# 3Rs resources and guidelines for zebrafish

### **Adrian Smith**

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# National Consensus Platform for the Replacement, Reduction and Refinement of Animal Experiments



a competence centre for the 3RS

# International consensus meetings

Harmonisation of the Care and Use of:
Fish (2005)
Wildlife (2008)
Fish (2009)
Agricultural animals (2012)

http://norecopa.no/consensus-meetings

All presentations and consensus statements are on the internet: a lasting resource

# An overview of existing guidelines for handling, bleeding, administration and identification techniques

Penny Hawkins, Research Animals Department, RSPCA

www.norecopa.no/norecopa/vedlegg/Fish-guidelines.pdf

## Global update on guidelines for fish research

Gilly Griffin, Canadian Council on Animal Care (CCAC)

www.norecopa.no/fish2009

# Guidelines for anaesthesia and analgesia of fish

Gidona Goodman, University of Edinburgh

www.norecopa.no/fish2009

# Compendium in Laboratory Animal Science for Fish Researchers

edited by
Trond Brattelid & Adrian J. Smith





Norwegian School of Veterinary Science & Norecopa June 2011 So what do we have for zebrafish...?

What qualifies as a guideline? Who decides? Every IACUC protocol?

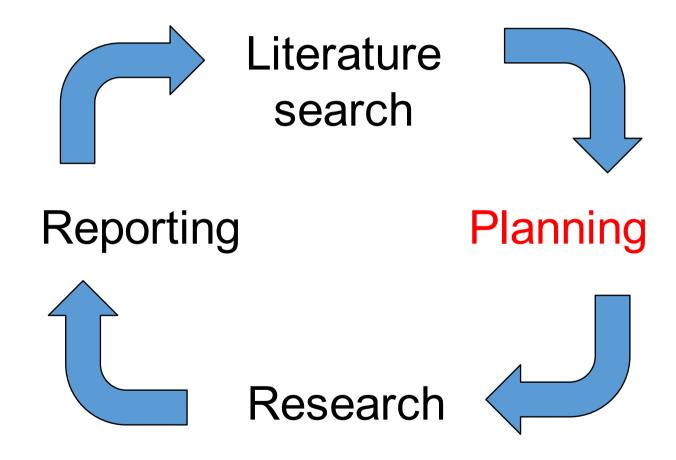
Norecopa had that question in mind when we produced our database 3R Guide (3RGuide.info).



# Zebrafish: Husbandry, Care and Welfare

21st to 22nd of April 2016

TI 1 01 4 11		
Thursday 21 April		
Registration		
Welcome and opening addresses		09:45 - 10:00
How to Run a Facility (Carole V	Vilson)	10:00 - 10:30
Coffee Break		10:30 - 11:00
Current Practices Practical Se	ssions and Group Discussion	11:00 - 12:00
Housing (Heather Callaway)	Handling and Sexing (Jenna Hal	kkesteeg)
Feeding (Matt Wicks)	Environment Corichment (Kar	ren Dunford)
Recommendations and Guidel	ines (Carole Wilson)	12:00 - 12:30



# What does Europe have to offer?

# European Convention ETS123 for the Protection of Vertebrate Animals Used for Scientific or Other Purposes

'The Convention is designed primarily to reduce both the number of experiments and the number of animals used for such purposes... Animals to be experimented on should be selected on the basis of clearly established quantitative criteria and must be well cared for and spared avoidable suffering whenever possible.'

# Appendix A: Guidelines for the accommodation and care of animals (revised, with species-specific guidelines from 2007).

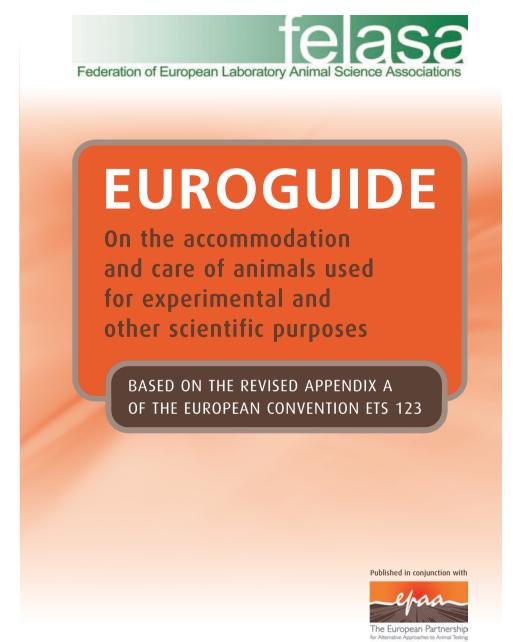
'Species-specific guidance on rainbow trout (Oncorhynchus mykiss), Atlantic salmon (Salmo salar), tilapiine cichlids, zebra fish (Danio rerio), sea bass (Dicentrarchus labrax), Atlantic halibut (Hippoglossus hippoglossus), Atlantic cod (Gadus morhua), turbot (Scophthalmus maximus), African catfish (Clarias gariepenus) is available in the background document elaborated by the Group of Experts.

Further advice on the requirements of these and other species should be sought from expert specialists and care staff to ensure that any particular species needs are adequately addressed.'

The FELASA library contains documents other than workinggroup reports, guidelines, recommendations or policy documents. These can be found under the respective tabs.

Part B of Appendix A: Scientific background. No Part B for fish!

>> ETS123 - Appendix A: guidelines for accommodation and care of animals (adopted version).
Background information on the draft proposal for species-specific provisions presented by Groups of Experts for: amphibians, birds, cats, dogs, ferrets, non-human primates, reptiles, rodents and rabbits.



http://www.felasa.eu/media/uploads/Euroguide\_official\_publication.pdf

7 pages mention Zebra finches, nothing on Zebrafish...

### EU Directive 2010/63 on the Protection of Animals Used for Scientific Purposes

### ETS 123 again!

Further advice on the requirements of these and other species should be sought from expert specialists and care staff to ensure that any particular species needs are adequately addressed.

# "Fish" (all 30,000 spp.)!

#### Fish

### 11.1. Water supply and quality

Adequate water supply of suitable quality shall be provided at all times. Water flow in re-circulatory systems or filtration within tanks shall be sufficient to ensure that water quality parameters are maintained within acceptable levels. Water supply shall be filtered or treated to remove substances harmful to fish, where necessary. Water-quality parameters shall at all times be within the acceptable range that sustains normal activity and physiology for a given species and stage of development. The water flow shall be appropriate to enable fish to swim correctly and to maintain normal behaviour. Fish shall be given an appropriate time for acclimatisation and adaptation to changes in water-quality conditions.

### 11.2. Oxygen, nitrogen compounds, pH, and salinity

Oxygen concentration shall be appropriate to the species and to the context in which the fish are held. Where necessary, supplementary aeration of tank water shall be provided. The concentrations of nitrogen compounds shall be kept low.

The pH level shall be adapted to the species and kept as stable as possible. The salinity shall be adapted to the requirements of the fish species and to the life stage of the fish. Changes in salinity shall take place gradually.

### 11.3. Temperature, lighting, noise

Temperature shall be maintained within the optimal range for the fish species concerned and kept as stable as possible. Changes in temperature shall take place gradually. Fish shall be maintained on an appropriate photoperiod. Noise levels shall be kept to a minimum and, where possible, equipment causing noise or vibration, such as power generators or filtration systems, shall be separate from the fish-holding tanks.

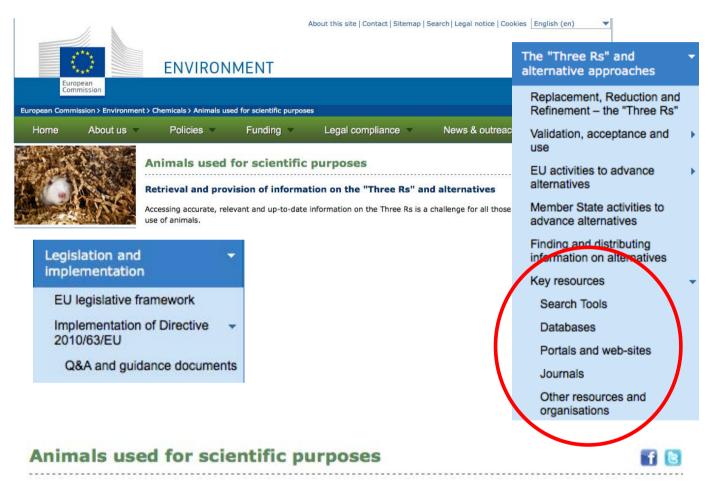
### 11.4. Stocking density and environmental complexity

The stocking density of fish shall be based on the total needs of the fish in respect of environmental conditions, health and welfare. Fish shall have sufficient water volume for normal swimming, taking account of their size, age, health and feeding method. Fish shall be provided with an appropriate environmental enrichment, such as hiding places or bottom substrate, unless behavioural traits suggest none is required.

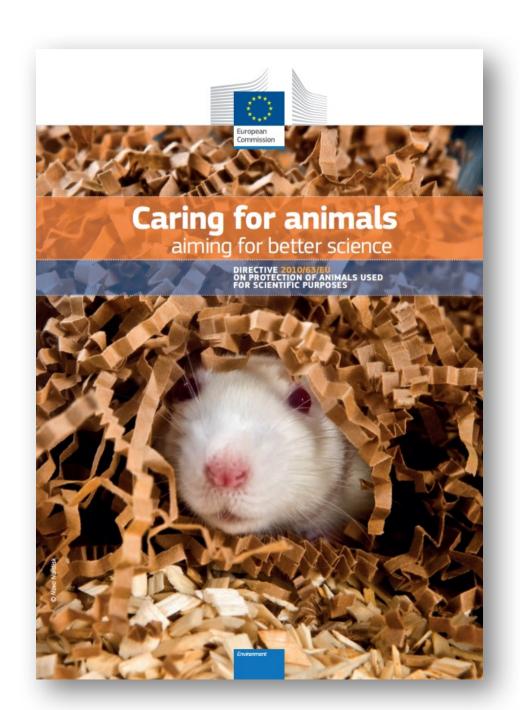
### 11.5. Feeding and handling

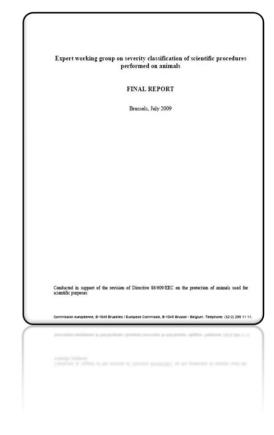
Fish shall be fed a diet suitable for the fish at an appropriate feeding rate and frequency. Particular attention shall be given to feeding of larval fish during any transition from live to artificial diets. Handling of fish shall be kept to a minimum.

# ec.europa.eu/animals-in-science



Opinions of European Commission Expert Committees related to the use of animals in experiments







# Expert Working Group report on severity classification

http://ec.europa.eu/environment/chemicals/lab\_animals/pdf/report\_ewg.pdf





# Guidance on the severity classification of procedures involving fish

Report from a Working Group convened by Norecopa

Designed to be a supplement to the EU Working Group report on the same subject, which is most relevant for traditional lab animals

P Hawkins, N Dennison, G Goodman, S Hetherington, S Llywelyn-Jones, K Ryder and AJ Smith

Laboratory Animals, 45: 219-224, 2011

www.norecopa.no/categories

# Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes

Refers to RSPCA Guidance

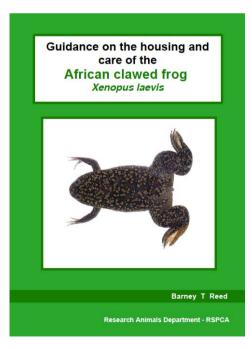
Presented to Parliament pursuant to Section 21 (5) of the Animals (Scientific Procedures) Act 1986

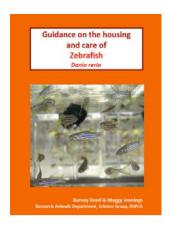
# Guidance on the housing and care of Zebrafish

Danio rerio



Barney Reed & Maggy Jennings Research Animals Department, Science Group, RSPCA





This report aims to improve the welfare of zebrafish by:

- facilitating understanding of zebrafish behaviour and thus a better appreciation of their requirements;
- highlighting current potential welfare and ethical concerns relating to the breeding, supply, housing and care of zebrafish;
- arriving, where possible, at consensus based on available evidence and sound scientific argument for appropriate environmental and care conditions for keeping zebrafish in the laboratory environment;
- providing recommendations for improving health, welfare and egg quality, for reducing the potential for stress and suffering, and for reducing the number of animals used;
- in areas where current knowledge is sparse or inconclusive, stimulating discussion and research to identify 'good practice'.

- 1. Introduction
- 2. Background information on zebrafish
  - Natural geographic range and habitat
  - Species characteristics
  - Use in research and teaching
- 3. Supply and transport
  - Source
  - Transport considerations
  - Quarantine
- 4. Housing and care
  - Lighting
  - Noise and other disturbances
  - Humidity
  - Water provision
  - Tank housing
  - Identification and marking techniques
  - Group housing
  - Catching and handling
  - Food type and feeding regime
  - Environmental enrichment
  - Assess of health and disease prevention

# Guidance on the housing and care of Zebrafish



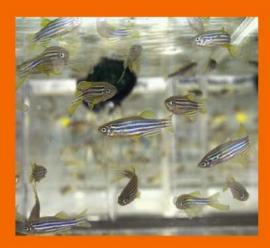
Barney Reed & Maggy Jennings Research Animals Department, Science Group, RSPCA

62 pages, including 8 pages with literature references

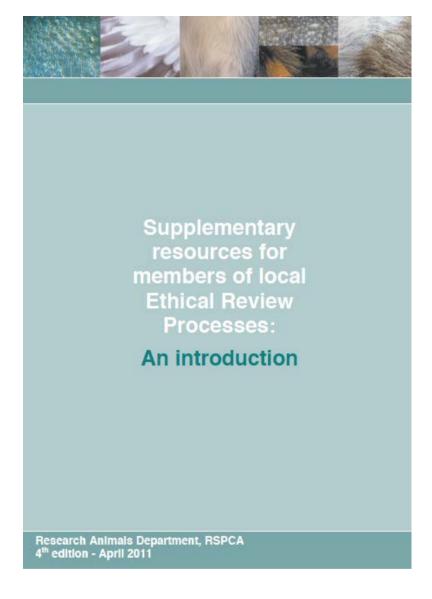
- 5. Scientific procedures
  - Egg harvesting
  - Transgenesis
  - Mutagenesis
  - Genotyping
  - Cryopreservation
  - Blood collection
  - Injections
  - Analgesia and anaesthesia
  - Humane killing
- 6. Training of animal care staff and users
- 7. Concluding comments
- 8. References

### Guidance on the housing and care of Zebrafish

Danio rerio



Barney Reed & Maggy Jennings Research Animals Department, Science Group, RSPCA



African clawed frogs (Xenopus laevis)

Cattle

Dogs

Domestic fowl

Ducks and geese

**Ferrets** 

Guinea pigs

Hamsters

Mice

**Pigeons** 

Pigs

Quail

**Rabbits** 

Rats

Sheep

Zebra finches

'They are primarily intended for members of ethical review committees, though should also be useful for animal technologists, scientists and laboratory animal veterinarians'.

# FELASA Guidelines under development

# Zebra fish: housing, husbandry, and health monitoring recommendations

This is a joint FELASA/ COST action BM0804 EuFishBioMed.

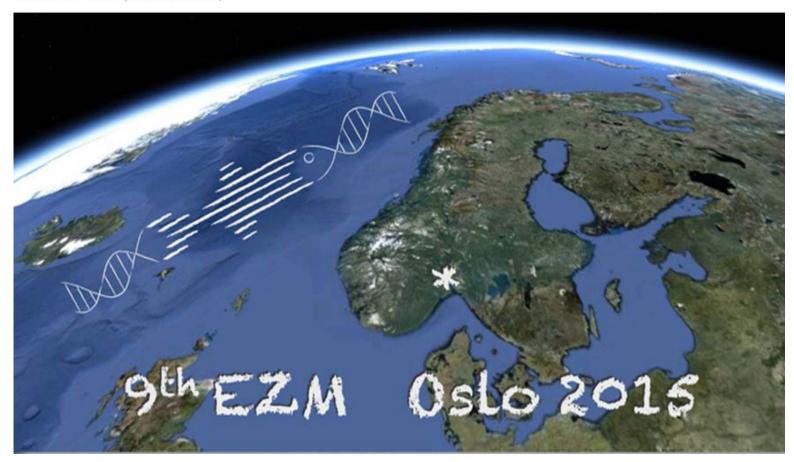
# Synopsis

The zebra fish (Danio rerio) has become a very popular and useful animal model in recent years. Zebra fish can replace mammalian models in a variety of scientific fields. Their mode of reproduction and the transparency of the embryos make them a unique tool for research. Many research institutions are adapting facilities and are educating personnel to be able to work with a species which in most cases is very different of the ones used traditionally. Information in the literature that can help professionals at research institutions to cope with this new challenge is spread and not easy to find. Some of the knowledge is in 'commercial' text about products and is sometimes not scientifically based. Of special interest are housing environment, husbandry practices, including health monitoring, veterinary care and experimental procedures.

http://www.felasa.eu/working-groups/working-groups-present/zebrafish-housing-husbandry-and-health-monitoring-recommendations

### Zebrafish 2015

28. June - 02. July / Oslo, Norway



http://www.ccnorway.no/zebrafish

# World Congresses on Animal Use in the Life Sciences and Alternatives

Important 3R-drivers and disseminators of information:

wc9prague.org (2014)

891 abstracts, 49 countries, 1000 participants (the next one is in September 2017 in Seattle)

Abstract book:

http://www.altex.ch/ALTEX-Proceedings/Current-Proceedings.97.html



OPEN Options

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Advancing Biomedical Research with Zebrafish and Other Aquarium Fish Models

2005 -

**Zebrafish** introduced the new section TechnoFish, which highlights these innovations for the general zebrafish community.

### TechnoFish features two types of articles:

- TechnoFish Previews: Important, generally useful technical advances or valuable transgenic lines
- TechnoFish Methods: Brief descriptions of new methods, reagents, or transgenic lines that will be of widespread use in the zebrafish community

### Zebrafish coverage includes:

- Comparative genomics and evolution
- Molecular/cellular mechanisms of cell growth
- Genetic analysis of embryogenesis and disease
- Toxicological and infectious disease models
- Models for neurological disorders and aging
- New methods, tools, and experimental approaches

ZEBRAFISH Volume 00, Number 00, 2016 © Mary Ann Liebert, Inc. DOI: 10.1089/zeb.2015.1198

# 2016 Special Issue: Health Management & Biosafety

# Toward an Integrated Zebrafish Health Management Program Supporting Cancer and Neuroscience Research

Sandra Martins, Joana F. Monteiro, Maria Vito, David Weintraub, Joana Almeida, and Ana Catarina Certal

### Abstract

Zebrafish is already one of the most used model organisms in biomedical sciences and other research fields. It is therefore becoming increasingly important to assure that zebrafish maintained in laboratory aquaculture conditions are raised and housed under rigorous standards that promote health and welfare to guarantee the required quality and reproducibility of research data. Specifying the programs each facility is adopting would be the first step to achieve this by allowing other facilities to compare, improve, and discuss their protocols and fish performance. We provide in this article a detailed description of an integrated facility health management program, with protocols and readouts, fully designed and aimed at maximizing fish health, welfare, and performance for research.



### Abstract

The presence of subclinical infection or clinical disease in laboratory zebrafish may have a significant impact on research results, animal health and welfare, and transfer of animals between institutions. As use of zebrafish as a model of disease increases, a harmonized method for monitoring and reporting the health status of animals will facilitate the transfer of animals, allow institutions to exclude diseases that may negatively impact their research programs, and improve animal health and welfare. All zebrafish facilities should implement a health monitoring program. In this study, we review important aspects of a health monitoring program, including choice of agents, samples for testing, available testing methodologies, housing and husbandry, cost, test subjects, and a harmonized method for reporting results. Facilities may use these recommendations to implement their own health monitoring program.

# Guidelines for health and welfare monitoring of fish used in research

R Johansen<sup>1</sup>, J R Needham<sup>1,2</sup>, D J Colquhoun<sup>3</sup>, T T Poppe<sup>4</sup> and A J Smith<sup>1</sup>

<sup>1</sup>Norwegian School of Veterinary Science, Laboratory Animal Unit, PO Box 8146 Dep., 0033 Oslo, Norway; <sup>2</sup>The Microbiology Laboratories, North Harrow, Middlesex HA2 7RE, UK; <sup>3</sup>Section of Fish Health, National Veterinary Institute, PO Box 8156 Dep., 0033 Oslo, Norway; <sup>4</sup>Department of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Science, PO Box 8146 Dep., 0033 Oslo, Norway

### Summary

The aim of this paper is to provide background material necessary for the development of international guidelines for the health and welfare monitoring of fish used in research. It provides an overview of present guidelines and discusses why more detailed and species-specific guidelines are needed. A major issue within fish research is to document the situation today and point out areas where improvements are needed.

Keywords Fish; health; welfare; monitoring; guidelines

Guidelines for monitoring and reporting the general health status and welfare of fish used in research are sparse compared with those available for mammalian laboratory animals. Despite the fact that there are more fish species than all other vertebrate species combined and that fish are studied in almost all biological disciplines (Powers 1989), most guidelines for fish encompass all species and all types of research [Casebolt et al. 1998]. There is a great need for more species-specific guidelines for health and welfare monitoring. In some cases, these guidelines may also have to be specific to the scientific topic where they are to be used.

The number of fish used in research is increasing, due both to the rapid expansion in the fish farming industry and an increased use of fish as model organisms in basic research and chemical testing [Kane et al. 1996]. The debate on whether to use fish or mice models started over 25 years ago [Dawe & Couch 1984]. Rodent models are now frequently being replaced by fish models

(May et al. 1987a, Powers 1989, DeTolla et al. 1995).

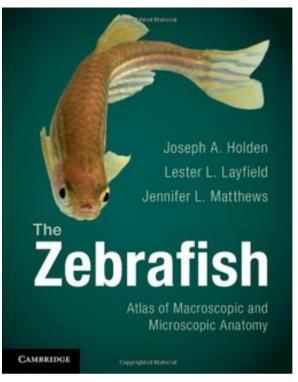
Guidelines and legislations are often more liberal towards the use of fish than mammals. This can be illustrated by the lack of focus on humane endpoints in fish models [Ryder 2005]. LD<sub>50</sub> testing is, for example, no longer allowed on mammals, but remains in use for fish [Braunbeck et al. 2004].

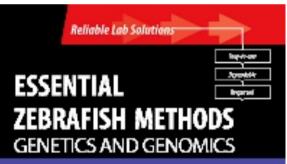
Even the reporting of numbers of fish used, and the type of research for which they are used, is confused by a lack of common international practice. Harmonization in this field is important to avoid the transfer of research from countries with high standards to those with lower ones. In Europe, all fish species and sizes are reported in the same statistical groups and the research disciplines reported are very general. Figure 1 shows, for example, an analysis of the use of live fish in Norway in 2004. This makes it difficult to monitor what fish are actually used for in research.

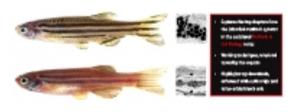
Reporting of the health and welfare of fish used in research is often sparse (Brattelid & Smith 2000), and may include general statements such as 'Healthy fish from a

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Needs to be followed up by species-specific guidelines

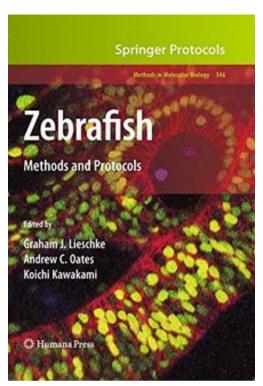


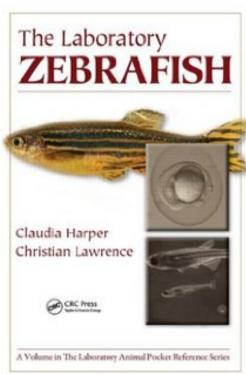


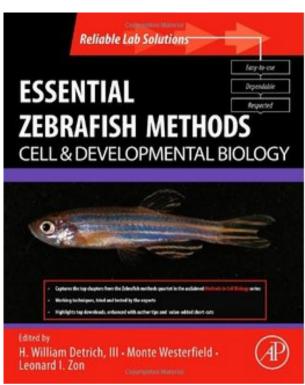


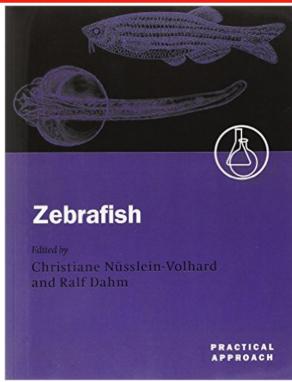
H. William Detrich, III - Monte Westerfield -Leonard I. Zon













# Aquaculture

Volume 269, Issues 1-4, 14 September 2007, Pages 1-20



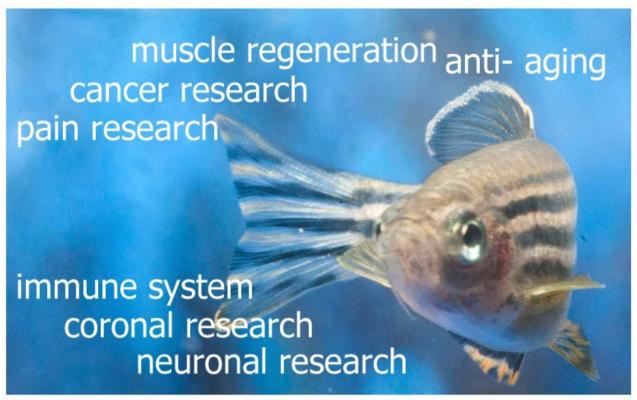
# The husbandry of zebrafish (Danio rerio): A review

Christian Lawrence . ×

### Abstract

The zebrafish (Danio rerio) has recently emerged as a pre-eminent vertebrate biomedical research model. The same favorable characteristics that have contributed to its popularity as a model of human disease and development; i.e. high fecundity, small size, rapid generation time, optical transparency during early embryogenesis, have also long endeared it to investigators in numerous other disciplines, including animal behavior, fish physiology, and aquatic toxicology. Despite this, the scientific rigour of zebrafish husbandry techniques is poorly developed. While there is a considerable body of literature on zebrafish that has both direct and indirect relevance to their husbandry, this information is from disparate sources, and little of it is has been applied to developing standard protocols. This review is an attempt to integrate the available scientific information related to zebrafish biology and culture into an overview of the field that can be used to improve the efficiency with which this important model animal is used in research. The review also highlights those areas in which further studies are needed.

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The zebrafish is a commonly used model organism in the biological sciences, similar to the mouse or rat. Zebrafish can grow to 4-5 cm and are easily handled in aquatic systems. Since their discovery for use in research in Prague in the 1980's, this fish has been established in many laboratories worldwide. The generation time of the zebrafish, from embryo to a breeding adult fish, is very short. Due to their transparent nature, developing Zebrafish embryos are very well suited for imaging purposes. There are very strict rules you have to follow if you want to work with laboratorial animals.

We will provide Information that describes all aspects of research with zebrafish and other fish models.

European Society for Fish Models in Biology and Medicine (EUFishBioMed) http://www.eufishbiomed.kit.edu





Home

#### - Home Scientific community

#### - Political Issues

Recommended Guidelines for Zebrafish

White Papers

Stress assessment in Zebrafish EULoS

#### Recommended Guidelines for Zebrafish

### EU Directive 2010 63. Experiments with Zebrafish

The Directive 2010/63/EU revising Directive 86/609/EEC on the protection of animals used for scientific purposes was adopted on 22 September 2010. It is firmly based on the principle of the Three Rs, to replace, reduce and refine the use of animals used for scientific purposes. European scientist working with zebrafish have been consulted and involved in the development of common guidelines after what stage experiments with zebrafish should be considered animal experiments in regulatory terms:

[ENGLISH] [SPANISH] [FRENCH] [GERMAN] [GREEK] [ISLANDIC]

### Recommended Guidelines for Zebrafish Husbandry Conditions

This guidelines are a joint FELASA (Federation of Laboratory Animal Science Associations) EuFishBioMed project and have been translated into seven laguages:

[ENGLISH] [FRENCH] [GERMAN] [ITALIAN] [GREEK] [DUTCH] [NORWEGIAN]

Please find below maintenance condtions for zebrafish (in German) written recently (Sept. 2014) by several German groups to support the ongoing discussions by German animal welfare associations to derive uniform recommendations for whole Germany.

Husbandry conditions (German)

https://www.eufishbiomed.kit.edu/59.php





















# British Association of Zebrafish Husbandry

BAZH is a volunteer non-profit making pan-institutional society that showcases the extent of zebrafish husbandry issues and research across the UK. Established in 2010 as an informal hub for all members of the zebrafish community, from researchers, vets, legislators, and animal technologists, to meet, discuss, and collate ideas, research and knowledge. BAZH holds biannual seminars on a vast range of topics, organises social events, and publishes newsletters, as well as hosting a range of media communication formats. If you want to get involved in any capacity, such as present a talk, contribute to newsletters, websites, etc. please get in touch.

# <u>Papers</u>

### **Papers**

Abi-Ayad, A., Kestemont, P. 1994. Comparison of the nutritional status of goldfish (Carassius auratus) larvae fed with live, mixed or dry diet. Aquaculture 128: 163–176

Alabaster, J.S., Lloyd, R., 1980. Water Quality Criteria for Freshwater Fish. Butterworth. 297 pp.

Alsop, D., Matsumoto, J., Brown, S., Van Der Kraak, G. Retinoid requirements in the reproduction of zebrafish. 2007. General and comparative endocrinology 156:51 -62

American Veterinary Medical Association (AVMA) (2007) 'AVMA guidelines on euthanasia' http://www.avma.org/issues/animal\_welfare/euthanasia.pdf.

Andrews C (1000) Exachinator field in The LIEAM Handbook on the Care and



www.zebrafish.org

# ZIRC Health Monitoring SOPs

Sentinel Fish Program

Daily Monitoring of Fish Morbidity and Mortality SOP

Fixing Zebrafish for Histopathology

ZIRC PCR protocol for P. neurophilia

**D** pdf

D pdf







SOP #:

Vet-0001

Revision #:

2.0

Last Update:

7/31/15

#### Monitoring of Fish Morbidity and Mortality

#### D. Procedures

Fish exhibiting the behavioral and physical signs below should be removed. If you
have time to remove the fish, go to step 3 or 4. If you do not have time to remove
the fish immediately, place a red flag on the front of the tank and write the number
of affected fish and observed clinical signs on the flag (i.e. "1 skinny").

Behavioral Abnormalities	Physical Abnormalities
Fish at surface or near water inlet	Color change
Rapid breathing/opercular movements	Weight loss
Sluggish movements/lethargy	Exophthalmia/pop-eyes
Flashing/rubbing on tank surfaces	Distended abdomen
Circling, twirling, spinning	Skeletal deformity
Loss of equilibrium	Mass/swelling
	Hemorrhage/redness
	Gas bubbles
	Protruding scales
	Fin erosion or lesion
	Skin ulceration or lesion



#### The Zebrafish Book

http://zfin.org/zf\_info/zfbook/zfbk.html

# ZFIN Protocol Wiki

Created by Jonathan Knight, last modified on Mar 24, 2014

#### Welcome to the Protocols Wiki

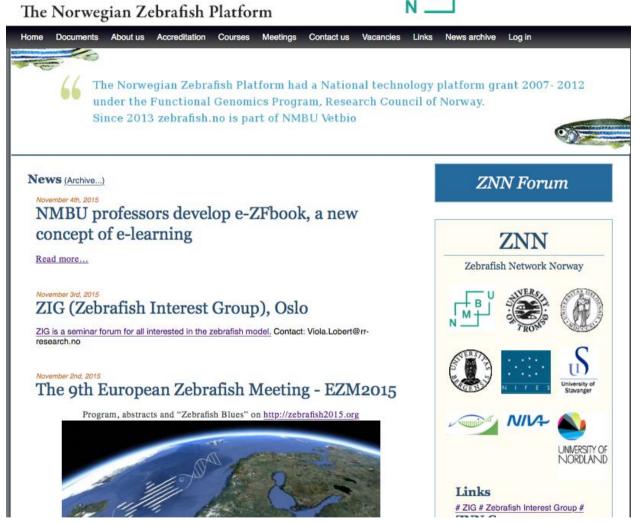
This is where zebrafish researchers can share experimental protocols and tips with the rest of the research community. Protocols are organized into sections corresponding to the chapters of The Zebrafish Book, 5th edition (4th edition on-line). Feel free to add new protocols to the appropriate section or add comments to any existing protocol.

https://wiki.zfin.org/display/prot/ZFIN+Protocol+Wiki

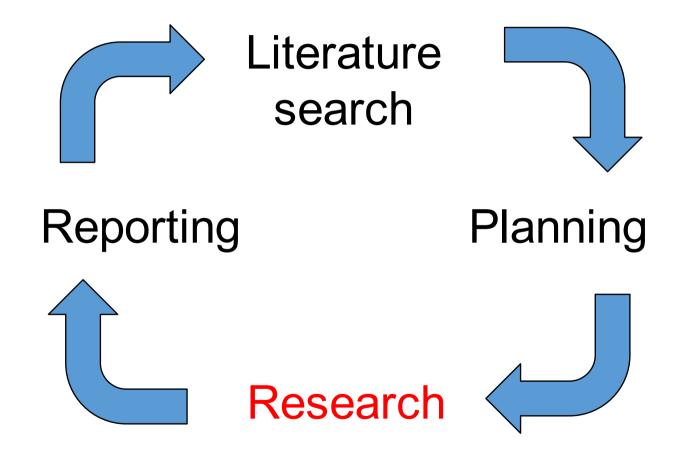




Norwegian University of Life Sciences



Zebrafish.no



# Frequent challenges with research on non-traditional species

- Absence of standardised animals
- Health, housing and welfare monitoring
- Recognition of pain, suffering, distress



http://ichef-1.bbci.co.uk/news/660/me dia/i mages/69786000/jpg/\_69786238\_69786233.jpg

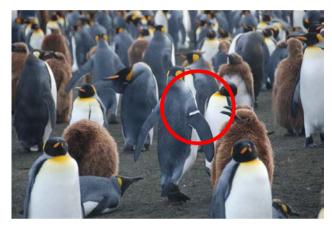


- "Normal" mortality
- Refinement of procedures
- Techniques for anaesthesia and humane killing

# "Simple" techniques?



Photo: T. Poppe, NMBU



http://blogs.discovermagazine.com/notrockets cience/2011/01/12/flipper-bands-impair-penguin-survival-and-bree ding-success/#.VLU6\_8Y7\_wo

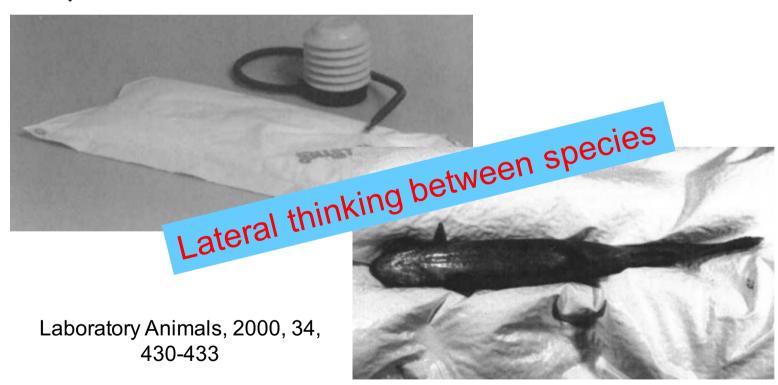


Photo: NMBU

# Methods of positioning fish for surgery or other procedures out of water

#### Trond Brattelid & Adrian J. Smith

Laboratory Animal Unit, Norwegian School of Veterinary Science, PO Box 8146 Dep., N-0033 Oslo, Norway







film.oslovet.norecopa.no

## Harm-Benefit Assessment

# No numerical 'formula'



- AND A SPECTRUM OF VIEWS
- The harm-benefit assessment involves value judgements, which depend on individual opinions on:
  - The nature and likelihood of the benefit
  - The value placed on animal life may depend on species, numbers
  - The nature and level of suffering
  - 4. Controversy around particular procedures
  - 5. The fate of the animals

## An useful additional (but largely unknown) tool...

Carol M. Newton (1925-2014)



National Library of Medicine

# The three S's

- Good Science
- Good Sense\*
- Good Sensibilities\*

\*We can do this ourselves without scientific literature!

Carol M Newton, quoted in Rowsell HC (1977): The Ethics of Biomedical Experimentation in The Future of Animals, Cells, Models, and Systems in Research, Development, Education, and Testing pp. 267-281, National Academy of Sciences, Washington, D.C., ISBN 0-309-02603-2.

# **Critical** anthropomorphism



= *empathy* + objective, knowledge-based consideration of what is likely to be significant to the animal

# Do you need more guidelines?



https://commons.wikimedia.org/wiki/File:Truman\_pass-the-buck.jpg



## Thanks to our main sponsors:

- Dag S. Stiansen Foundation
- Laboratory Animals Ltd.
- Ministries of Agriculture and Fisheries
- Nordic Society Against Painful Experiments
- Norwegian Research Council
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