

Zebrafish Facility Inspections and Accreditation

Lead by: Erik Sanders B.S. RALAT

Aquatics Lab Services LLC

1112 Nashville Street

St. Peters, MO 63376

+1-314-724-3800

www.aquaticslabservices.com

aquaticslabservices@icloud.com

7th Annual International
Zebrafish Husbandry Course



Inspections!

1. Who Inspects us and why?
2. What are the inspectors looking for?
 - compliance with licence conditions
 - local enforcement through appropriate committees
 - appropriately resourced (building infrastructure and staffing)
 - process and continuous improvement
3. Beware - disparities between inspectors and the different regulatory bodies

Who Inspects us and why?

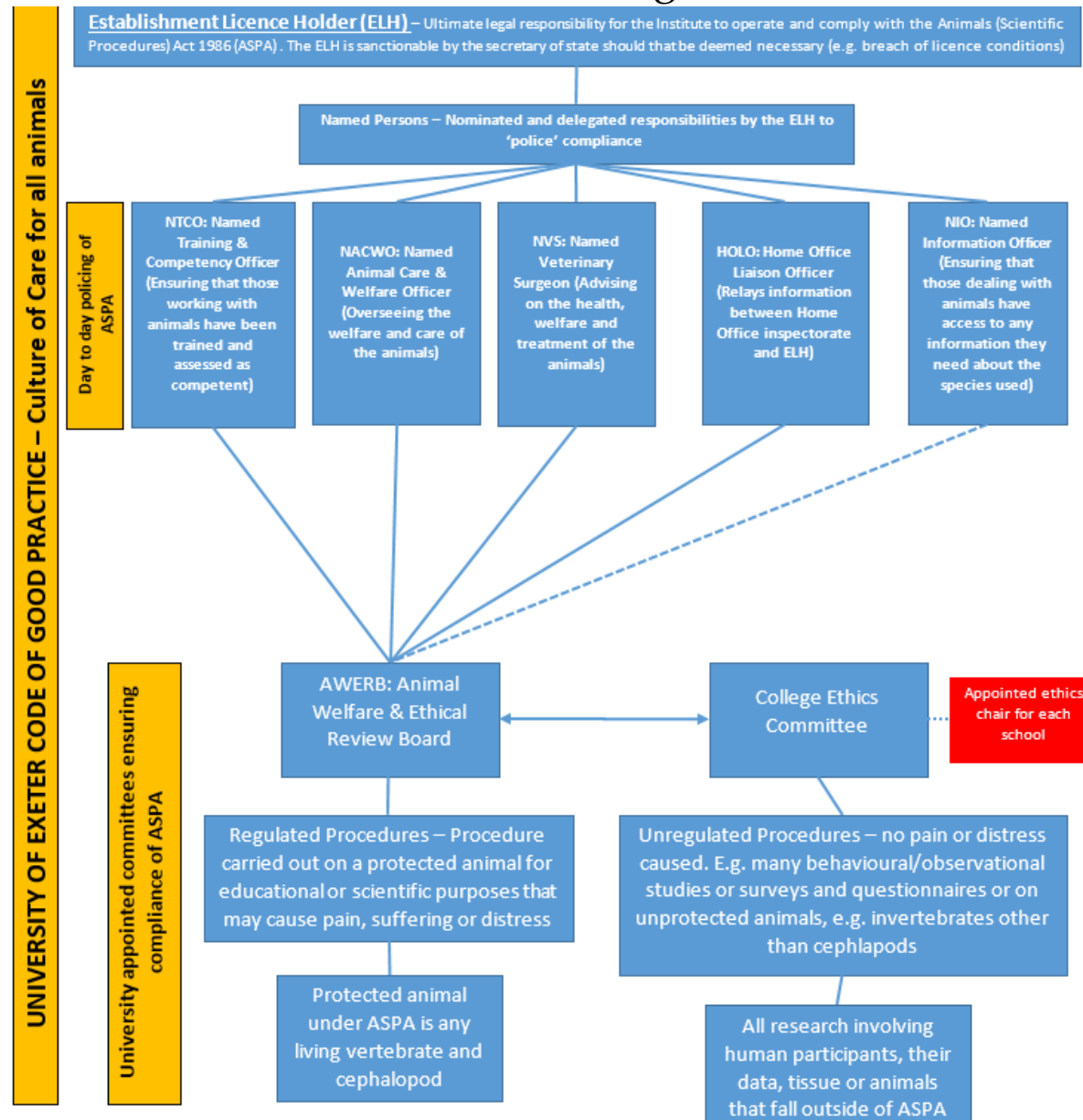




What are the
Inspectors looking for?

Compliance of licence conditions through local enforcement

- Internal Committees & Named Persons are in place
- Evidence that these committees meet regularly
- Evidence that information communicated to where it needs to go!



Facilities are appropriately resourced!

- Establishment takes seriously the requirements needed for an animal facility
- Appropriate investment in building infrastructure
 - biosecurity
 - primary services
 - life-support systems
 - back-up power/ system resilience
 - designated rooms for animal work (STH, LTH and SEP)
 - sufficient storage – i.e. no clutter in dedicated animal rooms
 - flow of the facility

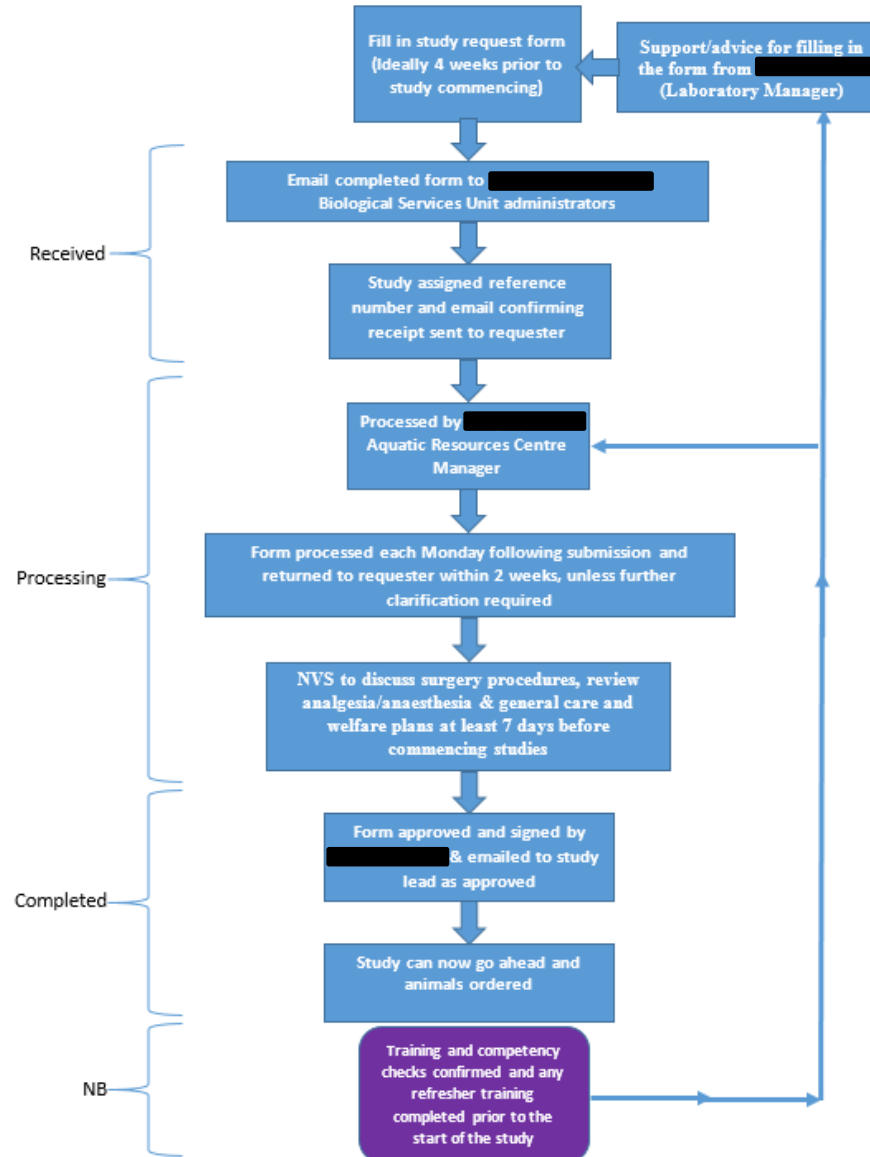
Facilities are appropriately resourced!

- Appropriate investment in staffing
 - number of staff
 - staff hold appropriate positions
 - 365 day cover
- Facility Managers (NACWO) have appropriate authority with academic colleagues
 - good engagement between technicians and academics
- Insufficient staffing puts facility at risk – lose multiple checkpoints needed to prevent breaches of licence
- NVS can help request extra staffing if necessary

Process and continuous improvement!

- Appropriate processes are in place on the ground and that they are being actioned
- Planning!! – starts with a study request form

Biological Services Unit (BSU) – Study Request Form



This form must be completed prior to animals being ordered/ or a study starting.

Cells in grey are for Animal Unit Management to complete after form received.

Ref Number	(Animal Unit to allocate)
Study Title	
Campus/ College/ School	
Telephone	
Email	
Site/ Animal Unit where work taking place:	<input type="checkbox"/> LSI BSU <input type="checkbox"/> Hatherly BSU <input type="checkbox"/> GP BSTL Rodent Facility <input type="checkbox"/> GP ARC <input type="checkbox"/> WSL Aquarium <input type="checkbox"/> WSL Room 127 <input type="checkbox"/> Penryn Fish Lab <input type="checkbox"/> Penryn (Other – please state) <input type="checkbox"/> Off Site (Please State)
	Details:

APPROVALS (Must be completed before animals arrive/ study starts)

Role	Name	Signed	Date
PI/ Project Lead			
Primary PIL Holder			
Primary Animal Carer (if not under ASPA)			
Animal Unit Management			
Lead Technician (Animal Unit Staff)			

Some sections of this form will not be applicable, please score out as appropriate

Once this form has been approved ANIMAL UNIT Management will contact for any requirement for a pre-study meeting.

Ethical Approval

Has the study received Ethical Approval?

ASPA	Y/N	Date:	Please complete ASPA section below
Non- ASPA	Y/N	Date:	Please attach ethical approval to this form.
Sch1/ Tissue	Y/N	Date:	Please attach ethical approval to this form.

ASPA DETAILS (Complete if applicable):

PROJECT LICENCE (PPL)

PPL No:		Protocol		Licence holder:	
Expected Severity:		PPL Contact Nos:			
Actual severity assessed by:	(If this is not completed the PI/ Project lead is expected to complete these severity assessment records)				
Authorisation for study to be performed under project licence: The Study Request form signed by the Project Licence holder confirms: all details have been confirmed as correct and allowed under the Project Licence indicated. The appropriate staffing resources are available to perform the requested work. The study is authorised to commence on the date that has been requested.					
A copy of the PPL must be available in the animal unit for all PILs working on this project.					
Signed (PPL Holder):			Date:		

Is the PPL available in the animal unit?	Y/N (Animal Unit manager to complete)
Have all PIL holders read the PPL?	Y/N (PPL holder to complete)

ADVERSE EFFECTS and END POINTS:

(please outline adverse effects and the expected END POINT authorised under the PPL from your project, so that ANIMAL UNIT Staff/ NACWOs are aware)

PROCEDURES (PIL and Sch1) and COMPETANCIES**What licenced techniques or Sch1 will be performed under this study?**

Procedure/ Sch1	Route	Procedure code* (See responsibilities below)
		A
		B
		C
		D
		E
		F

RESPONSIBILITIES (PILs and Sch1):

Name	*Procedure Codes performed	PIL Number	Competent/ In Training	Supervision by?	Training Record Checked (Sig Animal Unit Staff)

STUDY ANIMALS:

Requested Animal Arrival Date (7 days prior to study start)	
Experiment start date (Day 0):	Expected end date

DO YOU REQUIRE?

(1) New animals from a supplier or import of colony for breeding?	Y/N	If Y, please detail supplier/ establishment in ANIMAL ORDER REQUEST below.
(2) New animals from the wild	Y/N	If Y, please detail origin/ permissions in ANIMAL ORDER REQUEST below. <i>Addition permissions may be required from Fish Health Inspectorate (Fish/ Shell Fish), Natural England (for trapping protected mammals) or DEFRA advice may be applicable for restricted movements (i.e. avian flu)</i>
(3) Animals held within Animal Unit currently (i.e. transgenic ZF or Mice)	Y/N	If Y, please provide detail of animals to be used and permissions of owner (if not yourself).
Details:		

ANIMAL ORDER REQUEST:

Animals or colony for breeding*			
Species	Breed/ Strain	Sex	Weight/ Age/ Life Stage
Establishment of Origin or Animal Supplier or Location from the Wild:		Health Screen obtained?	(Y/N and details)
Please indicate any Special requirements: (eg. Disease status / vaccination history)			
Number animals required		Details	

***Please note importation of a colony of animals requires other authorisation and forms to be completed and health screens approved by the NVS. Depending on the decision, animals maybe quarantined or redervied prior to entry to the animal units. Please speak to a senior member of animal unit staff regarding this.**

ANIMAL KEEP REQUEST: (note if details are left blank standard husbandry will be applied)

Housing/ Tank type requested	
Tank/ Cage size and animal number per tank/cage	
Bedding/ Water/ Feed/ Enrichment requirements or additional requests:	

FISH REQUIREMENTS*:

Light regime:	
Water type (de-chlorinated/Reverse Osmosis):	
Water source (Flow through, Recirculation or Static):	
Any use of gases:	
Do you require sole occupancy of a room:	
Dosing system (e.g. peristaltic pumps):	
Specialist Equipment (oxygen probe, pH meter etc):	
* Please list appropriate references:	

VETERINARY ATTENTION

In the event of animals needing veterinary attention do you wish to be notified?*	
State restrictions on veterinary treatments (these may be indicated in the PPL)?	

+In an emergency where animal welfare is at risk it may not be possible to seek authorisation from the client before veterinary attention is sought.

FATE OF ANIMALS:

Please state expected fate of animals (i.e. Sch1/ Rehome/ Transfer to another facility/ release into the wild)	
Expected fate of animals found dead (do you require the carcass?) <i>Condition 18 notification to the Home Office could be required</i>	

HEALTH & SAFETY:

Please indicate if your study involves any of the following hazards:

Hazard	Used ?	If Yes please indicate approval status (by relevant H&S committee)
Transgenic Animals		Registration page for GM use: http://lifesciences.exeter.ac.uk/healthsafety/gmoprojects/register/
Infectious materials		
Radiation/ Lasers		

COSHH/Risk Assessments

Please indicate COSHH/ RA you will be using (and provided to the ANIMAL UNIT), prior to commencement of Study.

Title	RA/COSHH	Signature ANIMAL UNIT Staff

CONTAINMENT/ BIOSECURITY/ PPE REQUIRED

Indicate if any measures/ precautions need to be in place (above normal requirements):

Are any biosecurity measures / disease precautions required?
Waste disposal requirements? Of consumables/ of carcasses?
PPE required (in addition to standard)?

Plan of STUDY/ EXPERIMENT (This MUST be completed):
Please use extra pages as necessary

Please detail the study/ experiment plan, in particular timelines for your study:

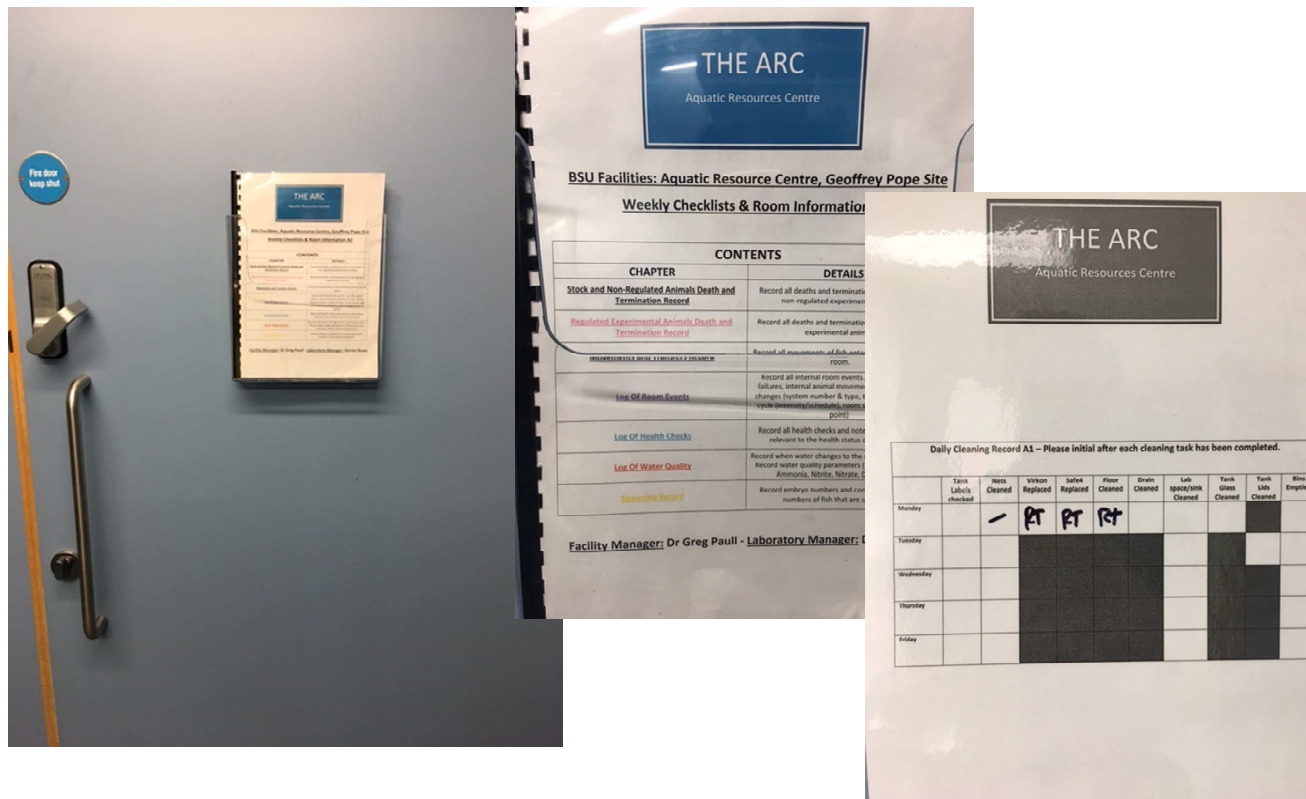
Approval of Study request form is done by signatures on the first page of this form, with the PPL signature for approval of licenced procedures. A study cannot commence without this approval.

This indicates that all information is correct, that PIL's are trained and competent and the animal unit has the required resources to run the study.

Unless agreed otherwise, studies will be billed for monthly.

Process and continuous improvement!

- Room management



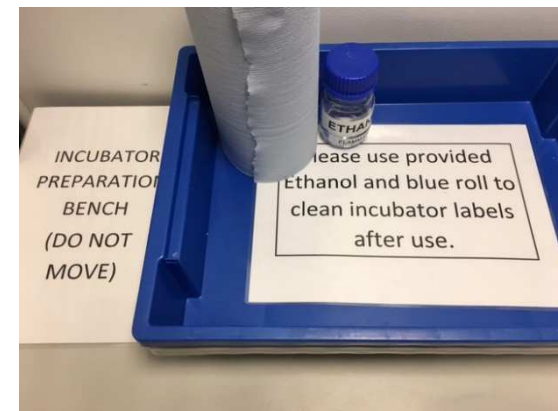
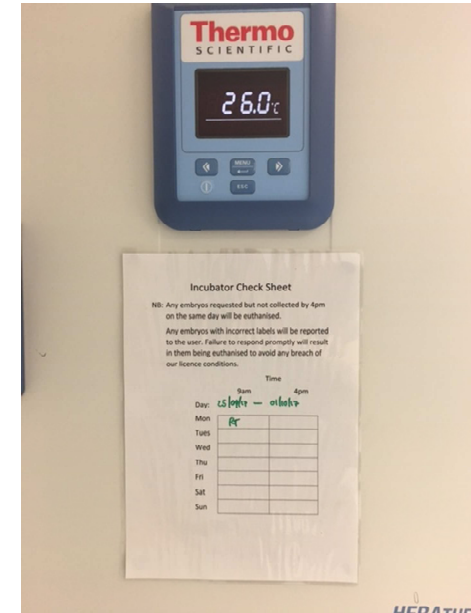
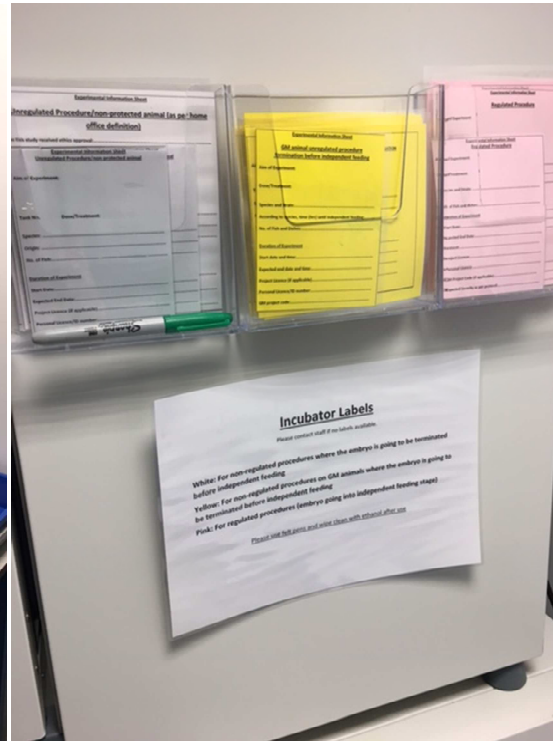
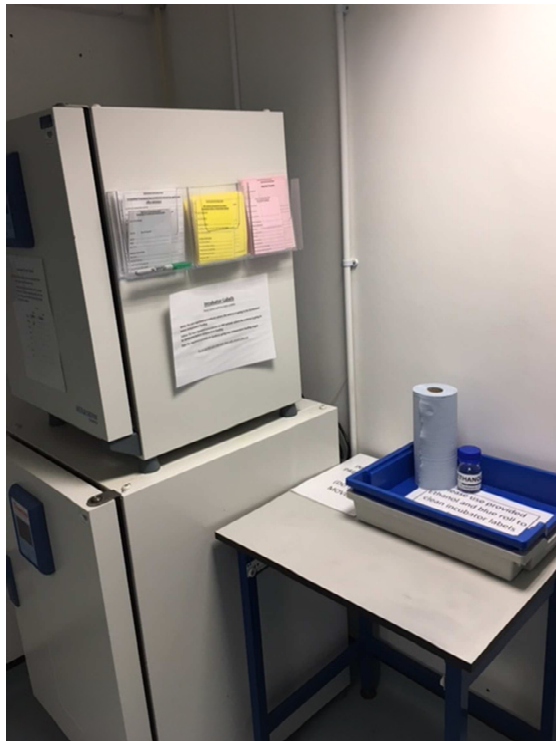
Process and continuous improvement!

- Tank labelling system



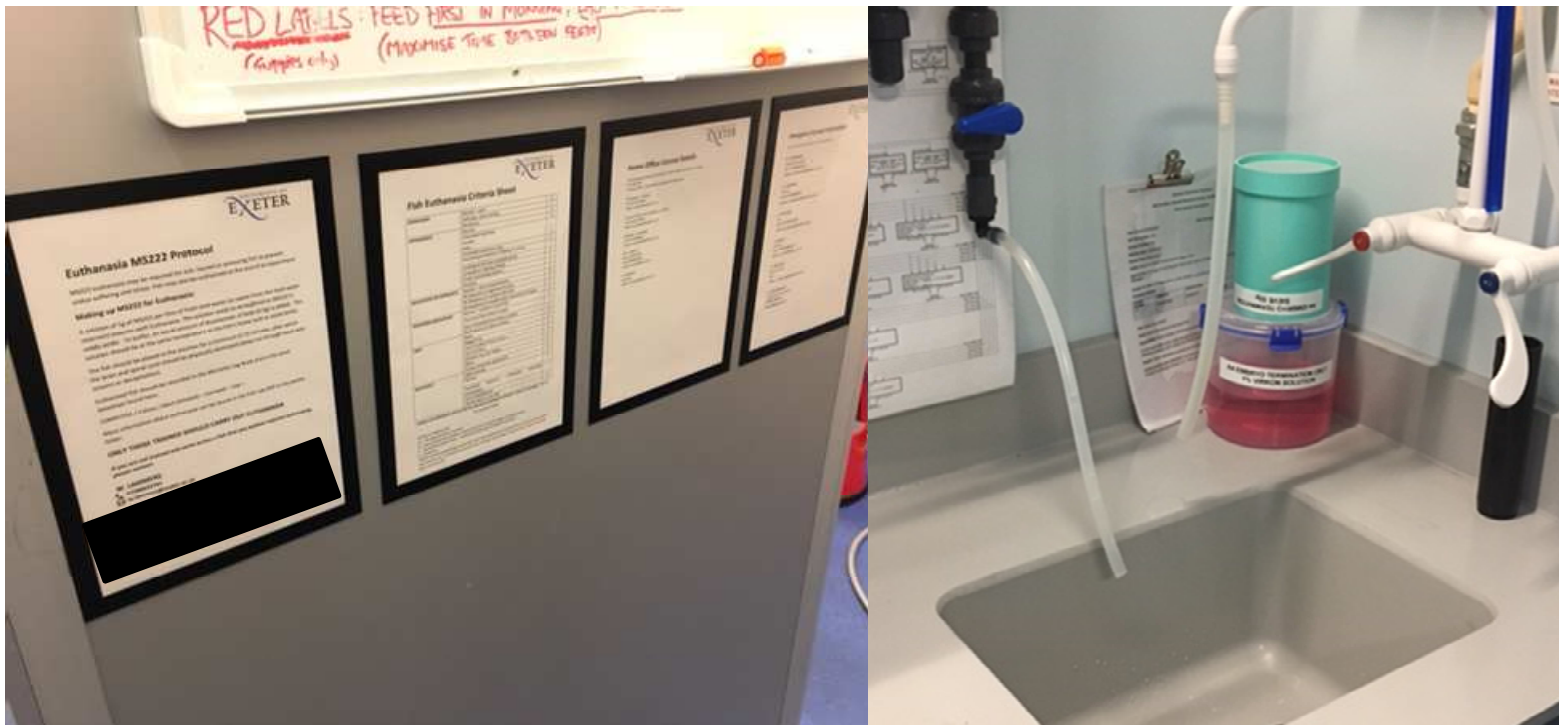
Process and continuous improvement!

- Relevant information at work stations!



Process and continuous improvement!

- Relevant information at work stations!



What about the animals?

- Interestingly the inspector hasn't mentioned the animals yet....often the case
 - health status tends to be left to the NACWO and NVS
 - unless a problem has been raised or is obvious on inspection
 - questions often around tank numbers or size, notably if a significant change is seen – e.g. Exeter moving from standard 40L tanks to 3.5 and 8L tanks.....
 - questions around 'culture of care' generally
- Inspectors are often getting an overall feel for the facility and how it operates
- NACWO must have a good relationship with the project licence holders



Disparities between inspectors and the different regulatory bodies

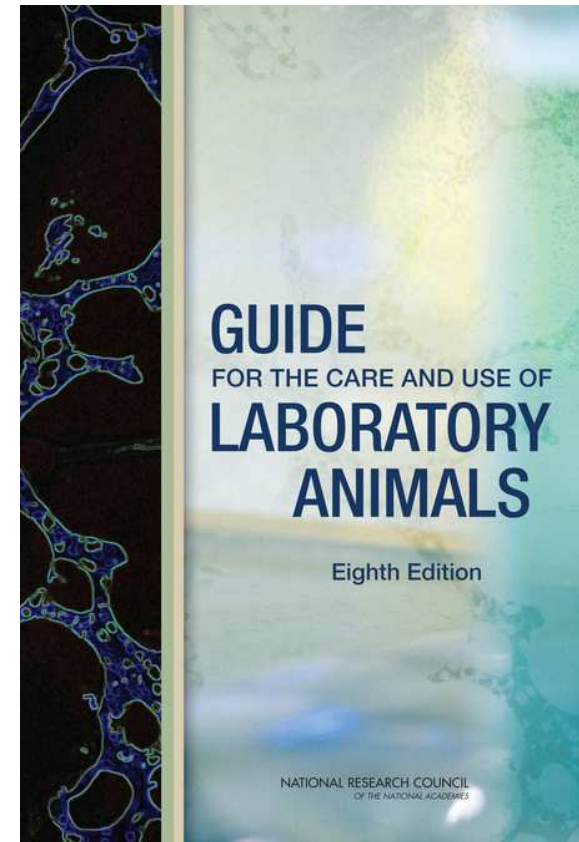
Inspections!

- Often inspectors have differences in how they want things done ☹
And/or they focus on different aspects of the facility
 - Problem is that they are only able to advise!
 - New inspector wanted different tank labels to the previous inspector
 - New inspectors often harder on older facilities as they are not aware of the history of the establishment
 - Building a new facility can put an old one at risk
 - Project Licence applications: structure differs greatly between inspectors
- Do not presume different regulatory bodies speak to each other!
 - HO licence allows us to re-home animals, e.g. SeaBass to a public aquaria (3R's) - Fish Health Inspectorate licence no longer permits this unless we have additional biosecurity measures in place
- Best advice is to have a direct phone line to your inspectors and engage with them at a personal level – they are not actually scary!

Biannual Institutional Level Inspections are carried out by:

- 1) Internal Animal Care and Use Committee (IACUC)
- 2) Oversight Veterinarian
- 3) Environmental Health and Safety Office

The guide and the Office of Laboratory Animal Welfare (OLAW) are used as the standard of measure for compliance.



Internal Inspections

The Problem?

The Guide is largely void of actual mandates regarding aquatic species.

Location

Construction

Water Quality

Life Support System

Temperature, Humidity, Ventilation/Illumination/Noise

Primary Enclosure

Environmental Enrichment, Social Housing

Food

Substrate

Sanitation, Cleaning, and Disinfection

Waste Disposal

Pest Control

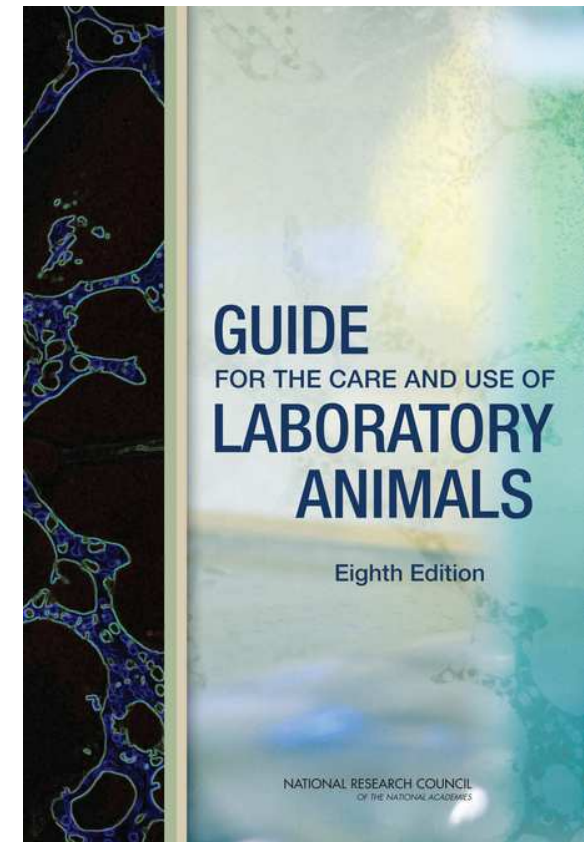
Emergency, Weekend, and Holiday Animal Care

Identification

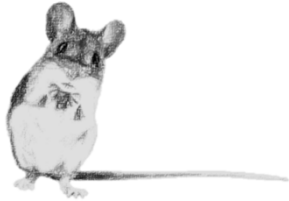
Record Keeping

Storage

Personnel



Internal Inspections



Temperature Recommendations from “The Guide”

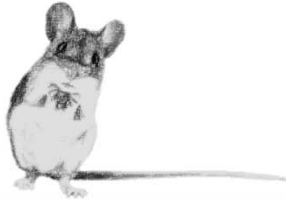
TABLE 3.1 Recommended Dry-Bulb Macro environmental Temperatures for Common Laboratory Animals

Animal	Dry-Bulb Temperature	
	° C	° F
Mouse, rat, hamster, gerbil, guinea pig ^a	20-26	68-79
Rabbit	16-22	61-72
Cat, dog, nonhuman primate	18-29	64-84
Farm animals, poultry	16-27	61-81



Temperature requirements are based on the natural history of the species and can vary depending on life stage (Green 2002; Pough 1991; Schultz and Dawson 2003). Water temperature may be controlled at its source, within the life support system, or by controlling the macroenvironment.

Internal Inspections



Housing Density Recommendations from “The Guide”

TABLE 3.2 Recommended Minimum Space for Commonly Used Laboratory Rodents Housed in Groups*

Animals	Weight, g	Floor Area/Animal, ^a in. ² (cm ²)	Height, ^b in. (cm)	Comments
Mice in groups ^c	<10	6 (38.7)	5 (12.7)	Larger animals may require more space to meet the performance standards.
	Up to 15	8 (51.6)	5 (12.7)	
	Up to 25	12 (77.4)	5 (12.7)	
	>25	≥15 (≥96.7)	5 (12.7)	
Female + litter		51 (330) (recommended space for the housing group)	5 (12.7)	Other breeding configurations may require more space and will depend on considerations such as number of adults and litters, and size and age of litters. ^d



Space recommendations and housing density vary extensively with the species, age/size of the animals, life support system, and type of research (Browne et al. 2003; Green 2009; Gresens 2004; Hilken et al. 1995; Matthews et al. 2002). In the United States, for example, adult zebrafish (*Danio rerio*) in typical biomedical research settings are generally housed 5 adult fish per liter of water (Matthews et al. 2002), but this housing density varies when breeding and for housing younger animals (Matthews et al. 2002). This guidance is not necessarily relevant for other species of fish, and may change as research advances (Lawrence 2007)

Confounding Problems



IACUC Committee is comprised of:

The IACUC must have a minimum of three members, one of whom is a Chairman appointed by the institution. The appointed members must be qualified to regulate animal care at that institution. The IACUC must also include **a veterinarian with training or experience with experimental animals, someone with no relation with the institution except for serving on the IACUC, a scientist with experience using experimental animals, and a nonscientist.** A member may cover more than one of these roles. If there are more than three members then no more than three may be affiliated with the same department at the institution.

Actual Quotes from Inspections.....

Where do you store the bedding?

How often do you take all these pipes apart and bleach them?

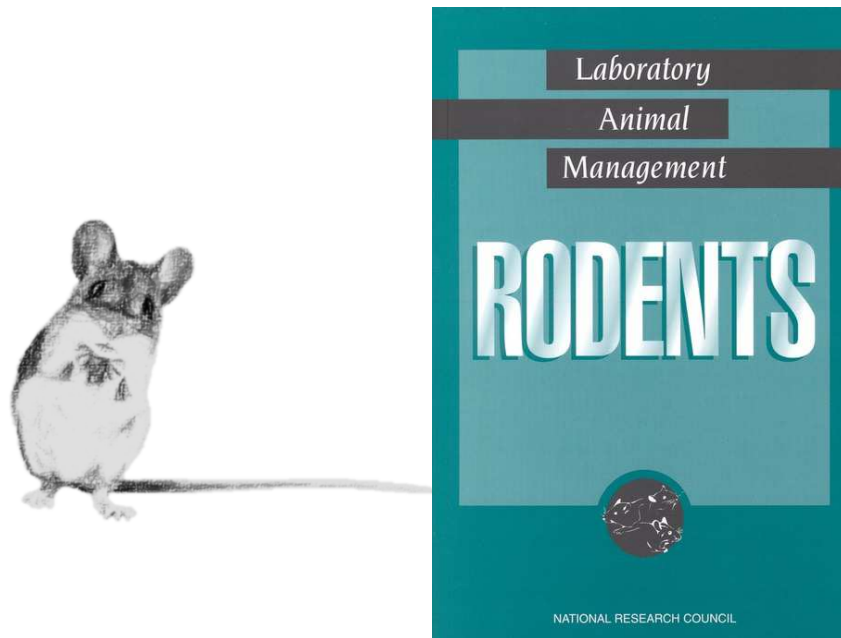
Your fish water failed the ATP test.

*The **ATP test** is a process of rapidly measuring actively growing microorganisms through detection of adenosine triphosphate, or **ATP**.*

Additional Resources for Inspectors and Vets

American College of Laboratory Animal Medicine “BLUE BOOK”

The Blue Book is a species specific operating manual, written by experts in the field, that gives specific details pertaining to the care and use of a Research Model



Voluntary Inspections



The **Association for Assessment
and Accreditation of Laboratory
Animal Care** International

AAALAC International is a private, nonprofit organization that promotes the humane treatment of animals in science through voluntary accreditation and assessment programs.

Voluntary

Institution is open to inspections

Detailed program description

Annual reporting

AAALAC status must be renewed every three years

<https://www.aaalac.org/accreditation/RefResources/ZebrafishReference.pdf>

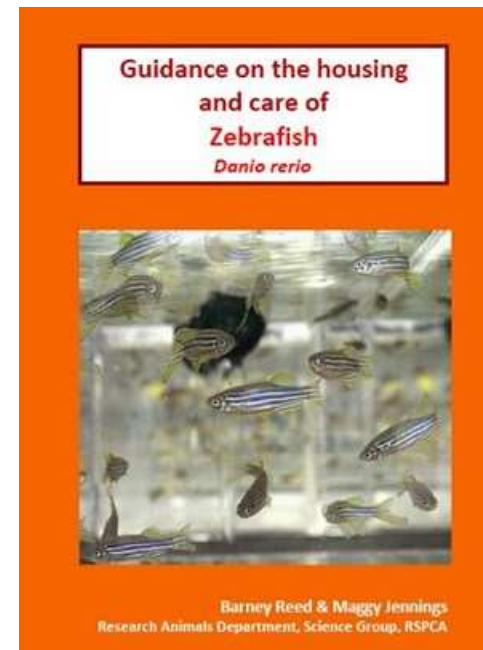
Voluntary Inspections



The **Association for Assessment and Accreditation of Laboratory Animal Care** International

Inspection based on an care and use manual specific to the species

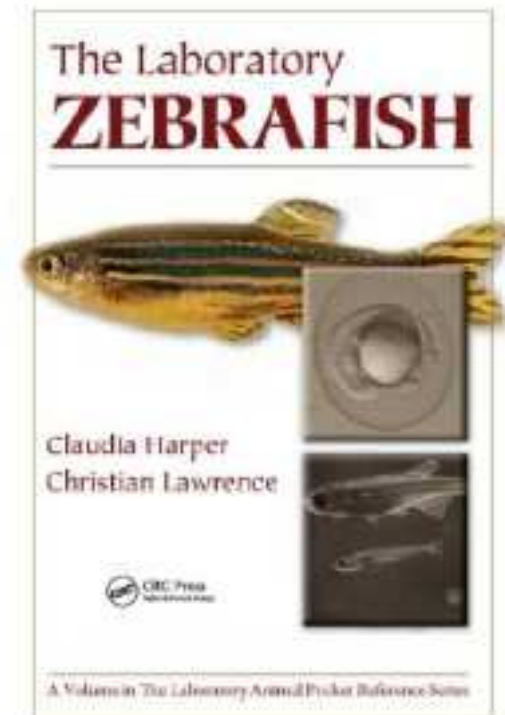
Inspection team is comprised of 3 primary inspectors and one alternate, but at least one person who has aquatics experience.



<https://www.aaalac.org/accreditation/RefResources/ZebrafishReference.pdf>

What Do You Do?

- You will often be your institutions expert
- Back up your husbandry practices with publications and manuals (current!!)
- Offer to share these resources with your oversight committees, inspectors and veterinarians
- Look to other organizations like AAALAC for guidance on inspection preparation.



CCAC Site Visits (not inspections!!)

- Occur every 3 years announced
- Evaluate the *function of the animal care committee* and animal care program based on CCAC guidelines and standards
 - Housing and enrichment
 - Anesthesia and euthanasia
 - Protocol review process
 - Post-approval monitoring
 - Researcher training
 - Physical plant and facilities



CANADIAN COUNCIL ON ANIMAL CARE
CONSEIL CANADIEN DE PROTECTION DES ANIMAUX

Ontario Inspections

- Every 1-2 years, unannounced
- Evaluate *animal care committee* and facilities based on the Ontario Animals for Research Act
 - Animal use protocols
 - Anesthesia
 - Euthanasia
 - Transportation
 - Physical plant and facilities



Ministry of Agriculture,
Food and Rural Affairs