should be performed to GCP standards. The design, execution and evaluation of protocols are key factors for the assessors. In many cases there could be significant improvements in the results produced by improved study design and better consultation with the regulators to ensure the right questions are addressed.

Frequent mistakes seen include lack of appropriate lab or semi-field studies prior to large field trials and insufficient information provided in protocols or discrepancies between protocols and reports. Study designs are frequently flawed. Such flaws include incorporating too many variables within one

trial, so that results are difficult to interpret, or taking inadequate numbers of types of samples (e.g. only testing 5-10 fish out of 10,000 for the presence of sealice in a treatment trial or testing the duration of immunity post-vaccination for only 2-3 weeks rather than for a prolonged period). Such designs are not suitable for purpose so the regulators can not use the data and this is expensive and "wastes" the fish used. The opportunity to follow up outstanding questions when the fish stocks used are still available is also frequently not used.
The following references were given: http://www.emea.eu.int/ and http://vich.eudra.org/

Anaesthesia and humane killing

Tor Einar Horsberg, Norwegian School of Veterinary Science

It is important to identify the purpose of the use of anaesthesia. Such chemical restraint may be required for immobilisation (e.g. weighing, grading, artificial spawning) or to provide relief from pain (e.g. cannulations). The different drugs commonly referred to as anaesthetics do not all provide equivalent levels of analgesia, sedation and immobilisation and this must be considered when choosing an agent. Important considerations include the species (influences behaviour when the agent is applied, the gill surface area to body weight ratio and metabolism), the animal's size, the nutritional status, environmental parameters (e.g. temperature, pH, oxygen saturation and water pressure) and duration of application.
Handling of fish should be done with wet latex not rough rubber gloves (or wet hands). A wet chamois leather can help in retaining moisture. Constant temperature can be maintained by irrigation of the skin. Fish should be protected from sound and light.
Common methods of humane killing are destruction of the brain by stunning, followed by spinal severance or overdose of anaesthetic for large numbers of fish. For slaughter CO_2 gas (with or without chilling) or electroanaesthesia followed by bleeding are commonly used, but the use of CO_2 in particular may have welfare issues.

"Inhalation" agents (agents applied by immersion):

In Norway, 90-95% of anaesthesia is using the "-aines" (tricaine, metacaine, benzocaine) which work by blocking sodium channels therefore stabilise excitable membranes. It is assumed, at least in salmonids, that this mechanism of action provides an analgesic effect. Different dose rates of these agents can be used to provide sedation for transport, short-term anaesthesia and long term anaesthesia. The margin of safety for salmonids is very good with these agents, but is very narrow for flat fish and marine white fish. The side effects seen with these drugs are mainly related to the hypoxic state they induce and include a decreased respiratory rate, increased haematocrit and increased cortisol, glucose, lactate and anions. These can be minimised by the provision if well oxygenated water. The provision of emergency first aid is possible if fish stop respiring under anaesthesia by flushing water through the mouth/ gills via a mouthpiece and by the use of cardiac compression (max. 10 compressions/ minute).
Metomidate/ etomidate are hypnotic agents (only produce sleep) and therefore should be combined with local anaesthetic/ analgesia for invasive procedures. These drugs have limited effects on heart rate and respiratory rate and therefore tend to be safe. They have a good margin of safety in marine species. They are not licensed for use in food-producing animals.
Carbon dioxide is a general anaesthesia and is currently the only anaesthetic agent that may be used during euthanasia of food fish. There are concerns about its use due to the excitation/ panic-like state it induces on application.
Isoeugenol (Aqui-S) is a clove oil derivative. It causes sedation and anaesthesia and is used for handling procedures and slaughter in New Zealand and Australia. It has no food animal licence in Europe yet, but it is hoped that this may be achieved soon.

Injectable agents:

Used infrequently. Two examples are "Saffan" (alphaxalone and alphadolone), which may be given intraperitoneally, and ketamine, which is given intramuscularly. The onset of action of Saffan is around 15 minutes after injection and duration of action around 2 hours. It increases the respiratory rate.
Eels are very difficult to anaesthetise.

References:

1. Smith, L.S., Bell, G.R. (1964). A technique for prolonged blood sampling in free-swimming

salmon. J. Fish. Res. Bd. Canada 21: 711 - 717.
2. Brown, L.A. (1987). Recirculation anaesthesia for laboratory fish. Lab. Anim. 21: 210 - 215.
3. McFarland, W.N. (1959). A study of the effects of anesthetics on the behavior and physiology of fishes. Pub. Inst. Marine Sci. 6: 23 - 55.
4. Horsberg, T.E. (1994): Experimental methods for pharmacokinetic studies in salmonids. Ann. Rev. Fish Dis. 4: 345 - 358.

The use of anaesthetics and analgesia to implement the 3R's: practical examples

Anders Kiessling, Norwegian University of Life Sciences

Reduction of animal numbers can be achieved by being able to sample the same individual on a number of occasions rather than destructive sampling of a series of individuals. However, the subset of animals chosen in such a design must be representative of the whole group. It is know that there are genetic traits can interact with the environment. Specific interactions can only be investigated by experiments where there is follow-up of individuals. The use of better anaesthesia/ analgesia protocols may improve the speed of recovery after interventions. Data show three times faster recovery in salmon to normal feeding after vaccination by pre anaesthesia sedation using 0.5 ppm metomidate treatment for 15 min (i.e. only loss of reaction to ocular stimuli, but maintained swimming ability etc).

Repeat blood sampling is possible in the same fish, but there are issues relating to repeat caudal vessel sampling because of the risk of sampling from the artery rather than the vein and haematoma formation. Cannulation of blood vessels is one potential refinement to allow repeat sampling and to strengthen experimental design by allowing use of animals as their own control, such that the variability that is otherwise often seen in fish in terms of physiological parameters is minimised. Such procedures require invasive surgery and the speaker's grouphave investigated the use of pre-anaesthetic sedation, as there is evidence that benzocaine and related drugs are irritant, create a stress response after administration and reduce food intake for a period, particularly if combined with procedures such as vaccination. A paper is in preparation, which identifies optimal sedative/ anaesthesia combinations. The use of sedation appears to reduce the anaesthetic dose required and may decrease the physiological effects of potentially painful procedures. The use of local anaesthesia at the site of surgery to reduce nocioceptive output from the area is recommended. Irritation related to skin sutures has been noted and methods used by this group take steps to avoid skin sutures where possible. Work with non-steroidal anti-inflammatory drugs (NSAIDs) is ongoing, but the evidence of their effectiveness or otherwise is not yet conclusive.

Species-specific provision for fish in the European Convention for the protection of animals used for experimental and scientific purposes

Rod Wootten, Institute of Aquaculture, University of Stirling

Species-specific provisions for fish have been drafted as part of Appendix A of this Convention. They are guidelines and give general principles rather than being proscriptive, in part because so much is currently unknown about specific requirements for fish. More detailed information on commonly used species will be found in part B of the document. Recommendations include using stocking densities that allow for normal swimming and prevent adverse behaviour and the use of the least invasive marking methods possible. Environmental enrichment should be considered, but it must take into account the species behavioural needs, should be of a size and shape to allow proper swimming behaviour and must not interfere with water quality.

http://www.coe.int/t/e/legal_affairs/legal_co-operation/biological_safety%2C_use_of_animals/laboratory_animals/GT%20123%20%282004%29%201%20E%20Appendix%20A%20final%20for%20adoption%20DRAFT2.asp#TopOfPage

Laws, regulations and guidelines associated with fish research in Norway

Renate Johansen, Norwegian School of Veterinary Science

The Norwegian Reference Centre for Laboratory Animal Science and Alternatives is currently working on an online compendium of laboratory fish science. A chapter of this book brings together the regulations and guidelines that apply to the use of fish in research in Norway. It is hoped that, as an outcome from this meeting, the guidelines on health monitoring and welfare will be updated and extended.

Vaccine side-effects: an example of the conflict between guidelines and real life

Trygve Poppe, Norwegian School of Veterinary Science

Vaccination has helped to reduce the use of antibiotics within the fish industry and is now required within modern aquaculture systems in order to reduce deaths from disease. Vaccination prevents acute disease in most fish, but can cause chronic adverse effects that appear at a frequency which would be unacceptable in other species. This situation needs to be improved.

All salmonids in Norway are vaccinated against up to 5 pathogens (see below). There is a strong relationship between the effectiveness of the vaccines and the incidence of adverse effects, both being related to oil adjuvants. There is a potential ethical dilemma that vaccination saves the majority of fish from the suffering of acute disease, but that the side effects seen may cause long term chronic illness/ suffering in some individuals. The types of adverse effects seen include generalised granulomatous peritonitis, adhesions and melanisation of the peritoneal cavity. Anorexia and decreased growth rate is sometimes seen related to these pathologies and it is therefore likely that the fish are suffering or experiencing pain due to the lesions. Intermittently major adverse incidents related to vaccines have been seen with vaccines from all producers. The magnitude of the problem is unknown as the situation is usually resolved between the manufacturer and farmer and so it is under-reported. Reasons given for problems include small fish size, high temperatures, fish being too close to smoltification, low water quality and problems with individual vaccine batches. After more than 12 years the situation seems no closer to resolution and the speaker questioned whether this was acceptable.

Discussion:

A number of interesting points were raised during discussions. It was pointed out that animals that have received very powerful adjuvants (such as oily adjuvants) don't have normal immunological responses in further studies. A representative from one of the vaccine companies countered that there was a strong correlation between side effects and effectiveness and that vaccines had improved, with reduced side effects in the last 3-4 years. A paper has been produced (but is only published in Swedish) that shows that fish given eugenol by immersion after vaccination will immediately feed, compared to anorexia seen in untreated controls. If the eugenol treatment is removed, those fish revert to an anorexic state. The fact that immunisation, by its nature, a stimulation of the immune system and therefore tends to give inflammatory responses was raised. Most species receive vaccinations via subcutaneous or intramuscular routes, rather than the intraperitoneal route used in fish, with adverse effects related to the route of administration.

All Atlantic salmon in Norway are vaccinated against Furunculosis, Vibriosis and Coldwater vibriosis. Most are also vaccinated against winter ulcers and IPN (Infectious Pancreatic Necrosis). More information on one of the most commonly used vaccines may be found here:
http://www.pharmaq.no/Products/PDFs/ProdInfoSheets/AJ6-2ProdInfoSheet_2004-08-26.pdf For the latest news on vaccine-side-effects in Norway: Koppang EO, Haugarvoll E, Hordvik I, Aune L, Poppe TT (2005)
Vaccine-associated granolomatous inflammation and melanin accumulation in Atlantic salmon, Salmo salar L., white muscle. J Fish Dis 28:13–22.
http://www.aquamedicine.no/artikkel.asp?artikkel=137

Comments from personal discussions afterwards: Ian Bricknell stated that there is not a good correlation between the irritant nature of the vaccine/adjuvant and protection and its persistence. Such adverse effects seem to be much less prevalent in the UK where the use of oily adjuvants is less. Ian Bricknell has published on this subject and could be asked to contribute.

Challenges when designing models for infectious diseases

Lill-Heidi Johansen, Norwegian Institute of Fisheries and Aquaculture Research

The example of IPN was used to discuss the challenges of modelling infectious diseases. Host-pathogen-environmental interactions are very important factors in how/ whether infectious disease is expressed.

IPN challenge was developed primarily as a research tool and then secondarily to test vaccine efficacy. Resistance to the challenge model had increased significantly over recent years. The individual susceptibility (related to species, age, carrier condition, intercurrent diseases and genetics), the organism virulence (organism isolate, load required for infection, load of organism present at challenge, mechanism of virulence, etc.) and the environment ("stressors", sub-optimal conditions) all act together in how a disease is expressed. For example a 10°C drop in water temperature slows onset of disease expression, but increases mortalities. 3.5 hours of exposure has been shown to be optimal by this group. Carrier condition is common, but may be undetectable depending on the titre present. To develop a standard model of disease a lot of factors have to be optimised, including the genetic strain of the fish, the choice of temperature, the immersion time and the pathogen strain. The husbandry pre-challenge can significantly alter IPN disease expression, with reduced mortalities in lower densities and with higher flow rates. A lot of traits that may affect susceptibility have probably not been identified. It has been noted that fish that are suffering from infection have a preference for higher water temperatures and this is believed to be equivalent to the fever response in mammals.

There is published work from FRS Marine Laboratory Aberdeen (T. Bowden *et al.*) on this.

Wednesday 25th May:

Genetic qualities of research fish: availability and limitations

Unni Grimholt, Norwegian School of Veterinary Science

The situation in fish is dissimilar to that in rodents with respect to genetics. Whilst there are well-defined genetic strains in both, commonly used strains of mice tend to have stable a stable genetic pool, whilst, due to the high selection pressures in commercial fish production, the genetics are constantly changing for many species of fish. The use of well-defined, stable and appropriate genotypes has the potential to reduce variability between fish and therefore to reduce the numbers of animals required in studies.

The choice and availability of different genetic strains can significantly alter the outcome of studies, for example fish with high genetic resistance to IPN display cumulative mortality figures of 30% compared to over 70% in low resistance fish. In order to get good reproducibility between studies investigating host-pathogen interactions, a stable host population, a stable pathogen and a stable environment are required. Salmon pose a particular challenge due to the 4-year generational interval. Genetically defined milt can be stored for repeat studies, but the female genotype will generally be altering rapidly with each generation of fish, meaning fish will not be equivalent between studies. This problem has been overcome in zebra fish by the use of inbred and clonal lines, but these fish may not be a relevant model for some studies. Well defined genetic lines of other fish are becoming available, and these may help to significantly reduce animal numbers required for studies by reducing between fish variability, but such fish will not represent the current commercial genotype.

Aquagen is a Norwegian national breeding company.

Choosing the correct number of fish in laboratory and field experiments

Stig Larsen, Norwegian School of Veterinary Science

The use of either too few or too many animals per group in a study is unethical. Proper consideration and optimisation of study design prior to commencing the study, by consideration of issues such as the clinically relevant difference for an effect, the degree of heterogenicity of a population, the necessary detection level and the required significance level (which doesn't have to be 5% in all cases!). The observational methodology (e.g. binomial versus continuous observation) can significantly alter the group size required and should also be optimised. A large number of experimental designs are available such as cross-over, stratified, factorial, Latin square, adaptive and multiple cross-over. The speaker indicated that more complex designs and in particular designs such as sequential and response surface designs are under utilised. Such designs may create uneven group sizes, but target animals most efficiently and are statistically robust.

Note added by Home Office delegates UK: The importance of defining specific objectives for the study needs to be stressed. Training on what constitutes an experimental unit would assist in optimising experimental designs. EASY access to statisticians BEFORE experiments are designed would have significant effects on animal numbers and quality of research.

The 3 R's and behavioural studies in aquaculture: trade-off between possible animal suffering and relevance?

Jon-Erik Juell, Institute of Marine Research, Matre

Behavioural studies related to aquaculture often aim at understanding the preferences or priorities of the animals in order to optimise production and improve welfare, There is a balance between attempting to minimise animal numbers in such studies and properly reflecting the environment being modelled. Fish behaviour is highly dependent on circumstances. Numbers and density may alter social interactions such as aggression, dominance hierarchies, competition and schooling behaviour. Such work is also usually highly species specific. The use of artificial lighting within a commercial cage environment was given as an example of the potential for such behavioural studies.

At night salmon use the surface of the water more, but addition of lights changes the pattern of use, so that more of the depth of the cage is used.

Note added by Home Office delegates UK: It would be interesting to know what wild salmon do with depth, temperature and light to enable better interpretation of what is better for aquaculture animals.

How does industry address the 3 R's?

Kjersti Gravningen, Pharmaq

The European Pharmacopoeia sets out mandatory testing methods for new and existing products via monographs. Studies must be valid, use sufficient numbers of animals to obtain true differences between groups, must incorporate good statistical design and must be reproducible/ repeatable. As lab trial results may not reflect the field situation, both types of study are generally required. There is room for improvement by the regulators in the area of acceptance of in vitro work during product development. There is a need for urgent revision of the guidelines/ standards and methods to allow incorporation by industry of the 3 R's, for example by the revision of endpoints away from mortality, revision of requirement for batch testing despite production to GMP and consistency of results, for less prescriptive requirements for group sizes, and for consideration of science, e.g. required use of route of administration of disease challenge know to produce poorer results than an alternative

Requirement to test each antigen and to challenge with each pathogen seems unnecessary in all circumstances. Objectives should be set and science considered in order determining the best testing strategy. Very large numbers of fish are required for duration of protection assays, which again seem unnecessary except in a few circumstances.

There is a position paper EMEA/CVMP/865/03 which is being considered but has not yet been accepted which may revise these requirements. There is also the possibility that antibody or cell mediated immunity methods may be used (as they are in mammals) but validation is awaited.

Note added by Home Office delegates UK: Discussion with Regulator suggested that moribund fish as end point was acceptable. Validation work on immune testing of vaccine efficacy has begun in the UK, funded by APC. There is no doubt that there is scope here to make the biggest difference to fish suffering in research by negotiating with the competent authorities to make these guidelines a) objective-led and science based b) ethical and c) comply with other European legislation e.g. 86/609.

Alternative techniques in fish toxicity testing

Anders Goksoyr, University of Bergen

Currently, the environmental impact factor (EIF) of a compound is based on 96-hour acute toxicity data identifying the LC50. The relevance of such data can be questioned, as exposure to low doses over long time periods better reflect the actual situation that the environment is exposed to. There are a number of phases of possible responses by an organism to a potential toxin, ranging from normal homeostasis ("health") through compensation ("stressed") to reversible then irreversible disease to death. Identification of biological responses in the earlier phases (biomarkers) would appear to be a more suitable method to investigate EIF and would refine *in vivo* testing by the use of much earlier endpoints. *In vitro* alternatives are becoming available to replace some LC50 testing, e.g. primary cell cultures and reporter cells, but it essential to know and understand the limitations of each system.

The use of vitellogenin as a marker to detect feminisation due to oestrogenic compounds in the environment (e.g. in sewage sludge) was discussed. The use of environmental toxicogenomics and toxicoproteomics (using genomic/ protein profiles to characterise the signatures of toxic compounds in the environment) may also help to identify further biomarkers. Developments of such systems are ongoing via initiatives such as Easyring (**E**nvironmental **A**gent **S**usceptibilit**y** Assessment utilising existing and novel biomarkers as **R**apid Non-**in**vasive Testin**g** methods). *In vitro* techniques can be used to detect levels of compounds, but have the limitation that they can't identify how the response will be ranked in vivo, can't identify pro-compounds, can't mimic physiological responses, e.g. endocrine responses, and don't detect population effects such as transfer to offspring.

Note added by Home Office delegates UK: there has been a recent revision to the OSPAR guidelines, which reduces and refines fish use in acute toxicity testing, by using a staged approach with invertebrates, followed by a limit test in most cases. We as regulators need to know from the experts, when the in vitro tests are NOT applicable as well as when they are; in this case, why should in vivo endocrine disruptor tests for oestrogen like substances be allowed, when in vitro tests are available.

The care and use of zebrafish in modern genomic research

Peter Aleström, Norwegian School of Veterinary Science

Danio rerio is being increasingly used in research due to the fact that it has a fully sequenced genome, the ability to use fish of known genetic lines, the short generational time and well-characterised early development. The speaker identified the processes by which his laboratory had upgraded the zebrafish holding system with a recirculation system with 10% water change daily. A daily monitoring form has been adopted for early identification of problems, with weekly monitoring of water quality parameters. There is a need to consider disease introduction via bought in fish and the use of quarantine or chlorine bleaching embryos may help reduce the risks.

The use of Artemia as a live food had caused an outbreak of nematode infection in the fish. Decapsulation of Artemia, if feeding with it was to be continued had been recommended during a workshop at this conference to decrease the risk of parasite transfer. No environmental enrichment was added other than marbles required for breeding. Problems with water quality and algal growth were cited. Fugu and Medaka are likely to be fully sequenced in the near future, and salmon may follow.

Current methods for capture, handling and tagging wild fish

Niels Jepsen, Danish Institute for Fisheries Research

The only truly relevant method to study the behaviour of fish in the wild is by using wild rather than domesticated fish. Methods for studying behaviour include radio- and acoustic tags or passive transponders that allow study of survival, growth, reproduction, migration and interactions. Particular difficulties experienced include avoiding sample bias, the adverse effects related to capture, handling, anaesthesia and tagging and the stress of holding fish to ensure recovery after surgery. Individual species tolerate specific tagging methods differently and this must be considered when choosing techniques. Knowledge transfer between different disciplines within fish research is vital to improve methods.

Adverse effects from capture, tagging and handling include increased mortality and altered behaviour. Anaesthesia itself can cause mortalities, with more problems seen in cold weather, with repeated use and with stressed fish (which has a strong link to specific species). Consideration of where to place incisions should include factors associated with both species (e.g. behaviour that might affect the wound) and individual (e.g. specific anatomy). The speaker does not perform midline incisions, but usually uses a paramedian incision behind the pectoral fin. The speaker does not favour trailing antennae in most cases, as necrosis can be associated with the exit wound, probably due to movement of the antenna. External tags are fine for pelagic fish, but not for species like eels that hide in holes and crevices or fish that move through dense vegetation. The choice of sutures should reflect the individual situation, but monofilament interrupted is commonly used. The speaker has seen no evidence for positive effects from the use of prophylactic antibiotics. Smolts seem particularly stressed by holding after procedures and therefore the speaker usually releases these immediately. Other fish are usually held for around 24 hours to check they have recovered from surgery, based on evidence that cortisol (which peaks at 3 hours post-procedure) has returned to close to normal by 24 hours in most cases. Surgery can be complicated by the stage of sexual maturity of the fish and surgery on egg-bearing females may be difficult.

Main recommendations by the speaker:

- More exchange of experience, also negative
- More interdisciplinary work (Biology/Veterinary Medicine)
- Better options for funding for methodological studies
- Harmonization of guidelines, not rules
- Different regulations for studies of wild fish, with the purpose of species conservation and habitat rehabilitation.

Note added by Home Office delegates UK: Surgery in egg-bearing animals should be avoided unless absolutely necessary. We discussed the use of aseptic principles with the speaker after the presentation. He indicated that, although instruments start clean or sterile there are difficulties maintaining sterility in the field. Recapture rates of fish would appear to indicate no significant sepsis problems post-operatively, however the speaker has noted that a higher number of adverse effects/ infections seem to occur in his hands with the same procedures in fish held in facilities. This may indicate sub-optimal environmental conditions (stress/ higher pathogen load) and closer attention to asepsis may be required in a captive situation. Generally there is a need for studies documenting any effect of the degree of cleanliness of surgical procedures in fish.

Ethical slaughter of fish: practices from large-scale production of Atlantic salmon. Past, present and future methods

Ulf Erikson, SINTEF Fisheries and Aquaculture

In order to produce fish products of the best quality for eating, it is essential that fish are not stressed prior to slaughter. Harvesting regimes are therefore important for both welfare of animals and quality of product. In Norway, transport of fish to slaughter from commercial farms is typically by well-boat. This provides good sea-water quality, safe transport and, with gentle loading systems appears to have no adverse effects. Commercial slaughter methods themselves still need refinement. Future methods under investigation to replace CO_2 stunning/ chilling include percussion stunning, electrical stunning and the use of eugenol sedation.

Methods using CO_2 and chilling have been improved by reduction of CO_2 levels for stunning (70-150mg/l) after chilling (0-0.5°C) by re-circulated sea water system, prior to exsanguination. Use of high levels of CO_2 has been associated with exhaustion in the fish as evidenced by pH of white muscle. For a method to be considered humane, it should be "instant" and even the best of these methods still takes 2-3 minutes and fish holding times in the RSW chill can be 30-60 minutes. Percussion stunning appears to be a humane method of killing, but requires fish to be presented to the automatic stunner in a certain way and there may be issues of crowding/ handling stress during this process. The voltage, frequency and time are all crucial and require high levels of control in electrostunning. By comparison, the use of eugenol is very simple – it can just be added to the tank, but it currently carries a withdrawal time for food animals.

Note added by Home Office delegates UK: Reference which may be of value: Robb DHF & Kestin SC Methods used to kill fish: field observations and Literature reviewed in Animal Welfare 2002, 11, 269-282.

Ethics – different attitudes to fish welfare depending on the situation?

Cecilie Mejdell, Norwegian Council for Animal Ethics

Attitudes to animals depend in part on personal likes and dislikes; with familiarity, "cuteness", recognition of emotions, beauty and size all impacting on one's attitude, as well as any personal fears or disgust towards a species. In this context fish has few natural advantages, which may suggest that fish are more in need of protection through legislation than dogs and cats.

A moral judgement is more than just a personal opinion, a moral judgement may be discussed and defended, be supported by logical arguments (which are made in the light of an individual's own values/ ethical principles) and should always be supported by facts. Values are what one believes to be good and bad. Attitudes will reflect values held by the person, and most values are shared with others. However, priorities within a set of values will differ with time, between cultures and between individuals. Priority of values may also change within a person with age/ stage of life/ experience.

Principles and norms are about what's right and wrong – things that we ought to do or ought not to do. For example, it is wrong to cause harms to others, and it is considered especially bad to cause harm deliberately. An ethical dilemma occurs when two norms can't be met at the same time; e.g. telling the truth will hurt someone's feelings. The utilitarian argument of the greatest good to the many may need to be balanced with the belief of individual rights, where the end can not be considered to justify all means and individual integrity shouldn't be violated.

The concept of membership of moral communities was discussed with the concept of moral agents (who are expected to act morally) and moral patients (who are protected and benefit from moral acts but are not expected to act morally themselves, e.g. young children). Some believe sentient animals enter into the moral community as moral patients, i.e. we should be kind to animals because of the animal. Others believe that we have no direct moral duties to animals, just indirect duties. It may still be argued that we should be kind to animals, but the reason is that hurting animals may hurt other people's feelings, or may eventually lead to cruelty to human beings.

Facts should be part of an ethics debate on whether a particular use of animals is considered acceptable or not. Considerations may include the physiology and sentience of the particular species, the number of animals used, the harm caused and whether suffering can be relieved, necessity (food versus pleasure) and the human benefit, and if there are alternative ways to achieve this benefit.

These factors were discussed concerning the ethics of use of fish in commercial fisheries, aquaculture, angling, as pets (ornamental fish), and for research were discussed. Particularly relevant to experimental fish is the issue of deliberate harm, and the uncertain nature of the human benefit, which may or may nor occur in the future. This may in part explain the great public concern for research and experimental animals, though the number of animals used is very low compared to fisheries and aquaculture.

Note added by Home Office delegates UK: The ethical principles of the European legislation are utilitarian (the greatest good to the many at the expense of the few) but with an overriding principle of individual integrity and right e.g. individual suffering which cannot be relieved will necessitate the animal being killed, individual and not batch counting of animals for statistical evaluation.

Thursday 26th May:

The challenge of FELASA accreditation of courses in laboratory animal science for fish researchers

Adrian Smith, Norwegian School of Veterinary Science

The provision of appropriate training is highly desirable, but difficult to achieve. FELASA (Federation of European Laboratory Animal Science Associations, http://www.felasa.org) has identified 4 categories of people involved in animal research and has produced guidelines for the education and training of these. Category A is animal technicians (subdivided into 4 levels depending upon experience and responsibility), category B covers research technicians who perform procedures on animals, category C is researchers planning or conducting experiments, while category D is laboratory animal specialists. Accreditation of such training is now underway, but there are a number of issues that need resolved, such as how competence is assessed and whether category B competence automatically gives competence in category A. FELASA has recently appointed a working group to revise category A.

The benefits and problems associated with systems in a number of countries were discussed. The assessment of competency appears to be more advanced in industries such as the pharmaceutical sector compared to academia. The importance of continued professional development was discussed.

The COST initiative and fish research

Liv Jorun Reitan, National Veterinary Institute, Oslo

COST is the acronym for European **C**o-**o**peration in the field of **S**cientific and **T**echnical Research. It covers fundamental and applied research and is a system for research networking within Europe to facilitate exchanges between nationally funded research teams. A recent COST initiative is B24, lab animal science and welfare, implemented in 2004 (http://biomedicum.ut.ee/costb24). The aims include refining husbandry, welfare, procedures, the use of genetically altered animals and other new models, ethical evaluation and quality of training/ facilities, by providing evidence-based recommendations. The recommendations will be provided by a number of working groups via a web-based system. The working group on housing is addressing the housing of fish in research, with emphasis on natural requirements, biological needs, tank enrichment, development of techniques and the use of death as an endpoint.

National platforms for the 3 R's: what are they and how does the Swedish platform function?

Adrian Smith, Norwegian School of Veterinary Science
Staffan Jacobsson, Swedish Animal Welfare Agency

ECOPA (the European Consensus Platform for Alternatives, http://ecopa.vub.ac.be) was launched at the World Alternatives Congress in 1999. For it to approve a forum as a national platform for the 3R's there is the requirement for the involvement of representatives from the 4 stakeholders of academia, industry, government and animal welfare organisations. The idea of such platforms is to identify specific problems at a national level and to co-ordinate funding of research into solutions for these national problem areas. Swecopa is the Swedish platform. It is currently has 2 working groups. The first is addressing the use of alternatives to animals in education. The second related to the use of animals in chemical testing, with particular reference to the new REACH initiative:
http://europa.eu.int/comm/enterprise/reach/overview_en.htm

Summary of group work

Monitoring and reporting fish used in research

Suggested discussion topics were the criteria used for selection of fish, methods for avoiding pathogen introduction into facilities, how the health of fish before and during a study is ascertained and reported, and how fish welfare is monitored. Currently, the monitoring of health and welfare seems very dependent on visual inspection and growth criteria. Knowledge of the microbiological status of fish appears very limited in many studies and the health (or otherwise) of fish during a study is often based on mortality levels. Thus current criteria for selection, health and welfare appear limited and require refinement. Species and group specific guidelines require development.

Generally selection appears to be on fish that appear normal on visual inspection, that show a normal growth rate/ feed conversion efficiency prior to study (for the individual unit). Selection should be based on historical information, and sources should show consistently high production parameters and absent disease when possible or low incidence. Genetic selection may be important in some studies. Veterinary inspection and health certificates are used to indicate health in some instances. There can be conflicts in selection criteria between the scientists and the people that care for the fish. Selection criteria of fish may depend on the study aim: acquisition from research or farm facilities and the need for fish free from 1 or more diseases might reflect whether a study aimed to mimic commercial conditions/ was looking at the efficacy of a vaccine, etc.

Methods used for avoiding the introduction of pathogens include: pre-screening (gills, skin, faeces, kidney,) the use of quarantine, separating tank systems (including separate filtration, separate equipment such as nets and separate staffing), use of animals hatched on site (i.e. no introduction of fish; but this can have implications on the availability of some genetic types) and the use of fish of known vaccination history. The use of live feed, in particular Artemia in Zebrafish facilities, as a potential pathogen source was stressed, as was the concept of "sharing water is sharing pathogens". The importance of general hygiene, biosecurity, building design, footbaths, disinfectant selection, separate nets for each tank, clothes, boots, tools and materials used only for a single microbiological entity was highlighted. The effectiveness of UV sterilisation was questioned and ozonation proposed as more effective especially in the presence of particulates although halides need to be removed. The potential for sub-optimal water conditions due to supplementation with non-physiological salts was identified where zebrafish facilities use marine salt rather than a re-mineralisation protocol that reflects natural conditions. Limitations of current detection methods for many diseases can cause problems with identifying disease carrying fish and the use of "stress-testing" to precipitate latent disease was proposed as one method of disease detection, acknowledging that this would cause suffering for the fish tested by this means. Ethically it is difficult to justify such tests except for setting up new disease free colonies. SPF fish are currently difficult/ impossible to obtain for many, and it was considered there would be room in the market for a supplier of such fish, although there is the issue of what genetic stock they could/ should supply.

Health and welfare of fish appear to often be judged on similar criteria, including visual appearance, growth rate, incidence of abnormalities and mortalities. Such criteria would often be considered insufficient as welfare measurements in other species and significant suffering may be occurring where mortality alone is used as a criterion. Level and type(s) of monitoring may need to reflect study types. Species and group-specific guidelines are required.

Guidelines and procedures: do we have what we need?

Suggestions for discussions included identification of what guidelines were in place in institutions for the use of commonly used techniques such as anaesthesia, transport, blood sampling and killing of fish. The issue of whether sufficient guidelines are currently in place, the difficulties of comparing results from different trials, side effects of treatments such as vaccines and the use of GCP were also suggested topics. It was identified that there are a lot of guidelines/ information on procedures out there, but the difficulty was often getting to it. There is a need to pull these resources together so that current knowledge can be properly shared. It is easier to harmonise theoretical teaching (e.g. by databases if questions) than practical training. It is not reasonable to demand that a standard set of techniques are used; rather there should be guidelines on best, or at least acceptable, practice. A lack of funding is often more of a problem in resolving best practice issues rather than a lack of interest, although lack of knowledge is also significant. Scientists would benefit from education as to how to find specialist literature/ websites, etc.

Good practice for marking animals should be using minimally invasive procedures with the least stressful handling. The example of restraint of fish in tubes within water rather than the use of anaesthesia for PIT tagging was given. It was considered that all the commonly used marking methods were probably acceptable under some circumstances and that some form of recommendation as to what was best when would be very helpful. How fish are transported is very important. Measurement of cortisol levels in fish in well-boats shows that it is the handling before and after transport that is stressful and the transport in this case is a “rest period”. The means of transfer is highly important, with positive pressure pumping preferable to netting or suction. By comparison, there appears to be fewer injuries seen in fish that are transported in tanks by road when they are sedated. Oxygenation is essential during transport, but with care not to supersaturate the water. More guidelines are needed for acceptable volumes for blood samples, based on total blood volume of each species. The use of liquid nitrogen was considered to be an unsuitable method of euthanasia unless the animal was small and was either placed onto a metal plate or into isopropanol cooled in liquid nitrogen. Liquid argon is a more satisfactory method. Ian Bricknell has evidence to support this contention. The use of more than 1 agent during euthanasia may improve methods and the wording of Appendix A should be flexible enough to allow this.

It was stated that methods would need to be scientifically proven before changing practice. It should be remembered that the validation process is long and difficult, and such stringent evidence may not always be required before adoption of what are shown to be better methods. Starving for 4-5 days for fish was thought to be too much. 48 hours might be better. We have since heard that 20 degree-days (e.g. 2 days in water at 10°C) is sufficient to empty the gut and is used commercially in the UK.

Blood sampling volumes should probably be restricted to 1-5% of total blood volume (TBV), but TBV is not known for fish. For mammals it is about 65ml/kg. Syringes were reported to be better than vacutainers as they cause less haemorrhage.

The preparation of a “Marking methods” table could be considered – UK may have a document which is several pages long which may be of value in the foreseeable future. It is not yet ready for release.

Panel debate: Are the methods used in fish research today ethically acceptable?

The panel consisted of representatives of the four stakeholders in animal research:

- *Academia:*
  Erik Sterud, National Veterinary Institute, Oslo
- *Animal welfare organisations:*
  Live Kleveland Karlsrud, Norwegian Animal Welfare Alliance, Oslo
- *Industry:*
  Kjersti Gravningen, Pharmaq AS, Oslo
- *Regulatory authorities:*
  Bjørn Groven, Norwegian Animal Research Authority, Oslo

There was a lively discussion between the panel members and with the audience. A number of interesting points were raised. These included:

- is it acceptable to assign different values to individuals of the same or different species?
- the difficulty of obtaining grants to investigate the 3R's in fish compared to other species
- the need to eliminate the requirement by regulatory authorities for death as an endpoint in some studies
- the need for good regulation/ control systems to monitor use
- the issue of what is driving much research in fish, i.e. increased profitability in farming, and at what point this becomes unacceptable. Some aspects of this work address fish welfare, e.g. vaccination development, but others purely try and improve profits, e.g. reduction in time to smoltification and use of alternative food sources
- the need for better understanding of how fish perceive the world, as this information would help identify which studies are reasonable/ acceptable and allow optimal refinement of methods, minimising stress and therefore giving the best science.