How can we together better

PREPARE for fish research?

Adrian Smith

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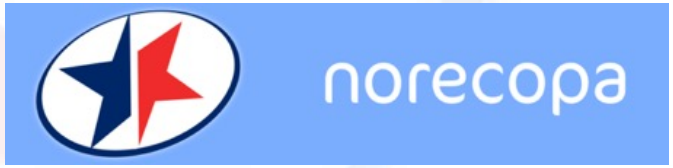
norecopa.no/170323



https://norecopa.no

Disclosures – it's my job to collect and disseminate guidelines...

- *Webmaster for the Norecopa site - information about 3R guidelines worldwide*
- *Lead author of several databases embedded in the website*
- *Lead author of the PREPARE guidelines for planning animal research*
- *Manager of the Refinement Wiki*

Thanks to many colleagues, including:

Aurora Brønstad, University of Bergen
Chris Noble, Nofima Tromsø
Gidona Goodman, University of Edinburgh
Susanna Lybæk, Norwegian Animal Protection Alliance
Tore Kristiansen, Institute of Marine Research, Bergen

Contributors to Norecopa's Refinement Wiki

Penny Hawkins and Chloe Stevens
especially for the fish section in PREPARE

Overview

- General comments
- Norecopa's website and PREPARE
- European legislation
- Health monitoring
- Procedures
- Anaesthesia, analgesia and humane killing
- Severity classification, humane endpoints and harm-benefit assessment
- Zebrafish
- How to keep updated

"Wild, wet and slippery: fish in research"

"Soft, furry and jumpy: mammals in research"

33,000 (?) v. 5,400 species

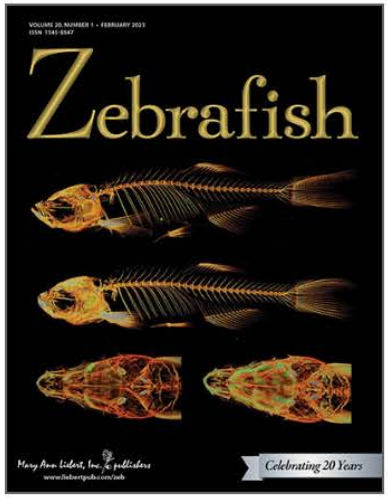
Guidelines: history repeats itself when developing new models

e.g. GA mice, nude mice, minipigs, isolators, IVC racks, cleaner fish, zebrafish:
Two sets of "guidelines" emerged, that address very different questions:

1. *Wow that was cool, **how** do you do it?*

2. *Hmm, **should** we really be doing this?*

*The first set are more **technical specifications** than **guidelines** in the 3R sense…*
The second set tend to be running fast in an attempt to catch up with the first set



Zebrafish

Editor-in-Chief: Stephen C. Ekker, PhD

ISSN: 1545-8547 | Online ISSN: 1557-8542 | Published Bimonthly | Current Volume: 20

Impact Factor: 2.229*

*2021 Journal Citation Reports™ (Clarivate, 2022)

CiteScore™: 4.2

The only peer-reviewed journal dedicated to the central role of zebrafish and other aquarium species as models for the study of vertebrate development, evolution, toxicology, and human disease.

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

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Two extra problems with fish have delayed progress:

- ***the debate about pain perception***

- ***Bambi-factor***

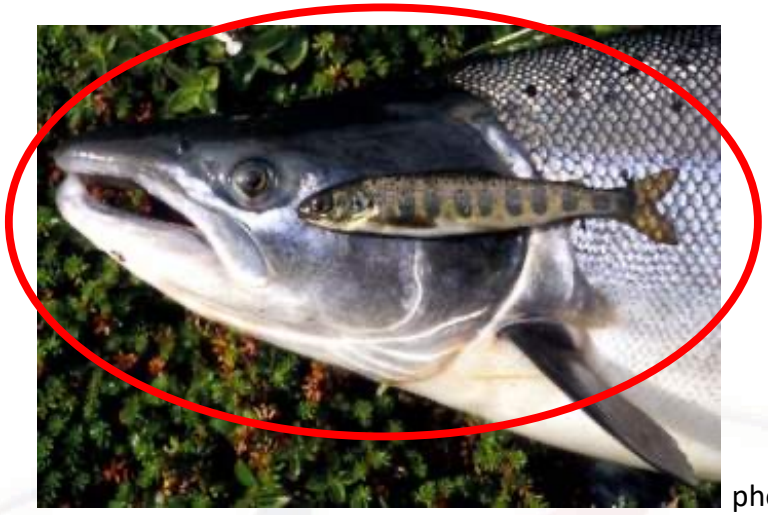
Blobfish voted world's ugliest animal

Deep-sea creature crowned winner of a competition to raise awareness of endangered and aesthetically challenged animals

The blobfish has been voted the world's ugliest animal. Photograph: Greenpeace/Rex Features

theguardian.com/environment/2013/sep/12/blobfish-world-ugliest-animal

To give guidance we have to know what's normal...



photos: T. Poppe

Physiological changes e.g. from freshwater to saltwater

Size differences between animals of the same age

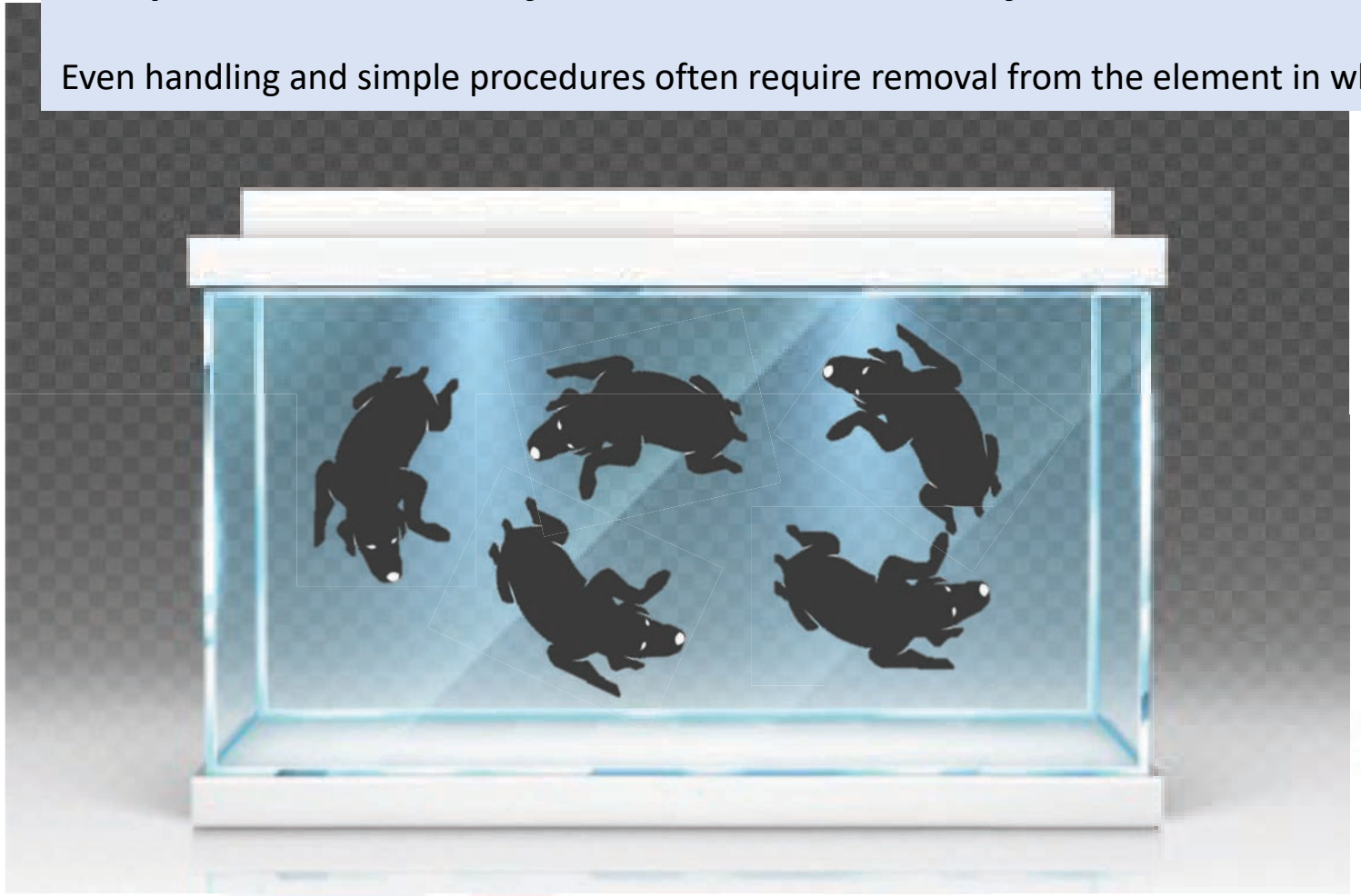
Housing needs: as individuals or in schoals?

Effects of drugs (e.g. anaesthetics and analgesics) at different temperatures

etc.

Fish procedures have often an additional set of harms

Even handling and simple procedures often require removal from the element in which they live and breathe



It's only fish...

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forskning.no

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”Simple” techniques – in a wet, viscous environment

photo: T. Poppe

- Drag forces
- Growth of seaweed, shells
- Infection risk
- Locomotory effects: prey and predation

”Contingent suffering”

indirect suffering

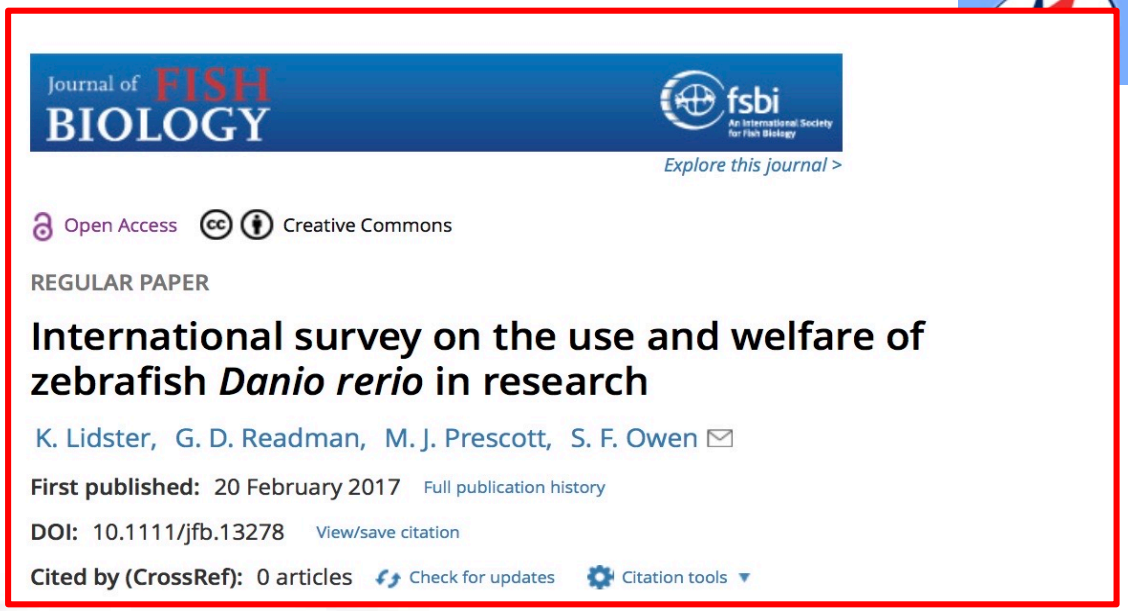
- fear
- boredom
- transport stress
- re-grouping

colourbox.com

tecniplast.it/usermedia/us/3-5LTankwithFish-small.jpg

Zebrafish?

Salmonids in tanks/nets?



Journal of FISH BIOLOGY

fsbi An International Society for Fish Biology

Explore this journal >

Open Access Creative Commons

REGULAR PAPER

International survey on the use and welfare of zebrafish *Danio rerio* in research

K. Lidster, G. D. Readman, M. J. Prescott, S. F. Owen

First published: 20 February 2017 Full publication history

DOI: 10.1111/jfb.13278 View/save citation

Cited by (CrossRef): 0 articles Check for updates Citation tools

98 responses from 22 countries

Concerns include:

- methods of anaesthesia and euthanasia
- Less invasive methods than fin clipping for genotyping
- Nutrition
- Stocking density
- Lighting

https://www.nc3rs.org.uk/news/international-survey-use-zebrafish-research-highlights-opportunities-refinement

'More guidelines for the fish industry than for research?

- *NRC nutritional requirements*
  norecopa.no/3r-guide/nrc-nutrient-requirements

- FAO Report on Welfare of Fishes in Aquaculture
  www.fao.org/publications/card/fr/c/CA5621EN

- RSPCA welfare standards for farmed Atlantic salmon
  science.rspca.org.uk/sciencegroup/farmanimals/standards/salmon

- FishWell Handbook on welfare indicators for Atlantic salmon
  nofima.no/fishwell/english

- Welfare Indicators for farmed rainbow trout (sister publication to FishWell)
  nofima.com/results/new-handbook-on-welfare-indicators-for-farmed-rainbow-trout

norecopa.no : an updated overview of global 3R resources

approx. 9,300 webpages
350,000 hits annually

7-8 detailed newsletters per year

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Databases & Guidelines

Published lists of resources are difficult to search and quickly become outdated. Lists on a website are easier to search, but do not enable the use of filters or intelligent search engines.
Norecopa has therefore constructed four databases, which together with all the text on this website can be searched simultaneously using the search field at the top of every page.

- 3R Guide: a global overview of **databases, guidelines, information centres, journals, email lists, regulations and policies** which may be of use when planning experiments which might include animals. A quick overview of all the guidelines can be accessed here. Norecopa has written several of these, including the PREPARE guidelines for planning animal research and testing.
- NORINA: a global overview of audiovisual aids and other items which may be used as **alternatives or supplements to animals in education and training** at all levels from junior school to University, including dissection alternatives and surgical simulators.
- TextBase: a global overview of **textbooks and other literature within laboratory animal science** and related topics.
- Classic AVs: a subset of NORINA covering **audiovisual aids that are based on older technology**.

These databases are updated regularly. Please give us feedback if you discover errors or omissions.

The Norecopa website also includes four other collections:

- NAL: a collection of literature references relating to the 3Rs from the US National Agricultural Library
- European Commission datasets:
  - 3Rs Knowledge Sources: over 800 resources collected by the Commission in 2016
  - 3Rs Education and Training Resources, over 560 items collected in 2018
  - Non-animal models for respiratory tract diseases, over 280 models identified in a literature review of over 21,000 publications

Here is an alphabetical global list of all the databases cited on the Norecopa website.

norecopa.no/databases-guidelines

links to over 70 other databases,
11 concerning fish

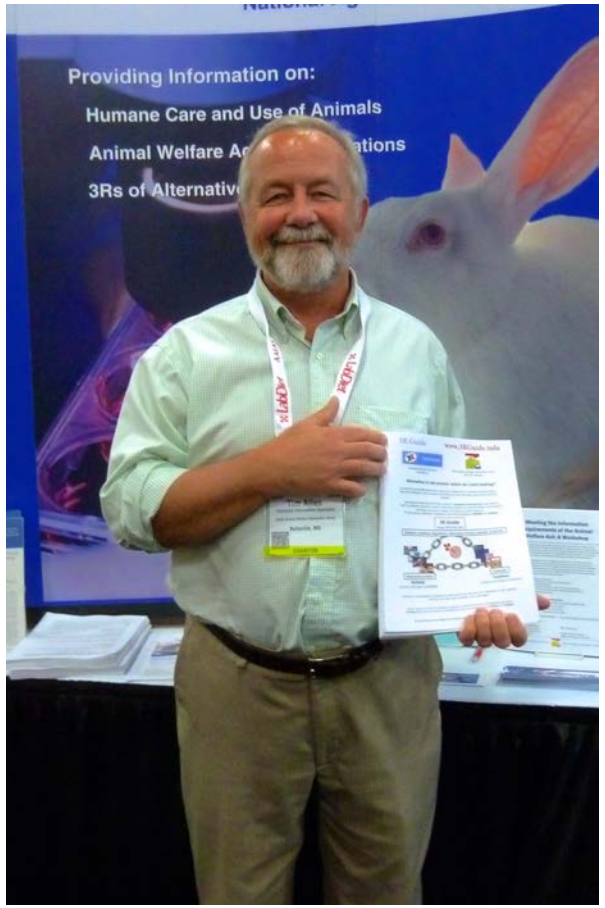
So what do we have for fish…?

What qualifies as a 3R-guideline? Who decides? Every protocol published on the web?

Norecopa had that question in mind when we compiled our database ***3R Guide***

Started as a collaboration with Dr. Tim Allen at AWIC

(Animal Welfare Information Center, National Agricultural Library, USA)



norecopa.no/3RGuide

Links to 419 guidelines (c. 35 on fish)

A good practice guide to the administration of substances and removal of blood, including routes and volumes

3R Guide database/c6721 (legacy id: 15079)

This paper provides the researcher in the safety evaluation laboratory with an up-to-date, easy-to-use set of data sheets to aid in the study design process whilst at the same time affording maximum welfare considerations to the experimental animals.

A guide to defining and implementing protocols for the welfare assessment of laboratory animals

3R Guide database/68ba4 (legacy id: 15065)

Eleventh report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement

A guide to the care and use of native Australian mammals in research and teaching

3R Guide database/502ff (legacy id: 15377)

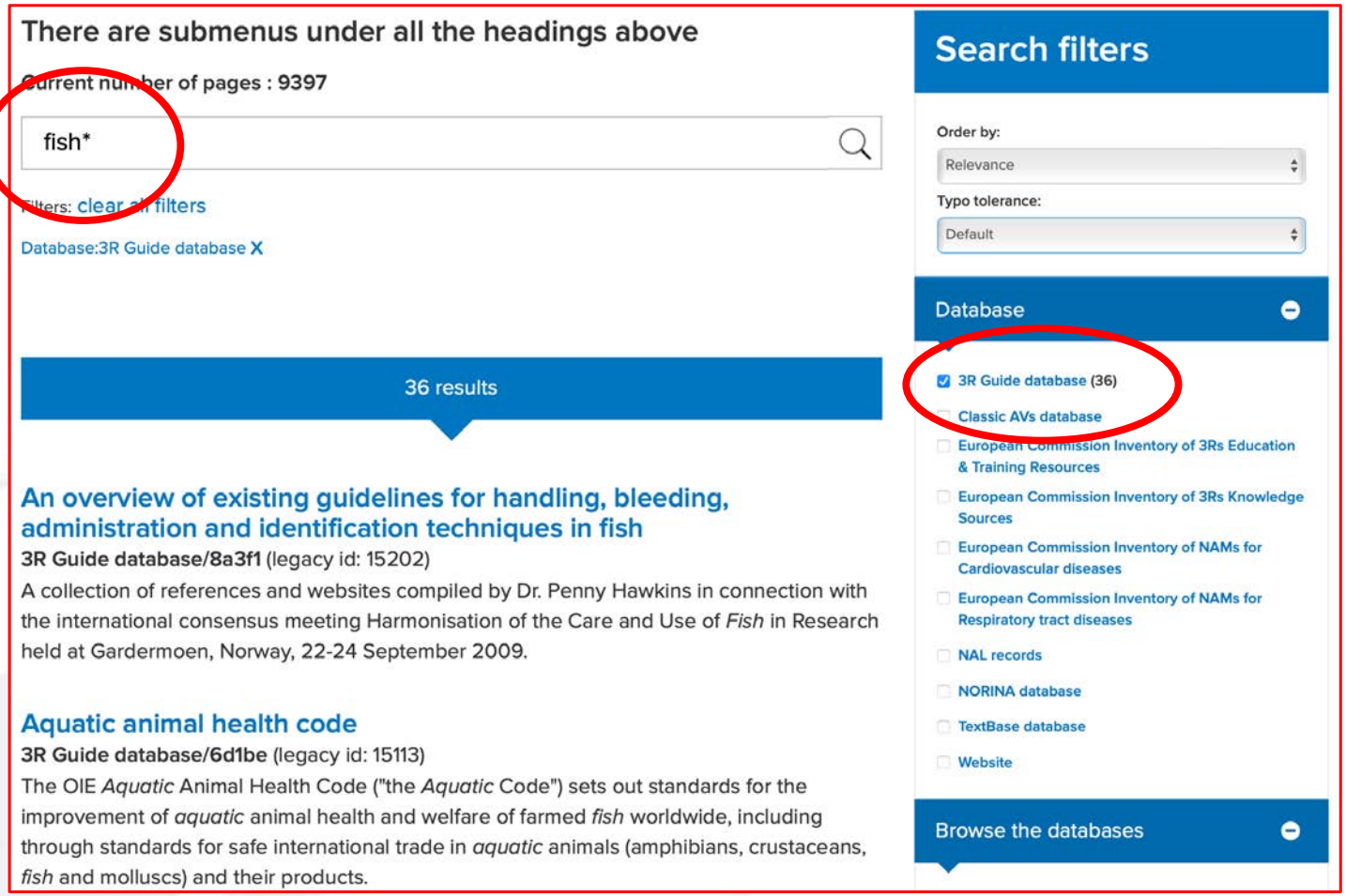
The Guide supports implementation of the Australian Code for the care and use of animals for scientific purposes (8th edition, 2013) and ensures that the specific and unique needs of Australian native mammals are met when these animals are used for scientific purposes.

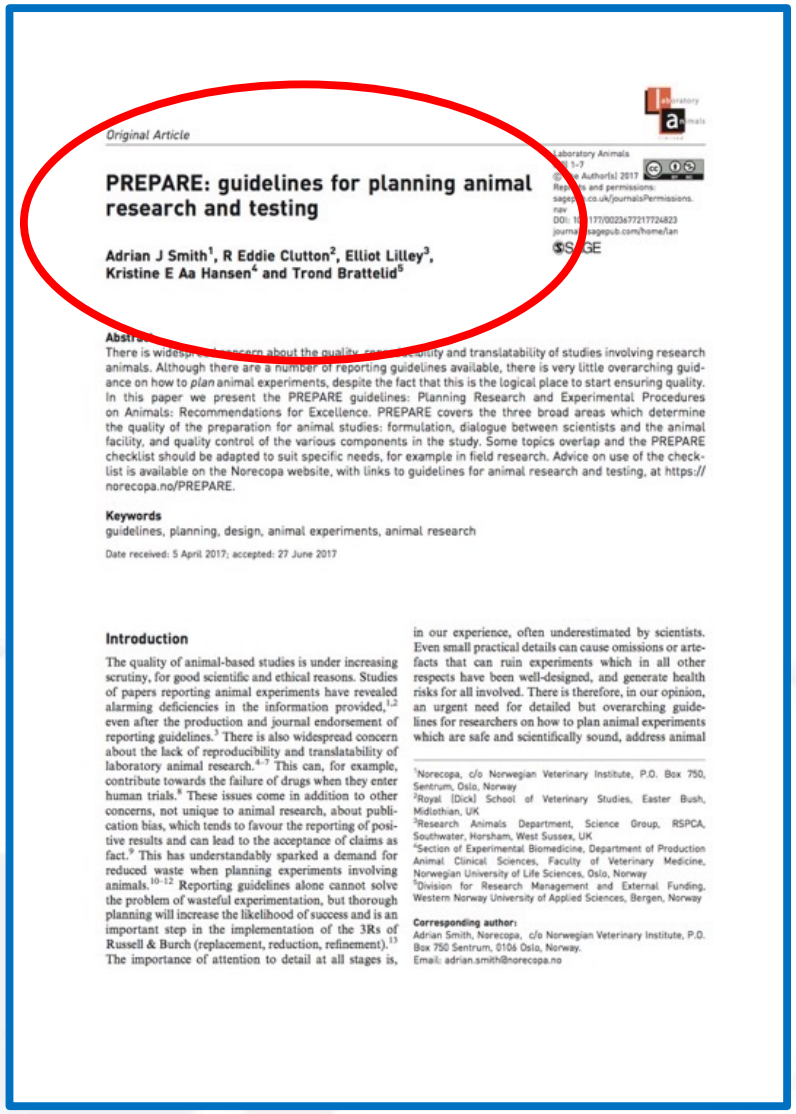
AAALAC Position Statements

3R Guide database/ef566 (legacy id: 15155)

In connection with its work of accreditation of animal care and use programmes, AAALAC International has issued position statements on a number of key elements in such a programme.

norecopa.no/3r-guide





Original Article

PREPARE: guidelines for planning animal research and testing

Adrian J Smith[1], R Eddie Clutton[2], Elliot Lilley[3], Kristine E Aa Hansen[4] and Trond Brattelid[5]

There is widespread concern about the quality, reproducibility and translatability of studies involving research animals. Although there are a number of reporting guidelines available, there is very little overarching guidance on how to *plan* animal experiments, despite the fact that this is the logical place to start ensuring quality. In this paper we present the PREPARE guidelines: Planning Research and Experimental Procedures on Animals: Recommendations for Excellence. PREPARE covers the three broad areas which determine the quality of the preparation for animal studies: formulation, dialogue between scientists and the animal facility, and quality control of the various components in the study. Some topics overlap and the PREPARE checklist should be adapted to suit specific needs, for example in field research. Advice on use of the checklist is available on the Norecopa website, with links to guidelines for animal research and testing, at https://norecopa.no/PREPARE.

Keywords
guidelines, planning, design, animal experiments, animal research

Date received: 5 April 2017; accepted: 27 June 2017

Introduction

The quality of animal-based studies is under increasing scrutiny, for good scientific and ethical reasons. Studies of papers reporting animal experiments have revealed alarming deficiencies in the information provided,[1,2] even after the production and journal endorsement of reporting guidelines.[3] There is also widespread concern about the lack of reproducibility and translatability of laboratory animal research.[4–7] This can, for example, contribute towards the failure of drugs when they enter human trials.[8] These issues come in addition to other concerns, not unique to animal research, about publication bias, which tends to favour the reporting of positive results and can lead to the acceptance of claims as fact.[9] This has understandably sparked a demand for reduced waste when planning experiments involving animals.[10–12] Reporting guidelines alone cannot solve the problem of wasteful experimentation, but thorough planning will increase the likelihood of success and is an important step in the implementation of the 3Rs of Russell & Burch (replacement, reduction, refinement).[13] The importance of attention to detail at all stages is, in our experience, often underestimated by scientists. Even small practical details can cause omissions or artefacts that can ruin experiments which in all other respects have been well-designed, and generate health risks for all involved. There is therefore, in our opinion, an urgent need for detailed but overarching guidelines for researchers on how to plan animal experiments which are safe and scientifically sound, address animal

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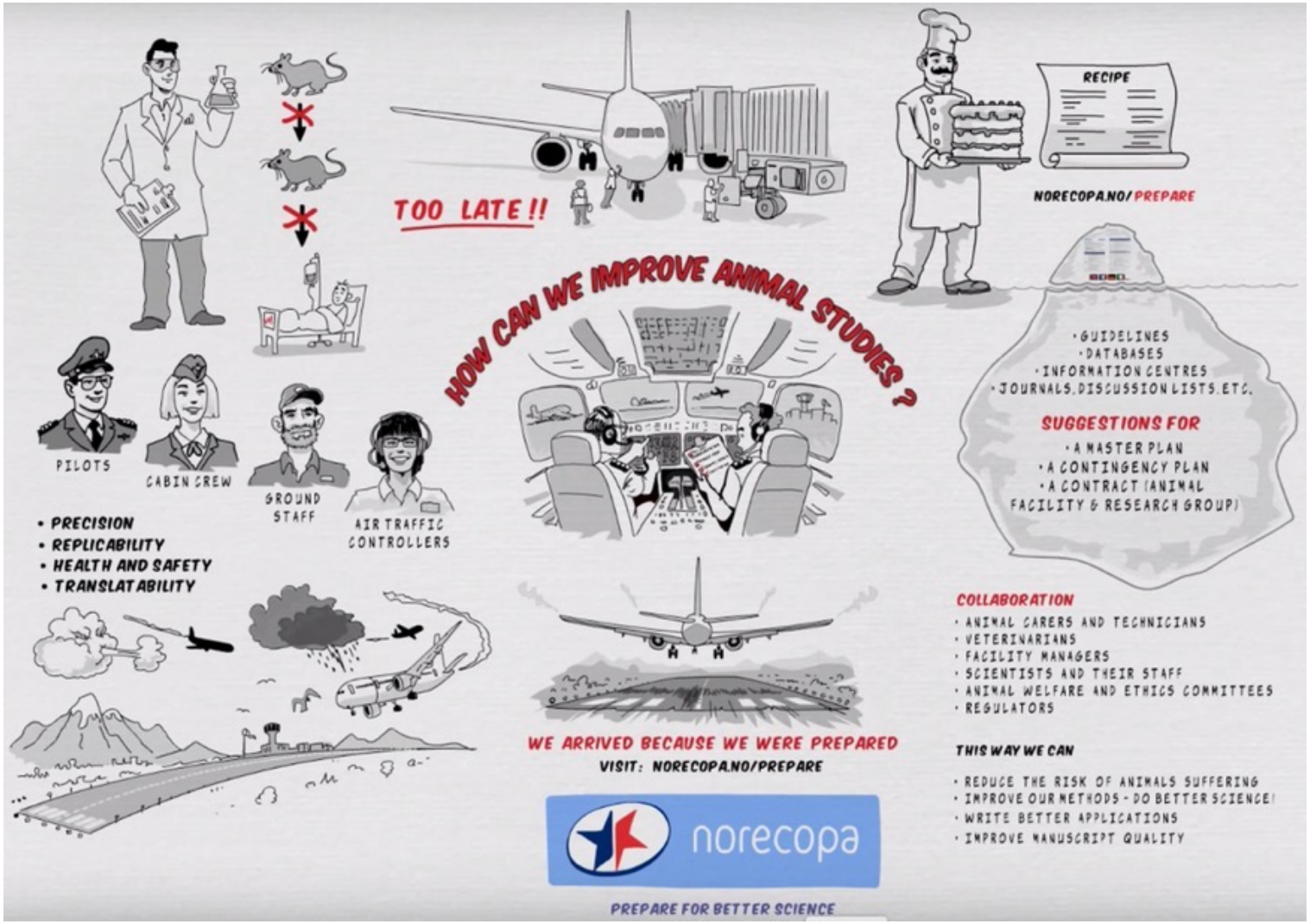
https://doi.org/10.1177/0023677217724823

Over 28,000 views/downloads from the journal website so far

Also downloadable from
norecopa.no/PREPARE



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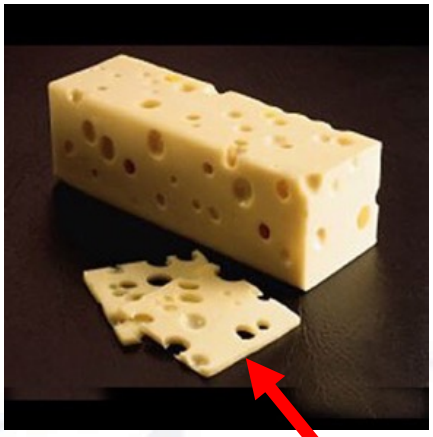
3-minute whiteboard film

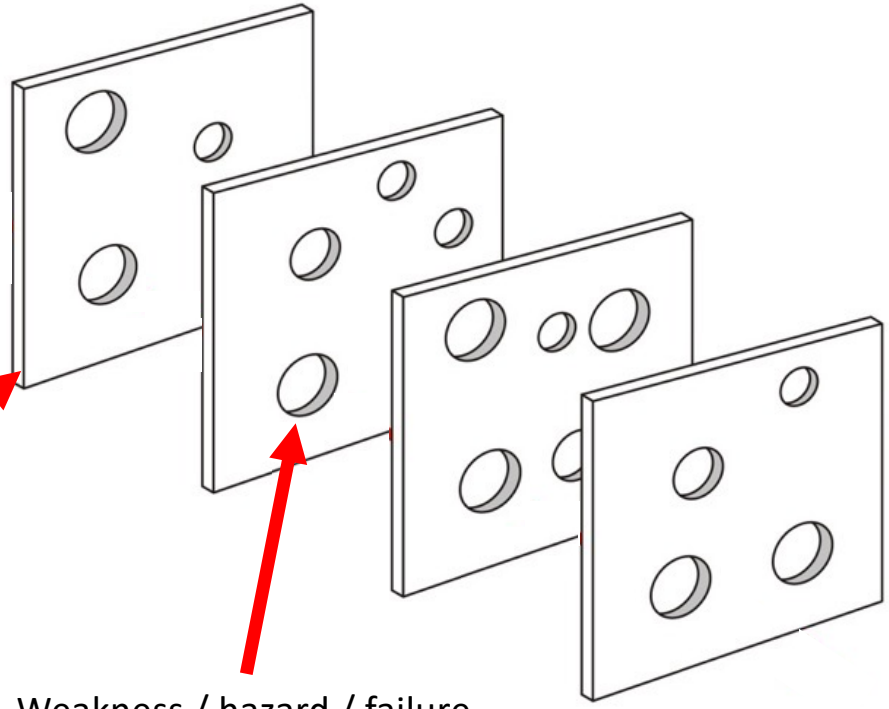
Emphasis on

- ✓ Collaboration (scientists/facility)
- ✓ Checklists
- ✓ Quality assurance
- ✓ Contingency plans

Threat and Error Management: attending to the small issues before they create a large one



eaugallecheese.com/Swiss-Cheese

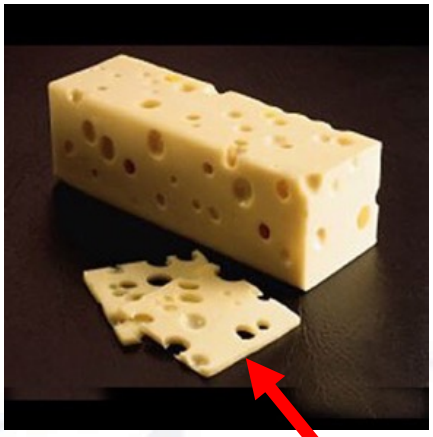


"Layer of defence"
or redundancy

Weakness / hazard / failure

wikipedia.org/wiki/Swiss_cheese_model

Threat and Error Management: attending to the small issues before they create a large one



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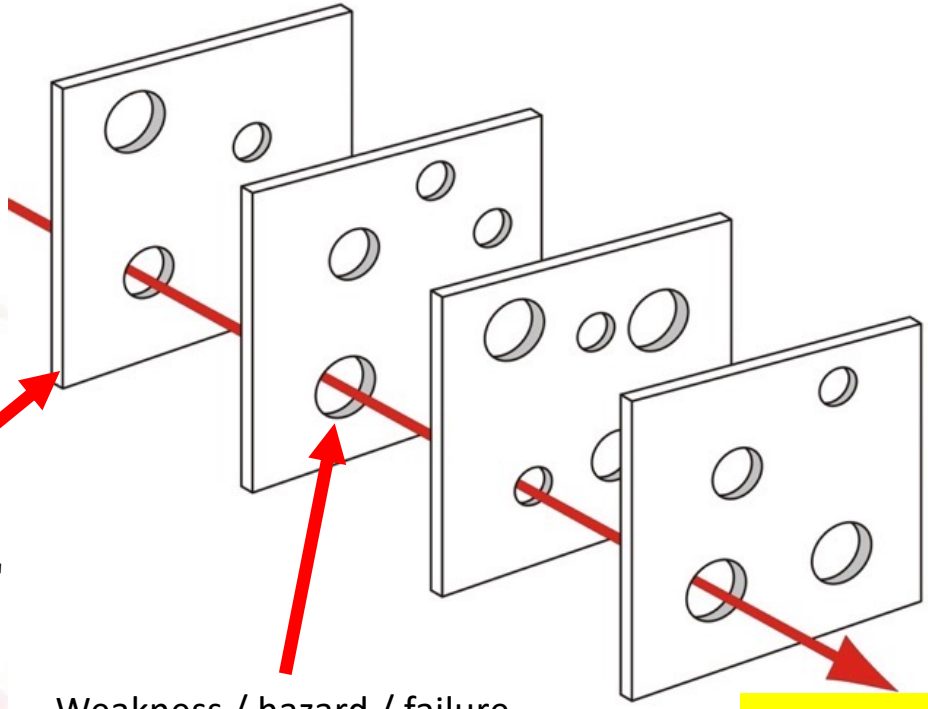


"Layer of defence"
or redundancy

Weakness / hazard / failure

Major incident

wikipedia.org/wiki/Swiss_cheese_model

Quality assurance and a culture of care at all levels in the animal facility

- SOPs describing good techniques, carried out by competent operators
- Checklist ("contract") between researcher and the facility
- The AAALAC Program Description template* can be a good overall performance checklist
  - Institutional policies on animal care and use
  - Animal environment, housing and management
  - Veterinary care
  - Physical plant
- A Master Plan as a weekly checklist for the whole facility during the year

*www.aaalac.org/programdesc/index.cfm

Photo: Naouel Gharbi
uib.no/en/zffac/54560/housing-system

norecopa.no/prepare/6-facility-evaluation/6a/general-principles

Contingency Plans for when things go wrong, based upon risk assessment

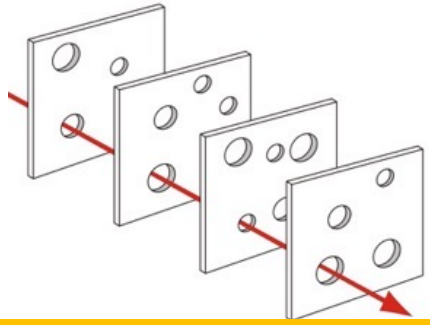
- Access to emergency services (police, fire, medical and veterinary help, security guards, personnel transport in cases of acute illness)
- Means of communicati… …s at all levels

Many of these were revised under Covid-19
norecopa.no/be-prepared

- allergic and …

Q&A | Published: 15 April 2020

COVID-19 Q&A: A fish facility down to its core



Temporary staff at weekends and holidays

- … and forms for reporting such injuries
- Firefighting, evacuation of personnel and animals
- Access to specialist services (e.g. ventilation system, plumbing, electrical installations, suppliers of equipment)
- Routines in cases of power failure, water leaks and (if applicable) natural disasters such as flooding
- Routines for emergency killing of animals
- Routines in cases of threats to the facility or personnel

https://norecopa.no/prepare/6-facility-evaluation/master-plan-and-sops/contingency-plan

PREPARE:

Planning **R**esearch and **E**xperimental **P**rocedures on **A**nimals: **R**ecommendations for **E**xcellence

PREPARE covers 15 topics:

Items in pink are not typically highlighted in reporting guidelines

Formulation of the study

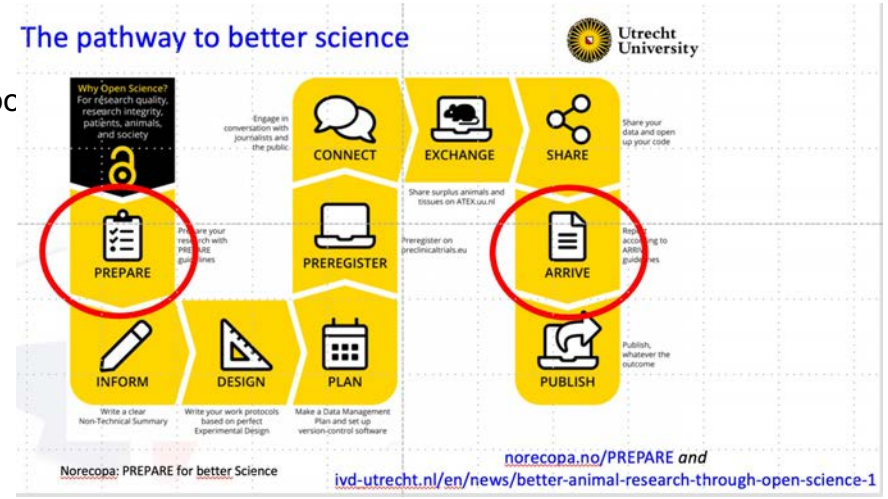
1. Literature searches
2. Legal issues
3. Ethical issues, harm-benefit assessment and humane endpc
4. Experimental design and statistical analysis

Dialogue between scientists and the animal facility

5. Objectives and timescale, funding and division of labour
6. Facility evaluation
7. Education and training
8. Health risks, waste disposal and decontamination

Methods

9. Test substances and procedures
10. Experimental animals
11. Quarantine and health monitoring
12. Housing and husbandry
13. Experimental procedures
14. Humane killing, release, reuse or rehoming
15. Necropsy



reddit.com

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norecopa.no/PREPARE/prepare-checklist

PREPARE

The PREPARE Guidelines Checklist

Planning Research and Experimental Procedures on Animals: Recommendations for Excellence

Adrian J Smith[a], R. Eddie Clutton[b], Elliot Lilley[c], Kristine E. Aa. Hansen[d] & Trond Bratteli[d]

[a]Norecopa, c/o Norwegian Veterinary Institute, P.O. Box 750 Sentrum, 0106 Oslo, Norway; [b]Royal (Dick) School of Veterinary Studies, Easter Bush, Midlothian, EH25 9RG, U.K.; [c]Research Animals Department, Science Group, RSPCA, Wilberforce Way, Southwater, Horsham, West Sussex, RH13 9RS, U.K.; [d]Section of Experimental Biomedicine, Department of Production Animal Clinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, P.O. Box 8146 Dep., 0033 Oslo, Norway; [e]Division for Research Management and External Funding, Western Norway University of Applied Sciences, 5020 Bergen, Norway.

PREPARE[1] consists of planning guidelines which are complementary to reporting guidelines [illegible]. PREPARE covers the three broad areas which determine the quality of [illegible]:

1. Formulation of the study
2. Dialogue bet[illegible]

[illegible] evolve as more species- and situation-specific guidelines are produced, and [illegible] Laboratory Animal Science progresses. [illegible] guidelines is available on the Norecopa [illegible] of animal

Topic	Recommendation
(A) Formulation of the study	
1. Literature searches	☐ Form a clear hypothesis, with primary and secondary outcomes. ☐ Consider the use of systematic reviews. ☐ Decide upon databases and information specialists to be consulted, and construct search terms. ☐ Assess the relevance of the species to be used, its biology and suitability to answer the experimental questions with the least suffering, and its welfare needs. ☐ Assess the reproducibility and translatability of the project.
2. Legal issues	☐ Consider how the research is affected by relevant legislation for animal research and other areas, e.g. animal transport, occupational health and safety. ☐ Locate relevant guidance documents (e.g. EU guidance on project evaluation).
3. Ethical issues, harm-benefit assessment and humane endpoints	☐ Construct a lay summary. ☐ In dialogue with ethics committees, consider whether statements about this type of research have already been produced. ☐ Address the 3Rs (replacement, reduction, refinement) and the 3Ss (good science, good sense, good sensibilities). ☐ Consider pre-registration and the publication of negative results. ☐ Perform a harm-benefit assessment and justify any likely animal harm. ☐ Discuss the learning objectives, if the animal use is for educational or training purposes. ☐ Allocate a severity classification to the project. ☐ Define objective, easily measurable and unequivocal humane endpoints. ☐ Discuss the justification, if any, for death as an end-point.
4. Experimental design and statistical analysis	☐ Consider pilot studies, statistical power and significance levels. ☐ Define the experimental unit and decide upon animal numbers. ☐ Choose methods of randomisation, prevent observer bias, and decide upon inclusion and exclusion criteria.

Topic	Recommendation
(B) Dialogue between scientists and the animal facility	
5. Objectives and timescale, funding and division of labour	☐ Arrange meetings with all relevant staff when early plans for the project exist. ☐ Construct an approximate timescale for the project, indicating the need for assistance with preparation, animal care, procedures and waste disposal/decontamination. ☐ Discuss and disclose all expected and potential costs. ☐ Construct a detailed plan for division of labour and expenses at all stages of the study.
[illegible]	[illegible]sical inspection of the facilities, to evaluate building and equipment standards and needs. [illegible]g levels at times of extra risk.
[illegible]	[illegible]ent competence of staff members and the need for further education or training prior
6. Health risks, waste disposal and decontamination	☐ Perform a risk assessment, in collaboration with the animal facility, for all persons and animals affected directly or indirectly by the study. ☐ Assess, and if necessary produce, specific guidance for all stages of the project. ☐ Discuss means for containment, decontamination, and disposal of all items in the study.
(C) Quality control of the components in the study	
9. Test substances and procedures	☐ Provide as much information as possible about test substances. ☐ Consider the feasibility and validity of test procedures and the skills needed to perform them.
10. Experimental animals	☐ Decide upon the characteristics of the animals that are essential for the study and for reporting. ☐ Avoid generation of surplus animals.
11. Quarantine and health monitoring	☐ Discuss the animals' likely health status, any needs for transport, quarantine and isolation, health monitoring and consequences for the personnel.
12. Housing and husbandry	☐ Attend to the animals' specific instincts and needs, in collaboration with expert staff. ☐ Discuss acclimatization, optimal housing conditions and procedures, environmental factors and any experimental limitations on these (e.g. food deprivation, solitary housing).
13. Experimental procedures	☐ Develop refined procedures for capture, immobilisation, marking, and release or rehoming. ☐ Develop refined procedures for substance administration, sampling, sedation and anaesthesia, surgery and other techniques.
14. Humane killing, release, reuse or rehoming	☐ Consult relevant legislation and guidelines well in advance of the study. ☐ Define primary and emergency methods for humane killing. ☐ Assess the competence of those who may have to perform these tasks.
15. Necropsy	☐ Construct a systematic plan for all stages of necropsy, including location, and identification of all animals and samples.

References

1. Smith AJ, Clutton RE, Lilley E, Hansen KEA & Brattelid T. PREPARE: Guidelines for Planning Animal Research and Testing. *Laboratory Animals*. 2017. DOI: 10.1177/0023677217724823.
2. Kilkenny C, Browne WJ, Cuthill IC *et al.* Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLoS Biology*. 2010; DOI: 10.1371/journal.pbio.1000412.

Further information
https://norecopa.no/PREPARE / post@norecopa.no / @norecopa

Animal welfare and Three Rs!

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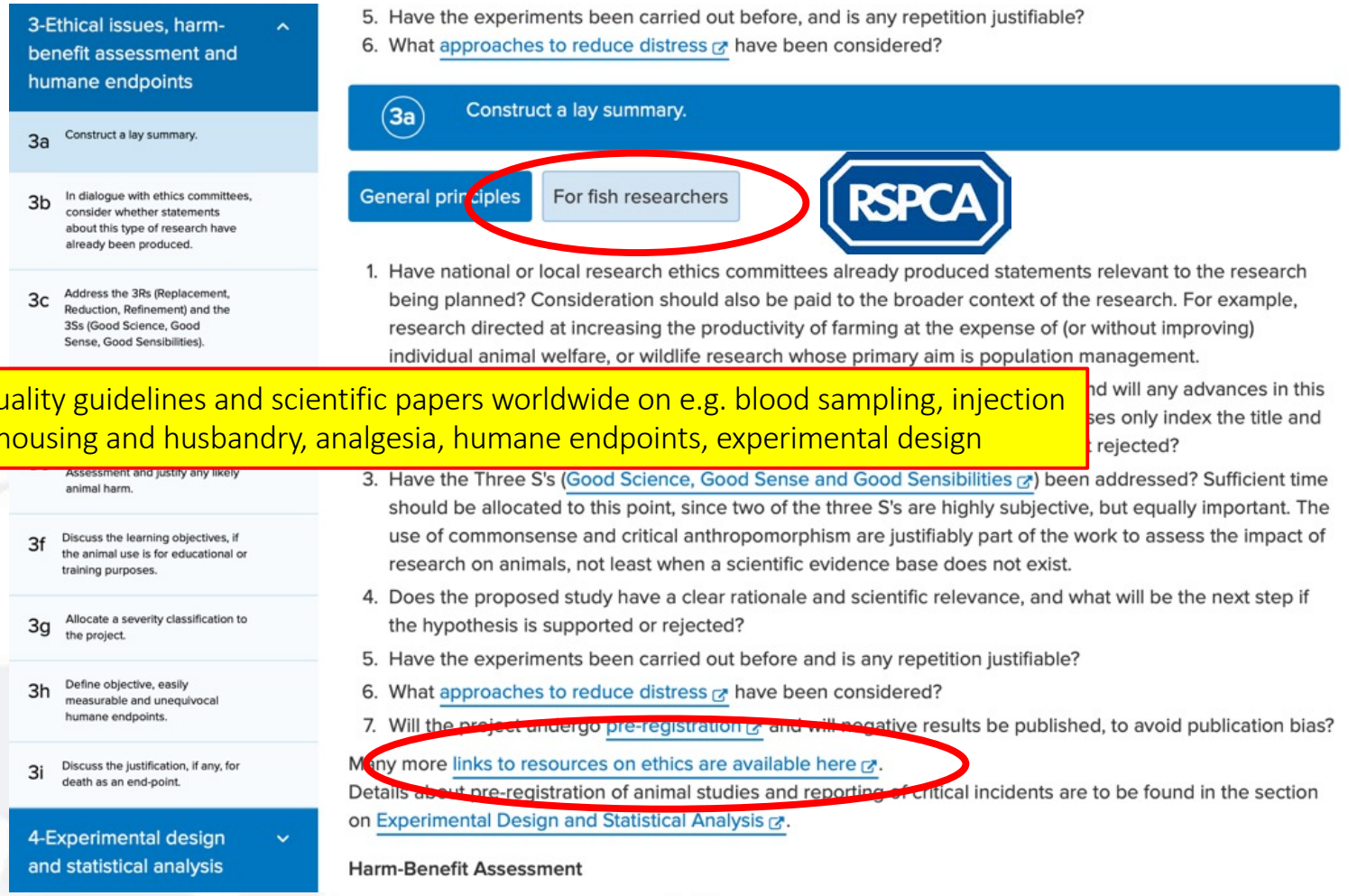
norecopa.no/PREPARE



Links to quality guidelines and scientific papers worldwide on e.g. blood sampling, injection volumes, housing and husbandry, analgesia, humane endpoints, experimental design

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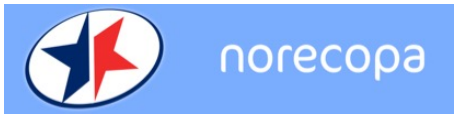
norecopa.no/PREPARE



1c Decide upon databases and information specialists to be consulted, and construct search terms.

General principles | For fish researchers

The editors and chapter authors of textbooks about fish are obvious candidates to be information specialists when designing fish experiments. Other sources include:

- The Zebrafish Information Network (ZFIN) database on model organisms and their publications
- EBSCO *Fish, Fisheries and Aquatic Biodiversity Worldwide* (FFAB) database
- FishBase - a global information system on fin fishes
- SeaLifeBase
- Fish Pathogens Database
- Aquatic Sciences and Fisheries Abstracts (ASFA)
- AGRIS
- ECOTOX
- The Alternatives section in the part of the Norecopa website on fish
- Fishwise Professional database
- An overview of global marine databases and resource centres

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norecopa

1a Form a clear hypothesis, with primary and secondary outcomes.

General principles | For fish researchers

There are approx. 100 textbooks about the care and use of fish in research in the TextBase database, which may be helpful when deciding upon the hypotheses in a fish experiment. The more general books include:

- The Laboratory Fish (ed. Ostrander, 2000)
- The Laboratory Zebrafish (Harper & Lawrence, 2010)
- Guidance on the housing and care of Zebrafish (*Danio rerio*) (Reed & Jennings, 2010)
- CCAC Guidelines: Zebrafish and other small, warm-water laboratory fish (2020)
- CCAC Guidelines on the Care and Use of Fish in Research, Teaching and Testing (2005)
- The Welfare of Fish (eds. Kristiansen *et al.*, 2020)
- The Physiology of Fishes (eds. Currie & Evans, 2020)

Books on more specific subjects are mentioned under the appropriate sections in PREPARE.

TextBase:

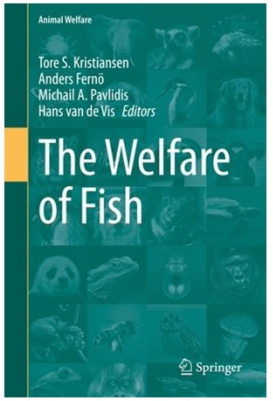
1,500 books related to LAS:

norecopa.no/textbase

The Welfare of Fish

By Tore S. Kristiansen, Anders Fernö, Michail A. Pavlidis & Hans van de Vis

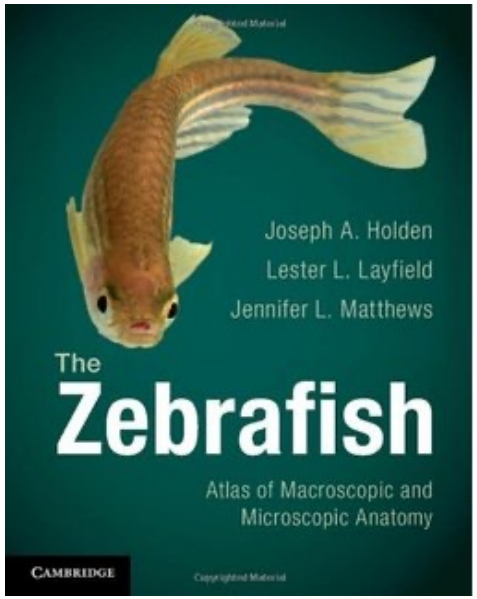
Record number: ef2a0

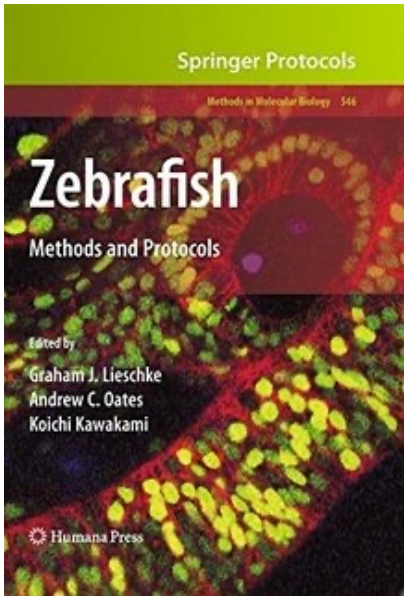


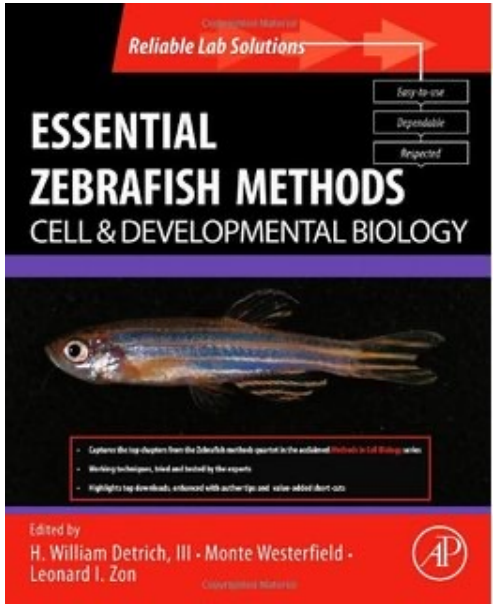
This book investigates how fish experience their lives, their amazing senses and abilities, and how human actions impact their quality of life. The authors examine the concept of fish welfare and the scientific knowledge behind the inclusion of fish within the moral circle, and how this knowledge can change the way we treat fish in the future. In many countries fish are already protected by animal welfare legislation in the same way as mammals, but in practice there is still a major gap between how we ethically view these groups and how we actually treat them. The poor treatment of fish represents a massive animal welfare problem in aquaculture and fisheries, both in terms of the number of animals affected and the severity of the welfare issues.

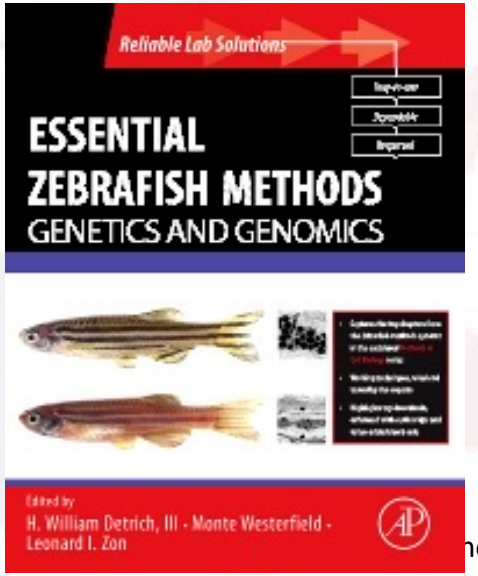
Thanks to its interdisciplinary scope, this book should appeal to professionals, academics and students in the fields of animal welfare, cognition and physiology, as well as fisheries and aquaculture management.

List of chapters:


Joseph A. Holden
Lester L. Layfield
Jennifer L. Matthews
The
Zebrafish
Atlas of Macroscopic and
Microscopic Anatomy
CAMBRIDGE


Springer Protocols
Zebrafish
Methods and Protocols
Edited by
Graham J. Lieschke
Andrew C. Oates
Koichi Kawakami
Humana Press


Reliable Lab Solutions
Easy-to-use
Dependable
Respected
ESSENTIAL
ZEBRAFISH METHODS
CELL & DEVELOPMENTAL BIOLOGY
Edited by
H. William Detrich, III · Monte Westerfield ·
Leonard I. Zon


Reliable Lab Solutions
ESSENTIAL
ZEBRAFISH METHODS
GENETICS AND GENOMICS
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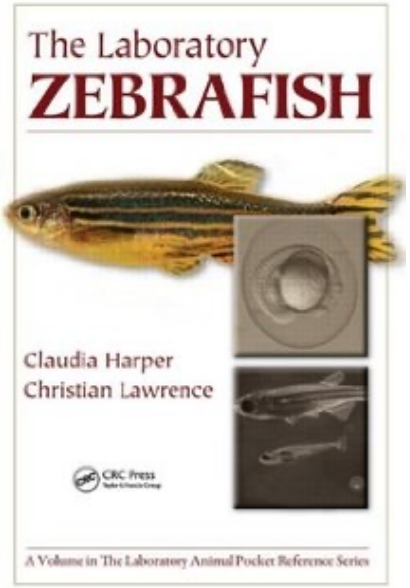
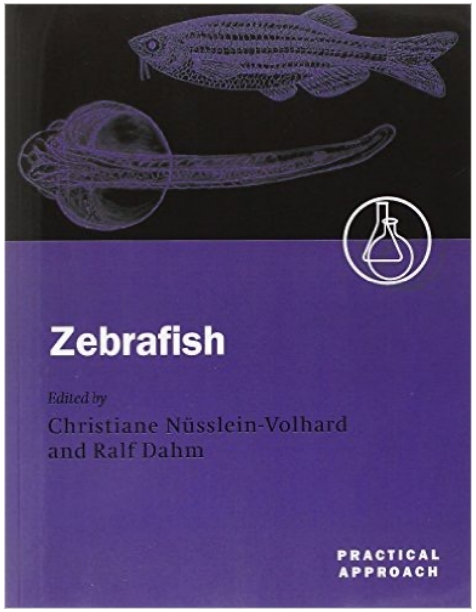
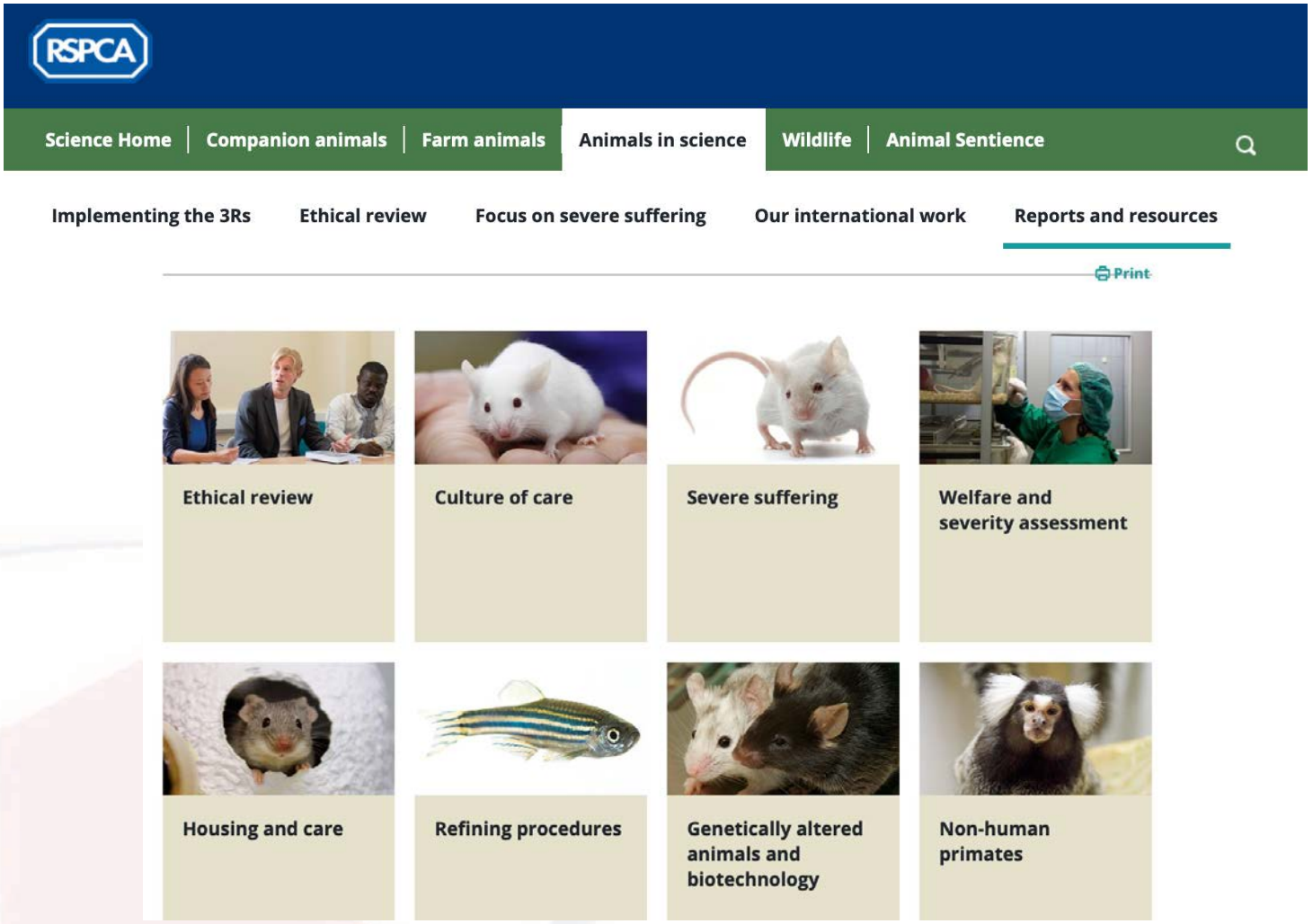
H. William Detrich, III · Monte Westerfield ·
Leonard I. Zon


The Laboratory
ZEBRAFISH
Claudia Harper
Christian Lawrence
CRC Press
A Volume in The Laboratory Animal Pocket Reference Series


Zebrafish
Edited by
Christiane Nüsslein-Volhard
and Ralf Dahm
PRACTICAL
APPROACH

Does anyone have something similar?

Resource hubs



science.rspca.org.uk/sciencegroup/researchanimals/reportsandresources

norecopa.no/meetings

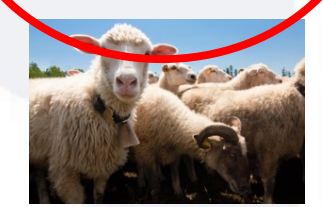
International consensus meetings

Harmonisation of the Care and Use of:

- *Fish (2005)*
- *Wildlife (2008)*
- *Fish (2009)*
- *Agricultural animals (2012)*
- *Wildlife (2017)*

All the presentations and consensus statements on the web: a lasting resource

Pdf files of 80+ presentations held at Norecopa's meetings



Norecopa: PREPARE for better Science

Webinars & Meetings

norecopa.no/meetings/presentations

Care and Use of Fish in Research			
What can Norecopa do for fish researchers?	Adrian Smith	Norecopa	2017
European legislation on environmental enrichment and what this means for fish	Penny Hawkins	RSPCA, UK	2017
Social enrichment and requirements for the tank rearing of Atlantic Salmon	Jonatan Nilsson	Institute of Marine Research, Norway	2016
Social enrichment for Atlantic salmon	Jonaton Nilsson	Institute of Marine Research, Norway	2016
Detection and alleviation of pain in fish	Lynne Sneddon	University of Liverpool, UK	2015
Fish and fish robots: how can they help to understand each other?	Marja Kruusmaa	Tallinn University of Technology	2014
Experiences from the inspection of fish research facilities in the UK	Kathy Ryder	Home Office Inspectorate, UK	2009
Research needs within fish welfare - presentation of a report on the status in Norway	Trond Brattelid	NIFES, Norway	2009
Do we have practical positive and negative welfare indicators for fish that we can use in a research/farm setting?	John Avizienius	RSPCA, UK	2009
Health monitoring of fish used in research - progress?	Anne Ramstad	VESO Vikan, Norway	2009
Husbandry and environmental enrichment - what do fish need and has there been much progress?	Gareth Readman	AstraZeneca, UK	2009
Telemetry in fish - update	Øyvind Aas-Hansen	Nofima Marin, Norway	2009
Challenges in fish research: the protectionist's view	Anton Krag	Norwegian Animal Protection Alliance	2009
Update on the EU directive and the CoE convention	Bente Bergersen	Norwegian Food Safety Authority	2009
Global update on guidelines for fish research	Gilly Griffin	CCAC, Canada	2009
Vaccine testing: can we reduce fish numbers and/or avoid fish use?	Kjetil Fyrand	PHARMAQ AS, Norway	2009

Webinars & Meetings

norecopa.no/meetings/presentations

Guidelines for anaesthesia and analgesia of fish (guidelines)	Gidona Goodman	Edinburgh University, UK	2009
Proposed revisions to methods of killing fish used in research in the UK	Robert Hubrecht	UFAW, UK	2009
The challenges of using humane endpoints in fish research	Kathy Ryder	Home Office Inspectorate, UK	2009
An overview of existing guidelines for handling, bleeding and administration techniques (Guidelines)	Penny Hawkins	RSPCA, UK	2009
The challenge of arranging accredited courses for fish researchers and technicians	Aurora Brønstad	University of Bergen, Norway	2009
Field work and lab studies: two sets of standards?	Grete Bæverfjord	Nofima Marin, Norway	2009

Norecopa's 3R Prize – has highlighted fish 3R-research










norecopa.no/about-norecopa/3r-prize

- **European legislation**

European Convention ETS123 for the Protection of Vertebrate Animals Used for Scientific or Other Purposes (1986)

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 (Part B) elaborated by the Group of Experts

Part B for fish is not available!
Part B for other species is available from FELASA:

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

» 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.

coe.int/en/web/conventions/full-list?module=treaty-detail&treatynum=123

felasa.eu/about-us/library/

rm.coe.int/168007a445

EU Directive 2010/63, Annex III, 'Species specific' section on Fish (all species!)

The water flow shall be appropriate ? to enable fish to swim correctly and to maintain normal behaviour.

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. ?

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. ?

'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'. (ETS 123)

EU Directive 2010/63, Annex III, 'Species specific' section on Fish (all species!)

11. 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 photo-period. 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.

Detailed stocking densities for

- *Aquatic urodeles (salamanders, newts)*
- *Aquatic anurans (frogs, toads*
- *Semi-aquatic anurans*
- *Semi-terrestrial anurans*
- *Arboreal anurans*
- *Aquatic chelonians (tortoises, turtles)*
- *…*

10. Reptiles

Table 10.1.

Aquatic chelonians

Body length (*) (cm)	Minimum water surface area (cm^2)	Minimum water surface area for each additional animal in group holding (cm^2)	Minimum water depth (cm)	Date referred to in Article 33(2)
up to 5	600	100	10	1 January 2017
Over 5 to 10	1 600	300	15	
Over 10 to 15	3 500	600	20	
Over 15 to 20	6 000	1 200	30	
Over 20 to 30	10 000	2 000	35	
Over 30	20 000	5 000	40	

(*) Measured in a straight line from the front edge to the back edge of the shell.

There is more about zebra finches than zebra fish...

CONSULTATION | Closed

SCHEER - Call for information in support of a targeted revision of Annexes III and IV of Directive 2010/63/EU on the protection of animals used for scientific purposes

PAGE CONTENTS

Details

Status	CLOSED
Opening date	25 January 2023
Deadline	27 February 2023, 23:59 (CET)

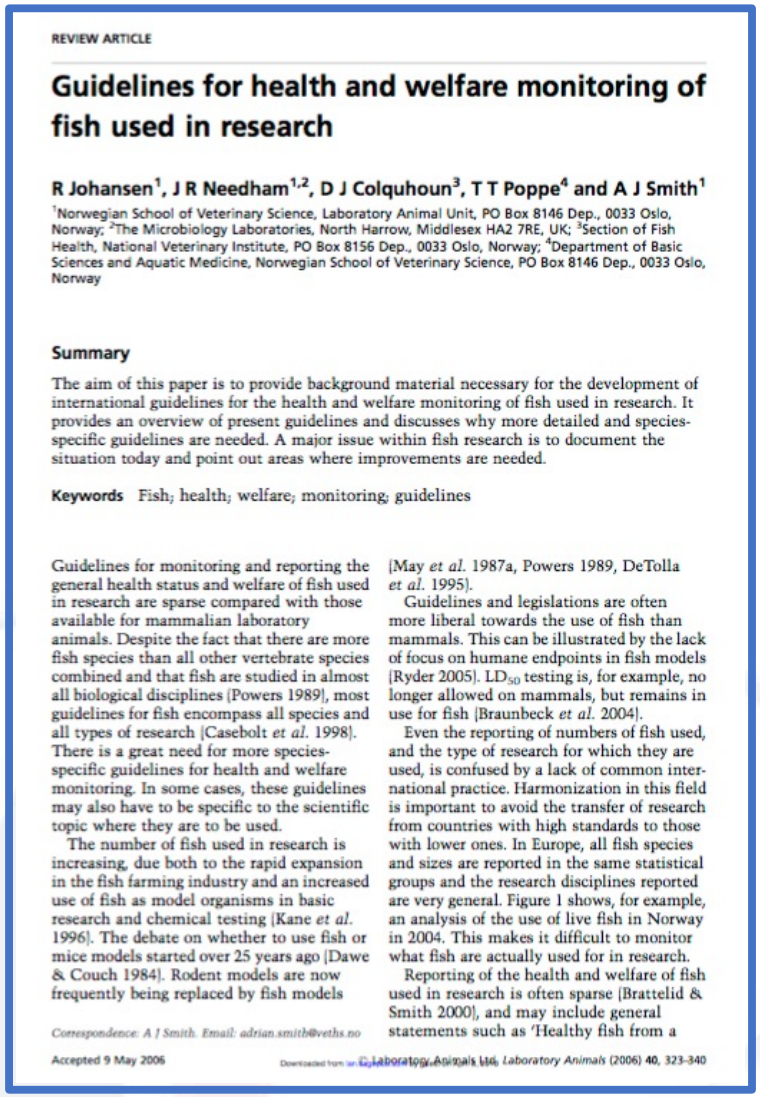
- standards of accommodation and care to safeguard the welfare of zebrafish when kept in captivity for scientific purposes: key parameters and their respective ranges for the housing; maximum stocking densities

- methods of killing appropriate for animal bred, supplied or used in scientific procedures: conditions and limitations of the hypothermic shock

https://health.ec.europa.eu/consultations/scheer-call-information-support-targeted-revision-annexes-iii-and-iv-directive-201063eu-protection_en

ec.europa.eu/animals-in-science

- **Health monitoring**



REVIEW ARTICLE

Guidelines for health and welfare monitoring of fish used in research

R Johansen[1], J R Needham[1,2], D J Colquhoun[3], T T Poppe[4] and A J Smith[1]

[1]Norwegian School of Veterinary Science, Laboratory Animal Unit, PO Box 8146 Dep., 0033 Oslo, Norway; [2]The Microbiology Laboratories, North Harrow, Middlesex HA2 7RE, UK; [3]Section of Fish Health, National Veterinary Institute, PO Box 8156 Dep., 0033 Oslo, Norway; [4]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_{50} 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

Correspondence: A J Smith. Email: adrian.smith@veths.no

Accepted 9 May 2006

Needs to be followed up by species-specific guidelines

http://lan.sagepub.com/content/40/4/323.full.pdf

Recommendations for Health Monitoring and Reporting for Zebrafish Research Facilities

Chereen Collymore, Marcus J. Crim, and Christine Lieggi

Published Online: 28 Jun 2016 | https://doi.org/10.1089/zeb.2015.1210

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.

ZEBRAFISH
Volume 00, Number 00, 2016

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,[1] Joana F. Monteiro,[1] Maria Vito,[1] David Weintraub,[2]
Joana Almeida,[1] and Ana Catarina Certal[1]

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.

http://online.liebertpub.com/doi/pdfplus/10.1089/zeb.2015.1198

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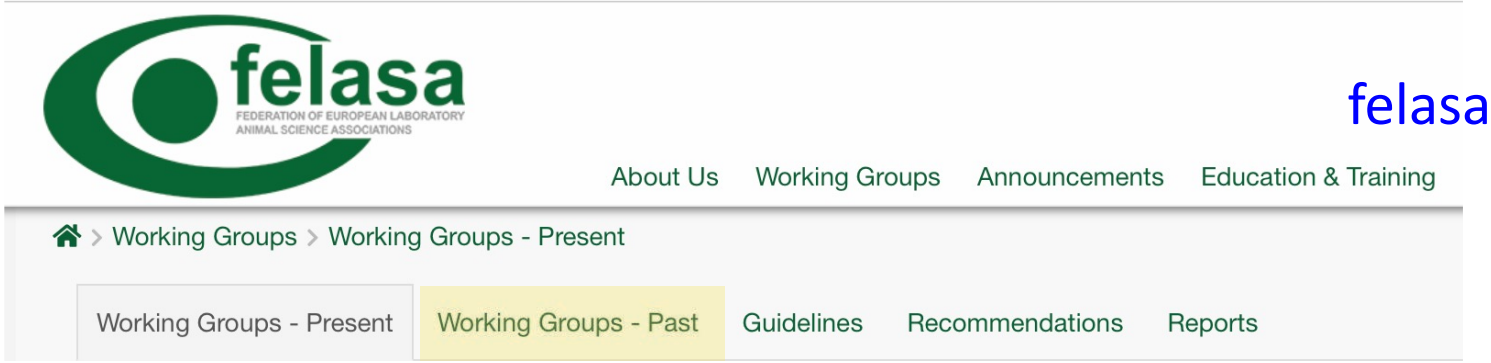

felasa.eu/working-groups

Health monitoring of fish in research (FELASA/AALAS, JP Mocho)

PART 1: Establish the Health Status

FELASA-AALAS Recommendations for Monitoring and Reporting of Laboratory Fish Diseases and Health Status, with an Emphasis on Zebrafish (Danio Rerio). Mocho JP, Collymore C, Farmer SC, Leguay E, Murray KN, Pereira N. Comp Med. 2022. 72(3):127-148. doi: 10.30802/AALAS-CM-22-000034.

PART 2: Exchange fish safely

FELASA-AALAS Recommendations for Biosecurity in an Aquatic Facility, Including Prevention of Zoonosis, Introduction of New Fish Colonies, and Quarantine. Mocho JP, Collymore C, Farmer SC, Leguay E, Murray KN, Pereira N. Comp Med. 2022. 72(3):149-168. doi: 10.30802/AALAS-CM-22-000042.

Survey

A FELASA Working Group Survey on Fish Species Used for Research, Methods of Euthanasia, Health Monitoring, and Biosecurity in Europe, North America, and Oceania. Mocho, J.-P. and von Krogh, K. Biology 2022, 11, 1259. https://doi.org/10.3390/biology11091259

felasa.eu/working-groups/past/id/5

- **Procedures**

norecopa.no/education-training/films-and-slide-shows

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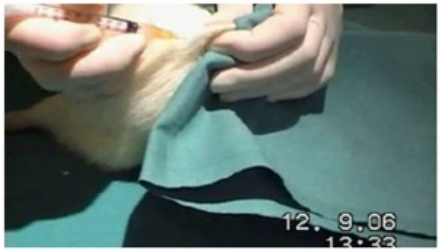
Rat s.c. injection
Norecopa | 1,380 views

Rat i.p. injection (method 2)
Norecopa | 1,280 views

Anatomia de la rata
Norecopa | 977 views

Testing anaesthetic depth in the chicken
Norecopa | 598 views

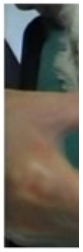
Blood collection from the saphenous vein in the mouse
Norecopa | 6,777 views



Subcutaneous injection in the rat - Technique 1
Norecopa | 2,249 views

Blood san
Norecop

Blood san
Norecop



Blood san
Norecop

Fish?

Blood sa pig

Intravenous injection in a rabbit
Norecopa | 2,025 views

Lifting a rabbit
Norecopa | 2,420 views

Subcutaneous injection in the rabbit
Norecopa | 1,479 views

Subcutaneous injection in the chicken
Norecopa | 1,806 views

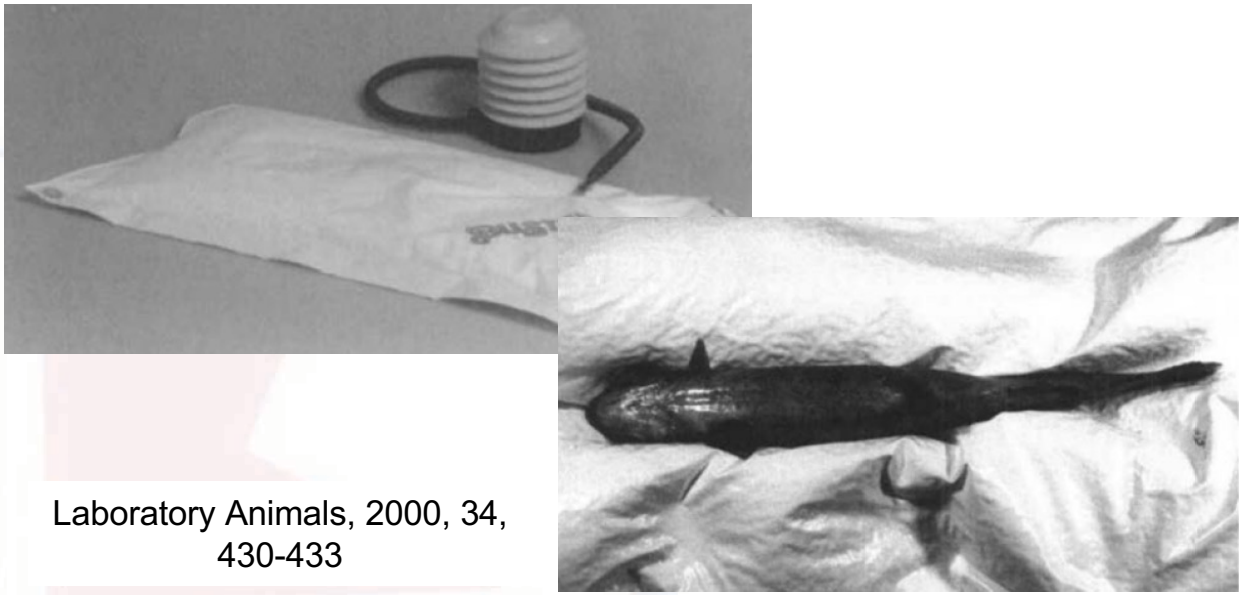
Immobilisation of the rabbit
Norecopa | 2,072 views

Norecopa: PREPARE for better Science

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

Trond Brattelid & Adrian J. Smith

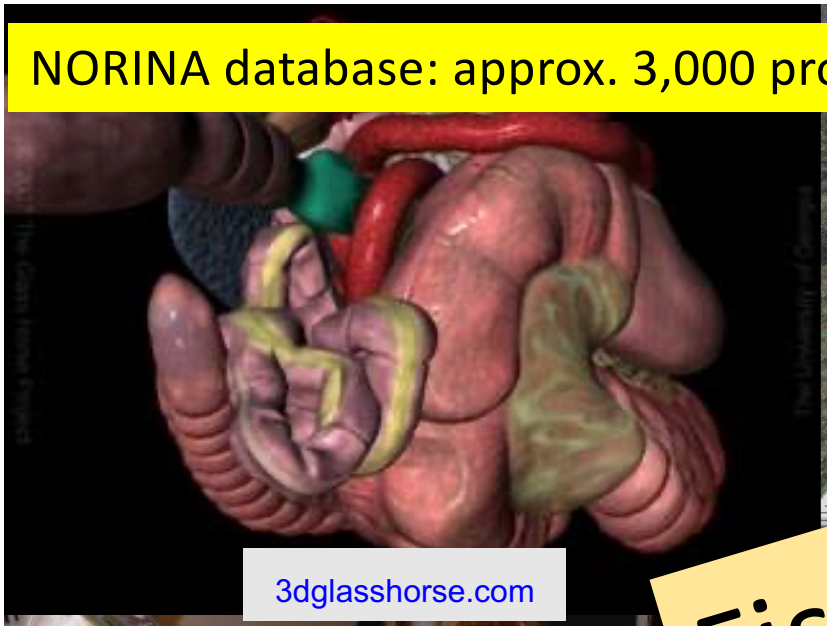
Laboratory Animal Unit, Norwegian School of Veterinary Science, PO Box 8146 Dep., N-0033 Oslo, Norway



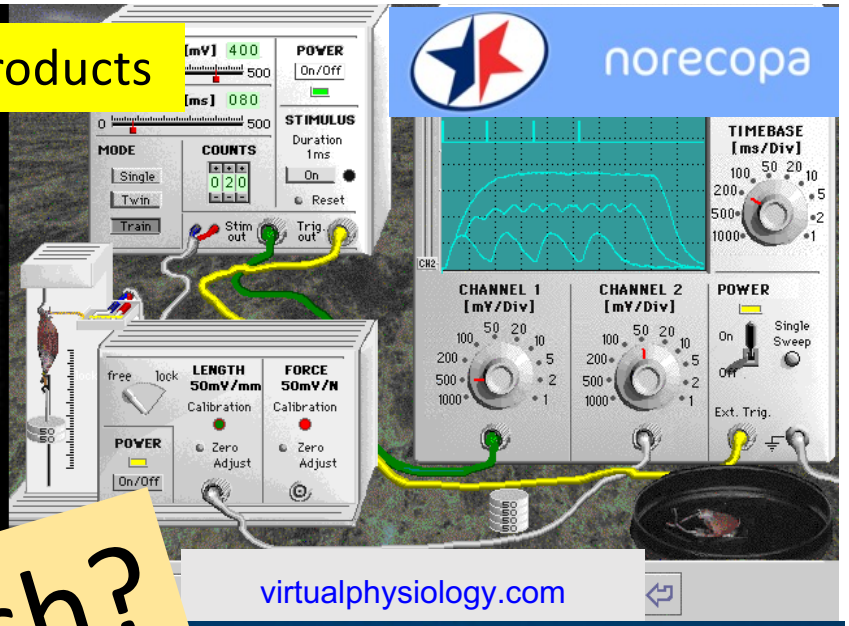
Laboratory Animals, 2000, 34, 430-433

norecopa.no/species/fish/handling

NORINA database: approx. 3,000 products



3dglasshorse.com



virtualphysiology.com

Fish?

rescuecritters.com

limbsandthings.com

RESEARCH ANIMAL TRAINING

researchanimaltraining.com

Articles ⌄ eModules ⌄ Log in

Training resources for animal research

National Legislation (EU1)
Understand the national and international legal and regulatory framework within which projects involving animals are constructed and managed and of the legal responsibilities of the people involved.

Ethics, Animal Welfare and the 3Rs (EU2)
Identify the ethical and welfare issues raised by the use of animals in scientific procedures and understand the basic principles of the 3Rs.

Basic and Appropriate Biology (EU3)
Discover the basic principles of animal behaviour, care, biology and husbandry.

Animal Care, Health and Management (EU4)
Examine information on various aspects of animal health, care and management including, environmental controls, husbandry practices, diet, health status and disease.

Recognition of Pain, Suffering and Distress (EU5)
Identify the normal condition and behaviour of experimental animals and differentiate between a normal animal and one which is showing signs of pain, suffering or distress.

Humane Methods of Killing (EU6.1)
Learn the principles of humane killing including descriptions of the different methods available and information to help you compare the methods permitted to determine the most appropriate method.

Minor Procedures without Anaesthesia (EU7)
An introduction to the theory relating to minor procedures and information about appropriate methods of handling, restraint, appropriate techniques for injection, dosing and sampling relevant to the species.

Anaesthesia for Minor Procedures (EU20)
Guidance and information for individuals who, during their work with animals, will need to apply sedation or short-term anaesthesia for a brief period and mild pain level procedure.

eModules

Fish?

Click to access

eModule – Recognition and Prevention of Pain, Suffering and Distress (EU5)

Click to access

eModule – Humane Methods of Killing (EU6)
ACCESS

Click to access

eModule – Design of procedures and projects (level 1) (EU10)
ACCESS

Click to access

eModule – Design of procedures and projects (level 2) (EU11)
ACCESS

Click to access

…he Severity …ssessment Framework (EU12)
ACCESS

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eModule – Anaesthesia for Minor Procedures (EU20)
ACCESS

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eModule – Pre-Anaesthetic Preparations (EU21-1)
ACCESS

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eModule – Choosing an Anaesthetic (EU21-2)
ACCESS

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eModule – Anaesthetic Monitoring and Intraoperative Care (EU21-3)
ACCESS

Click to access

eModule – Anaesthetic Breathing Systems, Airway Management and Neuromuscular Blocking Agents (EU21-4)
ACCESS

Click to access

eModule – Anaesthetic Management and Preventing Problems (EU21-5)
ACCESS

Click to access

eModule – Post Anaesthetic Care (EU21-6)
ACCESS

Click to access

eModule – Project Evaluation (EU25)

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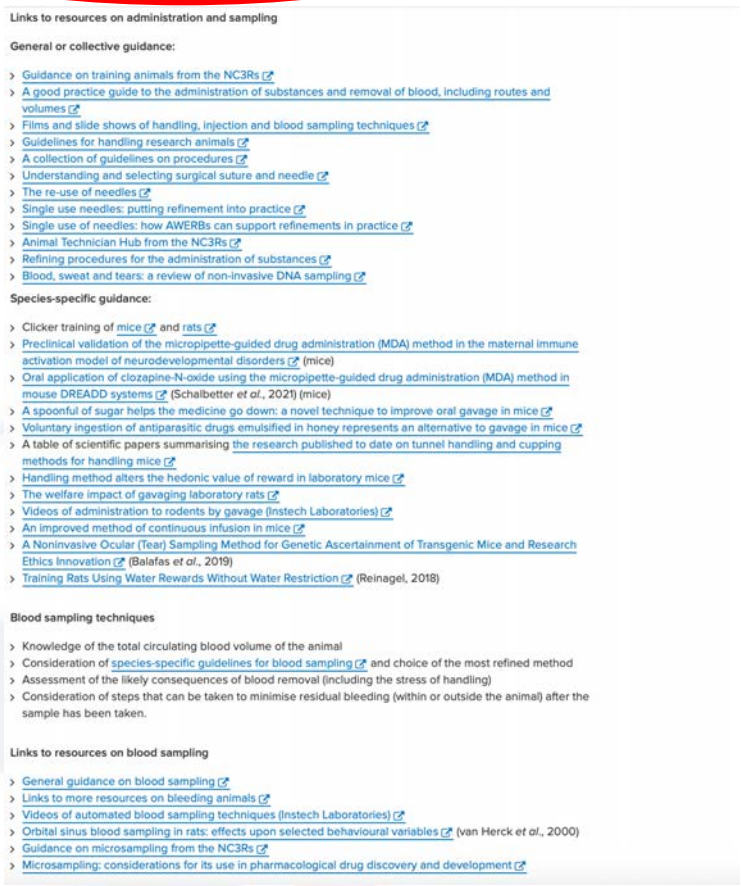
Administration and sampling



Links to resources on administration and sampling

General or collective guidance:

- Guidance on training animals from the NC3Rs
- A good practice guide to the administration of substances and removal of blood, including routes and volumes
- Films and slide shows of handling, injection and blood sampling techniques
- Guidelines for handling research animals
- A collection of guidelines on procedures
- Understanding and selecting surgical suture and needle
- The re-use of needles
- Single use needles: putting refinement into practice
- Single use of needles: how AWERBs can support refinements in practice
- Animal Technician Hub from the NC3Rs
- Refining procedures for the administration of substances
- Blood, sweat and tears: a review of non-invasive DNA sampling

Species-specific guidance:

- Clicker training of mice and rats
- Preclinical validation of the micropipette-guided drug administration (MDA) method in the maternal immune activation model of neurodevelopmental disorders (mice)
- Oral application of clozapine-N-oxide using the micropipette-guided drug administration (MDA) method in mouse DREADD systems (Schalbetter *et al.*, 2021) (mice)
- A spoonful of sugar helps the medicine go down: a novel technique to improve oral gavage in mice
- Voluntary ingestion of antiparasitic drugs emulsified in honey represents an alternative to gavage in mice
- A table of scientific papers summarising the research published to date on tunnel handling and cupping methods for handling mice
- Handling method alters the hedonic value of reward in laboratory mice
- The welfare impact of gavaging laboratory rats
- Videos of administration to rodents by gavage (Instech Laboratories)
- An improved method of continuous infusion in mice
- A Noninvasive Ocular (Tear) Sampling Method for Genetic Ascertainment of Transgenic Mice and Research Ethics Innovation (Balafas *et al.*, 2019)
- Training Rats Using Water Rewards Without Water Restriction (Reinagel, 2018)

Blood sampling techniques

- Knowledge of the total circulating blood volume of the animal
- Consideration of species-specific guidelines for blood sampling and choice of the most refined method
- Assessment of the likely consequences of blood removal (including the stress of handling)
- Consideration of steps that can be taken to minimise residual bleeding (within or outside the animal) after the sample has been taken.

Links to resources on blood sampling

- General guidance on blood sampling
- Links to more resources on bleeding animals
- Videos of automated blood sampling techniques (Instech Laboratories)
- Orbital sinus blood sampling in rats: effects upon selected behavioural variables (van Herck *et al.*, 2000)
- Guidance on microsampling from the NC3Rs
- Microsampling: considerations for its use in pharmacological drug discovery and development

General principles | **For fish researchers**

See also:

- Anaesthesia of fish
- Anaesthesia and analgesia of zebrafish specifically
- Pain and suffering in fish
- Sampling of fish
- Surgery on fish

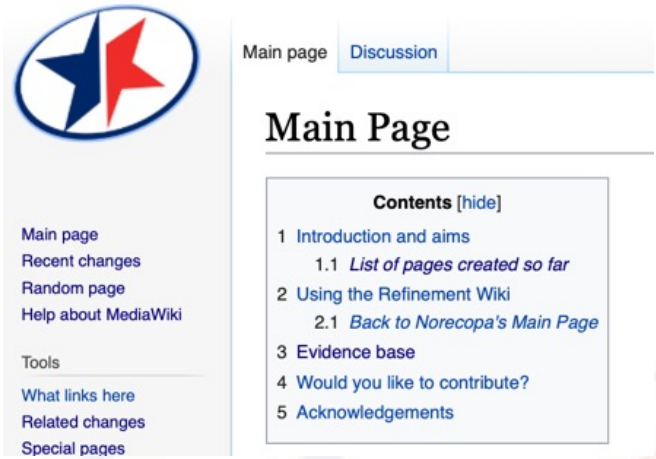
The RSPCA has arranged two meetings entitled *Focus on Fish*, the first one on 23 February 2021 and the second on 23 February 2022. These contained several relevant presentations. The 2021 meeting had a session on Fish Welfare, including fish behaviour and using analgesia in fishes.

The Norwegian National Committee has sent a recommendation to the regulatory authorities to validate a method for obtaining DNA from fish using skin and mucus material. This is described here in Norwegian.

Resources

- An overview of existing guidelines for handling, bleeding, administration and identification techniques in fish (Penny Hawkins, 2009)
- Skin swabbing for DNA sampling of zebrafish (NC3Rs site)
- Refined methods of DNA collection in fishes (summary by the RSPCA, 2021)
- Breacker *et al* (2017) A low-cost method of skin-swabbing for the collection of DNA samples from small laboratory fish. *Zebrafish*, 14(1): 35-41. A short summary is also available.
- Ding *et al*. (2023): Comparative assessment of plasma cortisol and fecal corticoid metabolites (FCM) of Atlantic salmon (*Salmo salar* L.) subjected to acute- and long-term stress
- Tilley *et al* (2020): Skin swabbing is a refined technique to collect DNA from model fish species. *Scientific Reports*, 10, 18212.
- Roques *et al* (2010): Tailfin clipping, a painful procedure: Studies on Nile tilapia and common carp. *Physiology & Behavior*, 101(4): 533-540.
- A collection of training templates (CCAC website)
- Black (2000): Collection of Body Fluids, in *The Laboratory Fish*, Academic Press
- Ganassin, Schirmer & Bols (2000): Cell and Tissue Culture, in *The Laboratory Fish*, Academic Press
- Black (2000): Routes of Administration for Chemical Agents, in *The Laboratory Fish*, Academic Press
- Johnson (2000): Surgical Techniques, in *The Laboratory Fish*, Academic Press
- Kleinow (2000): Autoradiography of Fishes, in *The Laboratory Fish*, Academic Press
- Faustino *et al* (2017) Mechanisms of social buffering of fear in zebrafish. *Scientific Reports*, 7: 44329.
- Aerts *et al* (2015) Scales tell a story on the stress history of fish. *PLOS One*, 10(4): e0123411.
- Animal Research Advisory Committee Guidelines on Zebrafish and Zebrafish Facilities from the NIH, USA

The Refinement Wiki



Susanna Louihimies

wiki.norecopa.no

Born from the knowledge that a lot of good ideas on refinement circulate on discussion forums, but never get published.

Designed to be

- a portal for rapid publication and dissemination of these ideas
- a place to identify experts on specific refinement techniques

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Blood sampling of rainbow trout

Poster Title: ***Non-lethal blood sampling from Rainbow trout in the laboratory and in situ***

Co-authors: J. Anderson, S. Pollard, C.D. Tyson, J. Guchardi, D.B.D. Simmons

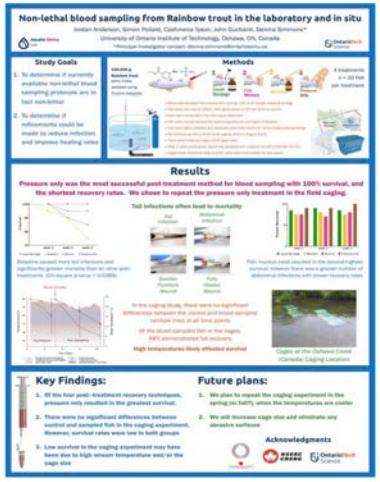
Aquatic Omics Lab, Ontario Tech University, Oshawa, ON, Canada

The use of humane methods in animal research is an internationally recognized priority, but few Environmental Effects Monitoring programs use non-lethal methods with fish, and the ones that do are normally limited to behaviour, morphology and reproduction.

The goal of the study shown in this poster was to determine the impacts and survival of larger bodied fish after sampling small volumes of blood for the purpose of using non-lethal methods in Environment Effects Monitoring.

Overall, we observed 90% survival among all treatments; the most effective approach was the pressure-only (100% survival), while the post-treatment with the largest impact on fish survival was the use of betadine (75% survival).

This study has now been published under Open Access. The paper was part of a larger special topic on non-lethal methods in environmental toxicology.



We plan to repeat this test in the natural environment, and will post more information later.

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Refinement Wiki

- Acclimatisation
- Adrian Smith
- Alphaxalone
- Anaesthesia in neonates
- Analgesia
- Asepsis
- Blood sampling of hamsters
- Blood sampling of pigs
- Blood sampling of rainbow trout
- Breeding strategies for mice
- Clicker training
- Contingency plans
- Decapitation
- Detecting early onset of clinical signs in the mouse model of Covid-19
- Detection of pain and distress in mice
- EMLA cream
- Embryo transfer
- Experimental Autoimmune Encephalomyeltis (EAE)
- Facial expression analysis
- Food crunchers
- General discusson on use of analgesics
- Genotyping mice
- Habituation training
- High-fat diets
- Hot Bead Sterilisers
- Housing nude mice
- Housing research fish
- Humane endpoints
- Hydrodynamic gene delivery
- Intra-ocular injections
- Intranasal administration
- Intraperitoneal injection
- Intraperitoneal pentobarbitone
- Ketamine and alpha-2 agonist combinations
- Long-term anaesthesia in rodents
- Lumpfish
- Main Page
- Marble Burying Test
- Metabolic cages
- Minipumps
- Montanide adjuvant
- Mouse Grimace Scale
- Mouse handling
- Nest building material
- Oestrus suppression in ferrets
- Pneumocystis murina
- Recapping needles
- Rotarod Test
- Screening cell lines
- Sedation of cattle
- Splenectomy
- Sterilisation of instruments
- TTEAM and TTouch
- Tail vein injection
- Tramadol
- Transport stress
- Tumour cell implant into mammary fat pad
- Ulcerative Dermatitis in Mice
- Water quality
- Xenopus laevis
- Zebrafish swabbing

The Zebrafish Book

http://zfin.org/zf_info/zfbook/zfbk.html

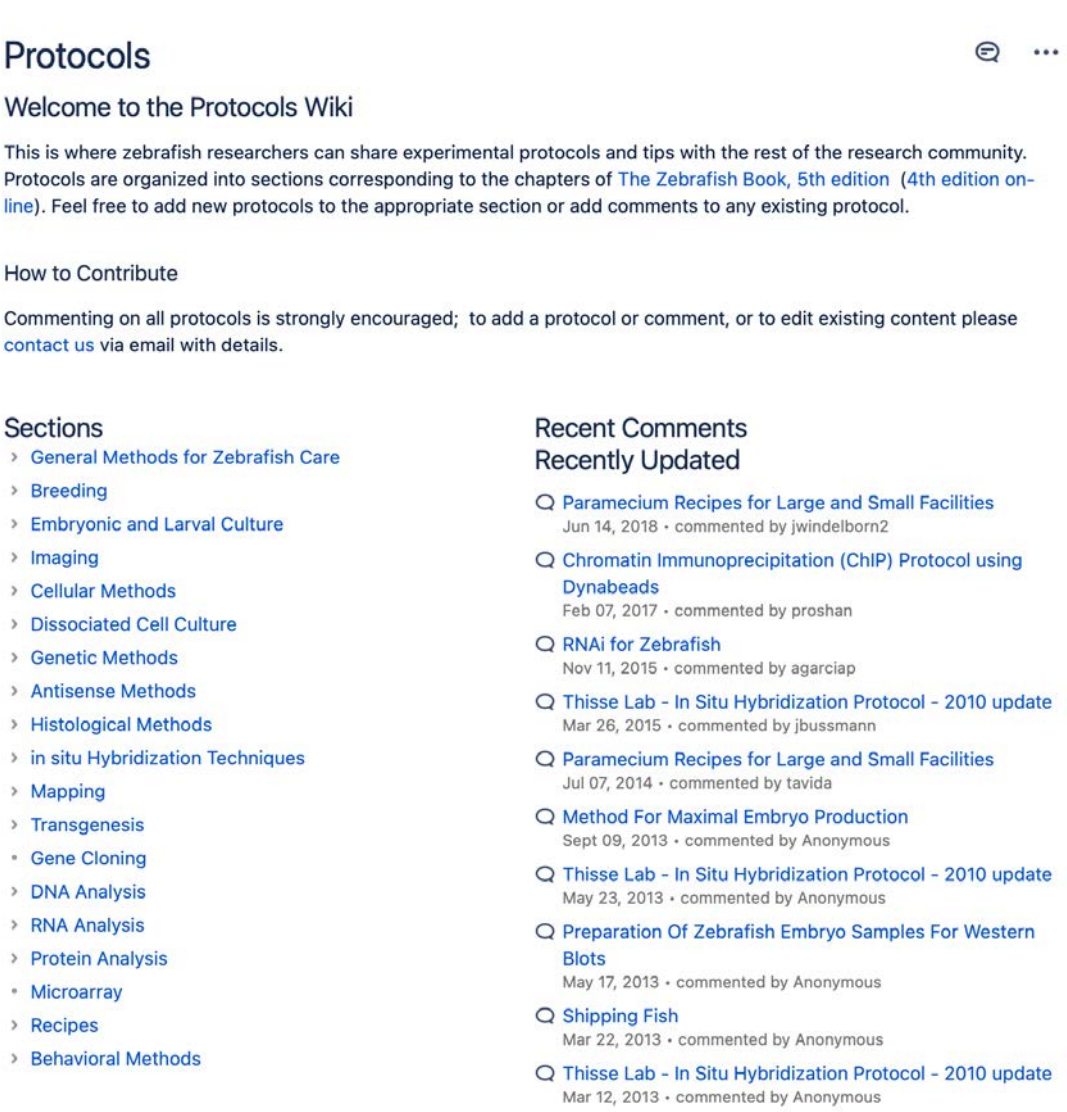
ZFIN Protocol Wiki

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.

zfin.atlassian.net/wiki/spaces/prot/overview



Protocols

Welcome to the Protocols Wiki

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How to Contribute

Commenting on all protocols is strongly encouraged; to add a protocol or comment, or to edit existing content please contact us via email with details.

Sections

- General Methods for Zebrafish Care
- Breeding
- Embryonic and Larval Culture
- Imaging
- Cellular Methods
- Dissociated Cell Culture
- Genetic Methods
- Antisense Methods
- Histological Methods
- in situ Hybridization Techniques
- Mapping
- Transgenesis
- Gene Cloning
- DNA Analysis
- RNA Analysis
- Protein Analysis
- Microarray
- Recipes
- Behavioral Methods

Recent Comments
Recently Updated

- Paramecium Recipes for Large and Small Facilities
  Jun 14, 2018 • commented by jwindelborn2
- Chromatin Immunoprecipitation (ChIP) Protocol using Dynabeads
  Feb 07, 2017 • commented by proshan
- RNAi for Zebrafish
  Nov 11, 2015 • commented by agarciap
- Thisse Lab - In Situ Hybridization Protocol - 2010 update
  Mar 26, 2015 • commented by jbussmann
- Paramecium Recipes for Large and Small Facilities
  Jul 07, 2014 • commented by tavida
- Method For Maximal Embryo Production
  Sept 09, 2013 • commented by Anonymous
- Thisse Lab - In Situ Hybridization Protocol - 2010 update
  May 23, 2013 • commented by Anonymous
- Preparation Of Zebrafish Embryo Samples For Western Blots
  May 17, 2013 • commented by Anonymous
- Shipping Fish
  Mar 22, 2013 • commented by Anonymous
- Thisse Lab - In Situ Hybridization Protocol - 2010 update
  Mar 12, 2013 • commented by Anonymous

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zfin.atlassian.net/wiki/spaces/prot/overview

Norecopa: PREPARE for better Science

Home > Office of Continuing Education and Professional Development > Certificates and Courses > Experimental Fish

Experimental Fish

Health and Welfare of Fish in Research
An online training program

Certificate Overview

As fish become increasingly important as experimental animals, there is a growing need for training researchers in the principles of laboratory fish care and welfare and the proper care and handling techniques associated with these animals.

The Atlantic Veterinary College at the University of Prince Edward Island, in partnership with the UPEI Office of Continued Education and Professional Development, have designed this online training program to comply with the requirements outlined by the Canadian Council on Animal Care, American Association of Laboratory Animal Science (AALAS), Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC), and Animal Welfare Committee of the American Veterinary Medicine Association with special reference to aquatic animal welfare and the specific needs of laboratory fish as research models.

Register today!

Office of Continuing Education and Professional Development

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- Information for Participants
- Participant Testimonials
- Custom Group Training
- Experimental Fish
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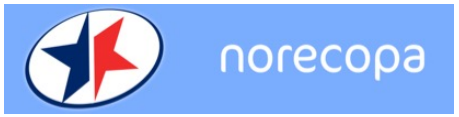
Objectives

Experimental Fish will instruct participants on:

- The role and structure of the Canadian Council on Animal Care (Canadian Version)
- The regulations, policies and guidelines that oversee the care, handling, and human treatment of animals in the United States (American Version)
- The ethical issues surrounding experimental animal use
- Factors (e.g. stress, disease, husbandry) affecting research
- Anesthesia and euthanasia for fish
- Considerations of selection and set-up of laboratory holding systems
- Water quality parameters
- Feed and nutrition for fish
- Aquatic animal identification and monitoring
- Recognizing pain, stress, and distress in aquatic animals
- Interdependence of health factors for aquatic animals
- Humane endpoints
- Physical, chemical, and biological hazards in aquatic animal facilities

upei.ca/professional-development/certificates/experimental-fish



International Zebrafish and Medaka Course (IZMC)

International Zebrafish and Medaka Course (IZMC)

International Zebrafish and Medaka Course (IZMC)

European Zebrafish Resource Center (EZRC), near Karlsruhe, Germany

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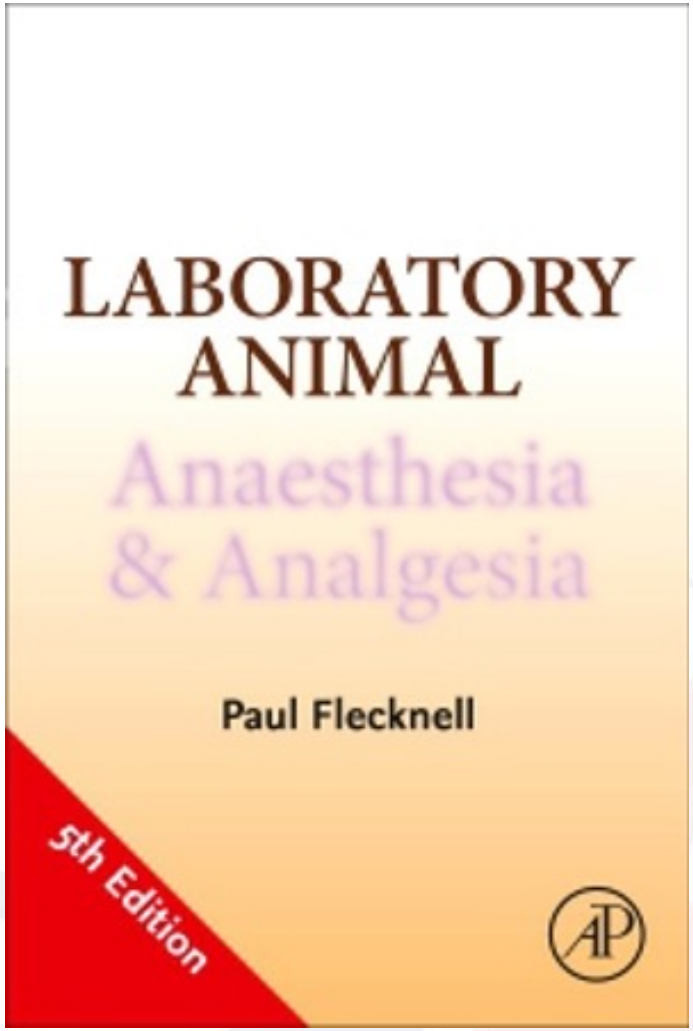
izmc.ezrc.kit.edu/english/index.php

Other courses in Laboratory Animal Science and related fields:

- Education and training from ESLAV/ECLAM
- Information about residency programmes approved by ECLAM
- Overview of institutions offering E-learning modules in laboratory animal science
- ETPLAS (European and Training Platform for Laboratory Animal Science)
- Overview of FELASA-accredited courses in Europe and beyond
- Dipl.SVLAS - postgraduate recognition as Diplomate Specialised Veterinarian in Laboratory Animal Science awarded by SAVIR (Swiss Association of Veterinarians in Industry and Research)
- International Course in the Care and Use of Laboratory Animals, online/Crete, April/May/June 2022
- Course in Animal Experimentation: Welfare, Legislation and Ethical Committees and Evaluation of Projects, Universitat Autònoma de Barcelona, 15 February - 30 April 2022
- Courses on Recognition, Prevention and Alleviation of Pain and Distress in Laboratory Animals (Flecknell et al., Newcastle University)
- International course in Laboratory Animal Science, Crete, April-June 2021
- Courses in Laboratory Animal Science at the University of Zurich
- Curriculum for undergraduate and Masters students on the use of research animals, organised by the British Pharmaceutical Society and partners
- M.Sc. in Laboratory Animal Science at Aachen University
- International Course in Laboratory Animal Science, Utrecht
- Courses in Laboratory Animal Science at the Universitat Autònoma de Barcelona (UAB), including Master in Laboratory Animal Science & Welfare, ECLAM Residence and Certificate LAS&M

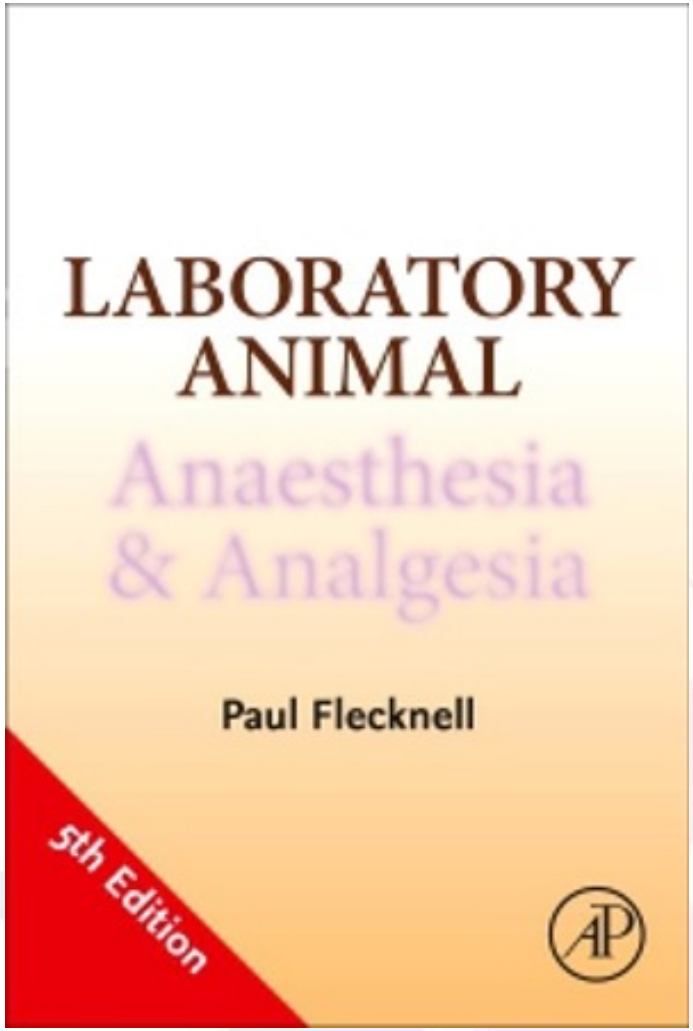
norecopa.no/education-training/courses

- **Anaesthesia, analgesia and humane killing**



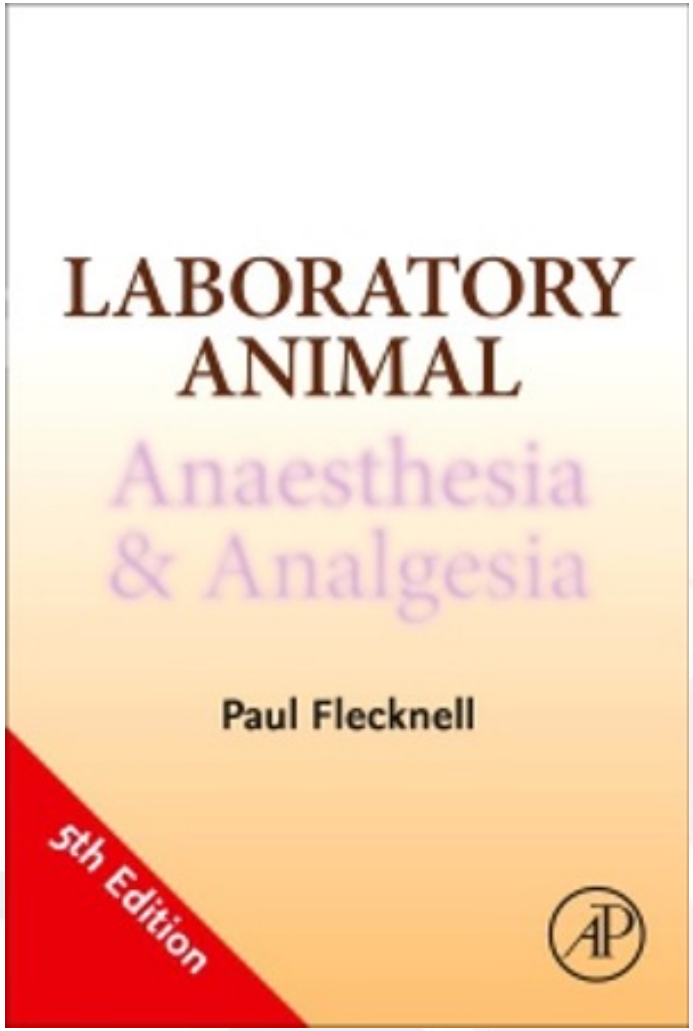
Brief descriptions of anaesthetic techniques for fish, amphibia, reptiles and birds have also been included, to provide some basic guidance for dealing with these species.

norecopa.no/textbase/laboratory-animal-anaesthesia-and-analgesia-5th-edition



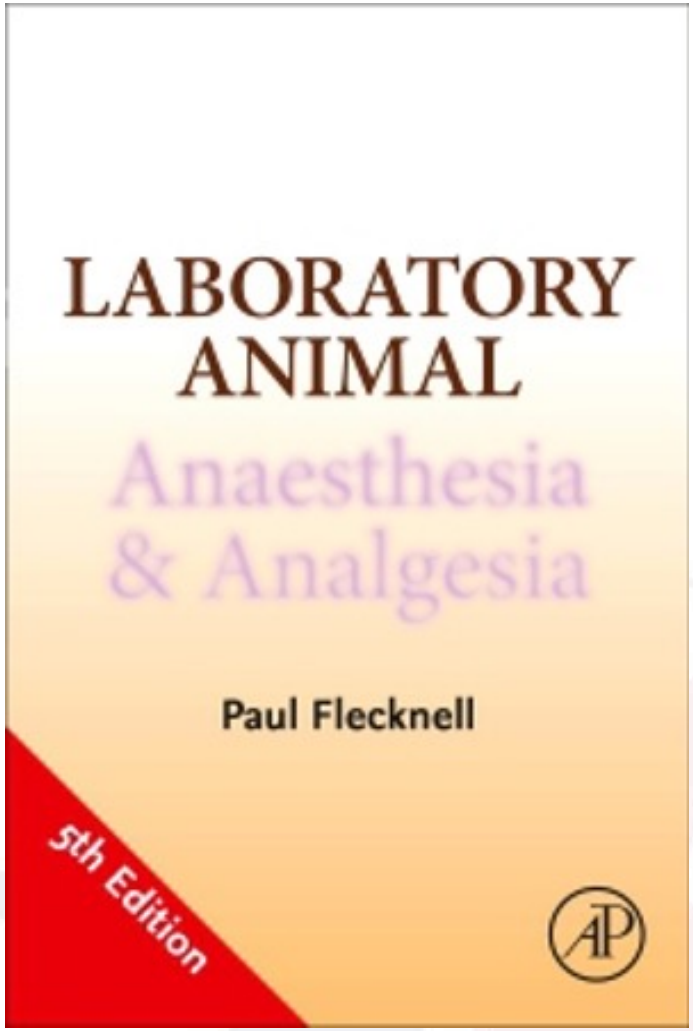
Fish, reptiles, amphibia, and cephalopods

Pain assessment in these groups of animals is particularly challenging. Some information related to signs of pain in fish is available, although these behaviours are mainly related to experimental studies using defined noxious stimuli (Sneddon, 2020). Laboratory-based studies of nociceptive responses have been undertaken in reptiles and amphibia (Coble et al., 2011; Stevens, 2011; Vachon, 2014) and cephalopods (Crook et al., 2013, reviewed by Walters, 2018) and more complex behavioural responses measured in octopus (Crook, 2021). However, assessment of postprocedure pain remains highly subjective (Fiorito et al., 2015; Lambert et al., 2019; Whittaker et al., 2021; Williams et al., 2019).



Fish

Fish are most easily anaesthetized by immersion in anaesthetic solution. Since these animals may be sensitive to sudden changes in pH and temperature, it may be advisable to use some of the water from their normal tank to fill the anaesthetic chamber (Sneddon, 2012). The induction tank should be aerated using a standard aquarium pump and airstone. Following induction of anaesthesia, the fish can be removed from the solution of anaesthetic and wrapped in moist gauze to prevent desiccation, and any procedure should be undertaken rapidly. For some procedures, it is possible to position the fish so that its gills remain



submerged in anaesthetic solution. Alternatively, a more complex system, in which oxygenated anaesthetic solution is passed over the gills, can be constructed. A simpler recirculating system has been described (Longley, 2008). It is important to minimize handling of the fish during anaesthesia, since the skin is easily damaged, resulting in infections postoperatively. Fish should be fasted for 24–48 h prior to anaesthesia, as they may vomit and this can interfere with gill function.

The signs of onset of anaesthesia in fish have been described in detail (Sneddon, 2012) and differ significantly from mammals. Briefly, after loss of equilibrium and muscle tone, and onset of very shallow opercular movements, the response to pressure on the muscles at the tail base is reduced, but not abolished. At this stage, the fish can be removed from the anaesthetic solution and surgery or other manipulations carried out. If surgical stimuli cause muscle spasms, then either the fish can be returned to the anaesthetic solution, or additional solution can be dripped or sprayed over the gills, for example, by placing a drip in the buccal cavity. Overdosage is indicated by loss of regular opercular movements and occasional exaggerated respiratory movements. Cardiac arrest follows in 1–2 min unless the fish is resuscitated. This can be achieved either by flushing the mouth, and hence the gills, with fresh water, or by placing the fish in a tank of fresh water and moving it back and forth with its mouth open.

General anaesthesia

Injectable agents

Tricaine methanesulphonate (MS222): Tricaine is used for induction and maintenance of anaesthesia of a wide range of fish species (Carter et al., 2011; Topic Popovic et al., 2012). It is administered as a 25–300 mg/L solution, by immersion; the concentration used determines the depth of anaesthesia. Most small to medium-sized fish (e.g. goldfish, trout) require 100 mg/L for surgical anaesthesia. Anaesthesia is induced in around 2 min, and recovery occurs about 5 min after removal from the anaesthetic solution. The anaesthetic solution should be buffered before use, using sodium bicarbonate. The effects of MS222 have been evaluated in zebra fish embryos for both short- and long-term (24 h) immobilization (Rombough, 2007). MS222, in common with several other anaesthetics has been shown to be aversive in fish, and etomidate has been recommended as an alternative (Readman et al., 2013).

Etomidate: Etomidate has been shown to be nonaversive in fish (Readman et al., 2013) and to produce safe and effective anaesthesia (Kazuń and Siwicki, 2012). Dose rates vary between species, but are generally 2.0–4.0 mg/L (Amend et al., 1982).

Benzocaine: Benzocaine should be administered as a freshly prepared solution of 200 mg benzocaine in 5 mL acetone, which when added to 8 L of water provides a solution of 25 ppm (25 mg/L). This concentration is sedative, enabling minor manipulations to be undertaken. Higher concentrations (50 ppm, 50 mg/L) induce surgical anaesthesia.

Anaesthesia and analgesia in fish

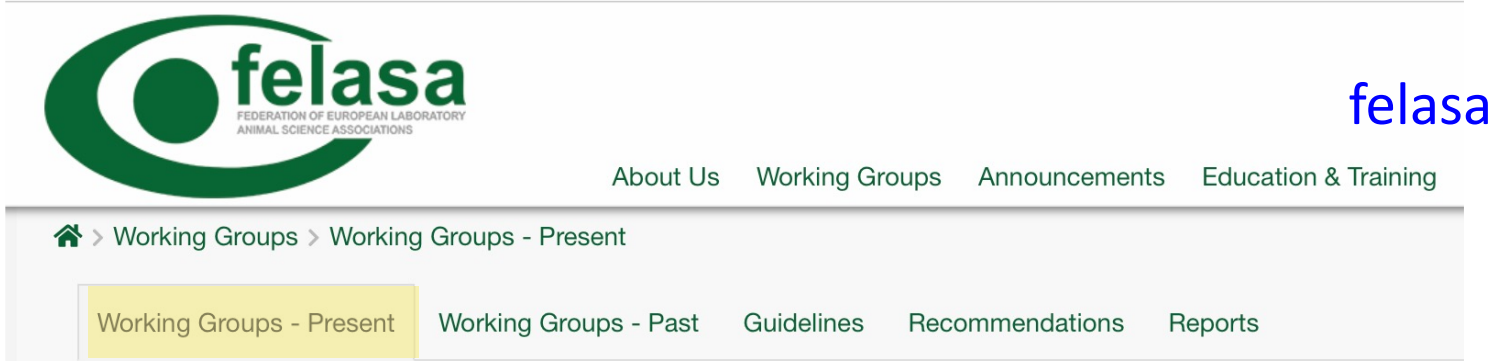
This webpage is a collection of links to papers and guidelines on anaesthesia and analgesia in fish.
Anaesthesia and analgesia of zebrafish is covered on a separate page.
General principles of anaesthesia and analgesia

Suggestions for additions are welcome and should be sent to post@norecopa.no.
Some links on Norecopa's webpages on cephalopods and on other aquatic species may also be relevant.
Anesthesia for fish in aquaculture research: presentations from a course in Bergen (6 March 2018)

References

- Leyden *et al.* (2022): Efficacy of Tricaine (MS-222) and Hypothermia as Anesthetic Agents for Blocking Sensorimotor Responses in Larval Zebrafish
- Schroeder *et al.* (2021): Anaesthesia of laboratory, aquaculture and ornamental fish: Proceedings of the first LASA-FVS symposium
- Martins T *et al.* (2019): Anaesthetics and analgesics used in adult fish for research: A review.
- Chatigny *et al.* (2018): Updated review of fish analgesia.
- Anaesthetics and analgesics used in adult fish for research: a review (Martins *et al.*, 2018)
- Readman GD *et al.* (2017): Species specific anaesthetics for fish anaesthesia and euthanasia
- Vila Pouca C & Brown C (2017): Contemporary topics in fish cognition and behaviour
- Sneddon LU (2015): Pain in aquatic animals
- Sneddon LU et al. (2014): Defining and assessing animal pain
- Physiological and behavioural evaluation of common anaesthesia practices in the rainbow trout (Pounder *et al.*, 2018)
- An in-tank method of anaesthesia with MS222 is described in the paper *Individual Monitoring of Immune Response in Atlantic Salmon Salmo salar following Experimental Infection with Infectious Salmon Anaemia Virus (ISAV)* (Collet *et al.*, 2015)

norecopa.no/species/fish/anaesthesia-and-analgesia



felasa.eu/working-groups

- Methods of humane killing of laboratory fish

Article

A FELASA Working Group Survey on Fish Species Used for Research, Methods of Euthanasia, Health Monitoring, and Biosecurity in Europe, North America, and Oceania

Jean-Philippe Mocho [1,*] and Kristine von Krogh [2]

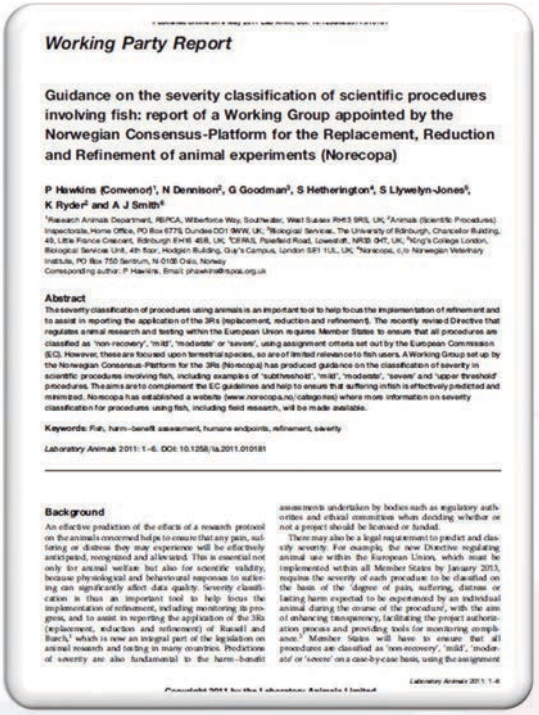
felasa.eu/working-groups/present/id/7

- Pain management in zebrafish (Lynne Sneddon)
- Severity classification in zebrafish and their larvae (Almut Koehler)

- **Severity classification, humane endpoints and harm-benefit analysis**

From **3R-Guide**

norecopa.no/3r-guide



Working Party Report

Guidance on the severity classification of scientific procedures involving fish: report of a Working Group appointed by the Norwegian Consensus-Platform for the Replacement, Reduction and Refinement of animal experiments (Norecopa)

P Hawkins (Convenor)[1], N Dennison[2], G Goodman[3], S Hetherington[4], S Llywelyn-Jones[5], K Ryder[2] and A J Smith[6]

Abstract

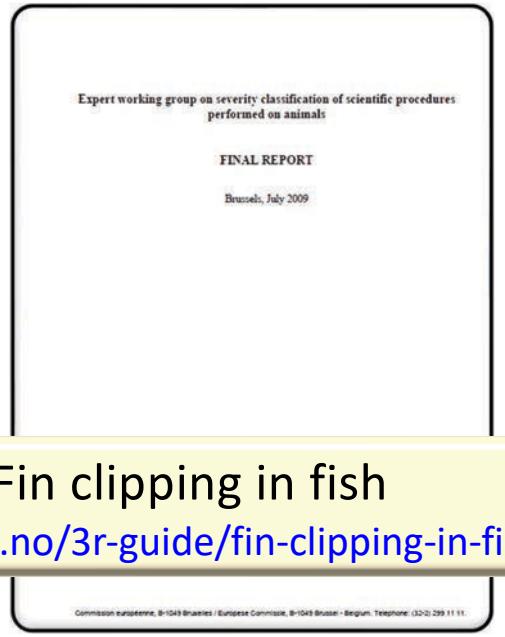
Keywords: Fish, harm–benefit assessment, humane endpoints, refinement, severity

Laboratory Animals 2011; 1–6. DOI: 10.1258/la.2011.010181

Background

Guidance on the severity classification of procedures involving fish

Report from a Working Group convened by Norecopa



Expert working group on severity classification of scientific procedures performed on animals

FINAL REPORT

Brussels, July 2009

Fin clipping in fish

norecopa.no/3r-guide/fin-clipping-in-fish

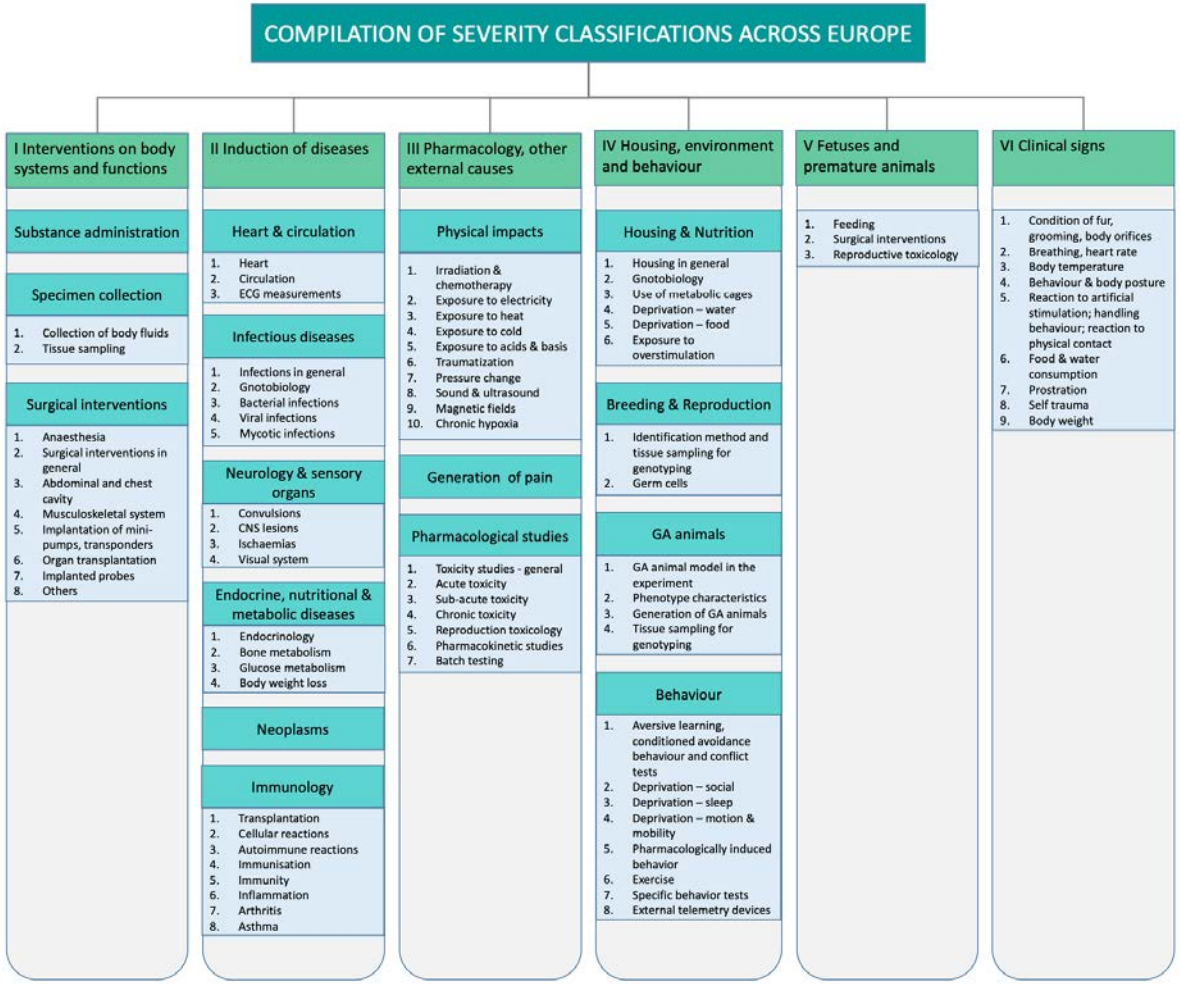
http://ec.europa.eu/environment/chemicals/lab_animals/pdf/report_ewg.pdf

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

Laboratory Animals, 45: 219-224, 2011

norecopa.no/categories

norecopa.no/severity



Norecopa: PREPARE for better Science

Working Party Report

Laboratory Animals
0(0) 1–13

DOI: 10.1177/00236772231156031
journals.sagepub.com/home/lan

Defining piscine endpoints: Towards score sheets for assessment of clinical signs in fish research

L Andersen[1], A Rønneseth[2], MD Powell[3] and A Brønstad[4]

Abstract

The seminar 'Severity and humane endpoints in fish research' organized by the University of Bergen, the Industrial and Aquatic Laboratory, together with Fondazione Guido Bernadini, took place on 4 October 2019 in Bergen, Norway. The seminar was followed by a workshop, 'Establishing score sheets and defining endpoints in fish experiments', held on 28 January 2020, also in Bergen. The purpose of the seminar was to raise awareness about fish ethics together with severity classification and humane endpoints in fish studies, using examples from farmed fish, mainly salmonids and lumpfish. The overall aim of the workshop was to better define humane endpoints in fish experiments, as well as to discuss suggestions for development and use of score sheets for assessing clinical signs related to endpoints. Endpoints for fish should not only be based on what we know about fish diseases and the lesions they induce but should also take into consideration knowledge about fish species and life stage, fish anatomy, physiology and the general state and behaviour of the fish. For this reason, to reinforce that endpoints should come from the animal's perspective and needs, we renamed humane endpoints for fish to piscine endpoints. This paper reports the main messages from the workshop sessions including advice on development and use of score sheets.

Keywords

Moribund, categorization, scoring systems, severity assessment, humane endpoints, animal welfare, refinement

Date received: 12 September 2022; accepted: 4 January 2023

Establishing score sheets and defining endpoints in fish experiments

Program

8:00-9:00	Opening registration and coffee
9:00-9:45	Aurora Brønstad, University of Bergen (UoB): Introduction to humane endpoints in fish experiments
	Linda Andersen, ILAB & Anita Rønneseth, UoB: Outline for the workshop and score sheet development
Coffee refill	
10:00	Mark Powell, Marineholmen RASLab AS: From algae to jelly fish- when Atlantic salmon experiments don't go as planned!
10:25	Karin Pittman, UoB: Skin- and gill biopsies of Atlantic salmon
10:50	Trond Isaksen, NORCE: Tolerance of juvenile Atlantic salmon (*Salmo salar* L.) to dissolved gas supersaturation in freshwater
11:15	Gyri Haugland: Development of anaesthetic protocols for lumpfish (*Cyclopterus lumpus* L.)
11.40	Carolina Gutierrez Rabadan, Swansea University: Development and validation of an Operational Welfare Index for farmed lumpfish.
Lunch: 12-13:00	
13:00-15:45	Work in groups, discussions and feedback to participants
15:45	Summary: issues raised during the workshop

Seminar, Bergen, 2020

uib.no/en/rg/animalfacility/132071/establishing-score-sheets-and-defining-endpoints-fish-experiments

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Current concepts of Harm–Benefit Analysis of Animal Experiments – Report from the AALAS–FELASA Working Group on Harm–Benefit Analysis – Part 1 by Aurora Brønstad, Christian E Newcomer, Thierry Decelle, Jeffrey I Everitt, Javier Guillen, and Kathy Laber.

Recommendations for Addressing Harm–Benefit Analysis and Implementation in Ethical Evaluation – Report from the AALAS–FELASA Working Group on Harm–Benefit Analysis – Part 2 by Kathy Laber, Christian E Newcomer, Thierry Decelle, Jeffrey I Everitt, Javier Guillen, and Aurora Brønstad.

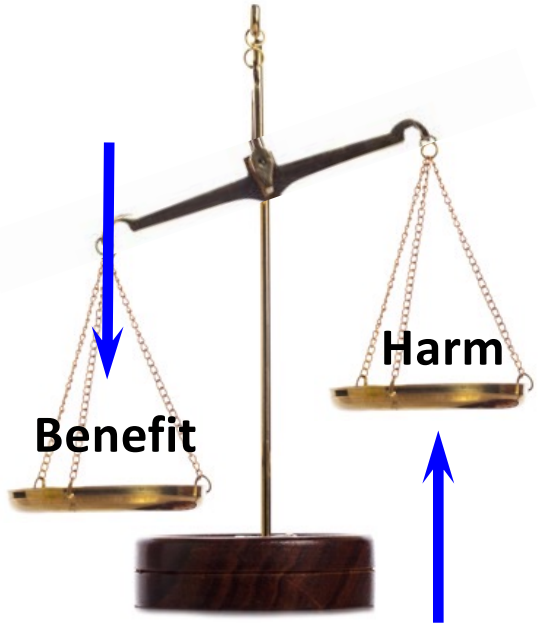
norecopa.no/more-resources/harm-benefit-assessment

We can work to tip the balance

***The 3 Rs* to minimise the harm:**

- *Replace the unnecessary experiments*
- *Reduce the number of animals used*
- *Refine the conditions for the animals*

norecopa.no/3R
norecopa.no/3S
norecopa.no/3V



***The 3 Ss* - your commonsense and your heart**

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

***The 3 Vs* to increase the validity of the experiment:**

- *Construct Validity (can the model answer the question?*
- *Internal Validity (has the experiment been correctly designed?)*
- *External Validity (are the results translatable to the target group?)*

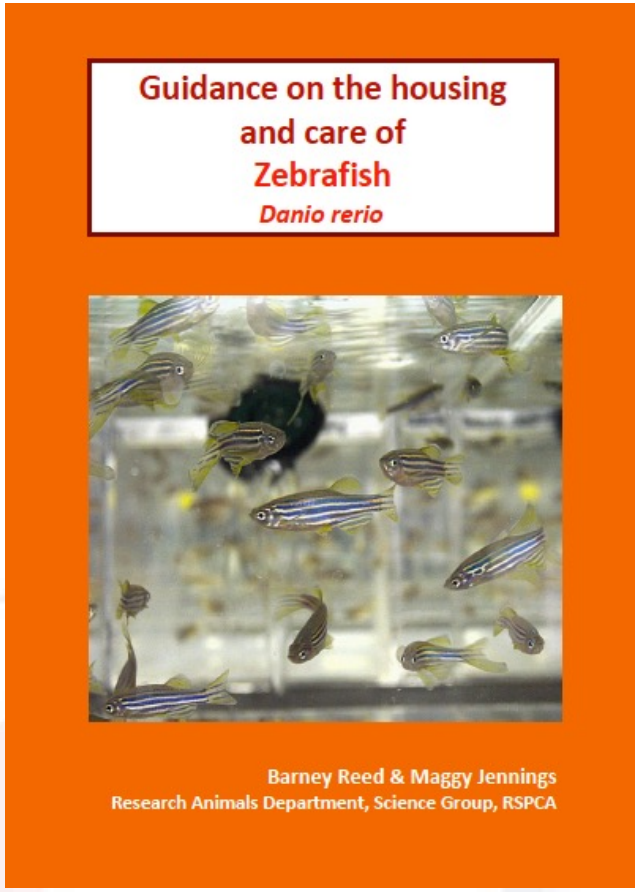
- **Zebrafish**

Zebrafish

Zebrafish (*Danio rerio*) are being used in increasing numbers within molecular biology, developmental biology, neurobiology, genetics, cancer research and drug discovery, due to the low costs of maintaining them, their short generation interval, the transparency of the embryos and the ability to manipulate the genome.

- General references
- Housing and welfare
- Health monitoring
- Anaesthesia and analgesia
- Procedures
- Euthanasia
- Other literature and media articles
- Organisations and resource collections
- Meetings
- Zebrafish facilities

norecopa.no/species/fish/zebrafish



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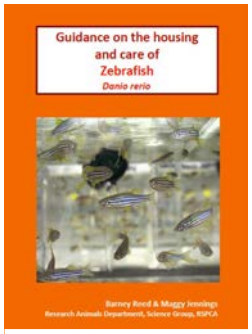
science.rspca.org.uk/sciencegroup/researchanimals/implementing3rs/refininghousing



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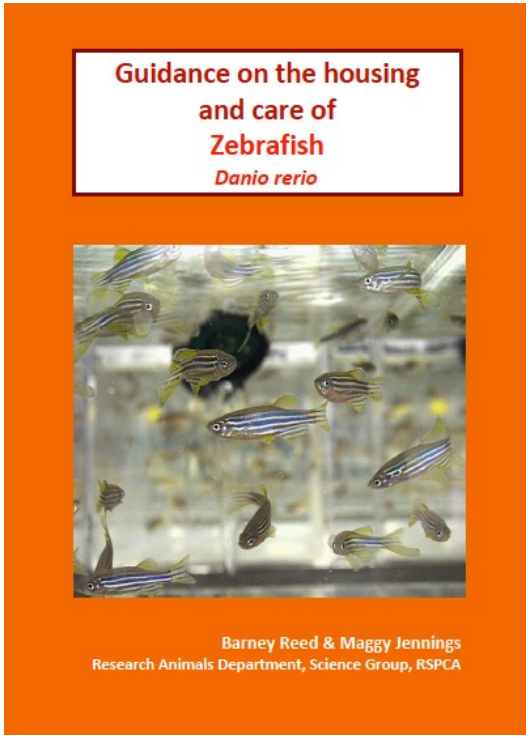
This report aims to improve the welfare of zebrafish by:

- 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'.

http://science.rspca.org.uk/sciencegroup/researchanimals/implementing3rs/housingandcareaquaticspecies

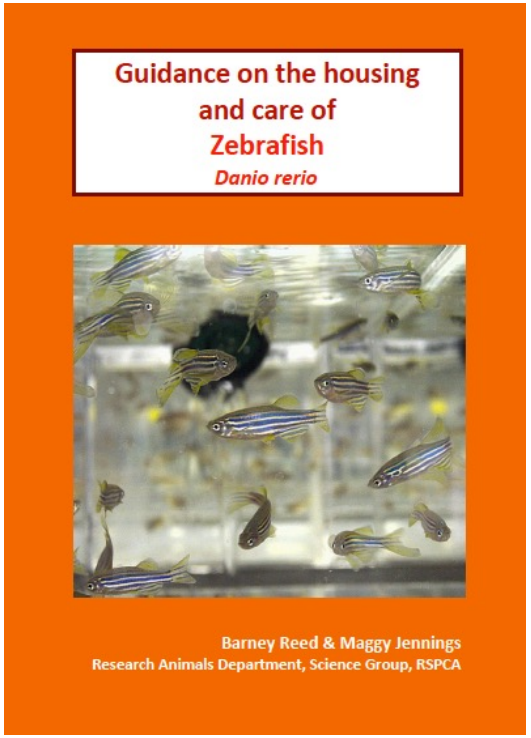
1. Introduction
2. Background information on zebrafish
   - Natural geographic range and habitat
   - Species characteristics
   - Use in research and teaching
3. Supply and transport
   - Source
   - Transport considerations
   - Quarantine
4. Housing and care
   - Lighting
   - Noise and other disturbances
   - 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



62 pages, including
8 pages with literature references

http://science.rspca.org.uk/sciencegroup/researchanimals/implementing3rs/housingandcareaquaticspecies

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



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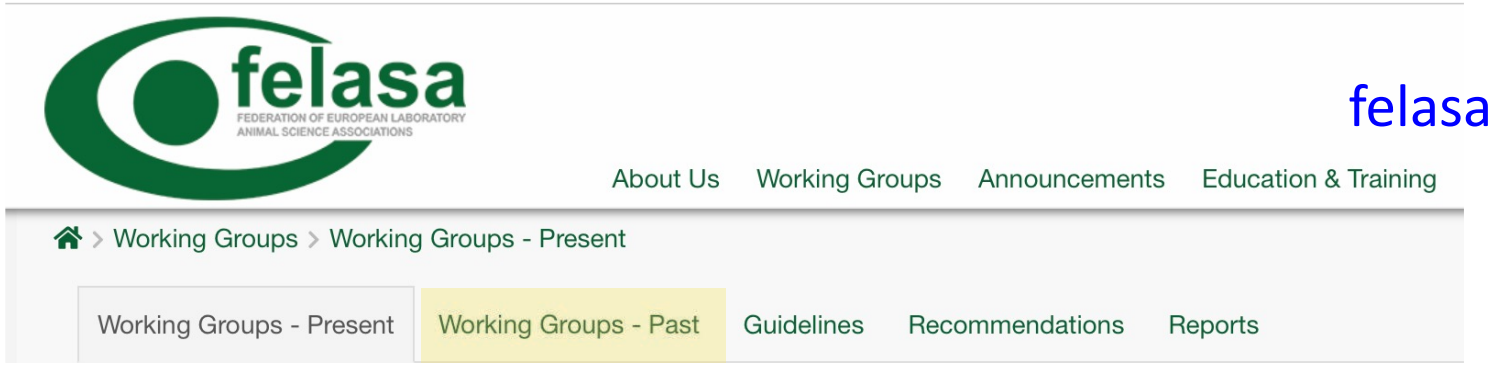

felasa.eu/working-groups

Zebra fish: housing, husbandry and health monitoring recommendations (Peter Aleström)

Publications

Zebrafish: Housing and husbandry recommendations Aleström P., D'Angelo L., Midtlyng P.J., Schorderet D.F., Schulte-Merker S., Sohm F., Warner S. Lab Anim. 2019. https://doi.org/10.1177%2F0023677219869037

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wikipedia


CIRS-LAS Portal
Critical incident reporting system in laboratory animal science
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norecopa.no/meetings/meetings-calendar

focusonfish.co.uk

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