



LAS-Learning

# Trainees Instructions

## EU Module 3.1 - Basic and appropriate biology - Species specific: Zebrafish

Development of interactive e-learning modules on specific areas of the Education & Training framework facilitating implementation of DIR 2010/63/EU

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### Authors:

Ana Maria Valentim  
Lynne U. Sneddon

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# 1. Module Description

## 1.1 Overview

This module - Basic and appropriate biology - Species specific: Zebrafish - will introduce you to the fascinating world of zebrafish, providing insights into their care to ensure they remain healthy in experiments, thereby producing reliable data. Fish are vertebrate animals protected under EU scientific legislation, and zebrafish have become a popular experimental model. Understanding their biology is essential for maintaining their health and welfare.

This module will cover seven topics: Biological functioning of zebrafish, Zebrafish development, Zebrafish behaviour, Zebrafish in the laboratory, Good husbandry practice, Common events in the aquaria, and Zebrafish strains.

If you are visiting the site independently, you should consult the EC Training and Education framework [guidance document](#), which provides an overview of training requirements for individuals with different responsibilities under their relevant national legislation. Additional education and training may be necessary to meet national or institutional requirements.

This document offers suggestions for supporting your training. Each module aligns with the learning outcomes specified by the EU Education & Training framework for laboratory animal science. This module was developed by Lynne U. Sneddon and Ana Maria Valentim, two prominent researchers in animal welfare and pain perception in aquatic animals, mainly fish with extensive experience researching, publishing, and training. In addition, an international Reflection Group panel has further refined the content, while Nuno H. Franco managed overall coordination.

*Your collaboration and feedback are greatly appreciated, as the module is currently in the testing phase.*

## 1.2 Topics Covered

- Biological functioning of zebrafish
- Zebrafish development
- Zebrafish behaviour
- Zebrafish in the laboratory
- Good husbandry practice
- Common events in the aquaria
- Zebrafish strains

## 1.3 Learning Objectives

- Understand the physiological systems and biological processes that underpin zebrafish health and development.



- Recognise the stages of zebrafish embryogenesis and growth, and their relevance to developmental biology studies.
- Identify and analyse typical zebrafish behaviors, including social interactions, foraging, and reproductive activities.
- Apply best practices for maintaining zebrafish in laboratory settings, ensuring ethical standards and reliable research outcomes.
- Implement effective husbandry techniques to promote zebrafish welfare and experimental validity.
- Recognise and manage typical occurrences in zebrafish aquaria, such as breeding and health issues, to maintain a stable environment.
- Differentiate between various zebrafish strains and understand their specific applications in research.

To support your ongoing learning, each module concludes with a curated list of recommended readings and cited references. Whenever possible, these references are linked to facilitate further exploration.

## 2. Prerequisites and Requirements

No specific prior knowledge is required. However, a basic understanding of searching bibliographic databases and a background in laboratory animal science and/or non-animal methods can be advantageous.

This module is designed to guide you step by step, eliminating the need for prior study.

### 2.1 Requirements

- Completion of lessons and understanding of learning objectives.
- Participation in knowledge checks and assessments to evaluate understanding.

## 3. Grading and Completion

Grading will be based on the successful completion of knowledge checks and assessments provided at the end of each module. Upon finishing all parts of the module, students will receive a certificate of completion. If proof of completion for a specific section is required, learners may be asked to take a screenshot of the screen, as each part's completion is indicated. This ensures transparency and verifiability of progress.

## 4. Textbooks and Reading Materials

The “**References and Further Reading**” lesson provides most references and readings. They comprise scientific articles, sections of books, websites, and videos. Clicking on any link will open a new window to download or visualise the additional material. Several links to further resources can also be found in the module contents to better guide the reader.



## 5. Course Program

The module is organised into eight chapters, with lessons and learning objectives as follows:

| Chapter                                    | Lesson | Learning objectives   |
|--|--------|---|
| <b>Introduction</b>                        | 1      | Get to know the learning objectives   |
| <b>Biological functioning of zebrafish</b> | 2-12   | Basic anatomy and physiology<br>Cardiovascular system<br>Respiratory system<br>Digestive system<br>Nervous system<br>Reproductive system<br>Sensory systems<br>Skin<br>Osmoregulation<br>The genetics of the zebrafish<br>Assess your knowledge |
| <b>Zebrafish development</b>               | 13-17  | Introduction<br>Embryogenesis<br>Larval stage<br>Juvenile and Adult Stages<br>Assess your knowledge   |
| <b>Zebrafish behaviour</b>                 | 18-23  | The Natural Environment and Geographical Habitat<br>Mating Behaviour<br>Social behaviour<br>Stress and anti-predatory behaviours<br>Sexual dimorphism<br>Assess your knowledge  |
| <b>Zebrafish in the laboratory</b>         | 24-29  | Animal care and management in the European legislation<br>Aquatic systems<br>Filtration setup and maintenance<br>Freshwater Chemistry and Physical Parameters<br>Impact of management and research on zebrafish<br>Assess your knowledge        |
| <b>Good husbandry practice</b>             | 30-43  | Husbandry and handling<br>Physical parameters for zebrafish husbandry<br>Stocking density and individual housing<br>Feeding zebrafish<br>Breeding<br>Rearing zebrafish<br>Environmental enrichment<br>Biosecurity<br>Anaesthesia and analgesia  |



|                                     |       |   |
|-------------------------------------|-------|---|
|                                     |       | Health monitoring<br>Record Keeping<br>Human health and safety<br>Impact of Husbandry on experimental data<br>Assess your knowledge |
| <b>Common events in the aquaria</b> | 44-46 | Transport<br>Common laboratory procedures<br>Assess your knowledge  |
| <b>Zebrafish strains</b>            | 47-50 | Strains<br>Strain differences<br>Genetic modifications<br>Assess your knowledge   |
| <b>Summary and knowledge check</b>  | 51-53 | Module summary<br>List of references and further reading<br>Assess your knowledge   |

Table 1 - Learning objectives per parts and lessons.

## 5.1 Progress Tracking

Once you begin working through a module, your progress is tracked, and you can break off and resume your studies at any point. Once the module is completed, the trainee can access any section to refresh their understanding of a topic.

## 5.2 Module Structure

The module is divided into several parts, and although they were designed to be followed sequentially, they can also be taken iteratively. Please note that a certificate of completion is only issued to learners who complete all module parts. If proof of completion for a specific part of the module is required, the trainee should provide a snapshot of the screen because the module indicates progress after each part is completed.

## 5.3 In-Depth Explanation Lesson by Lesson

| <b>Lesson</b> | <b>Title</b>                 | <b>LO</b> | <b>Explanation</b>   |
|---------------|------------------------------|-----------|--|
| <b>1</b>      | Introduction                 |           | Short introduction with 1 photo  |
| <b>2</b>      | Basic anatomy and physiology | 3.1.1     | Image and short introduction<br>Zebrafish anatomy with 1 image           |
| <b>3</b>      | Cardiovascular system        | 3.1.1     | Image and short introduction<br>Zebrafish anatomy and circulatory system |



|           |                               |       |   |
|-----------|-------------------------------|-------|---|
|           |                               |       | Schematic summary of the regenerative process after cryoinjury in the zebrafish heart with 1 image/diagram  |
| <b>4</b>  | Respiratory system            | 3.1.1 | Image and short introduction<br>Oxygen uptake in zebrafish with 1 image   |
| <b>5</b>  | Digestive system              | 3.1.1 | Image and short introduction<br>Evolution of the digestive system with 1 image  |
| <b>6</b>  | Nervous system                | 3.1.1 | Image and short introduction<br>Peripheral and central nervous system<br>Schematic representation of the embryonic brain and simplified representation of the adult brain and main domains in 1 image |
| <b>7</b>  | Reproductive system           | 3.1.1 | Image and short introduction<br>Reproductive biology: differences between male and female zebrafish with 1 image  |
| <b>8</b>  | Sensory systems               | 3.1.1 | Text descriptions of the sensory systems: Vision, Hearing, The lateral line, Olfactory system, Taste, Touch and pain  |
| <b>9</b>  | Skin                          | 3.1.1 | Image and short introduction<br>Text description of zebrafish epidermis with 1 image  |
| <b>10</b> | Osmoregulation                | 3.1.1 | Image and short introduction<br>Text description on osmotic balance   |
| <b>11</b> | The genetics of the zebrafish | 3.1.1 | Image and short introduction<br>Text description of zebrafish genome  |
| <b>12</b> | Knowledge-check               |       | Assesses progress and knowledge acquired during the chapter   |
| <b>13</b> | Introduction                  | 3.1.1 | Short introduction<br>Early development of the zebrafish embryo with 1 diagram  |
| <b>14</b> | Embryogenesis                 | 3.1.1 | Image and short introduction<br>Description of the production of eggs and their development<br>Video on zebrafish development   |
| <b>15</b> | Larval stage                  | 3.1.1 | Image and short introduction<br>Characterisation of the larval stages: Text for early larvae, 1 image, Text for larval development and Text for larval behaviour                                      |
| <b>16</b> | Juvenile and adult stages     | 3.1.1 | Zebrafish stages (juvenile and adult) with 1 image  |



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|-----------|--|-------|--|
| <b>17</b> | Knowledge-check  |       | Assesses progress and knowledge acquired during the chapter  |
| <b>18</b> | The Natural Environment and Geographical Habitat       | 3.1.1 | Image and short introduction<br>Information on zebrafish habitat and distribution with 2 images  |
| <b>19</b> | Mating Behaviour                                       | 3.1.1 | Image and short introduction<br>Mating courtship with a diagram<br>The behaviour of both males and females during breeding<br>Differences in the female body with 1 image                                      |
| <b>20</b> | Social behaviour                                       | 3.1.1 | Image and short introduction<br>Social behavior of zebrafish with 1 video  |
| <b>21</b> | Stress and anti-predatory behaviours                   | 3.1.1 | Stress<br>Fear and anti-predatory behaviour with 1 diagram with information points   |
| <b>22</b> | Sexual dimorphism                                      | 3.1.1 | Image and short introduction<br>Characteristics of males and females with 1 image with information points  |
| <b>23</b> | Knowledge-check  |       | Assesses progress and knowledge acquired during the chapter  |
| <b>24</b> | Animal care and management in the European legislation | 3.1.2 | Image and short introduction<br>Zebrafish and the law in Europe  |
| <b>25</b> | Aquatic systems  | 3.1.2 | Image and short introduction<br>Modern self-cleaning aquariums<br>Stand-alone units with 1 image<br>Large, centralised units<br>Special aquatic systems with 2 images  |
| <b>26</b> | Filtration setup and maintenance                       | 3.1.2 | Image and short introduction<br>Filtration systems with stacked cards<br>Filter maturity<br>Reverse osmosis process with 1 diagram<br>Ultraviolet sterilisation with 1 image of the recirculating water system |
| <b>27</b> | Freshwater Chemistry and Physical Parameters           | 3.1.2 | Image and short introduction<br>Good husbandry conditions with 2 images  |
| <b>28</b> | Impact of management                                   | 3.1.2 | Image and short introduction   |





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|           | t and research on zebrafish                 |                | Maintenance and management of zebrafish colonies and research practices  |
| <b>29</b> | Knowledge-check                             |                | Assesses progress and knowledge acquired during the chapter  |
| <b>30</b> | Husbandry and handling                      | 3.1.3          | Image and short introduction<br>Recommendations for handling zebrafish   |
| <b>31</b> | Physical parameters for zebrafish husbandry | 3.1.2          | Text description on physical parameters for zebrafish husbandry  |
| <b>32</b> | Stocking density and individual housing     | 3.1.3          | Image and short introduction<br>Text analysis on how many zebrafish are in a tank  |
| <b>33</b> | Feeding zebrafish                           | 3.1.5          | Image and short introduction<br>Correct nutrition<br>Different forms of live feeds with 2 images   |
| <b>34</b> | Breeding                                    | 3.1.3<br>3.1.9 | Short introduction<br>How to breed zebrafish<br>Breeding programs<br>Breeding techniques with 2 images<br>Natural fertilisation - in-tank technique<br>Reasons for poor egg production with checkbox marking |
| <b>35</b> | Rearing zebrafish                           | 3.1.3          | Short introduction<br>How to rear zebrafish in a 6-step process  |
| <b>36</b> | Environmental enrichment                    | 3.1.3<br>3.1.6 | Image and short introduction<br>Environmental enrichment with 4 tabs and 2 dropdown texts<br>Testing enrichment types with 1 table   |
| <b>37</b> | Biosecurity                                 | 3.1.3<br>3.1.9 | Image and short introduction<br>Biosecurity strategy and health measures   |
| <b>38</b> | Anaesthesia and analgesia                   | 3.1.3          | Anaesthetics and analgesics  |
| <b>39</b> | Health monitoring                           | 3.1.3<br>3.1.9 | Image and short introduction<br>Monitoring for disease with description of non-infectious diseases and bacterial diseases  |
| <b>40</b> | Record Keeping                              | 3.1.9          | Image and short introduction<br>Keeping animal records with a list of elements to keep track of  |
| <b>41</b> | Human health and safety                     | 3.1.3<br>3.1.9 | Image and short introduction<br>Safe working with zebrafish with a list of potential zoonotic diseases   |



|           |  |                |   |
|-----------|--|----------------|---|
|           |  |                | Allergic reactions to zebrafish and how to protect yourself with 2 text tabs  |
| <b>42</b> | Impact of Husbandry on experimental data | 3.1.3<br>3.1.4 | Introduction<br>Confounding factors to zebrafish research   |
| <b>43</b> | Knowledge-check                          |                | Assesses progress and knowledge acquired during the chapter   |
| <b>44</b> | Transport                                | 3.1.2<br>3.1.9 | Image and short introduction<br>Required documentation for transport  |
| <b>45</b> | Common laboratory procedures             | 3.1.2<br>3.1.9 | Image and short introduction<br>Common laboratory procedures in zebrafish, their consequences and possible refinements in 1 table |
| <b>46</b> | Knowledge-check                          |                | Assesses progress and knowledge acquired during the chapter   |
| <b>47</b> | Strains                                  | 3.1.7          | Different strains of zebrafish  |
| <b>48</b> | Strain differences                       | 3.1.7          | Image and short introduction<br>Differences between strains description   |
| <b>49</b> | Genetic modifications                    | 3.1.8          | Different genetic modifications with 4 cards  |
| <b>50</b> | Knowledge-check                          |                | Assesses progress and knowledge acquired during the chapter   |
| <b>51</b> | Summary                                  |                | Module summary  |
| <b>52</b> | References and Further Reading           |                | References for additional materials   |
| <b>52</b> | Knowledge-check                          |                | Assesses progress and knowledge acquired during the module  |

Table 2 - Explanation lesson by lesson.

## 6. Target Audience

This module is intended for (bio)medical researchers, participants in laboratory animal science courses, university students, biology/medical teachers, animal welfare body members, regulators, and anyone interested in learning more about animal research ethics and the principles of Replacement, Reduction, and Refinement of animal use for scientific and educational purposes.

## 7. Notes

As this is a test run, we kindly request one feedback form for each tested module to ensure we gather thorough insights for every tested module. The majority of the modules are designed to complement other components of your training, and the content should be accessible even if you have relatively little experience working with laboratory animal science. Where appropriate, the introduction to the module suggests



pre-reading and suggested training that should be completed before continuing with the module.

## 7.1 Testers' Feedback Form

We would greatly appreciate your valuable insights and detailed feedback regarding the instructions provided. Your input will help us ensure clarity, accuracy, and overall effectiveness in conveying the necessary information.

[https://forms.uu.nl/universiteitutrecht/TestReview\\_LASLearning\\_instructions](https://forms.uu.nl/universiteitutrecht/TestReview_LASLearning_instructions)