

**Extract taken from the  
Code of Practice for the Housing and  
Care of Animals Bred, Supplied or  
Used for Scientific Purposes**

**Fish, Amphibians, Reptiles  
and Cephalopods**



**December 2014**



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

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## Executive Summary

This Code of Practice contains standards for the care and accommodation of animals at licensed breeder, supplier and user establishments in accordance with section 21 of the Animals (Scientific Procedures) Act 1986 as amended in 2012 (“ASPA”), which requires the Secretary of State to “issue codes of practice as to the care of protected animals and their use for regulated procedures”.

The purpose of this Code of Practice is to ensure that the design, construction and function of the installations and equipment of licensed establishments – along with their staffing, care and practices – allow procedures to be carried out as effectively as possible.

The key outcomes driven by this Code of Practice are:

- to promote good animal welfare through the provision of consistent, high quality care and accommodation;
- to support the generation of high quality, reliable scientific results through the reduction of environmental variables;
- to implement the principles of the 3Rs through using the minimum number of animals and causing the minimum degree of pain, suffering, distress or lasting harm.

It contains three sections covering both general and species specific indications:

- **Section 1** describes the legal minimum standards applicable now.
- **Section 2** describes the legal minimum standards applicable from 1 January 2017.
- **Section 3** provides non-mandatory advice covering a broader range of subjects than Sections 1 and 2.

Section 3 of this Code of Practice is based on Commission Recommendation 2007/526/EC (itself based on Appendix A to the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes), which remains in force and requires that “member states shall pay regard to the guidelines set out in the Annex to the Recommendation”.

Section 3 aims to promote a shared understanding between establishments and Home Office Inspectors of the manner in which the requirements of Sections 1 and 2 might be met. It will assist establishments to meet the requirements of Sections 1 and 2, as well as encourage the application of up-to-date evidence-based 3Rs approaches to accommodation and care. With advances in welfare and science, leading practice evolves. Additional advice may therefore be published separately by the Home Office over time, and Section 3 will be formally revised, as appropriate.

The advice set out in Section 3 is not prescriptive and need not provide the only means of compliance with the mandatory standards in Sections 1 and 2.

Establishments should seek to continually improve their standards of care and accommodation in line with the principles of the 3Rs, striving to adopt higher standards where practicable and applicable. Section 3 is neither intended as a training manual nor as a substitute for proper training, experience and expert advice.

**Animals in Science Regulation Unit, December 2014.**



## Introduction

### 1 Background

This Code of Practice (“CoP”) is issued under section 21 of the Animals (Scientific Procedures) Act 1986 as amended in 2012 (“ASPA”), which requires the Secretary of State to “issue codes of practice as to the care of protected animals and their use for regulated procedures”.

Article 22 of the Directive 2010/63/EU of the European Parliament and of the Council (22 September 2010) on the protection of animals used for scientific purposes requires that Member States ensure that “all establishments of a breeder, supplier or user have installations and equipment suited to the species of animals housed and, where procedures are carried out, to the performance of the procedures.”

Article 22 also requires that “Member States shall ensure that the relevant requirements as set out in Annex III are complied with.”

This CoP fulfils these requirements by meeting Annex III standards, while retaining higher UK CoP standards in force prior to 2010.<sup>1,2</sup> It sets out changes to legal minimum standards applicable from 1 January 2017.<sup>3</sup> Section 3 provides additional advice derived from Commission Recommendation 2007/526/EC<sup>4</sup> and other expert sources on how the requirements of Annex III might be met. This advisory section also encourages licensed establishments to continually improve their standards of care and accommodation in line with the principles of the 3Rs (replacement, reduction and refinement), striving to adopt higher standards where practicable and applicable. The Animals in Science Committee has been consulted in the drafting of this CoP.

This CoP primarily relates to the *care and accommodation* of animals. The Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 (“the Guidance”) relates to their *use* in scientific procedures and how to comply with the legislation.

### 2 Why does this CoP exist?

The purpose of the CoP is to ensure that the design, construction and method of functioning of the installations and equipment of licensed establishments – along with their staffing, care and practices – allow procedures to be carried out as effectively as possible, in order to obtain reliable results using the minimum number of animals and causing the minimum degree of pain, suffering, distress or lasting harm.

Some procedures are conducted under field conditions on free-living, self-supporting, wild animals, but such procedures are relatively few in number. The great majority of animals used in procedures are kept in facilities ranging from outdoor pastures to cages for small animals in an animal house. There are often highly conflicting interests between the scientific requirements and the needs of the animal. In this conflict, the basic physiological and ethological needs of the animals (freedom of movement, social contact, meaningful activity, nutrition, water) should be restricted *only for the minimum necessary period of time and degree*. Such restrictions should be reviewed by scientists, animal technicians and those competent persons charged with advisory duties in relation to the well-being of the animals before procedures are undertaken, to ensure that the extent of the compromise to animal welfare is minimised to a level consistent with the scientific objectives of the study.

1 Code of Practice for the Housing and Care of animals in Designated Breeding and Supplying Establishments. Pursuant to Animals (Scientific Procedures) Act 1986, 24 January 1995.

2 Code of Practice for the Housing and Care of animals used in scientific procedures. Pursuant to Animals (Scientific Procedures) Act 1986, 7 February 1989.

3 As set out in Annex III of the Directive 2010/63/EU of the European Parliament and of the Council (22 September 2010) on the protection of animals used for scientific purposes.

4 Commission Recommendation (18 June 2007) on guidelines for the accommodation and care of animals used for experimental and other scientific purposes (2007/526/EC).

### 3 To whom and where does this CoP apply?

If an establishment is licensed for breeding, supplying and/or using animals under section 2C of ASPA it must abide by the mandatory standards within Sections 1 and 2 of this CoP for all protected animals<sup>5</sup> held at the establishment that will be, are being or have been used for a licensed purpose.<sup>6</sup>

In Great Britain this CoP is administered by the Home Office. In Northern Ireland it is administered by the Department of Health and Social Services. Where the Code refers to the “Secretary of State” or “the Home Office” it means, in Northern Ireland, the Department of Health and Social Services.

### 4 When does the CoP come into force?

The standards in Section 1 of this CoP came into force through the amended legislation on 1 January 2013. The standards in Section 2 will come into force from 1 January 2017 and will supersede Section 1.

### 5 What does this CoP contain?

This CoP contains standards for the care and accommodation of animals at licensed breeder, supplier and user establishments.

**Section 1** describes the legal minimum standards applicable until 31 December 2016.

**Section 2** describes the legal minimum standards applicable from 1 January 2017.

**Section 3** provides advice covering a broader range of subjects than Sections 1 and 2. This section *does not* set out legal requirements.

Within each section is:

- A chapter containing information **relevant to all species**.
- **Species-specific** chapters containing information relevant only to those species.

“Care and accommodation”, used in the context of animals in science, covers all aspects of the relationship between animals and man. Its substance is the sum of material and non-material resources provided by man to obtain and maintain an animal in a physical and mental state where it suffers least and which promotes good science. It starts from the moment the animal is intended to be used in procedures, including breeding or keeping for that purpose, and continues until it is humanely killed (or rehomed or set free, as appropriate) after the completion of the procedure(s).

### 6 From where does the content originate?

#### 6.1 Section 1

Section 1 standards are taken from Annex III of the European Directive on the protection of animals used for scientific purposes,<sup>7</sup> but retains higher UK CoP standards in force prior to 2010,<sup>8,9</sup> in accordance with Article 2 of the Directive.

Some of the Annex III standards do not come into force until 1 January 2017. Where this is the case, standards from the previous UK Codes of Practice are quoted, for use until 1 January 2017. Standards that are changing are clearly labelled and cross referenced. The post-2017 standards are found in Section 2.

<sup>5</sup> A “protected animal” means any living vertebrate other than man and any living cephalopod.

<sup>6</sup> The **applying of regulated procedures** to protected animals; the **breeding** of animals listed in schedule 2 of ASPA with a view to their use in regulated procedures or the use of their tissues or organs for scientific purposes, or the breeding of protected animals (other than animals listed in schedule 2 of ASPA) primarily for these purposes; or the keeping of relevant protected animals which have been bred elsewhere and are to be **supplied** with a view to their use elsewhere in regulated procedures or the use elsewhere of their tissues or organs for scientific purposes.

<sup>7</sup> Annex III of the Directive 2010/63/EU of the European Parliament and of the Council (22 September 2010) on the protection of animals used for scientific purposes.

<sup>8</sup> Code of Practice for the Housing and Care of animals in Designated Breeding and Supplying Establishments. Pursuant to Animals (Scientific Procedures) Act 1986, 24 January 1995.

<sup>9</sup> Code of Practice for the Housing and Care of animals used in scientific procedures. Pursuant to Animals (Scientific Procedures) Act 1986, 7 February 1989.

On 1 January 2017 Section 1 will no longer apply.

## 6.2 Section 2

These standards are taken from Annex III of the European Directive on the protection of animals used for scientific purposes,<sup>10</sup> but retain higher UK CoP standards in force prior to 2010<sup>11,12</sup> in accordance with Article 2 of the Directive.

### 6.2.1 What are the changes?

Most of the standards for accommodation of animals are changed. There are some completely new standards, for example covering additional species.

Where standards have *not* changed, they are restated here, so that this section provides a complete guide to standards post-2017.

## 6.3 Section 3

The advice given in Section 3 is based on Commission Recommendation 2007/526/EC<sup>13</sup> (“the Recommendation”), which has been adapted to ensure the information provided is appropriate (specific and clear) in the context of the UK. Supplemental information has also been taken from the EU Expert Working Groups who advised on revision of Appendix A of convention ETS 123, from UK CoP standards in force prior to 2010<sup>14,15</sup> and other expert sources. The revised Appendix A was incorporated into the Recommendation in June 2007.

The Recommendation is still in force and part of the EU legislative framework. It was not repealed by Directive 2010/63/EU (“the Directive”) and Recital 5 of the Directive makes a specific reference to the Recommendation. The Recommendation provides more in-depth understanding of the housing and care obligations that are part of the Directive, and helps to assist all involved in meeting the requirements of Annex III. The Recommendation constitutes complementary guidance and is not binding under EU law.

Similarly, Section 3 of this CoP provides advice on how the requirements of Annex III might be met. This advisory section also encourages licensed establishments to continually improve their standards of care and accommodation in line with the principles of the 3Rs, striving to achieve better welfare outcomes where practicable and applicable.

# 7 How to use this CoP

*Chapter 1: Standards applicable to all animals* can be found in each section and lays out standards applicable to all species and the general principles to which you are expected to adhere (Sections 1 and 2) or consider (Section 3). You should also consult the relevant *species-specific chapters* of Sections 1 or 2 in order to ascertain what is expected of your establishment.

You should use the advice in Section 3:

- to help you to understand how the standards in Sections 1 and 2 might be met;
- to guide you where there are no standards applicable in Sections 1 or 2 for your species or situation;
- to help you improve your facilities or working practices, where applicable.

10 Annex III of the Directive 2010/63/EU of the European Parliament and of the Council (22 September 2010) on the protection of animals used for scientific purposes.

11 Code of Practice for the Housing and Care of animals in Designated Breeding and Supplying Establishments. Pursuant to Animals (Scientific Procedures) Act 1986, 24 January 1995.

12 Code of Practice for the Housing and Care of animals used in scientific procedures. Pursuant to Animals (Scientific Procedures) Act 1986, 7 February 1989.

13 Commission Recommendation (18 June 2007) on guidelines for the accommodation and care of animals used for experimental and other scientific purposes (2007/526/EC).

14 Code of Practice for the Housing and Care of animals in Designated Breeding and Supplying Establishments. Pursuant to Animals (Scientific Procedures) Act 1986, 24 January 1995.

15 Code of Practice for the Housing and Care of animals used in scientific procedures. Pursuant to Animals (Scientific Procedures) Act 1986, 7 February 1989.

Each section is colour coded to help clarify whether it is mandatory immediately, mandatory after 1 January 2017 or constitutes advice.

The CoP may specify different standards depending on whether the animals are being bred, supplied, used or kept in stock for scientific purposes. The following definitions apply:<sup>16</sup>

<b>Breeding animals</b>	Protected animals <sup>17</sup> in a breeding programme, or being kept for use in a breeding programme, where they and/or their offspring are subsequently intended for use in a regulated procedure, or their tissues or organs are intended for use for scientific purposes. This includes all females that have been mated, and stud males. It includes the breeding of genetically altered animals and harmful mutants authorised in project licences. <sup>18</sup>
<b>Supply animals</b>	Protected animals kept or which have been kept for the purpose of being supplied for use in a regulated procedure, or for the use of their tissues or organs for scientific purposes.
<b>Use animals</b>	Protected animals being or which have been used in a regulated procedure, or which are being or have been kept for use in a regulated procedure, other than where the regulated procedure is the breeding of genetically altered animals or harmful mutants.

Should behaviour or breeding problems occur or should you require further information, you should seek the advice of animal care staff, the Named Veterinary Surgeon, your local Inspector and/or other specialists.

Definitions of terms can be found in the glossary.

## 8 The CoP's legal status

### 8.1 Mandatory and advisory sections

Sections 1 and 2 provide the mandatory legal minimum standards required now and from 1 January 2017 respectively. Section 3 provides advice.

Section 3 does *not* contradict any standard provided in Sections 1 or 2. It *does*, however, provide advice in areas that are not covered in Sections 1 or 2, advice for protected species absent from those sections, and additional information about how Section 1 or 2 standards might be met.

### 8.2 Non-compliance with Sections 1 and 2

Section 21 (4) of ASPA states:

“A failure on the part of any person to comply with any provision of a code... shall not of itself render that person liable to criminal or civil proceedings but ... any such code shall be admissible in evidence in any such proceedings... and if any of its provisions appears to the court ... to be relevant ... it shall be taken into account [in determining the outcome of the case]”.

In addition, standard condition 4 of Establishment Licences describes the requirement to provide adequate and appropriate standards of care and accommodation. Those standards will be taken from this CoP, and therefore a failure to meet the mandatory (Section 1 or 2) standards of the CoP may constitute a breach of standard condition 4 of the Establishment Licence.

If an establishment fails to meet the minimum legal standards as described in Sections 1 and 2 of this CoP it is likely to be in breach of standard condition 4 of the Establishment Licence.

In addition, any breach of Sections 1 and 2 of this CoP will be presented as evidence in any relevant criminal or civil case or action for breach of licence conditions taken by the Secretary of State or others.

<sup>16</sup> Animals (Scientific Procedures) Act 1986 as amended in 2012, sections 2B and 15A (11).

<sup>17</sup> As defined in the Animals (Scientific Procedures) Act 1986 as amended in 2012, section 2B.

<sup>18</sup> When breeding genetically altered animals, an assessment must be made of any potential differences to the requirements for care and accommodation brought about by the genetic alteration. Where additional requirements are identified they must be provided, unless withholding them is authorised in the project licence.



### 8.3 Section 3 advice

The purpose of Section 3 is to promote a shared understanding between Establishments and Home Office Inspectors of the manner in which the requirements of Sections 1 and 2 might be met. In addition, the advice seeks to encourage establishments to promulgate high quality animal welfare and high quality science, which may go beyond the minimum requirements, where applicable.

Home Office Inspectors may utilise the advice in Section 3 to discuss and encourage best practice. With advances in welfare and science, leading practice evolves. Establishments should seek to continually improve their standards of care and accommodation in line with the principles of the 3Rs, striving to achieve better welfare outcomes, where practicable and applicable.

Whilst there is no legal requirement to make provisions above the minima in Sections 1 and 2, demonstration that an establishment has given due consideration to the advice in Section 3 or other relevant, evidenced-based advice would provide *positive* evidence of a good culture of care at that establishment. Other indicators, not related to this CoP, are also important in demonstrating the culture of care at an establishment.

Where an Inspector has significant concerns about animal welfare and/or the quality of science at a particular establishment, they may utilise the advice in Section 3, as well as other sources of expert opinion, to assist in identifying how welfare or scientific outcomes may be improved.

### 8.4 Specifying deviations from CoP Section 1 or 2 standards

#### 8.4.1 Specifying deviations from Sections 1 or 2

Where deviation from standards laid down in Sections 1 or 2 of this CoP is required for **scientific** reasons, authorisation for the deviation must be specified in the Project or Establishment Licence after local ethical review and on approval by the Secretary of State. Normally authorisation will only be given with specific justification.

Where deviation from standards laid down in Sections 1 or 2 of this CoP is required temporarily as a part of **treatment** the animal is receiving as recognised veterinary practice under the care of a veterinary surgeon, for example confinement in order to enforce rest, such a deviation will fall under the Veterinary Surgeons Act and will not require authorisation in the Project or Establishment Licence.

There are some circumstances when deviation from standards laid down in Sections 1 or 2 of this CoP is required for a short period on isolated occasions for **animal health or welfare** reasons. For example, temporary single housing of a social species may be required for a short time after weaning if there is a single mouse in a litter, prior to the animal being issued on procedure. Such deviations must be for the minimum period necessary, and may be specified by the agreement of the appropriate named persons (normally NVS and NACWO). Such agreements should be documented, and records made available to the Home Office Inspector on request.

Should the event become regular and/or predictable, for example because it becomes apparent that a certain strain of mouse has a high frequency of producing litters with single offspring, local authorisation may no longer be appropriate and Project or Establishment Licence authorisation may be required. However, in accordance with ASPA section 2(8) practices undertaken for the purposes of recognised animal husbandry<sup>19</sup> will not require licence authority and may be continued with the ongoing agreement of the appropriate named persons.

If there is any doubt as to what type of authorisation is appropriate you should seek the advice of your Home Office Inspector.

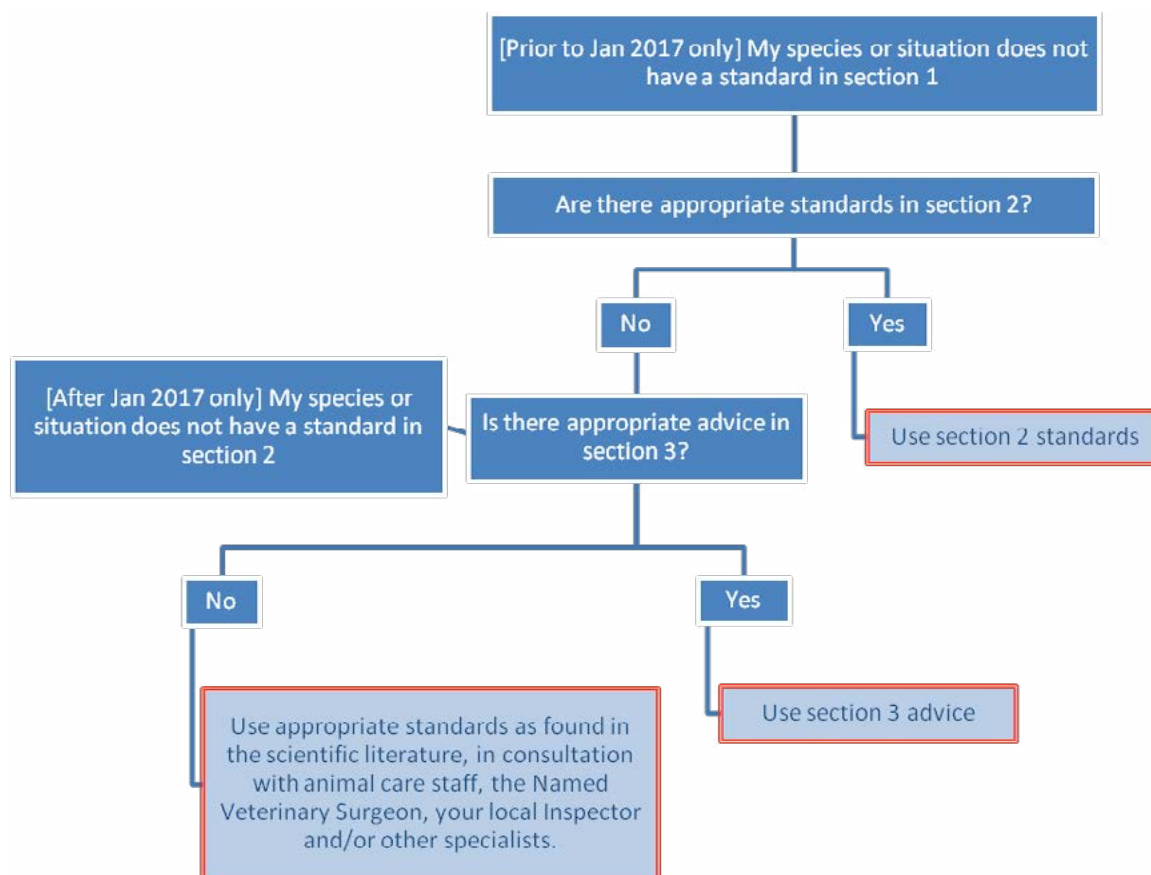
<sup>19</sup> "Recognised animal husbandry" refers to techniques currently widely practised in the UK. Such techniques will be found in literature such as the Defra Codes of Recommendation for the Welfare of Livestock, the RSPCA Research Animals Team's Guidance Notes (<http://science.rspca.org.uk/sciencegroup/researchanimals/ethicalreview/functionstasks/housingandcare>) and the UFAW Handbook (Hubrecht, R. & Kirkwood, J. [eds] (2010) 'The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals' 8th edn. Wiley-Blackwell, Oxford).

### 8.4.2 Specific circumstances within agricultural research

During agricultural research, when the aim of the project requires that the animals are kept under similar conditions to those under which commercial farm animals are kept, the keeping of the animals shall comply at least with the standards laid down in Council Directives 91/629/EEC, 91/630/EEC, 98/58/EC, 1999/74/EC and 2007/43/EC.<sup>20</sup> Authority to use Council Directive standards in place of these ASPA CoP standards should be specified in the Project or Establishment Licence after local ethical review and on approval by the Secretary of State.

### 8.5 Where standards do not exist in this CoP – the Standards Cascade

This CoP does not necessarily provide standards for all species and circumstances. Where standards are not given in Section 1 (for example for a particular species or particular circumstance) there is no legal minimum standard laid down. In this case establishments will be expected to adopt any relevant standards described in Section 2. Where no relevant standard is presented in Section 2, establishments will be expected to consider relevant advice in Section 3. Where no relevant advice is presented in Section 3, establishments will be expected to apply appropriate standards as found in the scientific literature, in consultation with animal care staff, the Named Veterinary Surgeon, your AWERB, your local Inspector and/or other specialists. From January 2017 Section 1 can be disregarded, and this cascade operates only for Sections 2 and 3. For the purposes of compliance, where this cascade has been used the standards within the red double-framed boxes will be treated as advice.



<sup>20</sup> Council Directive 91/629/EEC of 19 November 1991 laying down minimum standards for the protection of calves (OJ L 340, 11.12.1991, p. 28).  
 Council Directive 91/630/EEC of 19 November 1991 laying down minimum standards for the protection of pigs (OJ L 340, 11.12.1991, p. 33).  
 Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes (OJ L 221, 8.8.1998, p. 23).  
 Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens (OJ L 203, 3.8.1999, p. 53).  
 Council Directive 2007/43/EC of 28 June 2007 laying down minimum rules for the protection of chickens kept for meat production (OJ L 182, 12.7.2007, p. 19).

## 9 Inspections

Inspectors use the standards within this CoP to assess whether the care and accommodation provided by the establishment meet the requirements of ASPA and standard condition 4 of the Establishment Licence.

Failure to meet the legal minimum standards set out in Sections 1 and 2 is *likely* to result in non-compliance action.

The inspector's focus with Section 3 will be to advise you how you might best use the advice within it. As none of the advice in Section 3 is mandatory, you cannot be found in breach of Section 3.

Information within Section 3 may also be used as a trigger for discussions during the assessment of project licence applications. For example, should an investigator wish to hold animals at an unusually low or high temperature (outside the range advised in this CoP), this should be highlighted and discussed within the application. Similarly, barren environments devoid of enrichment, unusual flooring or bedding materials, social isolation, atypical feeding regimes and other such factors will require project licence authority if they may cause pain, suffering, distress or lasting harm, and the specific circumstances and justifications should be discussed with your Inspector.

For more information on inspections and Inspectors, please read 'Guidance on the Operation of ASPA' available on the 'Research and Testing Using Animals' website.

## 10 How the CoP will be reviewed and updated

As understanding of how best to care for animals evolves, the recommendations contained in this CoP may require updating. The Secretary of State will keep this CoP under review and will issue amendments as necessary.

If you would like to contribute to the next version, please send your comments to [ASRUBusinessSupport@HomeOffice.gsi.gov.uk](mailto:ASRUBusinessSupport@HomeOffice.gsi.gov.uk).



## Glossary

<b>Animals in Science Committee (ASC)</b>	The independent non-departmental public body set up under ASPA sections 19 and 20. It is responsible for providing impartial, balanced and objective advice to the Home Office on issues relating to ASPA.
<b>AWERB</b>	Animal Welfare and Ethical Review Body. Every licensed establishment is required by ASPA to have an AWERB. Its membership and activities are defined by ASPA.
<b>Body condition</b>	See condition scoring.
<b>Brooder lamp</b>	A heat lamp with a controllable or known temperature output.
<b>Condition scoring</b>	An objective system of evaluating an animal's body condition (amount of stored fat) and assigning a numeric value.
<b>Conspecifics</b>	Belonging to the same biological species.
<b>Cubicle</b>	Individual cow bedding spaces separated by half height and half length partitions. Usually located in loose housing cow accommodation in which the cow is free to wander at will.
<b>Enclosure</b>	The primary accommodation in which the animal is confined, for example a cage, pen, run or stall. It is the extent to which the enclosed animal can freely range.
<b>Environmental enrichment</b>	The practice of providing animals under managed care with stimuli such as natural and artificial objects which promote the expression of species-appropriate behavioural and mental activities.
<b>Establishment</b>	A place holding a licence that has been granted under section 2C of ASPA.
<b>Establishment Licence (PEL)</b>	A licence granted to a place under section 2C of ASPA.
<b>Fill and dump system</b>	A water cleaning system where there is no through flow of water. A proportion of water volume is extracted and replaced at regular intervals in order to keep the aquatic environment healthy. The volume and frequency of the exchange depends on the species requirements.
<b>Filter top cage</b>	A cage box with a wire or plastic structure on the top of the cage with filter element. These cages are housed in standard racks.
<b>Floor area</b>	The footprint of the enclosure. Whether or not shelving may contribute to floor area varies according to the species being kept and the space below the shelf. For more information, please see Section 3 Chapter 1 Advice applicable to all animals, paragraph 4.6.
<b>Flow system</b>	A continuous matched top up and outflow of water in and out of tanks in order to keep the aquatic environment healthy.
<b>Group housed</b>	Housed as two or more animals within the same enclosure.
<b>Harem (guinea pig)</b>	A group of guinea pigs – typically one male with one to ten females.
<b>Headweaving</b>	A stereotypical behaviour where the animal repeatedly moves its head from side to side, while keeping its body relatively still.
<b>Health status</b>	The presence or absence of infectious or non-infectious disease or abnormality in an individual or population.

<b>Height</b>	The vertical distance between the enclosure floor and the top of the enclosure. This height should apply over more than 50% of the minimum enclosure floor area prior to the addition of enrichment devices.
<b>Holding room</b>	Secondary accommodation in which the animal enclosure(s) may be located, as specified in the schedule of premises of the Establishment Licence.
<b>Inspector</b>	An inspector in the Animals in Science Regulation Unit appointed under ASPA section 18.
<b>Isolator</b>	A device that creates a barrier in order to maintain an internal condition (e.g. sterile or aseptic).
<b>IVC</b>	Individually ventilated cage. A sealed cage unit attached to a dedicated air-handling system.
<b>Mismothering</b>	Failure of maternal behaviour that places the offspring at increased risk of suffering and/or harm.
<b>NACWO</b>	Named Animal Care and Welfare Officer.
<b>Named Veterinary Surgeon (NVS)</b>	A member of the Royal College of Veterinary Surgeons, specified by the Establishment, to advise on the health, welfare and treatment of the animals.
<b>Open-water systems</b>	Enclosures within a larger body of water, for example a pond, lake, river or the sea.
<b>Pen</b>	An area enclosed, for example, by walls, bars or meshed wire in which one or more animals are kept. Depending on the size of the pen and the stocking density, the freedom of movement of the animals is usually less restricted than in a cage.
<b>Pet animal</b>	An animal kept by a human as a source of companionship or pleasure.
<b>POLE</b>	Place Other than a Licensed Establishment.
<b>Post-weaned stock</b>	Young animals that have been weaned from the dam being kept for a licensed purpose.
<b>Procedure</b>	A licensed procedure causes pain, suffering, distress or lasting harm greater than or equal to the insertion of a hypodermic needle in accordance with good veterinary practice.
<b>Procedure room</b>	A room allocated for use for sterile and/or non-sterile procedures as specified in the schedule of premises of the Establishment Licence.
<b>Production indices</b>	Measurements of animal growth and production (e.g. milk production).
<b>Project Licence (PPL)</b>	A licence granted under section 5 of ASPA.
<b>Protocol</b>	A series of experimental steps defined in the Project Licence (PPL).
<b>Re-use</b>	The use of an animal in a protocol which has already been used in a previous protocol, where a naive animal could be used in its place. More information on re-use is available in the 'Guidance on the Operation of ASPA' available on the 'Research and Testing Using Animals' website.
<b>Rooting</b>	The natural behaviour of pigs and other animals to turn over soil or litter with their noses in search of edible matter.

<b>Service room</b>	A room within the facility which does not house animals and is not used for procedures, but contributes to the running of the facility, for example store rooms, cleaning and washing areas and waste management areas.
<b>Single housed</b>	Housed as one animal per enclosure, where other animals are not able to physically occupy the same space. This definition applies regardless of the degree of contact (e.g. sight or smell) that the animal may have with others.
<b>Stud animal</b>	An animal used for breeding only.
<b>Supplying</b>	Selling or passing on animals for a purpose regulated under ASPA.
<b>Using</b>	Using an animal for a purpose regulated under ASPA.
<b>Wild animal</b>	A species or animal that has never been cared for or farmed by humans, and is not descended from domesticated individuals.





## Section 1, Chapter 1: Standards applicable to all animals

There are no changes to these standards pre-and post-January 2017.

### 1 The physical facilities

#### 1.1 Functions and general design

All facilities shall be constructed so as to provide an environment which takes into account the physiological and ethological needs of the species kept in them. Facilities shall also be designed and managed to prevent access by unauthorised persons and the ingress or escape of animals.

Establishments shall have an active maintenance programme to prevent and remedy any defect in buildings or equipment.

#### 1.2 Holding rooms

Establishments shall have a regular and efficient cleaning schedule for the rooms and shall maintain satisfactory hygiene standards.

Walls and floors shall be surfaced with a material resistant to the heavy wear and tear caused by the animals and the cleaning process. The material shall not be detrimental to the health of the animals and shall be such that the animals cannot hurt themselves. Additional protection shall be given to any equipment or fixtures so that they are not damaged by the animals nor do they cause injury to the animals themselves.

Species that are incompatible, for example predator and prey, or animals requiring different environmental conditions, shall not be housed in the same room nor, in the case of predator and prey, within sight, smell or sound of each other.

#### 1.3 General and special purpose procedure rooms

Establishments shall, where appropriate, have available laboratory facilities for the carrying out of simple diagnostic tests, post-mortem examinations, and/or the collection of samples that are to be subjected to more extensive laboratory investigations elsewhere. General and special purpose procedure rooms shall be available for situations where it is undesirable to carry out the procedures or observations in the holding rooms.

Facilities shall be provided to enable newly acquired animals of uncertain health status to be isolated until their health status can be determined and the potential health risk to established animals assessed and minimised.

There shall be accommodation for the separate housing of sick or injured animals.

#### 1.4 Service rooms

Store-rooms shall be designed, used and maintained to safeguard the quality of food and bedding. These rooms shall be vermin and insect-proof, as far as possible. Other materials, which may be contaminated or present a hazard to animals or staff, shall be stored separately.

The cleaning and washing areas shall be large enough to accommodate the installations necessary to decontaminate and clean used equipment. The cleaning process shall be arranged so as to separate the flow of clean and dirty equipment to prevent the contamination of newly cleaned equipment.

Establishments shall provide for the hygienic storage and safe disposal of carcasses and animal waste.

Where surgical procedures under aseptic conditions are required there shall be provision for one or more than one suitably equipped room, and facilities provided for postoperative recovery.

## 2 Environmental conditions

### 2.1 Ventilation and temperature

Insulation, heating and ventilation of the holding room shall ensure that the air circulation, dust levels, and gas concentrations are kept within limits that are not harmful to the animals housed, and are appropriate for the housing system in operation.

Temperature and relative humidity in the holding rooms shall be adapted to the species and age groups housed. The temperature shall be measured and logged on a daily basis.

Animals shall not be restricted to outdoor areas under climatic conditions which may cause them distress.

### 2.2 Lighting

Where natural light does not provide an appropriate light/dark cycle, controlled lighting shall be provided to satisfy the biological requirements of the animals and to provide a satisfactory working environment.

Illumination shall satisfy the needs for the performance of husbandry procedures and inspection of the animals.

Regular photoperiods and intensity of light adapted to the species shall be provided.

When keeping albino animals, the lighting shall be adjusted to take into account their sensitivity to light.

### 2.3 Noise

Noise levels, including ultrasound, shall not adversely affect animal welfare.

Establishments shall have alarm systems that sound outside the sensitive hearing range of the animals, where this does not conflict with their audibility to human beings.

Holding rooms shall, where appropriate, be provided with noise insulation and absorption materials.

### 2.4 Alarms

Establishments relying on electrical or mechanical equipment for environmental control and protection shall have a stand-by system to maintain essential services and emergency lighting systems as well as to ensure that alarm systems themselves do not fail to operate.

Heating and ventilation systems shall be equipped with monitoring devices and alarms.

Clear instructions on emergency procedures shall be prominently displayed.

## 3 Care of animals

### 3.1 Health

Establishments shall have a strategy in place to ensure that a health status of the animals is maintained that safeguards animal welfare and meets scientific requirements. This strategy shall include regular health monitoring, a microbiological surveillance programme and plans for dealing with health breakdowns and shall define health parameters and procedures for the introduction of new animals.

Animals shall be checked at least daily by a competent person.<sup>21</sup> These checks shall ensure that all sick or injured animals are identified and appropriate action is taken.

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<sup>21</sup> A person who has been trained and assessed as competent to perform daily routine health checks for the animals in question.

## 3.2 Animals taken from the wild

Transport containers and means of transport adapted to the species concerned shall be available at capture sites, in case animals need to be moved for examination or treatment.

Special consideration shall be given and appropriate measures taken for the acclimatisation, quarantine, housing, husbandry, care of animals taken from the wild and, as appropriate, provisions for setting them free at the end of procedures.

## 3.3 Housing and enrichment

### 3.3.1 Housing

Animals, except those which are naturally solitary, shall be socially housed in stable groups of compatible individuals. In cases where single housing is allowed<sup>22</sup> the duration shall be limited to the minimum period necessary and visual, auditory, olfactory and/or tactile contact shall be maintained where appropriate to the species, strain and sex. The introduction or re-introduction of animals to established groups shall be carefully monitored to avoid problems of incompatibility and disrupted social relationships.

### 3.3.2 Enrichment

All animals shall be provided with space of sufficient complexity to allow expression of a wide range of normal behaviour. They shall be given a degree of control and choice over their environment to reduce stress-induced behaviour. Establishments shall have appropriate enrichment techniques in place, to extend the range of activities available to the animals and increase their coping activities including physical exercise, foraging, manipulative and cognitive activities, as appropriate to the species. Environmental enrichment in animal enclosures shall be adapted to the species and individual needs of the animals concerned. The enrichment strategies in establishments shall be regularly reviewed and updated.

### 3.3.3 Animal enclosures

Animal enclosures shall not be made out of materials detrimental to the health of the animals. Their design and construction shall be such that no injury to the animals is caused. Unless they are disposable, they shall be made from materials that will withstand cleaning and decontamination techniques. The design of animal enclosure floors shall be adapted to the species and age of the animals and be designed to facilitate the removal of excreta.

## 3.4 Feeding

The form, content and presentation of the diet shall meet the nutritional and behavioural needs of the animal.

The animals' diet shall be palatable and non-contaminated. In the selection of raw materials, production, preparation and presentation of feed, establishments shall take measures to minimise chemical, physical and microbiological contamination.

Packing, transport and storage shall be such as to avoid contamination, deterioration or destruction. All feed hoppers, troughs or other utensils used for feeding shall be regularly cleaned and, if necessary, sterilised.

Each animal shall be able to access the food, with sufficient feeding space provided to limit competition.

## 3.5 Watering

Uncontaminated drinking water shall always be available to all animals.

When automatic watering systems are used, they shall be regularly checked, serviced and flushed to avoid accidents. If solid-bottomed cages are used, care shall be taken to minimise the risk of flooding.

<sup>22</sup> See Introduction paragraph 8.4: Specifying deviations from CoP Section 1 or 2 standards.

Provision shall be made to adapt the water supply for aquaria and tanks to the needs and tolerance limits of the individual fish, amphibian and reptile species.

### 3.6 Resting and sleeping areas

Bedding materials or sleeping structures adapted to the species shall always be provided, including nesting materials or structures for breeding animals.

Within the animal enclosure, as appropriate to the species, a solid, comfortable resting area for all animals shall be provided. All sleeping areas shall be kept clean and dry.

### 3.7 Handling

Establishments shall set up habituation and training programmes suitable for the animals, the procedures and length of the project.



## Section 1, Chapter 10: Fish

There are no changes to these standards pre- and post-January 2017.

### 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.

### 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.

### 3 Temperature, lighting and noise

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

### 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.

### 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.

## Section 2, Chapter 1: Standards applicable to all animals

There are no changes to these standards pre- and post- January 2017.

### 1 The physical facilities

#### 1.1 Functions and general design

All facilities shall be constructed so as to provide an environment which takes into account the physiological and ethological needs of the species kept in them. Facilities shall also be designed and managed to prevent access by unauthorised persons and the ingress or escape of animals.

Establishments shall have an active maintenance programme to prevent and remedy any defect in buildings or equipment.

#### 1.2 Holding rooms

Establishments shall have a regular and efficient cleaning schedule for the rooms and shall maintain satisfactory hygiene standards.

Walls and floors shall be surfaced with a material resistant to the heavy wear and tear caused by the animals and the cleaning process. The material shall not be detrimental to the health of the animals and shall be such that the animals cannot hurt themselves. Additional protection shall be given to any equipment or fixtures so that they are not damaged by the animals nor do they cause injury to the animals themselves.

Species that are incompatible, for example predator and prey, or animals requiring different environmental conditions, shall not be housed in the same room nor, in the case of predator and prey, within sight, smell or sound of each other.

#### 1.3 General and special purpose procedure rooms

Establishments shall, where appropriate, have available laboratory facilities for the carrying out of simple diagnostic tests, post-mortem examinations, and/or the collection of samples that are to be subjected to more extensive laboratory investigations elsewhere. General and special purpose procedure rooms shall be available for situations where it is undesirable to carry out the procedures or observations in the holding rooms.

Facilities shall be provided to enable newly acquired animals of uncertain health status to be isolated until their health status can be determined and the potential health risk to established animals assessed and minimised.

There shall be accommodation for the separate housing of sick or injured animals.

#### 1.4 Service rooms

Store-rooms shall be designed, used and maintained to safeguard the quality of food and bedding. These rooms shall be vermin and insect-proof, as far as possible. Other materials, which may be contaminated or present a hazard to animals or staff, shall be stored separately.

The cleaning and washing areas shall be large enough to accommodate the installations necessary to decontaminate and clean used equipment. The cleaning process shall be arranged so as to separate the flow of clean and dirty equipment to prevent the contamination of newly cleaned equipment.

Establishments shall provide for the hygienic storage and safe disposal of carcasses and animal waste.

Where surgical procedures under aseptic conditions are required there shall be provision for one or more than one suitably equipped room, and facilities provided for postoperative recovery.

## 2 Environmental conditions

### 2.1 Ventilation and temperature

Insulation, heating and ventilation of the holding room shall ensure that the air circulation, dust levels, and gas concentrations are kept within limits that are not harmful to the animals housed, and are appropriate for the housing system in operation.

Temperature and relative humidity in the holding rooms shall be adapted to the species and age groups housed. The temperature shall be measured and logged on a daily basis.

Animals shall not be restricted to outdoor areas under climatic conditions which may cause them distress.

### 2.2 Lighting

Where natural light does not provide an appropriate light/dark cycle, controlled lighting shall be provided to satisfy the biological requirements of the animals and to provide a satisfactory working environment.

Illumination shall satisfy the needs for the performance of husbandry procedures and inspection of the animals.

Regular photoperiods and intensity of light adapted to the species shall be provided.

When keeping albino animals, the lighting shall be adjusted to take into account their sensitivity to light.

### 2.3 Noise

Noise levels, including ultrasound, shall not adversely affect animal welfare.

Establishments shall have alarm systems that sound outside the sensitive hearing range of the animals, where this does not conflict with their audibility to human beings.

Holding rooms shall, where appropriate, be provided with noise insulation and absorption materials.

### 2.4 Alarms

Establishments relying on electrical or mechanical equipment for environmental control and protection shall have a stand-by system to maintain essential services and emergency lighting systems as well as to ensure that alarm systems themselves do not fail to operate.

Heating and ventilation systems shall be equipped with monitoring devices and alarms.

Clear instructions on emergency procedures shall be prominently displayed.

## 3 Care of animals

### 3.1 Health

Establishments shall have a strategy in place to ensure that a health status of the animals is maintained that safeguards animal welfare and meets scientific requirements. This strategy shall include regular health monitoring, a microbiological surveillance programme and plans for dealing with health breakdowns and shall define health parameters and procedures for the introduction of new animals.

Animals shall be checked at least daily by a competent person.<sup>23</sup> These checks shall ensure that all sick or injured animals are identified and appropriate action is taken.

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<sup>23</sup> A person who has been trained and assessed as competent to perform daily routine health checks for the animals in question.

## 3.2 Animals taken from the wild

Transport containers and means of transport adapted to the species concerned shall be available at capture sites, in case animals need to be moved for examination or treatment.

Special consideration shall be given and appropriate measures taken for the acclimatisation, quarantine, housing, husbandry, care of animals taken from the wild and, as appropriate, provisions for setting them free at the end of procedures.

## 3.3 Housing and enrichment

### 3.3.1 Housing

Animals, except those which are naturally solitary, shall be socially housed in stable groups of compatible individuals. In cases where single housing is allowed<sup>24</sup> the duration shall be limited to the minimum period necessary and visual, auditory, olfactory and/or tactile contact shall be maintained where appropriate to the species, strain and sex. The introduction or re-introduction of animals to established groups shall be carefully monitored to avoid problems of incompatibility and disrupted social relationships.

### 3.3.2 Enrichment

All animals shall be provided with space of sufficient complexity to allow expression of a wide range of normal behaviour. They shall be given a degree of control and choice over their environment to reduce stress-induced behaviour. Establishments shall have appropriate enrichment techniques in place, to extend the range of activities available to the animals and increase their coping activities including physical exercise, foraging, manipulative and cognitive activities, as appropriate to the species. Environmental enrichment in animal enclosures shall be adapted to the species and individual needs of the animals concerned. The enrichment strategies in establishments shall be regularly reviewed and updated.

### 3.3.3 Animal enclosures

Animal enclosures shall not be made out of materials detrimental to the health of the animals. Their design and construction shall be such that no injury to the animals is caused. Unless they are disposable, they shall be made from materials that will withstand cleaning and decontamination techniques. The design of animal enclosure floors shall be adapted to the species and age of the animals and be designed to facilitate the removal of excreta.

## 3.4 Feeding

The form, content and presentation of the diet shall meet the nutritional and behavioural needs of the animal.

The animals' diet shall be palatable and non-contaminated. In the selection of raw materials, production, preparation and presentation of feed, establishments shall take measures to minimise chemical, physical and microbiological contamination.

Packing, transport and storage shall be such as to avoid contamination, deterioration or destruction. All feed hoppers, troughs or other utensils used for feeding shall be regularly cleaned and, if necessary, sterilised.

Each animal shall be able to access the food, with sufficient feeding space provided to limit competition.

<sup>24</sup> See Introduction paragraph 8.4: Specifying deviations from CoP Section 1 or 2 standards.



### 3.5 Watering

Uncontaminated drinking water shall always be available to all animals.

When automatic watering systems are used, they shall be regularly checked, serviced and flushed to avoid accidents. If solid-bottomed cages are used, care shall be taken to minimise the risk of flooding.

Provision shall be made to adapt the water supply for aquaria and tanks to the needs and tolerance limits of the individual fish, amphibian and reptile species.

### 3.6 Resting and sleeping areas

Bedding materials or sleeping structures adapted to the species shall always be provided, including nesting materials or structures for breeding animals.

Within the animal enclosure, as appropriate to the species, a solid, comfortable resting area for all animals shall be provided. All sleeping areas shall be kept clean and dry.

### 3.7 Handling

Establishments shall set up habituation and training programmes suitable for the animals, the procedures and length of the project.



## Section 2, Chapter 10: Fish

There are no changes to these standards pre- and post-January 2017.

### 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.

### 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.

### 3 Temperature, lighting and noise

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

### 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.

### 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.



## Section 2, Chapter 11: Amphibians

### 1 Accommodation specifications

#### 1.1 Aquatic urodeles

All animals

Table 2-11-1

these are new standards

Body length (cm) measured from snout to vent	Minimum water surface area for one animal (cm <sup>2</sup> )	Minimum water surface area for each additional animal in group (cm <sup>2</sup> )	Minimum water depth (cm)
<10	262.5	50	13
10-15	525	110	13
15-20	875	200	15
20-30	1837.5	440	15
>30	3150	800	20

#### 1.2 Aquatic anurans

All animals

The following standards apply to holding (i.e. husbandry) tanks but not to those tanks used for natural mating and super-ovulation for reasons of efficiency, as the latter procedures require smaller individual tanks. Space requirements are determined for adults in the indicated size categories; juveniles and tadpoles shall either be excluded or dimensions altered according to the scaling principle.

Table 2-11-2

these are new standards

Body length (cm) measured from snout to vent	Minimum water surface area for one animal (cm <sup>2</sup> )	Minimum water surface area for each additional animal in group (cm <sup>2</sup> )	Minimum water depth (cm)
<6	160	40	6
6-9	300	75	8
9-12	600	150	10
>12	920	230	12.5

#### 1.3 Semi-aquatic anurans

All animals

Table 2-11-3

these are new standards

Body length (cm) measured from snout to vent	Minimum enclosure size for one animal (cm <sup>2</sup> )*	Minimum area for each additional animal in group (cm <sup>2</sup> )	Minimum enclosure height (cm)**	Minimum water depth (cm)
<5.0	1500	200	20	10
5.0-7.5	3500	500	30	10
>7.5	4000	700	30	15

\*One-third land division, two-thirds water division sufficient for animals to submerge.

\*\* Measured from the surface of the land division up to the inner part of the top of the terrarium; furthermore, the height of the enclosures shall be adapted to the interior design.



## 1.4 Semi-terrestrial anurans

All animals

Table 2-11-4

these are new standards

Body length (cm) measured from snout to vent	Minimum enclosure size for one animal (cm <sup>2</sup> )*	Minimum area for each additional animal in group (cm <sup>2</sup> )	Minimum enclosure height (cm)**	Minimum water depth (cm)
<5.0	1500	200	20	10
5.0-7.5	3500	500	30	10
>7.5	4000	700	30	15

\*Two-thirds land division, one-third water division sufficient for animals to submerge.

\*\* Measured from the surface of the land division up to the inner part of the top of the terrarium; furthermore, the height of the enclosures shall be adapted to the interior design.

## 1.5 Arboreal anurans

All animals

Table 2-11-5

these are new standards

Body length (cm) measured from snout to vent	Minimum enclosure size for one animal (cm <sup>2</sup> )*	Minimum area for each additional animal in group (cm <sup>2</sup> )	Minimum enclosure height (cm)**
<3.0	900	100	30
>3.0	1500	200	30

\*Two-thirds land division, one-third water division sufficient for animals to submerge.

\*\* Measured from the surface of the land division up to the inner part of the top of the terrarium; furthermore, the height of the enclosures shall be adapted to the interior design.



## Section 2, Chapter 12: Reptiles

### 1 Accommodation specifications

#### 1.1 Aquatic chelonians

All animals

Table 2-12-1

these are new standards

Body length (cm)*	Minimum water surface area for one animal (cm <sup>2</sup> )	Minimum water surface area for each additional animal in group (cm <sup>2</sup> )	Minimum water depth (cm)
<5	600	100	10
5-10	1600	300	15
10-15	3500	600	20
15-20	6000	1200	30
20-30	10000	2000	35
>30	20000	5000	40

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

#### 1.2 Terrestrial snakes

All animals

Table 2-12-2

these are new standards

Body length (cm) measured from snout to tail	Minimum floor area for one animal (cm <sup>2</sup> )	Minimum area for each additional animal in group (cm <sup>2</sup> )	Minimum enclosure height (cm)*
<30	300	150	10
30-40	400	200	12
40-50	600	300	15
50-75	1200	600	20
>75	2500	1200	28

\* Measured from the surface of the land division up to the inner part of the top of the terrarium; furthermore, the height of the enclosure shall be adapted to the interior design.

## Section 3, Chapter 1: Advice applicable to all animals

### 1 The physical facilities

#### 1.1 Functions and general design

When siting an animal house, consideration should be given to the activities in the adjacent buildings and any effect these may have on the welfare of the animals. An animal facility forming part of a larger complex should normally be designed to be self-contained. Wild, stray or pet animals should not be able to gain entry to any part of the animal house, including stores and personnel areas. Special care should be taken where drains and other services pierce the walls or floors to ensure that they have been properly proofed against rodents and other vermin.

Any special requirement for exercise or social contact for the species to be housed should incorporate adequate facilities sufficient for the activities carried out within it.

When substantial alterations to the premises are proposed, the Inspector should be consulted at an early stage.

##### 1.1.1 Security

Facilities that are part of a larger building complex should also be protected by appropriate security and building measures and arrangements that limit the number of entrances. Advice should be taken about security from Crime Prevention Officers, from the local police or other experts during the design of new facilities or modifications of existing premises.

#### 1.2 Holding rooms

The following are strongly recommended:

1. that all joints between door frames and walls etc. are sealed;
2. that floor-to-wall, wall-to-ceiling and wall-to-wall junctions are coved for easy cleaning;
3. that special attention is paid to junctions, including those with doors, ducts, pipes and cables;
4. where an inspection window is required in the door, that a flush fitted window is used to reduce maintenance;
5. that floors should be smooth, impervious and have a non-slippery (including when wet), easily washable surface, which can carry the weight of racks and other heavy equipment without being damaged;
6. that drains, if present, are adequately covered and fitted with a barrier, which will prevent vermin from gaining access or animals from escaping.

It is advisable to install services in such a way that they are either buried within the fabric of the building, boxed in or clear of the wall surface for easy cleaning. When the fabric of the building is penetrated, the holes created would normally be sealed.

It is recommended that holding rooms, where appropriate, have facilities for carrying out non-regulated procedures and manipulations.

Farm animals in pens generally require more robust wall and floor finishes. There should be no projections that could present a hazard to animals.<sup>25</sup> Where no Section 1 or 2 standards exist, farm animals which are kept in animal houses should be given at least as much room as recommended in other relevant legislation and Codes of Practice, for example those produced by Defra. For some procedures the standards of environment and housing required may be much higher than where animals are kept under farm conditions. Exercise areas should normally be provided for larger farm

<sup>25</sup> In order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 3.3.3.

animals but, in some cases, such facilities may be impracticable from an experimental, environmental, disease control or security point of view.

It is advisable to avoid housing some species within sight, sound or smell of each other because of their natural predator-prey relationships. The decision to allow/avoid particular combinations using particular housing systems should be evidence-based.

### 1.3 General and special purpose procedure rooms

At breeding or supplying establishments suitable facilities for making consignments of animals ready for dispatch should normally be available.

Animals inoculated with infective agents that are transmissible to man or to other animals held on the premises should be contained within an area reserved for this purpose which has been designed, built and maintained in accordance with appropriate health, safety and biosecurity regulations.

It is advisable that regulated procedures, surgery or euthanasia are not performed in rooms where animals are normally housed or where other conscious animals are undergoing procedures, where this may cause additional avoidable stress to the animals. There should be separate preparation areas for animals, equipment and staff.<sup>26</sup>

Sick or injured animals should normally be housed separately, unless being separated from their normal social group is more detrimental than beneficial to their recovery. The advice of the veterinary surgeon responsible for the animals' treatment should be followed.

#### 1.3.1 Surgical facilities

It is advisable that facilities used for aseptic surgery should meet the following minimum requirements.

- a. Surfaces and finishes within the surgical area should be designed so that they can be kept clean for use in sterile procedures.
- b. The surgical area should be large enough and arranged appropriately to allow correct aseptic technique.
- c. There should be separate areas for preparation of animals, surgery and recovery; these will preferably be separate rooms, although separate areas within the same room may be an appropriate alternative.
- d. There should be no open shelving in surgical areas. Ceiling-high wall cupboards or cupboards with sloped tops to prevent dust accumulation are preferred, should storage provision be essential.
- e. There should be a minimum of furniture in the surgical area so as to avoid the accumulation of dust and for ease of cleaning.

For detailed advice on aseptic technique, please see *LASA 2010 Guiding Principles for Preparing for and Undertaking Aseptic Surgery*.<sup>27</sup>

### 1.4 Service rooms

Special facilities may be required for storing and handling chemicals.

It is advisable that separate store-rooms and adequate space for clean cages, instruments and equipment are provided.

It is advisable that walls and floors are covered with a suitably durable surface material and the ventilation system has ample capacity to carry away the excess heat and humidity.

<sup>26</sup> Please see also Sections 1 and 2 Chapter 1 *Standards applicable to all animals* paragraph 1.3.

<sup>27</sup> *LASA 2010 Guiding Principles for Preparing for and Undertaking Aseptic Surgery. A report by the LASA Education, Training and Ethics section.* (M. Jennings and M. Berdoy Eds) [www.lasa.co.uk/publications.html](http://www.lasa.co.uk/publications.html) (accessed 27/5/14).

If on-site incineration of waste material is not possible or necessary, it is advisable that suitable arrangements are made for the safe disposal of such material, having regard to national and local regulation and by-laws. Special precautions are required with toxic, radioactive or infectious waste.

It is advisable that the general design and construction of circulation areas corresponds to the standards of the holding rooms. Consideration should be given to the width of the corridors to allow easy circulation of movable equipment.

## 2 Environmental conditions

Environmental variables can have a significant impact on animal welfare. Furthermore, experimental results may be influenced by environmental conditions. Animals should be kept under conditions that favour a consistency of response to scientific procedures. Unstable environmental conditions are likely to introduce avoidable variability into biological responses. To demonstrate any experimental response against such a variable background generates a requirement for greater animal usage if the result is to be statistically valid. Good control of variables such as ventilation, humidity, temperature, lighting and noise can therefore contribute both to good science and to the minimisation of animal use.

It is advisable that measures are in place to ensure that breakdowns in equipment or systems controlling the environment are remedied promptly. Consideration should be given to maintaining a stock of critical spare parts.

Conditions should not be detrimental to the welfare of the animals, unless authorised by the project licence.

### 2.1 Engineering standards, performance and welfare

The species-specific chapters within Section 3 provide suggested ranges for environmental parameters, for example temperature and relative humidity. The intention of providing these ranges is to indicate typical values that will normally satisfy the requirements of Sections 1 and 2 to provide “appropriate” environmental conditions.

However, whether the environmental conditions are or are not “appropriate” will ultimately be assessed by performance and welfare outcomes. Examples of indicators that may demonstrate unsuitable environmental conditions include, but are not limited to:

1. presence of respiratory disease in rats that may be attributable to low relative humidity;
2. decreased reproductive performance in mice that may be attributable to a noisy environment;
3. greasy coats in gerbils that may be attributable to high relative humidity;
4. increased aggressive behaviour in pigs that may be attributable to decreased environmental stimulation;
5. decreased growth rates in cattle that may be attributable to inadequate access to food of appropriate quality.

Where there is no impact on performance or welfare the ranges may be extended, with the agreement of appropriate named persons.

Even where the parameters in question are within the suggested ranges, if there appears to be, or may soon be an adverse impact on the animals, appropriate steps should be taken to adjust the parameters so as to avoid that adverse impact. Likewise, being within the suggested ranges *per se* does not necessarily guarantee that conditions are “appropriate” in every situation. Anticipation of potential welfare problems is as important as promptly dealing with them should they occur. Focussing on outcomes and solutions specific to your facility will ensure that your standards of accommodation and care are appropriate for your animals.

This principle should also be extended to other advice presented in Section 3 that could be interpreted as engineering standards.



## 2.2 Ventilation

The purpose of the ventilation system is to provide sufficient fresh air of an appropriate quality and to keep down the levels and spread of odours, noxious gases, dust and infectious agents of any kind. It provides for the removal of excess heat and humidity.

The air in the room should be renewed at frequent intervals. A ventilation rate of 15 to 20 air changes per hour of fresh or conditioned air distributed throughout the room is normally adequate for a fully stocked room of rodents or lagomorphs in open cages. However, in some circumstances, for example where stocking density is low, eight to ten air changes per hour may suffice. For cats, dogs and primates, 10 to 12 changes per hour may be adequate. In some cases, natural ventilation may suffice and mechanical ventilation may not even be needed. Re-circulation of untreated air should be avoided. However, it should be emphasised that even the most efficient system cannot compensate for poor cleaning routines or negligence. A smell of ammonia probably reflects overstocking, too little ventilation, inadequate cleaning, or a combination of these factors; the causes should be investigated and rectified. It is advisable that the optimum stocking density for each room for each species likely to be housed is calculated and readily available.

It is advisable that the ventilation system is designed so as to avoid harmful draughts and noise disturbance while delivering air as evenly as possible throughout the holding area. Please see paragraph 2.9 below for considerations for rooms holding Individually Ventilated Cages (IVCs), incubators etc.

The ventilation system can be used to create differential air pressures within the building as part of a “barrier system”. “Clean” areas are generally maintained at higher pressure and “hazardous” areas at lower pressure than those adjacent to them to minimise the leakage of “dirty” air into “cleaner” areas and the escape of airborne hazards into the air outside the premises. This is effective only if the supply air is itself clean or is suitably filtered to be free from contaminants.

## 2.3 Temperature

The subsequent species-specific sections give the range within which it is recommended that the temperature should be maintained. It should also be emphasised that the figures given in these sections apply only to adult, normal animals. New-born, young, hairless, newly operated, sick or injured animals will often require a much higher temperature level. The temperature of the premises should be regulated according to possible changes in the animals’ thermal regulation, which may be compromised due to special physiological conditions or to the effects of the procedures.

It may be necessary to provide a ventilation system having the capacity both to heat and cool the air supplied.

In user establishments precise control of the temperature experienced by the animal may be required, because the temperature is a physical factor which has a profound effect on the metabolism and behaviour of all animals, and therefore affects the validity of certain scientific outcomes. The target should be to maintain the temperature at which the animal is living in a band width of 4°C, the whole of the band lying within the optimal range indicated. For breeding in some species, however, a controlled daily fluctuation in temperature may be beneficial.

Outdoor areas provided for animals to exercise and interact cannot have strict temperature regulation.

## 2.4 Humidity

For some species, such as rats and gerbils, relative humidity may need to be controlled within a fairly narrow range to minimise the possibility of health or welfare problems, whereas other species, such as dogs, tolerate well wide fluctuations in humidity levels. As a general rule, prolonged periods below 40% or above 70% should be avoided.

## 2.5 Lighting

Exposure of some species or strains (especially albinos) to bright light should be avoided and darker areas for withdrawal should be available within the animal enclosures.

Consideration should be given to the inclusion of windows in holding rooms, since they are a source of natural light and can provide environmental enrichment for some species, especially non-human primates, dogs, cats, some farm animals and other large mammals.

The circadian “clock” of some species may be affected as much by light pulses of less than one second during the dark phase as by a long photoperiod; thus it may be important not to turn on lights during the dark period. On the other hand, intervals of darkness during the light period are far less disruptive. Where animals are maintained on reverse photoperiod, daily inspections of the animals should still be undertaken.

## 2.6 Noise

Noise can be a disturbing factor for animals. High noise levels and sudden noises can cause stress which, in addition to the welfare consequences for the animal, may influence experimental data. Background noise may be helpful in reducing the impact of unavoidable sudden noises.

Due consideration should be given to controlling noise levels within the hearing ranges of animals, including in some cases ultrasound (sound above the hearing range of the human being, conventionally taken to be sounds exceeding 20 kHz), particularly during their resting phase. This includes reducing noise due to human working practices as well as considering noise from equipment – for example alarms should be of a silent type. The layout of rooms and corridors can be major factors influencing the acoustic environment and this should be taken into account in their design.

## 2.7 Vibration

Animals such as rodents, amphibians and fish are very sensitive to vibration. Vibration can have a negative effect on reproductive efficiency and can have an influence on experimental results. It is therefore important to minimise vibration in the animal facility. For this reason, animal holding and procedure rooms should not be located adjacent to sources of vibration such as cage wash areas, lifts or busy corridors where cage racks are constantly being moved. Vibration is not normally an issue for large animals although minimising vibration may still be important in behaviour testing rooms.

Vibration is more likely to be an issue in animal facilities located in an upper level of a building rather than at ground level because of structural considerations. However animals in ground floor and basement facilities may be affected by vibration arising from major structural projects. The potential impact of vibration should be carefully evaluated when considering the use of portable buildings.

## 2.8 Alarms

A technologically dependent animal facility is a vulnerable entity. It is strongly recommended that such facilities are appropriately protected to detect hazards such as fires, the intrusion of unauthorised persons, and the breakdown of essential equipment, such as ventilation fans, air heaters or coolers and humidifiers.<sup>28</sup>

Carefully designed monitoring, alarms and call-out procedures will ensure that any faults can be quickly identified and promptly rectified, and that the minimum number of animals is impacted for the shortest possible time by any fault.

Care should be taken to ensure that the operation of an alarm system causes as little disturbance as possible to the animals.

## 2.9 Special environments (e.g. Individually Ventilated Cages (IVCs), incubators, etc.)

Biocontainment systems (e.g. Individually Ventilated Cages [IVCs] and isolators but excluding filter top cages) are designed to ensure that the key environmental welfare issues are catered for. Thus, they should provide and maintain an appropriate environment in terms of temperature, relative humidity, air quality and air velocity (i.e. avoidance of draughts).

<sup>28</sup> In order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 2.4.

It is important that alarms and their management effectively detect and deal with any problems should they arise. The appropriate degree of complexity of the alarm and length of response time will be dependent on the biocontainment system and the species being kept in it.

In facilities in which the ventilation system is unable to provide suitable air quality, biocontainment systems may offer a less expensive alternative to complete refurbishment. However, in such cases all the other environmental parameters will still need to be considered. For example, unless IVCs are connected to a system that provides heating or cooling they will be totally dependent on the room temperature. Rooms should therefore be capable of providing air of an appropriate temperature and humidity. Where biocontainment systems are used in rooms that do not meet code of practice requirements, particular consideration needs to be given to the potential for failure of the biocontainment unit itself, as failure in these circumstances is likely to have a greater/more rapid adverse impact on animal welfare.

## 2.10 Emergency response planning

Carefully considered contingency planning and emergency response plans are central to the management of the animal facility.<sup>29</sup> There are a number of aspects that should be thought through including what happens in the event of a major incident to the facility or locality (e.g. loss of power or water) and how emergency animal care and accommodation can be provided. However, in some cases the facilities or programme of work may be so compromised that destruction of the animals needs to be considered to prevent significant welfare issues developing. Therefore, plans should normally include arrangements for the emergency evacuation, housing, husbandry (including feeding and watering), and, where inevitable, the killing of animals. These plans should normally include assessments of the risks, their likelihood and mitigations as well as actions to be taken, by whom, in the event of temporary, longer-term disruption or catastrophic facility failures. They should normally reflect the requirements for the care of animals as described in Part 4 below.

## 3 Education and training

All persons handling animals or directly involved in caring for animals being bred, held or used for experimental or other scientific purposes should be appropriately educated and trained to the standard described in Section 9 of the Guidance on the Operation of the Animals (Scientific Procedures) Act 1986.

## 4 Care of animals

Animals within an animal facility are totally dependent on humans for their health and well-being. The physical and psychological state of the animals will be influenced by their local environment, food, water and the care and attention provided by the animal care staff and scientific personnel.

### 4.1 Supervision of procedures

Unconscious animals (i.e. where a protective gag reflex is absent) should be closely monitored. This includes animals undergoing imaging, in warming facilities, undergoing surgical preparation or during or after surgery.

### 4.2 Health

Animals of an appropriate known health status are an essential prerequisite for good animal welfare and good science. Intercurrent infection in the animal population may call into question the validity of information obtained from scientific procedures and make interpretation of results impossible.

The aim is to maintain animals in good health and physical condition, behaving in a manner normal for the species and strain and with a reasonably full expression of their behavioural repertoire, and amenable to handling.

<sup>29</sup> They are likely to be necessary to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 2.4.

The person responsible for the establishment should ensure regular inspection of the animals and supervision of the accommodation and care by a veterinarian or other competent person, normally a NACWO.

Should behavioural or breeding problems occur, or should further information on specific requirements for other species be required, advice should be sought from experts specialised in the species concerned and care staff or veterinary surgeon, to ensure that any particular species' needs are adequately addressed.

Regular health monitoring of all animals should be carried out, comprising the daily check, plus ongoing monitoring and evaluation to enable prompt interventions to safeguard the health of individuals and populations, as appropriate to the species and situation. Animals which are undergoing scientific procedures should be inspected at a frequency commensurate with the severity of the procedure and/or potential rate of change of the condition of the animal, and never less than once per day.

Because of the potential risk of contamination of animals and staff presented by the handling of animals, it is advisable that particular attention is paid to the institution of hygiene procedures and supervision of staff health.

### 4.3 Separation from the mother

Early weaning from the mother – if it may cause pain, suffering, distress or lasting harm – may be a regulated procedure. In such circumstances the Home Office should be consulted regarding whether Project Licence authority is required.

### 4.4 Transport of animals

#### 4.4.1 Legislation and responsibilities

**Prior to entry to a licensed establishment**, an animal's welfare during transport is governed by applicable international, EU and UK law.<sup>30</sup> The enforcement authority in England, Wales and Scotland is the Animal and Plant Health Agency (APHA), and in Northern Ireland the Department of Agriculture and Rural Development.

**Once an animal enters a licensed establishment** and becomes a protected animal under ASPA its welfare during any subsequent transport is *additionally* regulated under ASPA. Both project licence holders and establishment licence holders have responsibilities relating to transport of protected animals.<sup>31</sup>

Under ASPA, the person legally accountable for the transport of the animals (the sending establishment licence holder) has the overall accountability for the planning, carrying out and completion of the whole journey, regardless of whether duties are subcontracted or delegated to other parties during transport. The sending establishment licence holder should check that all necessary arrangements are in place to fulfil standard condition 4(6) of their establishment licence – namely that the conditions under which a protected animal is transported are appropriate for the animal's health and well-being.

Once the animals have been received at their destination, legal accountability for their care transfers to the receiving establishment licence holder, or, in the case of establishments that are not licensed, the animal is discharged from ASPA.

The person in charge of the welfare of the animals has direct physical responsibility for the care of the animals during transport. Such a person may be the attendant or the driver of a vehicle if fulfilling

<sup>30</sup> Animals should be transported in accordance with the principles of the European Convention on the Protection of Animals during International Transport (ETS No. 65 and ETS No. 193), having regard to the Resolution on the acquisition and transport of laboratory animals, adopted by the May 1997 Multilateral Consultation of the Parties to Convention ETS No. 123 and, to EU Regulation (EC) 1/2005 on the protection of animals during transport, implemented in England by The Welfare of Animals (Transport) (England) Order 2006 and by parallel legislation in Scotland, Wales and Northern Ireland.

<sup>31</sup> Establishment Licence Standard Conditions 4(6), 23(a) and 23(b). Project Licence Standard Condition 24(a) and section D of the licence itself.

the same role. It is considered good practice for the person in charge of the welfare of animals being transported to be aware of the special needs of the animals in their care.

#### 4.4.2 Journey planning

For animals, transportation is a stressful experience which should be mitigated as far as possible. The following principles should apply to all animal movements, from short journeys by vehicle within scientific establishments to international transportation.

It is advisable that the route is planned in order to ensure that the transport is carried out efficiently to minimise journey time, from loading to unloading, and to avoid delays in order to limit any stress and suffering of the animals. Care is needed to ensure that animals are maintained under suitable environmental conditions for the species, and that measures are taken to minimise sudden movements, excessive noise, or vibration during transport. Both sender and recipient should agree the conditions of transport, departure and arrival times to ensure that full preparation can be made for the animals' arrival.

It is advisable that containers for travel:

- a. confine the animals in comfortable hygienic conditions with minimal stress for the duration of the journey;
- b. contain sufficient food and water or moisture in a suitable form;
- c. contain sufficient bedding so that animals remain comfortable and in conditions close to their thermo-neutral zone;
- d. be of such a design and finish that an animal will not damage itself during loading, transport and whilst being removed from the container;
- e. be escape-proof, leak-proof and capable of being handled without the animals posing a risk to handlers;
- f. be designed to prevent or limit the entry of micro-organisms, where appropriate;
- g. be designed so that they can be thoroughly disinfected between shipments, if intended to be reusable;
- h. allow sufficient ventilation;
- i. allow visual inspection of the animals without compromising their microbiological status (where appropriate);
- j. be clearly labelled.

#### 4.4.3 Dispatch

The sender should ensure that the animals are examined and found to be fit for transport before being placed in the transport container. Animals that are incompatible should be appropriately separated for transport.

Animals that are sick or injured should not normally be considered fit for transport, except for those whose sickness or injury is such that transport will not cause additional suffering, or where the transport is under veterinary supervision for, or following, veterinary treatment.

Sick or injured animals may also be transported for experimental or other scientific purposes approved by the Home Office if the illness or injury is part of the research programme. No additional suffering should be imposed by the transport of such animals, and particular attention should be paid to any additional care which may be required. A competent person, normally a veterinarian or NACWO, should confirm that such animals are fit for the intended journey. Pregnant and post partum animals (and their offspring) need special care.

#### 4.4.4 Reception

It is good practice to ensure that on arrival at their destination the animals are removed from their transport containers and examined by a competent person, normally a veterinarian or NACWO, with the least possible delay. It is recommended that after inspection, the animals are transferred to clean cages or pens and supplied with food and water as appropriate.

Animals which are sick, injured or otherwise out of condition, should be examined by a veterinary surgeon. These animals should be provided with veterinary treatment as appropriate or, if deemed necessary, promptly killed by a humane method, in line with the principles set out in Section 6 of the Guidance on the Operation of the Animals (Scientific Procedures) Act 1986.

### 4.5 Quarantine, acclimatisation and isolation

The objectives of quarantine and isolation periods are:

- a. to protect other animals in the establishment;
- b. to protect man against zoonotic infection; and
- c. together with an acclimatisation period, to foster good scientific practice.

According to the circumstances, these periods may vary and are either determined by national regulations or a competent person, normally the NVS.

#### 4.5.1 Quarantine

Quarantine is defined as a period of housing newly introduced or reintroduced animals separately from existing animals in the establishment to establish the state of health of the animals and to prevent the introduction of disease. Such a period is recommended when the health status of the animal is not known.

#### 4.5.2 Acclimatisation

A period of acclimatisation is usually needed to allow animals to recover from transport stress, to become accustomed to a new environment and to husbandry and care practices. Even when the animals are seen to be in good health, it is strongly advisable for them to undergo a period of acclimatisation before being used in a procedure. The time required depends on several things, such as the stress to which the animals have been subjected. This in turn depends on several factors such as the duration of the transportation, the age of the animal and degree of change of the social environment. It should also be taken into account that international transport may necessitate an extended period of acclimatisation due to disturbance of the diurnal rhythm of the animals. Acclimatisation periods should be agreed by the NVS, NACWO and investigator to ensure good animal welfare and science outcomes.

#### 4.5.3 Isolation

A period of isolation of diseased animals is intended to reduce the risk of infecting other animals or humans. It is advisable to house separately or in isolation any animal suspected of posing such a risk, for an appropriate period of time.

### 4.6 Housing and enrichment

Restricted environments can lead to behavioural and physiological abnormalities and affect the validity of scientific data. It is a requirement that animals should be able to exercise “a degree of choice over their environment”.<sup>32</sup> This could be achieved, for example, by having a variety of resting places in the enclosure, or by the animal being able to construct its own nest or resting place.

Consideration should be given to the potential impact of the type of accommodation, and that of the environmental and social enrichment programmes, on the outcome of scientific studies, in order to avoid the generation of invalid scientific data and consequential animal wastage.

<sup>32</sup> Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 3.3.2.

It is recommended that the design of housing and enrichment strategies should take into account the need to observe the animals with minimum disruption and to facilitate handling. Consideration should be given to the potential conflict between the need for regular observation of animals and the importance of avoiding disturbances that may have a negative impact on their welfare. Mandatory minimum animal enclosure sizes and space allowances are included for most species routinely used in research programmes in Sections 1 and 2 of this CoP.

The shape of the enclosure and the furniture provided may be as important to the animal as overall size. Room should be allowed for growth of the animals. Some animals continue to grow into old age although they may become less active.

Unless otherwise specified, additional surface areas provided by enclosure additions, such as shelves and mobile enrichment devices, should be regarded as being in addition to the recommended minimum floor areas.

Single housing should only occur on animal health or welfare grounds, unless it is permitted by Project Licence authority. The need for single housing on health or welfare grounds should be agreed with a competent person, normally a veterinarian or NACWO. There are some occasions when stud males need to be housed singly (e.g. to prevent fighting). In such circumstances, additional resources should be targeted to the welfare and care of these animals. In such cases, the duration should be limited to the minimum period necessary and, where possible, visual, auditory, olfactory and tactile contact should be maintained where appropriate to the species, strain and sex. The possibility of social housing can be promoted by purchasing compatible individuals when procuring animals of gregarious species.

Consideration should be given to housing animals so that they can be easily inspected. Some procedures may require a more restrictive system of housing to cater for special requirements imposed by experimental procedures, for example, the need to collect excreta or expired air, or the use of radioactive isotopes. Such housing should be used for the minimum time only and be authorised as appropriate.<sup>33</sup>

As animals grow, the adequacy of the housing or enclosure, in particular in relation to size and area, should be monitored to ensure that it continues to meet the requirements described in Sections 1 and 2 of this Code of Practice.

#### 4.6.1 Enrichment

In addition to social activities, enrichment can be achieved by allowing and promoting physical exercise, foraging, manipulative and cognitive activities, as appropriate to the species. It is advisable to allow the animals to exercise at every possible opportunity. Forms of enrichment should be adaptable so that innovation based on new understanding may be incorporated.

It is strongly recommended that the staff responsible for animal care understand the natural behaviour and biology of the species, so that they can make sensible and informed choices on enrichment. They should be aware that all enrichment initiatives are not necessarily to the advantage of the animal and therefore should monitor their effects and adjust the programme as required.

### 4.7 Feeding

The behavioural requirements for some species include foraging, so for animals of these species it is strongly advised that the opportunity for foraging is given. Roughage is an important component of the diet for some species of animals, as well as a means of satisfying some behavioural needs.

It is recommended that food is packed in containers that provide clear information on the identity of the product and its date of production. Where a commercially manufactured product is used, an expiry date should be clearly defined by the manufacturer. It is strongly advised to adhere to such expiry dates.

<sup>33</sup> See Introduction paragraph 8.4: Specifying deviations from CoP Section 1 or 2 standards.

Store-rooms should normally be cool, dark, dry, adequately ventilated and vermin and insect-proof. It is advisable to store perishable food like greens, vegetables, fruit, meat and fish in cold rooms, refrigerators or freezers.

If moist food is used, or if the food is easily contaminated with for example water or urine, daily cleaning is normally necessary.

In some circumstances, food intake may need to be controlled to avoid obesity.

Where withholding of food is necessary for experimental or safety reasons, such as prior to anaesthesia, care should be taken that deprived animals are not stressed by exclusion from food whilst other animals around them are fed. Removing deprived animals to another cage or room may be less stressful than leaving them with the fed animals.

Consideration should be given to providing diets for disease-free animals that have been treated to destroy vegetative organisms, parasites, pests and spores. Autoclaving or irradiation may be required. Where special diets containing chemicals for testing have been used, the nutritional consequences of the preparation and storage of the diet should be considered.

#### 4.8 Watering

Water is a vital resource to all animals. However, water is also a potential vehicle for micro-organisms, and due consideration should be given to arranging the supply so that the contamination risk is minimised. It is important that sufficient watering points (drinkers) are available.

Consideration should be given to the design and use of watering systems to ensure the provision of an adequate quantity of water of suitable quality. In addition, watering systems may carry a risk of flooding in the event of failure. It is strongly recommended that systems are designed to ensure that as few animals as possible are affected by any such failures. At least daily checks and regular cleaning are recommended to ensure blockages or leakages are detected and to reduce the spread of infection.<sup>34</sup> A diligent programme of maintenance and renewals is required.

Emergency provisions should be made in case pipes freeze or supplies otherwise fail.<sup>35</sup>

#### 4.9 Flooring, substrate, litter, bedding and nesting material

Various materials are commonly placed into the animal enclosure to serve the following functions: to absorb urine and faeces, and thus facilitate cleaning; to allow the animal to perform certain species-specific behaviour, such as foraging, digging or burrowing; to provide a comfortable, yielding surface or secure area for sleeping; to allow the animal to build a nest for breeding purposes.

Certain materials may not serve all of these needs, and it is therefore important to provide sufficient and appropriate materials. Any such materials should be dry, absorbent, dust-free, non-toxic and free from infectious agents or vermin and other forms of contamination. Materials derived from wood that have been chemically treated, or containing toxic natural substances, as well as products which cannot be clearly defined and standardised, should be avoided. Hydrocarbons present in wood-derived bedding have been shown to be capable of inducing cytochrome P450-dependent microsomal enzyme systems of animals resulting in altered drug metabolism.

In general, changes to the type of bedding material used should be avoided mid-study. Where changes are unavoidable, the investigators should be informed with as much notice as possible.

#### 4.10 Cleaning

The standard of a facility, including good husbandry, depends very much on good hygiene. It is strongly recommended that a very high standard of cleanliness and order is also maintained in holding, washing and storage rooms. Adequate routines for the cleaning, washing, decontamination and, when necessary, sterilisation of enclosures and accessories, bottles and other equipment should be established and carried out.

<sup>34</sup> In order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 3.5.

<sup>35</sup> In order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 3.5.



Consideration should be given to the design of these cleaning and disinfection regimes so as they are not detrimental to animal health or welfare. It is advisable that clear operating procedures, including a recording system, are in place for the changing of bedding in animal enclosures.

It is advisable that there should be regular cleaning and, where appropriate, renewal of the materials forming the ground surface in animal enclosures to avoid them becoming a source of infection and parasite infestation.

Odour-marking is an important form of behaviour in some species, and cleaning disturbances will cause some degree of social disruption. Cleaning regimes should have regard for these behavioural needs. Decisions on frequency of cleaning should be based on the type of animal enclosure, the type of animal, the stocking density, and the ability of the ventilation system to maintain suitable air quality.

#### 4.11 Handling

The quality of care animals are given may influence not only breeding success, growth rate and welfare but also the quality and outcome of experimental procedures. Accustoming animals to competent and confident handling during routine husbandry and procedures reduces stress both to animals and personnel. For some species, for example dogs and non-human primates, a training programme to encourage co-operation during procedures can be beneficial to the animals, the animal care staff and the scientific programme. For certain species, social contact with humans should be a priority. However, in some cases, handling should be minimised (e.g. wild animals).

Staff caring for animals are expected at all times to have a caring and respectful attitude towards the animals in their care, and to be proficient in the handling and restraint of the animals. Where there is evidence of a welfare benefit for the animals concerned, staff time should be set aside for talking to, handling, training and grooming animals.

#### 4.12 Humane killing

Personnel allowed to kill animals should be suitably trained. All humane methods of killing animals require expertise, which can only be attained by appropriate training. Section 9.5 of the Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 sets out the training requirements for those killing animals. Animals should be killed using a method as set out in Section 6 of the same Guidance.

Careful consideration should be given to the minimisation of stress both for the animals to be killed and for those around them. Where practicable and appropriate, killing should take place away from the immediate presence of other animals. Animals to be killed should be handled carefully to ensure that fear and anxiety are minimised as much as possible.

Careful consideration should also be given to the health and well-being of staff charged with undertaking this activity.

#### 4.13 Records

Records of source, use, retrospective severity of procedures and final disposal of all animals bred, kept for breeding, or for subsequent supply for use in scientific procedures should be used not only for statistical purposes but, in conjunction with health and breeding records, can also be used as indicators of animal welfare and for husbandry and planning purposes.

#### 4.14 Identification

In some instances, it is necessary for animals to be individually identified, for example, when being used for breeding purposes or scientific procedures, to enable accurate records to be kept. The method chosen should be reliable and cause the minimum pain and discomfort to the animal when applied and in the long term. Sedatives or local anaesthetics and analgesics should be used if necessary. Staff should be trained in carrying out the identification and marking techniques. For more information see the Guidance on the Operation of the Animals (Scientific Procedures) Act 1986.

### 4.15 Specific requirements of breeding animals

Housing restricts an animal's ability to exercise choice and, therefore, has to provide for as many of their needs as can reasonably be met. The environmental needs of breeding animals are likely to differ from those of stock and experimental animals for four main reasons.

1. Breeding animals typically have longer lives than those used in experiments and the female is exposed to the stresses of reproduction. Hence, particular attention needs to be given to designing an environment that takes account of the animal's behavioural as well as physiological needs.
2. Animals give birth during the time of the day when they are usually quiescent and will often seek or create a secure place for parturition and the raising of offspring; typically a nest or den in the case of rodents, cats, dogs and birds. Such behaviour is strongly motivated. The breeder should ensure that the animal's need for privacy is considered. This can be achieved by the provision of nesting material, nest boxes or a secluded and sheltered area within the pen or cage. Nesting material also allows the animal to partially control its own environment (e.g. noise, temperature and humidity). Given the means for controlling its own microenvironment, the appropriate range of room temperatures may be wider than would otherwise be the case.
3. In some species when breeding stock are housed in social groups, subordinates and females that have just given birth may be vulnerable to social stresses. Extra care should be taken to prevent and monitor aggression and to separate individuals if necessary. Single housing for social species should only be considered on animal health or welfare grounds, unless it is permitted by Project Licence authority. Objects can act as barriers within the pen and allow animals greater control over their social interactions.
4. The needs of infants are different from those of adults. For example, they may have differing space and temperature requirements from adult animals. An adequately complex social and physical environment during development is needed to produce normal adults.



## Section 3, Chapter 10: Fish

This chapter must be read in conjunction with the *Introduction* and *Section 3 Chapter 1: Advice applicable to all animals*.

### 1 Introduction

A wide variety of fish species are used for experimental purposes and these have a diverse range of habitats, behaviour and environmental and husbandry requirements.

Fish are ectothermic animals and highly sensitive to their particular aquatic environment. They react very rapidly to stress with immediate physiological consequences that can be relatively long-lasting and such changes, as well as having obvious welfare implications, will also impact upon experimental results.

Investigators and animal care staff should acquaint themselves with the biology, behaviour and ecology of the proposed experimental fish species, to ensure that appropriate facilities and husbandry procedures are in place before animals are obtained. Species-specific guidance should be sought from expert specialists and care staff to ensure that any particular species' needs are adequately addressed.

### 2 Environmental conditions

#### 2.1 Water supply

It is essential that an adequate water supply of suitable quality is provided at all times. Water flow in re-circulatory systems or filtration within enclosures should be sufficient to remove suspended solids and wastes and to ensure that water-quality parameters are maintained within acceptable levels. Monitoring systems should be in place to ensure that fish are provided with an appropriate quantity of water of appropriate quality.<sup>104</sup>

#### 2.2 Water quality

Water quality is the most important factor in maintaining the well-being of fish and in reducing stress and the risk of disease. The definition of acceptable ranges is complicated in that optimum conditions are not well defined for many species and that the requirements of individual species may vary between different life stages, for example larvae, juveniles, adults, or according to physiological status, for example metamorphosis, spawning, feeding, previous history of exposure.

Fish show varying degrees of adaptability to changing water-quality conditions. Some degree of acclimatisation may be necessary and this should be carried out for a period appropriate for the fish species in question.

As most fish species cannot function well in water containing a high level of suspended solids, it is advisable to maintain these within an acceptable range. Where necessary, water supply to facilities should be appropriately filtered to remove substances harmful to fish and to maintain suitable water physico-chemical parameters.

##### 2.2.1 Oxygen

Required oxygen concentration will vary according to species of fish, its size, feeding level and amount of handling, as well as the environmental temperature, carbon dioxide concentration and salinity of the water.

##### 2.2.2 Nitrogen compounds

Ammonia and urea are the main excretory products of fish. Dissolved urea, as well as food and faeces, are converted to inorganic compounds such as ammonia and phosphate. Ammonia will be

<sup>104</sup> These recommendations are considered to be necessary in order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraphs 2.4 and 3.5.



further converted into nitrite and nitrate. Ammonia and nitrite are toxic to fish and their accumulation should be avoided by increasing flow rate, reducing stocking density or temperature, or biofiltration.

Susceptibility to the effects of ammonia varies between fish species and in general marine and younger fish are more susceptible. The toxic form of ammonia is unionised ammonia, the amount of which depends not only on total ammonia concentration but also on pH, salinity and temperature.

### 2.2.3 Carbon dioxide

Carbon dioxide is produced by fish during respiration and dissolves in water to form carbonic acid, thus lowering the pH. Accumulation of carbon dioxide can be a problem at a high stocking density if pure oxygen is used instead of air to maintain the oxygen content in the water. Although high concentrations of free carbon dioxide can be fatal to fish this is most unlikely to be a problem under normal housing conditions. However, care should be taken that water-supply systems, particularly in the case of groundwater-based systems, do not introduce harmful quantities of carbon dioxide into the enclosures.

### 2.2.4 pH

Acceptable pH levels depend on many water-quality factors, for example, carbon dioxide and calcium. As far as possible, pH should be kept stable as any changes in pH will influence other water-quality parameters. In general pH tends to be lower in fresh water than in salt water. If necessary, supply water should be buffered. In the wild pH is rarely constant, but fluctuates within a range, often diurnally and certainly seasonally.

### 2.2.5 Salinity

Salinity requirements of fish will vary according to whether they are marine or freshwater in origin or adapted. Some species are able to tolerate a wide range of salinity. In others salinity tolerance may vary according to life stage. Changes in salinity should be made at a rate appropriate for the purpose and species.

## 2.3 Temperature

At high temperatures it may be necessary to provide supplementary aeration or oxygenation of enclosure water. Changes in temperature should be made at a rate appropriate for the purpose and species. Increasing water temperature without de-gassing the water can cause super-saturation with carbon dioxide and other gases, leading to gas bubble disease.

## 2.4 Lighting

Many fish require light for feeding and other behavioural activities. Fish should be maintained on an appropriate photoperiod as far as possible since the day/night cycle influences the physiology and the behaviour of fish. Many fish species should not normally be kept in bright light, although some tropical species naturally encounter very bright light. As appropriate for the species, it is advisable that lighting is subdued or tanks covered. Abrupt changes in light should be avoided as far as possible, and phased sunrise and sunset is recommended, as appropriate for the species.

## 2.5 Noise and vibration

Fish can be acutely sensitive to sounds and vibrations, even at very low levels. Fish reared in a particular environment will adapt to the stimuli presented there and may become stressed if moved to unfamiliar surroundings. It is advisable to take reasonable steps to avoid sudden noise and vibration. It is advisable to situate plant in a separate room to tanks wherever possible.

# 3 Health

Appropriate attention should be paid to hygiene within experimental facilities. The health of fish is intimately bound up with their environmental and husbandry conditions. Deficiencies in environmental and husbandry conditions cause stress that often results in disease, and any attempt to control disease should give these areas due consideration. Fish health management is almost always



concerned with populations rather than single individuals, and it is advisable to design control measures accordingly.

### 3.1 Hygiene and disinfection

Thorough consideration should be given to the appropriate cleaning and disinfection of fish-holding facilities, including associated pipework. In closed systems, cleaning and disinfection should be compatible with maintenance of optimal microbiological conditions. Staff should take precautions to prevent cross-contamination between fish enclosures. Equipment, for example nets, should normally be cleaned and disinfected between uses. For tanks that are not on the same flow-through system, wherever possible separate equipment (e.g. nets) should be used to prevent cross-contamination.

### 3.2 Quarantine

Newly introduced stocks, both from farmed and wild fish, should be given an appropriate quarantine period, as far as possible separate from existing stocks. It is advisable to closely monitor the fish during quarantine and any disease problem which arises should be treated or the stock destroyed. It is advisable to procure farmed fish from reputable suppliers and as far as possible have a verified health status.

## 4 Housing, enrichment and care

### 4.1 Housing

Fish behaviour will influence stocking density and schooling or territorial behaviour should be considered. Measures should be taken to avoid or minimise aggression without otherwise compromising animal welfare. Acceptable stocking density for a given species will vary depending on water flow and current, water quality, as well as fish size, age, health and feeding method. For most species, it is advisable to keep fish in groups of the same-sized individuals to minimise the risk of injuries or cannibalism.

### 4.2 Enrichment

Environmental enrichment is often necessary to take account of behavioural traits, for example, in reproduction or predation. Examples of such needs include provision of hiding places for wrasse, flowing water for salmonids or substrate such as sand for some flatfish. Care is needed to ensure that environmental enrichment does not adversely affect water quality.

### 4.3 Fish-holding facilities

Fish can be maintained in land-based enclosures in dedicated buildings or in external areas, or in enclosures in open-water systems. Where practical, these should have controlled access and be arranged to minimise disturbance of the fish, and to facilitate maintenance of suitable environmental conditions.

### 4.4 Land-based enclosures

The materials used to construct the enclosures should be non-toxic, durable and with a smooth internal surface to prevent abrasions to the fish. Enclosures should be of an appropriate size to accommodate the required stocking density of fish and should be able to receive the necessary water flow. Enclosures should be of an appropriate shape to accommodate the behavioural needs and preferences of the particular experimental fish species; for example, circular enclosures are usually most appropriate for salmonids. Enclosures should be designed to prevent escape.<sup>105</sup>

It is advisable that, where possible, enclosures are self-cleaning to aid removal of waste products and surplus food.

<sup>105</sup> These recommendations are considered to be necessary in order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraphs 3.3.2 and 3.3.3.



## 4.5 Open-water enclosures

Fish, especially marine species, may be kept in large floating enclosures. The enclosure dimensions, including depth, should permit active swimming and shoaling of the fish. Mesh size should permit good water exchange while preventing escape of fish. Enclosures should be designed to minimise the risk of attack by predators. Enclosures should be rigged so as to prevent their shape distorting in tidal flows or running water and thus trapping fish.<sup>106</sup>

## 4.6 Feeding

Fish may be fed either on artificial diet or fresh/frozen natural food. Artificial diet is preferable, providing it meets the nutritional requirements of the species, and is acceptable to the fish. However, some fish species or life stages will not consume artificial diets. Artificial diets tend to have less impact on water quality, although some live natural diets (such as daphnia) are unlikely to compromise water quality and have the advantage that uneaten food need not be removed from the water.

Feeding rate and frequency will depend on a number of factors including temperature, size and maturity. As high temperature increases the metabolic rate, feeding level should also be increased. It may not always be necessary to feed fish daily. Presentation of diet is also very important to ensure adequate feeding. Consideration should be given to the number of meals per day, the age of the fish, the water temperature and the size of the pellet or food fragment offered. The feeding regime, palatability and the presentation of food should be designed to ensure that all fish obtain sufficient food.<sup>107</sup>

## 4.7 Cleaning

All enclosures should be kept free of detrimental accumulations of suspended waste products or uneaten feed. Excess accumulation may affect water quality and thus damage fish health. It is advisable to regularly treat and clean enclosures to prevent fouling and reduced water exchange. Consideration should be given to eliminating the risk of back-flushing and consequent fouling of enclosure water and increase in the risk of infection. If enclosures are not self-cleaning, waste material should be siphoned off as necessary, generally as soon as possible after feeding. It is advisable to clean the sides and bottom of enclosures regularly to avoid detrimental build-up of algae and other detritus.\* Care should be taken to minimise stress during cleaning.

\*Certain species may benefit from controlled algal build-up to facilitate grazing behaviour. Cleaning regimes should therefore be sensitive to species-specific needs.

## 4.8 Handling

Fish may be severely stressed by handling and careful consideration should be given to keeping this to the minimum possible. Fish should not be handled in air, or if this is unavoidable the duration of exposure to air should be kept to an absolute minimum. When catching fish, methods of handling that allow fish to remain submerged in water are likely to reduce exertion, anoxia, stress and physical damage. Transferring fish in a vessel containing water or a darkened, water-filled scoop are believed to be better practice than netting. If a net must be used, careful consideration should be given to the frame and mesh size of the net. Knotted net mesh should be avoided as this is considered to usually be detrimental to the health of the animal.<sup>108</sup> Appropriate consideration should be given to disinfecting and rinsing nets in clean water before use. It is strongly advised to ensure that netting is done as gently and efficiently as possible.

Out of water fish should ideally be handled with wet gloves or wet hands and on a moist surface to avoid scale and mucus loss. Particular attention should be paid to handling practices to avoid desiccation, suffocation and other injury.

<sup>106</sup> These recommendations are considered to be necessary in order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraphs 3.3.2 and 3.3.3.

<sup>107</sup> In order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 3.4.

<sup>108</sup> In order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 3.3.3.



Where required, fish should be kept under anaesthetic for as short a time as possible and be placed in clean aerated water for recovery. An effective concentration of anaesthetic should be maintained throughout the procedure.

#### 4.9 Humane killing

In most cases, fish are preferably killed by either:

- an overdose of anaesthetic using appropriate route and anaesthetic agent for the size and species; when killed by immersion, fish should be left in the anaesthetic solution for at least five minutes following the cessation of opercular movement and/or vestibulo-ocular reflex (VOR); or
- concussion of the brain by striking of the cranium.

Death should be confirmed, for example, by physical destruction of the brain or exsanguination.

#### 4.10 Records

It is advisable to maintain records on appropriate water-quality parameters, and the origin, number and species of fish held.

#### 4.11 Identification

It is not always necessary or feasible to individually identify all fish within a facility. If it is necessary to mark fish for identification purposes, subcutaneous dye injection is considered the least invasive method of marking. Careful consideration is needed before more invasive methods such as fin clipping or tagging are used. Mechanical tagging should normally not be used unless no other method is suitable. Marking should generally be carried out under anaesthesia in order to ease handling and minimise the risk of injury, morbidity and stress.

### 5 Source, capture and transport

Please note: zebrafish – being schedule 2 species – must have been bred for use in procedures unless authorisation is given to the contrary in the Project Licence.

Unless licence authority permits it, fish used for experimental or other scientific purposes should be bred and reared in captivity. However, it is recognised that there are significant difficulties in breeding many species of fish in captivity and therefore this may not be possible. Where animals must be taken from the wild, care should be taken to prevent injury and stress to them during capture, loading, transportation and unloading.

Where wild-caught animals are intended to be used in regulated procedures, the Project Licence holder must take all reasonable steps to ensure that they are caught by a competent person. Injuries, illness or death of animals on or shortly after receipt may indicate unsuitable source, capture or transport. If such problems arise, the Project Licence holder should review these arrangements before obtaining more animals from that source. The importation of fish into the UK requires authority from other regulators.

Prior to transportation of significant distance, it is advisable to deprive fish of food for a period sufficient to allow the gut to clear and reduce faecal contamination of the transport system. This period will vary according to species, with smaller fish having much shorter clearance times than larger fish. Consideration should therefore be given to species, length of transport and water-quality factors when calculating the food deprivation schedule.

Care should be taken to prevent injury and stress to fish during capture, loading, transportation and unloading. Abrupt temperature changes, periods of hypoxia and any deterioration in water quality due to excretory products should be avoided.



## 6 Further reading

Comprehensive advice regarding zebrafish can be found in the RSPCA's document, "Guidance on the housing and care of zebrafish, *Danio rerio*"<sup>109</sup>.

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109 Reed, B. & Jennings, M. (2010, updated 2011) "Guidance on the housing and care of zebrafish, *Danio rerio*" Research Animals Group, Science Department, RSPCA. <http://www.rspca.org.uk/sciencegroup/researchanimals/reportsandresources/housingandcare> (accessed 27/5/14).





## Section 3, Chapter 11: Amphibians

This chapter must be read in conjunction with the *Introduction* and *Section 3 Chapter 1: Advice applicable to all animals*.

### 1 Advice applicable to all amphibians

Please note: Frogs of the species *Xenopus laevis*, *Xenopus tropicalis*, *Rana temporaria* or *Rana pipiens* – being schedule 2 species – must have been bred for use in procedures unless authorisation is given to the contrary in the Project Licence.

Having regard to the Convention on International Trade in Endangered Species (CITES) and Commission Regulation 1158/2012, where possible, any amphibians used for experimental or other scientific purposes should be bred and reared in captivity. Purpose-bred animals should be used in preference to animals taken from the wild.

#### 1.1 Environmental conditions

Amphibian species inhabit a vast range of different habitats under climatic conditions ranging from snow to desert. Advice on environmental requirements should be sought from expert specialists and care staff to ensure that any particular species' needs are adequately addressed.

##### 1.1.1 Ventilation

Careful consideration should be given to the design of enclosures for amphibians to ensure that they are adequately ventilated. With increasing body weight, lung respiration dominates. The water in enclosures of aquatic housed amphibians should be filtered, circulated, and aerated as appropriate for the species and life stage.<sup>110</sup> *Xenopus* spp. rarely inhabits highly oxygenated (flowing) water in the wild. Therefore, air stones are not necessary for *Xenopus*. If the purpose of air stones is to reduce the fouling of the water, then it is advisable to tackle this by more frequent water changes.

##### 1.1.2 Temperature

Amphibians are ectothermic. Areas of different temperature and humidity are beneficial, to allow amphibians to seek their preferred microenvironment. Some amphibians, when exposed to frequent fluctuations in temperature and humidity within the enclosure, may be severely stressed and may be more prone to health problems. Room and water temperatures should be controlled as appropriate for the species and scientific requirements.

Hibernation in amphibians may be induced or interrupted by regulating light-dark cycles and room temperature. Before inducing hibernation in captivity, animals should normally be in good health and body condition. In animals used for breeding, a state of near winter torpor (for example dim light to darkness and low (e.g. 4°C) room temperature) may be simulated where appropriate. Under these conditions, animals can be kept without feeding for as long as four to five months. Restoration of pre-hibernation environmental conditions will induce activity and mating behaviour.

##### 1.1.3 Humidity

Amphibians do not drink but absorb moisture through their skin. Water loss is an especially critical problem in captive terrestrial and semi-terrestrial amphibians, as proper hydration is essential to the normal function of the amphibian skin. Areas of different humidity within the enclosure – or different humidity over time – are beneficial. Even desert-adapted amphibians should ideally have access to a humid environment.

##### 1.1.4 Lighting

It is advisable to use photoperiods reflecting the natural cycle from where the animals originate. Light levels in the enclosures are recommended to be consistent with that expected to be encountered

<sup>110</sup> In order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 3.5.



under natural conditions. It is advisable to ensure that both semi-terrestrial and aquatic housed animals have the opportunity to withdraw to shaded areas within the enclosure.

Incorporating a UV element is important for ensuring the health of some amphibians, such as many anurans. However, it should be noted that the mortality of some amphibians can be positively correlated with solar UV radiation. Furthermore, in some species, ambient levels of UV-B cause embryonic mortality in nature or cause deformities in amphibian embryos. Careful species-specific assessment of lighting needs is therefore required.<sup>111</sup>

### 1.1.5 Noise

Amphibians are very sensitive to noise (airborne stimuli) and vibration (substrate-borne stimuli) and are disturbed by any new, unexpected stimulus. It is advisable, therefore, to minimise such extraneous disturbances.

### 1.1.6 Alarms

Adequate alarm systems are necessary if circulation systems are used and/or aeration is required.<sup>112</sup>

## 1.2 Health

See Section 3 Chapter 1: Advice applicable to all animals.

## 1.3 Housing, enrichment and care

### 1.3.1 Housing

Group housing of amphibians is advisable, for instance to improve feeding and reduce fear responses. For example, in *Xenopus spp.* group feeding promotes feeding frenzies inducing all animals to feed. At very low stocking densities such frenzies may not occur and food is frequently not eaten.

To avoid cannibalism in certain species (particularly among larval *Ambystoma spp.* and *Scaphiopus spp.*), it is advisable to maintain animals in small groups. Cannibalism in groups can be reduced by grading animals according to their size. *Xenopus laevis* froglets and young adults can be particularly prone to cannibalism, which can be reduced by separating them into groups of similar-sized individuals. Cannibalism can also be eliminated by providing adequate food. For both *Scaphiopus* and *Ambystoma*, larval cannibalism is often facultative, as response to both high density and reduced availability of alternative food. Almost any larval caudate will graze the limbs of conspecifics.

### 1.3.2 Enrichment

The terrestrial habitat of amphibians should normally be enriched, including, for example, branches, leaves, pieces of bark, stones or other suitable man-made materials.<sup>113</sup> Amphibians benefit from environmental enrichment in different ways: for example, inclusions allow animals to hide, and provide visual cues important for spatial orientation. It is recommended that the side walls of the terraria are textured to provide a structured surface.

The provision of hiding places/shelters that are appropriate to the amphibian's needs is recommended, because they can reduce stress. For example, in *Xenopus spp.* a tube of ceramic or plastic composed of a non-leaching material may be provided. Refuges should be inspected regularly for sick or injured animals. A dark floor to the tank may enhance the sense of security of the animals.

Materials used for enrichment devices should not be detrimental to the health of the amphibians. Unless designed for single use only, devices should ideally be made of materials that withstand chemical sterilisation or autoclaving. Enclosures and enrichment should have smooth surfaces and rounded edges to minimise the risk of injury to the amphibian's skin.<sup>114</sup>

111 In order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 2.2.

112 In order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 2.

113 In order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 3.3.2.

114 In order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 3.3.3.



### 1.3.3 Dimensions and flooring

See Section 3 Chapter 1: Advice applicable to all animals.

### 1.3.4 Feeding

The majority of amphibians are carnivores with food preferences for living small invertebrates (such as larvae, insects and worms), although larvae are filter feeders. It is advisable to maintain captive animals on their natural foods or on foodstuffs approximating those of their natural diets – adults can be given fish, meat or commercial diets. The required feeding frequency is related to environmental conditions, such as temperature and light intensity. Daily feeding is not advisable for adult animals in fill-and-dump systems, but once to three times weekly to satiation at each feeding is recommended. Commercial diets are available and, where used, the manufacturer's recommendations should be followed.

### 1.3.5 Water quality

For aquatic and semi-aquatic amphibians, water quality, including the concentration of ammonia and the pH level in the water, should be regularly monitored.<sup>115</sup>

Ammonia is excreted by amphibians and its concentration will depend on the water-handling regime. A level of 5 mg/l would be cause for concern, although the degree of potential harm increases sharply with the pH of the water, as there is more ammonia and less  $\text{NH}_4^+$  at higher pH.

### 1.3.6 Substrate, litter, bedding and nesting material

See Section 3 Chapter 1: Advice applicable to all animals.

### 1.3.7 Cleaning

In order to avoid disease, it is advisable to carefully clean the terrestrial and aquatic areas in the terraria to remove dirt, excrement and food particles as appropriate. However, aggressive detergents should be avoided. The microbiome of amphibian skin provides a significant degree of innate immunity to the individual. Captive settings are known to reduce microbiome diversity, which can increase susceptibility to infections, for example with chytrid fungi. Cleaning regimes should therefore be designed carefully, taking this into account.

Amphibians explore their enclosures and will choose a favourite place (e.g. a stone or piece of bark) for drinking from or sleeping on. Amphibians become used to their enclosure. Therefore after cleaning it is important to return all enrichment to its original position.

### 1.3.8 Handling

Amphibians can be easily damaged. Care is required during handling, which should be kept to a minimum. Many species and some life stages respond negatively to latex gloves. Bare hands that retain soap or hand wash residues can also be hazardous during handling. Appropriate training is recommended.

### 1.3.9 Humane killing and anaesthesia

Pain perception in amphibians is likely to be analogous to mammals because amphibians possess neural systems for transmitting pain from peripheral receptors to the brain and antinociceptive mechanisms to modulate pain. Invasive, potentially painful procedures should be accompanied both by analgesia and anaesthesia, once an effective, safe protocol is identified.

It is important to consider that the tolerance to drugs may depend on the season. As amphibians' skin accounts for a significant portion of normal gaseous exchanges, in anaesthetised animals, in which lung respiration is reduced or interrupted, the skin should always be kept moist, for example with a wet tissue.

<sup>115</sup> In order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 3.5.



### 1.3.10 Records

See Section 3 Chapter 1: Advice applicable to all animals.

### 1.3.11 Identification

There are many types of amphibian used and different marking methods may be appropriate for different species. Where animals need to be identified individually there are a number of suitable methods such as transponders, tank labels for individually housed animals, photography of amphibians with complex markings or monitoring pigment or wart configurations. Chemical markings may be absorbed through the skin, possibly causing toxic effects. This may therefore require Project Licence authority. Toe clipping causes more than momentary pain or distress and therefore requires PPL authority. If the method you are considering using is not listed above contact your Inspector for advice.

## 1.4 Transport

For transport, careful consideration should be given to ensuring the provision of sufficient air and moisture and, if necessary, appropriate devices to maintain the required temperature and humidity during the journey.

## 2 Additional advice applicable to aquatic amphibians

### 2.1 Environmental conditions

#### 2.1.1 Ventilation

See Part 1: Advice applicable to all amphibians.

#### 2.1.2 Temperature

See Part 1: Advice applicable to all amphibians.

#### 2.1.3 Humidity

See Part 1: Advice applicable to all amphibians.

#### 2.1.4 Lighting

See Part 1: Advice applicable to all amphibians.

#### 2.1.5 Noise

See Part 1: Advice applicable to all amphibians.

#### 2.1.6 Alarms

See Part 1: Advice applicable to all amphibians.

### 2.2 Health

See Part 1: Advice applicable to all amphibians.

### 2.3 Housing, enrichment and care

#### 2.3.1 Housing

Aquatic amphibians such as *Xenopus laevis* or amphibian larvae are housed in tanks and aquaria. These may be equipped with a gentle flow-through water system for the circulation of uncontaminated (for example, dechlorinated) water, a heating device to maintain suitable temperatures, and a compressed air supply. Care is needed to ensure that aeration does not cause injury to the animals.



or undue stress due to excessive water turbulence. Unless a proper flow system is in place, it is advisable to renew the water in the enclosures with water of an appropriate quality about twice a week.

For *Xenopus* spp., systems with regular changes of water (fill-and-dump systems) are sufficient for maintaining appropriate water quality (such as minimising levels in ammonia).

### 2.3.2 Enrichment

See Part 1: Advice applicable to all amphibians.

### 2.3.3 Dimensions and flooring

It is advisable to avoid long, narrow enclosures since they may restrict locomotor activity and social behaviour such as feeding frenzies.

### 2.3.4 Feeding

See Part 1: Advice applicable to all amphibians.

### 2.3.5 Water quality

See Part 1: Advice applicable to all amphibians.

### 2.3.6 Substrate, litter, bedding and nesting material

See Part 1: Advice applicable to all amphibians.

### 2.3.7 Cleaning

See Part 1: Advice applicable to all amphibians.

### 2.3.8 Handling

See Part 1: Advice applicable to all amphibians.

### 2.3.9 Humane killing and anaesthesia

See Part 1: Advice applicable to all amphibians.

### 2.3.10 Records

See Part 1: Advice applicable to all amphibians.

### 2.3.11 Identification

See Part 1: Advice applicable to all amphibians.

## 3 Additional advice applicable to semi-aquatic and semi-terrestrial amphibians

### 3.1 Environmental conditions

#### 3.1.1 Ventilation

See Part 1: Advice applicable to all amphibians.

#### 3.1.2 Temperature

See Part 1: Advice applicable to all amphibians.



### 3.1.3 Humidity

See Part 1: Advice applicable to all amphibians.

### 3.1.4 Lighting

See Part 1: Advice applicable to all amphibians.

### 3.1.5 Noise

See Part 1: Advice applicable to all amphibians.

### 3.1.6 Alarms

See Part 1: Advice applicable to all amphibians.

## 3.2 Health

See Part 1: Advice applicable to all amphibians.

## 3.3 Housing, enrichment and care

### 3.3.1 Housing

It is advisable to keep semi-aquatic and semi-terrestrial amphibians in enclosures consisting of two parts: a terrestrial and an aquatic part. The aquatic area of the terrarium should ideally allow animals to submerge. Unless a flow-through system is used, it is advisable to renew water at least twice a week. It is advisable to remove aquatic habitats during hibernation.

It is advisable to cover each terrarium to prevent escape. Where the walls of the terrarium are transparent it is advisable to paint or otherwise make them opaque. However, care should be taken to ensure that such measures do not prevent the access required to check the animals.

### 3.3.2 Enrichment

Additions to the interior design can include: soft-foamed plastic material on the floor near the pool area, stones, pieces of artificial bark material, artificial branches/leaves and shelves.

### 3.3.3 Dimensions and flooring

See Part 1: Advice applicable to all amphibians.

### 3.3.4 Feeding

See Part 1: Advice applicable to all amphibians.

### 3.3.5 Water quality

See Part 1: Advice applicable to all amphibians.

### 3.3.6 Substrate, litter, bedding and nesting material

It is advisable to avoid fine sawdust and any other related small-particle substrate, as it affects the sensitive body skin, harbours pathogens and is difficult to clean and re-use.

### 3.3.7 Cleaning

See Part 1: Advice applicable to all amphibians.

### 3.3.8 Handling

See Part 1: Advice applicable to all amphibians.



### 3.3.9 Humane killing and anaesthesia

See Part 1: Advice applicable to all amphibians.

### 3.3.10 Records

See Part 1: Advice applicable to all amphibians.

### 3.3.11 Identification

See Part 1: Advice applicable to all amphibians.

## 4 Additional advice applicable to arboreal amphibians

### 4.1 Environmental conditions

#### 4.1.1 Ventilation

See Part 1: Advice applicable to all amphibians.

#### 4.1.2 Temperature

See Part 1: Advice applicable to all amphibians.

#### 4.1.3 Humidity

See Part 1: Advice applicable to all amphibians.

#### 4.1.4 Lighting

See Part 1: Advice applicable to all amphibians.

#### 4.1.5 Noise

See Part 1: Advice applicable to all amphibians.

#### 4.1.6 Alarms

See Part 1: Advice applicable to all amphibians.

### 4.2 Health

See Part 1: Advice applicable to all amphibians.

### 4.3 Housing, enrichment and care

#### 4.3.1 Housing

Having regard for the behaviour of different arboreal species, every effort should be made to allow for this by the provision of appropriate structures for climbing and resting. Given the climbing abilities of arboreal amphibians, measures should be taken to prevent them escaping from their enclosures. In addition, it is advisable to provide water in which they can submerge themselves or seek greater humidity. If water dishes are used, they should ideally be arranged in such a way that they are easy for the amphibians to enter and leave.

#### 4.3.2 Enrichment

See Part 1: Advice applicable to all amphibians.



#### *4.3.3 Dimensions and flooring*

See Part 1: Advice applicable to all amphibians.

#### *4.3.4 Feeding*

See Part 1: Advice applicable to all amphibians.

#### *4.3.5 Water quality*

See Part 1: Advice applicable to all amphibians.

#### *4.3.6 Substrate, litter, bedding and nesting material*

See Part 1: Advice applicable to all amphibians.

#### *4.3.7 Cleaning*

See Part 1: Advice applicable to all amphibians.

#### *4.3.8 Handling*

See Part 1: Advice applicable to all amphibians.

#### *4.3.9 Humane killing and anaesthesia*

See Part 1: Advice applicable to all amphibians.

#### *4.3.10 Records*

See Part 1: Advice applicable to all amphibians.

#### *4.3.11 Identification*

See Part 1: Advice applicable to all amphibians.





## Section 3, Chapter 12: Reptiles

This chapter must be read in conjunction with the *Introduction* and *Section 3 Chapter 1: Advice applicable to all animals*.

### 1 Advice applicable to all reptiles

This chapter provides details of the basic housing and care conditions recommended for reptilian species commonly used for scientific purposes. Specific procedures may require the use of certain other species which do not fall into these categories, such as semi-aquatic, arboreal or rock-climbing reptiles. Should behavioural or breeding problems occur, or should further information on specific requirements for other species be required, advice should be sought from experts and care staff specialised in the species concerned, to ensure that any particular species' needs are adequately addressed.

Having regard to the Convention on International Trade in Endangered Species (CITES) and Commission Regulation 1158/2012, where possible, reptiles used for experimental or other scientific purposes should be bred and reared in captivity. Purpose-bred animals should be used in preference to animals taken from the wild.

#### 1.1 Environmental conditions

##### 1.1.1 Ventilation

Enclosures for reptiles should be adequately ventilated. However, to prevent escape, ventilation holes should be screen-covered.<sup>116</sup>

##### 1.1.2 Temperature

Reptiles are ectothermic. In order to maintain their body temperatures, under natural conditions they will select microenvironments in which they can gain or lose heat. Therefore, enclosures should offer the animals areas of different temperature (a temperature gradient), which will help to avoid research-biasing stress and distress arising from inappropriate environmental conditions.<sup>117</sup>

Temperature requirements of different species vary considerably and may even fluctuate in the same species at different times of the year. In the laboratory, room and water temperatures should be controlled.<sup>118</sup> In many reptiles, sex determination and gonadal differentiation are temperature-dependent.

An incandescent lamp positioned over the platform provided as a resting area will allow basking reptiles to increase their body temperature. When the lights are turned off or are not used, a flat heating device may be inserted into the enclosure on which basking reptiles can lie to maintain body temperature. Where such heat sources are used and which allow direct contact between the animal and the source, the source temperature should not exceed 40°C in order to ensure that no injury is caused. Care should always be exercised when direct heat sources are used because even when there is the opportunity to move away from a focal heat source within an enclosure it is not uncommon for reptiles to remain in direct contact until third degree burns are inflicted. For this reason, heating devices should be thermostatically controlled and have appropriate guards to prevent animals from overheating and burning.

It is advisable to ensure that terraria of snakes or lizards from tropical biotopes are furnished with at least one warmth-plate.

<sup>116</sup> These recommendations are considered necessary in order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 3.3.3.

<sup>117</sup> These recommendations are also considered necessary in order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 3.3.2.

<sup>118</sup> In order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 2.1.



### 1.1.3 Humidity

Humidity levels are often best controlled by alterations to the ventilation rate. The provision of areas of different humidity (humidity gradient) is beneficial. Humidity should always be tailored to the species' requirement.

### 1.1.4 Lighting

Appropriate light and dark regimes for each species, life stage, and time of the year should be provided.<sup>119</sup> Reptiles should normally have the opportunity to withdraw to shaded areas within the enclosure. It is advisable that light or sun lamps are not the sole source of heat. The provision of UVA and UVB radiation is necessary to stimulate the animal's production of vitamin D.

### 1.1.5 Noise

Reptiles are very sensitive to acoustic noise (airborne stimuli) and/or to vibratory noise (substrate-borne stimuli) and are disturbed by any new, unexpected stimulus. Squamate reptiles (e.g. snakes) can be extremely sensitive to vibrations on the substrate: many "hear" by sensing ground vibrations through a mandibular connection to the inner ear. They detect predators by their footfalls, so staff should be aware of this when approaching cages. It is advisable to minimise noise and vibratory disturbances.

### 1.1.6 Alarms

Adequate alarm systems should be provided if ancillary heating devices and/or water circulation systems are used and/or aeration is required.<sup>120</sup> If alarms are used, it is advisable that they are "silent", so that they do not disturb the animals.

## 1.2 Health

See Section 3 Chapter 1: Advice applicable to all animals.

## 1.3 Housing, enrichment and care

### 1.3.1 Housing

Accumulation of as much information as possible on the ethological needs of the species is a necessary prerequisite when planning to accommodate reptiles in groups. Few reptiles naturally live in groups.

Enclosures and enclosure furniture should have smooth surfaces and rounded edges to minimise the risk of injury, and consideration should be given to the use of opaque materials for the most sensitive species.

To prevent collision with clear glass, it is advisable to pattern the side walls of the terraria to provide a structured surface.

### 1.3.2 Enrichment

The habitat of reptiles should be structured to include, for example, natural or artificial branches, leaves, pieces of bark and stones.<sup>121</sup> Reptiles benefit from environmental enrichment in different ways: for example, inclusions allow animals to hide, provide labels for visual and spatial orientation and will help to reduce stress.

### 1.3.3 Dimensions and flooring

See Section 3 Chapter 1: Advice applicable to all animals.

<sup>119</sup> In order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 2.2.

<sup>120</sup> In order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 2.4.

<sup>121</sup> In order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 3.3.2.



### 1.3.4 Feeding

It is advisable to maintain captive reptiles on their natural foods or commercial diets approximating those of their natural diets. Many reptiles are carnivores (all snakes and crocodiles, most lizards, and some turtles), but some are vegetarian and others are omnivores. Some species exhibit very narrow and specific feeding habits. Reptiles, except for some snakes, can be trained to feed on dead prey. Therefore, it should normally not be necessary to feed live vertebrates. Feeding of live vertebrates, if absolutely necessary, should only be undertaken under the direction of the Named Veterinary Surgeon, and following careful consideration of the Secretary of State's Standards of Modern Zoo Practice. When dead vertebrates are used, they should have been humanely killed using a method that avoids the risk of toxicity to the reptiles. Feeding regimes should be appropriate to the species, stage of development and husbandry system.

### 1.3.5 Watering

Drinking water should be provided for all reptiles. Water plays an important role in skin shedding and some reptiles drink from droplets on foliage so misting may be required. Some reptiles require sufficient water in which to bathe.

### 1.3.6 Substrate, litter, bedding and nesting material

A variety of substrates may be used for terraria, depending on the requirements of the species. It is advisable to avoid fine sawdust and any other small-particle substrate, as this may cause serious mouth or internal injuries or bowel obstruction, particularly in snakes.

### 1.3.7 Cleaning

In order to avoid disease, it is advisable to carefully clean the terrestrial and aquatic areas in the enclosure to remove dirt, excrement and food particles.

Aggressive detergents should be avoided. Reptiles explore their enclosures and will choose a favourite place (e.g. a stone or piece of bark) for drinking from or sleeping on, and they become used to their enclosure's layout. Therefore after cleaning it is important to return all enrichment to its original position.

### 1.3.8 Handling

Care is needed when handling reptiles, as they can be easily injured. For example, some lizards may shed their tails (autotomy) if handled in an inappropriate way, and other species can easily be traumatised. It is advisable that staff receive specialist training and follow specialist safe working practices when working with large, aggressive or venomous species.

### 1.3.9 Humane killing

An appropriate method of killing is by an overdose of a suitable anaesthetic.

### 1.3.10 Records

See Section 3 Chapter 1: Advice applicable to all animals.

### 1.3.11 Identification

Where animals need to be identified individually a number of suitable methods are available: transponders; enclosure labels for individually housed animals; monitoring individual skin patterns (according to colour, skin damages, etc.). Topically applied dye markings require renewal after skin shedding. Toe clipping is considered to cross the threshold for regulation and should not be done unless authorised by a Project Licence.



## 1.4 Transport

It is important to ensure that during transport reptiles are provided with adequate air and moisture. If necessary, appropriate consideration should be given to the provision of devices designed to maintain the required temperature and humidity.

# 2 Additional advice applicable to aquatic reptiles

## 2.1 Environmental conditions

### 2.1.1 Ventilation

See Part 1: Advice applicable to all reptiles.

### 2.1.2 Temperature

See Part 1: Advice applicable to all reptiles.

### 2.1.3 Humidity

See Part 1: Advice applicable to all reptiles.

### 2.1.4 Lighting

See Part 1: Advice applicable to all reptiles.

### 2.1.5 Noise

See Part 1: Advice applicable to all reptiles.

### 2.1.6 Alarms

See Part 1: Advice applicable to all reptiles.

## 2.2 Health

See Part 1: Advice applicable to all reptiles.

## 2.3 Housing, enrichment and care

### 2.3.1 Housing

Aquatic reptiles should normally be accommodated in tanks in which water is circulated, filtered and aerated. It is advisable to renew the water about twice per week, except in the case of flow-through systems. To minimise the bacterial contamination of the water, it is advisable that water temperatures do not exceed 25°C. It is recommended that water levels are sufficient for reptiles to submerge.

Platforms should be provided as a resting area onto which the reptiles can haul themselves or under which they can shelter. There should be two areas – one as a basking spot and the other in a cooler part of the enclosure. Such platforms should be made of suitable materials, such as wood, so that animals are able to get a purchase with their claws in order to pull themselves out of the water. Platforms should be replaced as necessary.<sup>122</sup>

### 2.3.2 Enrichment

See Part 1: Advice applicable to all reptiles.

<sup>122</sup> These recommendations are considered necessary in order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 3.3.2.



### 2.3.3 Dimensions and flooring

Platforms made of epoxy or polyurethane may not be appropriate as they are likely to deteriorate quickly under continuous warm temperatures.

### 2.3.4 Feeding

See Part 1: Advice applicable to all reptiles.

### 2.3.5 Water quality

See Part 1: Advice applicable to all reptiles.

### 2.3.6 Substrate, litter, bedding and nesting material

See Part 1: Advice applicable to all reptiles.

### 2.3.7 Cleaning

See Part 1: Advice applicable to all reptiles.

### 2.3.8 Handling

See Part 1: Advice applicable to all reptiles.

### 2.3.9 Humane killing and anaesthesia

See Part 1: Advice applicable to all reptiles.

### 2.3.10 Records

See Part 1: Advice applicable to all reptiles.

### 2.3.11 Identification

See Part 1: Advice applicable to all reptiles.

## 3 Additional advice applicable to terrestrial reptiles

### 3.1 Environmental conditions

#### 3.1.1 Ventilation

See Part 1: Advice applicable to all reptiles.

#### 3.1.2 Temperature

See Part 1: Advice applicable to all reptiles.

#### 3.1.3 Humidity

See Part 1: Advice applicable to all reptiles.

#### 3.1.4 Lighting

See Part 1: Advice applicable to all reptiles.

#### 3.1.5 Noise

See Part 1: Advice applicable to all reptiles.



### 3.1.6 Alarms

See Part 1: Advice applicable to all reptiles.

## 3.2 Health

See Part 1: Advice applicable to all reptiles.

## 3.3 Housing, enrichment and care

### 3.3.1 Housing

Terrestrial reptiles should normally be kept in enclosures consisting of an appropriate terrestrial part and an aquatic part. It is recommended that the water area of the terrarium allows animals to submerge. It is advisable to renew the water at least twice a week, except in the case of a flow-through system.

It is advisable that terraria are transparent, have tight seams with all holes securely screened, and be provided with well-fitted lids or doors that can be securely fastened by latches, hooks or clasps. For housing venomous snakes, it is important to also fulfil certain security criteria.

It is advisable to construct doors and lids so that the entire top or an entire end or side opens to facilitate cleaning (except in the case of venomous reptiles). For some species, it is advisable that all walls and the top are opaque, except the front wall. In cases of highly irritable or easily frightened reptiles, the clear wall can be provided with a removable covering. It is advisable to construct walls of a non-reflective material rather than glass. Many species of reptiles are stressed by their own reflection because they see it as a trespassing competitor that never goes away. Also having other reptiles within sensing distance can be very stressful because they may see the other reptiles as predators.

The provision of appropriate shelter is important for all terrestrial reptiles, both in which to hide and also sometimes to feed. A shelter-box, such as a tube of clay, simulates the darkness of a burrow.

### 3.3.2 Enrichment

The provision of appropriate structures to climb on, such as branches, is very important for arboreal snakes.

### 3.3.3 Dimensions and flooring

Adequate enclosure height is very important for arboreal snakes.

### 3.3.4 Feeding

See Part 1: Advice applicable to all reptiles.

### 3.3.5 Water quality

See Part 1: Advice applicable to all reptiles.

### 3.3.6 Substrate, litter, bedding and nesting material

See Part 1: Advice applicable to all reptiles.

### 3.3.7 Cleaning

See Part 1: Advice applicable to all reptiles.

### 3.3.8 Handling

See Part 1: Advice applicable to all reptiles.



### *3.3.9 Humane killing and anaesthesia*

See Part 1: Advice applicable to all reptiles.

### *3.3.10 Records*

See Part 1: Advice applicable to all reptiles.

### *3.3.11 Identification*

See Part 1: Advice applicable to all reptiles.



## Section 3, Chapter 13: Cephalopods

This chapter must be read in conjunction with the *Introduction* and *Section 3 Chapter 1: Advice applicable to all animals*.

### 1 Standards applicable to all cephalopods

#### 1.1 Introduction

A wide variety of cephalopod species are used for experimental purposes and these have a diverse range of habitats and behaviour. Knowledge of environmental and husbandry requirements is limited to a small number of species and may differ significantly between species. Investigators and animal care staff should acquaint themselves with the characteristics of the cephalopod species proposed for the work, and advice should be sought from cephalopod specialists and appropriately experienced care staff to ensure that any particular species' needs are adequately addressed and that appropriate facilities and husbandry procedures are in place before animals are obtained.

#### 1.2 Environmental conditions

Squid are pelagic, cuttlefish neo-benthic and octopus benthic and this should be considered for all housing and care. Specific conditions are not detailed in this code of practice but should be obtained from the relevant species-specific literature. A useful website is: <http://www.cephalopodresearch.org/>

##### 1.2.1 Water supply

It is essential that an adequate water supply of suitable quality for each species is provided at all times. Water flow in re-circulating systems or filtration within enclosures should be sufficient to remove suspended waste and to ensure that water-quality parameters are maintained within acceptable levels that sustain normal activity and physiology for a given species and individual.

Systems should be in place to ensure that water parameters are checked regularly. There should be an alarm system linked to flow and water level.<sup>123</sup>

##### 1.2.2 Temperature

Cephalopods are exothermic, highly adapted to the marine aquatic environment and are therefore unlikely to tolerate rapid or significant changes in the quality or temperature of the water in which they are housed. They react rapidly to environmental changes/external stimuli with immediate physiological consequences that can be relatively long lasting. Such changes, as well as having potential welfare implications, will also impact upon experimental results. They have limited convergence with fish and assumptions for housing, care and use of these animals based on fish, whilst appropriate in some circumstances, should be made with caution. Temperature should be maintained within the optimal range for the particular species and requires careful control. Appropriate chilling/heating equipment should be in place. Where water changes are performed on a larger scale temperature spikes are more likely, and these may cause adverse effects or even death.

##### 1.2.3 Water flow and quality

It is assumed that, as for other aquatic species, water quality is the most important factor in maintaining the health and well-being of cephalopods and in reducing stress and the risk of disease. Water-quality parameters should at all times be within the acceptable range that sustains normal activity and physiology for a given species and individual. The definition of acceptable range is complicated in that optimum conditions are not well defined for many species and it may not be possible to extrapolate between species. Optimum conditions may also vary between different life stages, e.g. paralarvae, juveniles, adults, or according to physiological status of the individual (e.g. females preparing to lay eggs).

Cephalopods eat a high protein diet. Ensuring protein levels do not build up in the system appears important. Protein skimmers and water flow should be suitably set up so that there is capacity to

<sup>123</sup> In order to meet the requirements of Sections 1 and 2 Chapter 1 *Standards applicable to all animals*, paragraph 2.4.





remove any ink. Ink could also act as an alarm substance and should be removed immediately to avoid stress to con- or closely related heterospecifics. Cuttlefish may release a considerable volume of ink. If capacity is insufficient to remove ink there will be a need to change the water in the system. Unless specifically required by the study, stimuli/manipulations likely to cause inking should be avoided where possible.

Cephalopods show little adaptability to changing conditions of water quality. Where animals need to be moved between tanks or systems it is therefore important to ensure water parameters are mirrored. Where necessary, water supply should be appropriately filtered to remove substances harmful to cephalopods and to maintain suitable water physico-chemical parameters. Cephalopods have well-developed olfaction/chemoreception. Although more research is needed, it is possible that they could be stressed if able to sense other animals which they consider rivals or predators, or which are ill or distressed, and consideration of their olfactory abilities should be made with respect to water supply and quality.

Water flow should enable cephalopods to maintain normal locomotion/behaviour. Cephalopods can use rapid expulsion of water through the funnel to power jet propulsion, which results in swift movement. Cuttlefish and squid have mantle adaptations to assist in other types of locomotion. Squid are in continuous motion due to their pelagic nature and water flows need to be high – in specialised tanks – to fulfil their requirements. Octopuses have a tendency to use their arms rather than jetting due to the reduced energy expenditure. Repetitive locomotion in cephalopods, such as jetting backwards continuously, is a sign of stress. Jetting can lead to collisions with the walls of the tank if animals are startled or there is insufficient space for escape reactions. Such jetting may cause permanent damage and retard growth.

### 1.2.3a Oxygen

Generally, cephalopods have high respiration rates and therefore oxygen levels are important for welfare. Oxygen concentration should be appropriate to the species and, based on other aquatic species, it is assumed that the required oxygen concentration will vary according to temperature, carbon dioxide concentration, salinity, feeding level and amount of handling. Cephalopods should generally be kept in highly aerated water and, where necessary, supplementary aeration of water should be provided. Specific levels have been reported for at least some species. Generally, levels should not be maintained significantly above 100% saturation as supersaturation may cause problems with gas exchange at the gills. This may be a particular issue with squid. Unlike some species of fish, cephalopods cannot facultatively breathe air so water flow should be checked more regularly than for other aquatic organisms. The provision of additional aeration for each tank may be required. This might be via the use of air stones, although consideration should be given to the possible effects of noise/vibration and the possibility of forming mucus bubbles and foaming if the protein level is high.

### 1.2.3b Nitrogen compounds

The accumulation of nitrogenous compounds should be avoided. Such accumulation may be reduced by more frequent cleaning of the tank, by increasing the flow of water known to be of low nitrate content, by reducing stocking density, or by increasing the quality of biofiltration in closed or semi-closed systems. Build-up of nitrogenous compounds may lead to behavioural changes and/or changes in skin colouration. At nitrate levels >80 mg/l, cuttlefish will be very agitated, will ink profusely and their skin tone may be dark. In addition, larvae and hatchlings become more vulnerable to bacterial disease. There is some anecdotal evidence which suggests ozone produced by some types of protein skimmers may have serious adverse effects on cephalopods. If such skimmers are used, there is the need for particularly close monitoring and it is generally advised that such systems be avoided where possible.

### 1.2.3c Carbon dioxide

Carbon dioxide is produced during respiration and dissolves in water to form carbonic acid, thus lowering pH. Since stability of pH is very important accumulation of carbon dioxide should be avoided. Situations which may increase carbon dioxide levels include high stocking density. Care should be taken that water supply systems, particularly groundwater-based systems if these are used as a basis for artificial seawater, do not introduce harmful quantities of carbon dioxide into the enclosures.



### 1.2.3d pH

Due to the effects of pH on the carriage of oxygen by blood pigments, cephalopods poorly tolerate low pH. Regular measurement and careful maintenance of pH is critical. Acceptable pH levels depend on many water-quality factors (e.g. carbon dioxide and calcium) and so control of water solutes and soluble gases is important. This may be a particular issue for establishments that use synthetic marine salts rather than natural seawater and closed filtration systems, especially where water changes are limited. Acceptable pH values for cephalopods are in the range of 7.8 to 8.2.

### 1.2.3e Salinity

Cephalopods are marine organisms, and therefore salinity is critically important with the level dependent on the natural salinity of the source of the animals. A typical range for European temperate species is 32–36 ppt.

### 1.2.3f Metals

Copper is highly toxic to all species. Since this metal may be present in certain treatments for fish and in pipework, it is essential cephalopods are not in same system as fish under such treatments or where there is copper pipework. Caution should be used when fitting out systems. Expert advice should be sought and non-toxic materials be used in all fittings.

### 1.2.4 Lighting

Cephalopods appear to have good vision and may be startled by unexpected movements. It appears common to house cephalopods in ambient photoperiod. Species-specific needs should be met, and allowing animals to choose an appropriate level of light may be ideal. Typically, opaque covers or darkened areas are provided and/or dark sides for tank housing are used for adults. Abrupt changes in light level or regime should be avoided as far as possible.

### 1.2.5 Noise

At least one study has suggested that cephalopods can detect low frequencies of sound. In line with other aquatic species, they appear to dislike vibrations such as drilling or banging on tank sides and may respond by inking. It is recommended that plant is housed in an area separate from the animal housing if possible. It is considered that animals are likely to benefit from a quiet environment in order to minimise disruption and that, at a minimum, sudden noises which could startle them should be avoided, for example the covers of tanks should be removed cautiously and reduction of noise considered in the design of covers and other equipment.

### 1.2.6 Alarms

Alarms are advisable, with water level/flow and low oxygen among parameters considered. The positioning of such alarms may need to be considered with care as animals may attack or remove probes.

## 1.3 Health

### 1.3.1 General health

The health of cephalopods is intimately bound up with their environmental and husbandry conditions. Investigations of poor health must therefore always check for deficiencies in the environment and husbandry practices and any defects must be addressed promptly.

It is not always easy to assess the well-being of cephalopods, but changes in appetite, feeding response, behaviours (such as social interactions, position in the water column and presence of abnormal or reduced normal movement), physical appearance (such as skin texture and colour) and respiratory rate or depth may provide early warning of developing problems. Currently, indicators of potential pain, suffering or distress have yet to be defined and agreed for each species and this is urgently required.



Colour change, inking and jetting are known to occur in response to the presence of a predator in the wild and to conspecifics during breeding. If these occur in the laboratory, it is possible that the animal is finding a stimulus stressful and every effort should be made to minimise or eliminate exposure to the stressor. Signs that animals require close checking include lack of cleaning behaviour (removal of sucker discs) in octopuses and reduction in movement in squid. Indicators of good health would appear to include animals holding position at the bottom of the tank during the day for cuttlefish, although it may depend on age and lifecycle stage (adults will frequently hover, particular during breeding period) and use of the den during the day for octopuses, with more active behaviour at night for both.

### 1.3.2 Disease and injury

Water quality is essential for minimising disease, particularly bacterial infections. Much of the disease in cephalopods captured and kept in captivity appears to be related to trauma. For example, evidence of traumatic lesions such as partial limb loss (assumed to occur in predator/prey interactions) or sucker-induced trauma from confrontations between individuals has been recorded in wild-caught animals. Although some of these injuries do not appear to necessarily disadvantage individuals or to cause them significant discomfort, they may increase the risk of secondary infections and their incidence should be minimised on principle.

Self-induced trauma known as “butt-burn” is seen in cuttlefish, caused by jetting against tank sides in escape responses. This results in severe, focally extensive, ulcerative dermatitis and cellulitis of the dorsal apex of the mantle. These lesions can lead to mortality. Tank design (shape, size and water flow) is important to minimise the risk of such injuries. Injuries can also occur when bringing together individuals for breeding. Deaths through agonistic interactions can occur, with females suffering in particular. In cuttlefish, a ratio of two males to one female to reduce male/mating aggression has been suggested.

Ulcerative skin disease and swelling of one or both eyes have been reported. Live food may be a source of parasites and these, and other infections may be a source of illness. Bacterial infections have been recorded in cuttlefish. As with other species, it is likely that stress may be a factor in the development of such diseases. It is important to review potential health issues with a veterinarian, or other suitably qualified individual if there is no veterinarian with relevant knowledge and experience, and to implement a preventative medicine programme as appropriate.

Where possible, sick animals should be isolated to reduce the potential spread of infection to others, and any dead animals removed immediately since these may shed vast numbers of infectious organisms. Care should be taken that any waste water from tanks of isolated animals does not contaminate water supplies to other animals or the environment. An investigation of disease outbreaks should not only include the immediate cause, e.g. infectious agent, but possibly underlying causes such as adverse water quality. Failure to correct such factors will often result in a further outbreak of disease.

With age, cephalopods undergo the natural process of senescence (a process where the body appears to “shut down” after mating and the animal begins to die). The clinical signs of animals in senescence include reduced or absent drive to eat, poor skin quality, cloudy eyes and changed behaviour. It should be possible to distinguish this state from an animal that is showing similar signs due to disease, and good record keeping and knowledge of the age (where possible) and general health of individuals may help to differentiate the two. It is unknown whether animals experience any form of pain or suffering during senescence but the precautionary principle should be applied and the process taken into account when determining humane endpoints for studies involving cephalopods. In general, animals showing signs of senescence should be humanely killed, unless there is sound scientific or animal welfare justification for keeping them alive. For example, it could be justifiable to allow a senescing female octopus to survive in order for her eggs to develop, if there was also a justified use for the eggs or hatchlings themselves. Otherwise, both the senescing female and the embryos should be humanely killed.



### 1.3.3 Hygiene and disinfection

Appropriate attention should be paid to hygiene within experimental facilities. Holding facilities, including associated pipework, should be regularly cleaned and disinfected. In closed systems cleaning and disinfection should be compatible with maintenance of optimal microbiological conditions of filters. Equipment (e.g. nets) should be disinfected and washed between uses and should not be shared between different water systems to prevent the potential spread of infectious diseases.

### 1.3.4 Quarantine and acclimatisation

Little is known about the quarantine requirements for cephalopods, but in principle animals caught at different times or from different sources should not be held in the same tank or in systems where water from their tank will flow subsequently through tanks holding other cephalopods. Equipment such as nets should not be shared between tanks for a period of time that will ensure that animals carrying disease can be identified. As with other species, ideally, quarantined animals should be kept in a separate room with its own access, disinfection procedures and equipment, but it is accepted that cephalopods may go into fixed groups on entry to a facility which will remain separated for life and a separate quarantine area may not be necessary under these circumstances. If warranted, effluent water from quarantined animals should also be sterilised before disposal by chemical means or UV or ozonation treatment.

During the quarantine period animals should be monitored closely for unusual signs or behaviour, and detailed examinations made of any individuals which are considered to be abnormal. Diseased animals should be treated or the stock destroyed. Quarantine is also useful in acclimatising animals to new surroundings before experimentation. Sometimes change of environment, particularly slight changes in temperature, can cause egg laying in cuttlefish and this may therefore be induced during quarantine. Should this occur, consideration should be given to the normal breeding cycle for the species in terms of the role of maternal care and whether egg laying will result in senescence of the adults, with appropriate action taken if required to safeguard the welfare of the animals.

## 1.4 Housing, enrichment and care

The natural behaviour of cephalopods will influence acceptable stocking density, schooling, predatory and territorial behaviour. The stocking density should be based on the total needs of the individual species in respect of environmental conditions, health and welfare. This includes the need to perform a range of behaviours including social behaviour, normal exercise and appropriate feeding behaviours. Acceptable stocking density for a given species will vary depending on water flow and current, water quality, animal size, age, health and feeding method. There is substantial literature on how best to keep the more commonly used cephalopods. There are specific practices to be avoided for specific cephalopods, for example bare glass tanks for cuttlefish.

Animals should have sufficient water volume and flow for normal locomotion/movement. Measures should be taken to avoid or minimise conspecific aggression and cannibalism, particularly in the breeding season or where there could be territory-related antagonism. Such measures should not alter the overall welfare of the animal.

### 1.4.1 Enclosures

Cephalopods can be maintained in a variety of types of enclosures within dedicated buildings, in outside enclosures or in open-water systems. Facilities should be arranged to minimise the disturbance to animals from the movement or work of personnel. Animals should not be held outside in conditions that would cause them physiological or psychological stress or distress.

The materials used to construct the enclosures should be non-toxic, durable and with a smooth internal surface to prevent abrasions to the animals. Octopuses have very well developed tactile and olfactory/chemosensory systems and tank design should take this into consideration. Enclosures should be secured to minimise the risk of escape by jetting (squid and cuttlefish) or climbing (octopus).

Enclosures should be of an appropriate size to allow individuals to maintain normal locomotion/behaviour, accommodate the required stocking density and should be able to receive the necessary water flow. Enclosures should be of an appropriate shape to accommodate the behavioural needs



and preferences of the particular species; for example, enclosures with rounded ends and with a large tank floor area are most appropriate for cuttlefish. Enclosures should be appropriately designed to aid removal of waste products and surplus feed. There appear to be particular difficulties in maintaining water quality in closed systems because of the water flows required, especially if removal of ink becomes necessary. Keeping squid in particular in such systems is likely to be highly problematic.

#### 1.4.2 Enrichment

There are significant differences in the needs of octopuses, cuttlefish, squid and nautilus, according to their different behavioural traits. Species- and stage-specific needs for water depth and flow, swimming space, conspecifics, substrate and hiding places should be taken into consideration. For example, a den is essential for octopuses and the needs for benthic and pelagic species are likely to differ, based on their natural habitats and behaviours. It has been suggested that cuttlefish benefit greatly from provision of fake or real seaweed which allows them to express their natural behaviours. Care is needed to ensure that environmental enrichment does not adversely affect water quality, but this should not impede the development of suitable measures to enhance the welfare of the animals.

#### 1.4.3 Feeding

Cephalopods are predators with complex multi-lobular brains, complex behaviours, good vision and the capability for rapid movement. Generally, they have a high metabolic rate, grow rapidly and are short-lived. This is an area where it is considered that cephalopods differ significantly from fish. Cephalopods are carnivorous, with a strong preference for a live prey diet and some species and stages may be difficult or impossible to feed using artificial diets. Whilst it may be ethically preferable to use artificial diet, providing it meets the nutritional requirements of the species (especially high protein requirement), and it is acceptable to the animals, studies to date would indicate that growth and possibly welfare of the animals may be reduced by the use of artificial diets. Feeding of non-protected live prey (e.g. crustaceans) at least once a week where animals are on artificial diets is generally considered important to allow cephalopods to express the full range of their natural behaviours.

It is important that cephalopods are fed at an appropriate feeding rate and frequency, and this will depend on a number of factors including species, size, maturity and the type of food offered. Presentation of diet is very important to ensure adequate feeding. Feeding regime, palatability and the presentation of food should ensure that all individual cephalopods kept in the same tank obtain sufficient food. Particular attention should be paid to the feeding of young or wild-caught animals.

#### 1.4.4 Handling

Cephalopods may be severely stressed by handling, which should therefore be kept to the minimum possible. When catching animals, nets should be avoided where possible. Buckets can be used to scoop out animals for transportation. Where nets cannot be avoided, nets with an appropriate frame and mesh size should be used. Knotted net mesh should be avoided as it may cause injury. Nets should be disinfected and rinsed in clean water before use. Animals should be kept in water at all times during netting as emersion in air will cause an elevated stress level. Training animals to enter a transfer container, possibly using food rewards, is likely to significantly reduce the stress of handling. Transport vessels should be covered, for example with a towel, to provide a dark environment. This helps keep the animal calm and reduces the risk of inking. Consideration should be given to whether training might be appropriate to minimise stress for any other procedures.

When animals are anaesthetised the duration should be as short as possible and the animals should be placed in clean aerated water for recovery. Research into efficacy and aversion of general anaesthesia in cephalopods is ongoing and it is important to regularly review the literature and adjust practice accordingly. Cephalopods exchange gases with the seawater by forcing water through their gills, using contraction of the mantle. When mantle movement is not possible, for example under deep general anaesthesia, there is a significant risk of the animal becoming anoxic. Where mantle movement is significantly reduced or stops assisted ventilation may be required. Cessation of breathing is highly undesirable and should lead to a review of the anaesthetic protocol. Particular attention should be paid to handling practices (including of anaesthetised animals) to avoid desiccation, suffocation and other injury. Cephalopods should be handled with wet gloves or wet



hands and on a moist surface to avoid damage. Cephalopods use haemocyanin, a copper-containing protein, to transport oxygen. As a result, their blood is colourless when adequately oxygenated, meaning that blood loss during procedures can be difficult to gauge.

#### 1.4.5 Humane killing

Cephalopods should be killed using approved methods in accordance with Schedule 1 of ASPA – the only specified method being an overdose of an anaesthetic using a route and an anaesthetic agent appropriate for the size and species of animal – unless an alternative method is authorised in the Project Licence. In all cases, appropriate measures should be taken to confirm that death has occurred. Current evidence for the humaneness of specific anaesthetic agents and of other methods of killing is very limited and the most up-to-date information available should be sought. Generally, however, the preferred method is an overdose of an agent producing rapid general anaesthetic effects with minimum aversive responses, by immersion. Cephalopods should be left in the anaesthetic solution for a period appropriate to the type and concentration of the anaesthetic. Death should usually be confirmed by physical destruction of the brain, although this may be difficult for some species. Exsanguination may be suitable, but there is little or no information on its effectiveness in these species. Octopuses, cuttlefish and squid have two branchial hearts that move blood through the capillaries of the gills. A single systemic heart then pumps the oxygenated blood through the rest of the body. Transection of the dorsal aorta would ensure cessation of blood flow to the brain irrespective of any residual cardiac activity following terminal general anaesthesia.

#### 1.4.6 Identification

It is not usually necessary or feasible to individually identify all cephalopods within a facility. Octopuses are generally housed individually whilst on study and therefore marking is usually unnecessary. Passive Integrated Transponder (PIT) tagging has been used in some species. Marking the shell or identifying unique patterning can be used for identification in nautilus. Squid and cuttlefish may be more problematic, but some methods have been described, at least for cuttlefish. Careful consideration is needed before the use of more invasive methods of tagging. Marking should generally be carried out under anaesthesia in order to ease handling and minimise the risk of injury, morbidity and stress.

#### 1.4.7 Cleaning

All enclosures should be kept free of waste products or uneaten feed. If these are allowed to accumulate, water quality and thus animal health will be adversely affected. Where practicable, enclosures should be regularly drained and cleaned to prevent fouling and reduced water exchange. There should be no risk of back-flushing and consequent fouling of enclosure water with potential risk of infection. Waste material should be siphoned off as necessary, generally as soon as possible after feeding. The sides and bottom of enclosures should be cleaned regularly to avoid build-up of detritus which could adversely affect the health of the animals. Care should be taken to minimise stress during cleaning and it is likely that in most cases animals will need to be moved out of the tank while it is done. Consideration should be given to how animals can best be transferred to a holding tank and how long they can be held there if moved.

### 1.5 Source, capture and transport

Unless licence authority permits it, cephalopods used for experimental or other scientific purposes should be bred and reared in captivity. However, it is recognised that there are significant difficulties in breeding many species of cephalopod in captivity and therefore this may not be possible. Where animals must be taken from the wild, care should be taken to prevent injury and stress to cephalopods during capture, loading, transportation and unloading.

Where wild-caught animals are intended to be used in regulated procedures, the Project Licence holder must take all reasonable steps to ensure that they are caught by a competent person who has been adequately trained and assessed in the appropriate techniques. Injuries, illness or death of animals on or shortly after receipt may indicate unsuitable source, capture or transport. If such problems arise, the Project Licence holder should review these arrangements before obtaining more



animals from that source. The importation of cephalopods into the UK requires authority from other regulators.

Animals should be kept in water of the same temperature as that in which they were caught and be submerged at all times (running water is preferable). Holding and transport containers should be covered with opaque material. Periods of hypoxia and any deterioration in water quality due to excretory products should be avoided.

If captive stock is to be transported, it is highly preferable that eggs rather than live animals are moved. If live captive stock must be transported, container type, oxygenation and temperature maintenance are critical. Animals should be transported over as short a time as possible and periods of more than 24 hours are not recommended. Successful transport has been achieved using large plastic bags with added oxygen, placed in polystyrene boxes and transported under temperature-control conditions. Food withholding prior to transport, as is practised with fish, is not required, although it is commonly practised by some to reduce the build-up of nitrogenous waste for short journeys. Magnesium chloride anaesthesia has been used to minimise potential distress during longer distance transport. The benefits of such interventions should be considered on a case-by-case basis for the species and specific journey being undertaken.