



LAS-Learning

# Course Organisers Instructions

EU Module 5 - Recognition of pain, suffering and distress - Species specific: Zebrafish

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

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# 1. Module Description

## a. Overview

This module, *Recognition of pain, suffering and distress - Species specific: Zebrafish*, will equip you with the knowledge to identify critical indicators of distress and pain in zebrafish, enabling you to assess their welfare. Studies have shown that various laboratory procedures, such as netting, fin clipping, tagging, surgery, and heart damage, can cause distress and/or pain in zebrafish.

This document provides guidance and suggestions to support the course organiser's efforts. Each module is aligned with the learning outcomes outlined in the EU Education & Training framework for laboratory animal science.

We recommend consulting the EC Training and Education framework [guidance document](#) if you are accessing the site independently. This resource offers an overview of training requirements for individuals with different responsibilities under their relevant national legislation.

Further reading and additional education and training may be necessary to meet national or institutional training requirements. At the end of each module, you will find a list of recommended further readings and references cited throughout the content. Links to these references are provided whenever possible.

This module was developed by Lynne U. Sneddon and Ana Maria Valentim, two prominent researchers in the field of animal welfare and pain perception in aquatic animals, particularly fish. Their work spans research, publications, and training in these fields. The module was further revised by an international Reflection Group panel, with coordination led by Nuno H. Franco.

*The module is currently in the testing phase. We appreciate your collaboration in integrating it into your courses and providing feedback. After completing a module, please fill out the form below with your feedback.*

## b. Learning Objectives

This module will equip participants with essential knowledge and skills to navigate animal research's ethical, legal, and practical aspects. The key learning objectives include:

2. Clinical evaluation.
3. How to recognise pain and distress.
4. Management of pain and distress: anaesthesia, analgesia and others
5. Humane endpoints and euthanasia
6. Severity classification.



## 2. Course Program

The module is organised into different chapters, with lessons and learning objectives for the participants as follows:

Chapter	Lesson	Learning objectives
<b>Introduction</b>	1-4	Principles of clinical evaluation Humane Endpoints Principles of pain management in zebrafish Assess your knowledge
<b>Clinical evaluation</b>	5-10	Get to know the learning objectives Normal zebrafish behaviour Clinical and behavioural signs to monitor Methodologies: Score sheets Methodologies: Analysis of Behaviour Other factors affecting behaviour Assess your knowledge
<b>How to recognise pain and distress</b>	11-15	Introduction - recognition of pain and distress Nociception and pain in zebrafish Distress in zebrafish Assess your knowledge
<b>Management of pain and distress: anaesthesia, analgesia and others</b>	17-21	Introduction - Anaesthesia and analgesia Anaesthetics Analgesics Management of pain and distress, examples Environmental Enrichment as a strategy to manage pain and distress Assess your knowledge
<b>Humane endpoints and euthanasia</b>	22-25	Introduction Humane Endpoints Euthanasia and humane killing Assess your knowledge
<b>Severity classification</b>	26-27	Severity classification of laboratory procedures Assess your knowledge
<b>Summary and knowledge check</b>	28-30	Module summary List of references and further reading Assess your knowledge

Table 1 - Learning objectives per parts and lessons.

### a. Progress Tracking

Once learners begin working through a module, their progress is automatically tracked. This allows them to pause and resume their studies at any point. Upon completing the



module, learners retain access to all sections, enabling them to revisit and review specific topics to reinforce their understanding.

## b. Model Structure and Implementation Guidance

The module is structured into several parts, which were designed to be followed in sequence but can also be taken iteratively based on the learner's needs. Please note that a **certificate of completion** is issued exclusively to learners who finish all parts of the module.

If proof of completion for a specific part is required, trainees can provide a screenshot of their progress, as the module tracks and displays the completion status after each part. This allows for flexibility while maintaining accountability for partial or full completion.

From a pedagogical perspective, each tutor is responsible for deciding which materials to use in face-to-face sessions, which parts learners should complete independently, and whether to mandate their completion. However, it is essential to consider the time required to complete the eModule or its parts to avoid overburdening learners.

We highly recommend completing the module to ensure it aligns with your course's content and scope. Familiarising yourself with the material will also enable you to engage more effectively with students on the various topics covered in the eModule.

## c. In-Depth Explanation Lesson by Lesson

Lesson	Title	LO	Explanation
1	Principles of pain management in zebrafish		Pain management in zebrafish represented in 1 photo, 1 quote and 1 list. "Pain - Modulation and Consequences" represented with 2 lists and 1 quiz. "Treatment of pain - the drug classes" represented with 1 list, 1 collapsible, 1 diagram and 1 table. "Analgesia - Pre-emptive and multimodal analgesic regimes" represented with 1 collapsible. "Non-pharmacological management of pain" represented with 1 interactive figure.
2	Knowledge-check		Assesses progress and knowledge acquired during the chapter.
3	Introduction		Short introduction
4	Normal zebrafish behaviour	5.1	Description of what is normal for a zebrafish represented with 1 video.
5	Clinical and behavioural signs to monitor	5.3	Body condition with 3 photos in a slack of 3 flip-cards and 1 figure. External morphology represented with 1 photo and 6 flip-cards.



			Eyes represented in 4 flip-cards. Gills represented in 2 flip-cards. Fins represented in 2 flip-cards. Behaviour represented in 1 interactive labelled figure.
6	Methodologies: Score sheets	5.3	Score sheets represented with a 3-step process, a 1 list and 1 timeline.
7	Methodologies: Analysis of Behaviour	5.3	Ethogram description. Decide on the sampling approach description with 1 list and 1 figure. Choose a recording technique represented in 1 list.
8	Other factors affecting behaviour	5.3	Factors that can affect behaviour are represented in 1 list.
9	Knowledge-check		Assesses progress and knowledge acquired during the chapter.
10	Introduction - recognition of pain and distress	5.1 5.2	Recognising and alleviating pain with 1 photo and 1 gif.
11	Nociception and pain in zebrafish	5.2	Recognising pain in zebrafish description. Criteria described for animals that feel pain, represented with a 1 list. Pain Assessment description. General Indicators represented in 1 table. Physiological Indicators description with 1 figure. Behavioural Indicators description with 1 interactive labelled figure and 1 figure.
12	Distress in zebrafish	5.2	Good working practice to avoid distress represented with 1 list and 12 flip-cards.
13	Knowledge-check		Assesses progress and knowledge acquired during the chapter.
14	Introduction - Anaesthesia and analgesia	5.2 5.6	Welfare in zebrafish description.
15	Anaesthetics	5.2 5.6	Anaesthesia description with 1 video and 1 figure in a 3 window tab, and a table. types of anaesthetics used in fish, represented with a slack of 5 flip-cards and 1 table.
16	Analgesics	5.2 5.6	Managing pain description with 1 photo, 1 slack of 3 flip-cards and 2 tables. Drug administration represented in 1 interactive labelled figure.
17	Management of pain and distress, examples	5.2 5.6	Examples of management of pain and distress represented in a 6-step process and 5 figures.



<b>18</b>	Environmental Enrichment as a strategy to manage pain and distress	5.2 5.6	Environmental enrichment represented with 1 photo and 1 slack of 3 flip-cards with 2 photos.
<b>19</b>	Knowledge-check		Assesses progress and knowledge acquired during the chapter.
<b>20</b>	Introduction	5.4	Introduction text on humane endpoints and euthanasia.
<b>21</b>	Humane Endpoints	5.4	Ending experiments to reduce or prevent suffering descriptions with 1 photo and a 1 tab with 2 windows. When a humane endpoint is reached, represented in 1 list.
<b>22</b>	Euthanasia and humane killing	5.4	Euthanasia represented with 2 lists. “When is euthanasia needed?” represented with 1 list. Considerations of euthanasia methods represented with 1 list and 1 process with 3 steps and 1 figure. Rapid cooling method represented with 1 interactive labelled figure. Euthanasia of larval stages represented with 1 list and 1 image. Confirmation of death represented with 1 list and 1 interactive labelled figure.
<b>23</b>	Knowledge-check		Assesses progress and knowledge acquired during the chapter.
<b>24</b>	Severity classification of laboratory procedures	5.5	“What a procedure is” represented with 2 quotes, 6 flip-cards and 1 table. Cumulative severity represented with a 1 list.
<b>25</b>	Knowledge-check		Assesses progress and knowledge acquired during the chapter.
<b>26</b>	Summary		Module summary
<b>27</b>	References and Further Reading		References for additional materials
<b>28</b>	Knowledge-check		Assesses progress and knowledge acquired during the module

Table 2 - Explanation lesson by lesson.



### **3.Participants' Profile**

This module is intended for scientists using zebrafish as a model species. Therefore, as zebrafish are used across a wide variety of scientific fields this course will interest (bio)medical researchers, laboratory animal science students, university students, biology/medical teachers, animal carers and technical staff, ethical board members, and anyone interested in learning more about pain and suffering in zebrafish and how to mitigate them.

No specific prior knowledge is necessary. However, a basic understanding of searching bibliographic databases and a background in laboratory animal science can be beneficial.





## 4.eModule

The eModule provides clear definitions, essential knowledge, and interactive components designed to enhance understanding of key animal ethics theories and develop critical thinking skills. Participants will learn to ethically frame and evaluate animal research from a broad perspective and a case-by-case approach.

The content and references are curated from expert sources, including researchers and information specialists, ensuring high-quality and reliable information. The module is presented dynamically, combining text, images, built-in exercises, and videos to engage learners effectively. It can be integrated into courses as homework or used during a lecture day. Many lessons are designed to deliver comprehensive information and understanding without additional in-class interaction.

### a. Limitations

The presented guidelines aim to ensure high standards of welfare and ethical responsibility; practical limitations and challenges must be acknowledged and addressed to implement them effectively. This often requires ongoing research, training, and resource allocation to overcome the barriers and improve the care and welfare of fish in research settings.

Basic knowledge and further reading are provided so the students can apply the gained knowledge to several situations in the animal facility during fish maintenance or experimental procedures. By being aware of these limitations, researchers can better design their studies, interpret their results, and apply appropriate controls to mitigate potential issues, thereby maximising the utility of zebrafish as a model organism. . Therefore, not every tool or method could be explained in detail within this eModule. However, references and further reading suggestions open vast possibilities for interested users to learn more about this topic.

### b. Blended Learning Approach

E-learning modules offer significant advantages, particularly for learners who may find it challenging to attend traditional intensive training sessions spanning several days. Such sessions can disrupt work schedules and limit participants' ability to balance learning with other responsibilities. While this eModule covers all required learning outcomes, we do not advocate entirely replacing face-to-face teaching (or "live" online discussion sessions) with e-learning. Instead, we recommend a blended learning approach (hybrid or mixed-mode learning). This approach combines the flexibility of e-learning with the engagement of interactive, live sessions, ensuring that learners receive the necessary information while accommodating those who require greater flexibility.

The modules are split into short, manageable lessons, allowing participants to integrate learning activities into their daily schedules seamlessly.



## 5. Implementing Blended Learning Strategies

### Flipped Classroom Arrangement

Before face-to-face classes, learners are introduced to the course contents (for example, by completing our e-learning modules). You can recommend that learners take the whole course (and request a certificate of completion) or focus on specific lessons or chapters.

This approach can:

- Familiarise learners with the content in advance, helping them better understand complex concepts.
- Prepare and motivate learners to engage more actively in their learning and during face-to-face classes.
- Harmonise learners' knowledge levels before in-person classes.
- Provide sufficient background knowledge for group work, allowing for more focused and productive discussions.
- Provide a starting point for interactive discussion.

### Consolidate Learning and Prepare for Exams

The courses are designed to align with the learning outcomes of traditional laboratory animal science courses. Learners can use each module to study and prepare for the final exam. Additionally, the built-in quizzes allow learners to test their knowledge and track their progress.

### Address Expertise Gaps in Your Facility

Gathering expertise across all subjects covered in the EU-functions modules can be challenging, especially in smaller establishments. This may hinder the ability to deliver training that meets all outcomes of the Education and Training framework to a high standard. Using these modules as a basis, tutors and learners can access quality reference material that could mitigate such gaps and ensure education and training are up to standard.

### Use Modules as Teaching Resources

Tutors can integrate various components—such as text, videos, images, interactive exercises, and quizzes—into their teaching activities. This not only boosts engagement but also caters to different learning styles. For each module, we provide suggestions for topics that can be incorporated into interactive discussion sessions.



## 6. 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.

The additional materials provide more information on specific topics, tools, and resources. They are ideal for learners who wish to expand their knowledge or gain a more comprehensive understanding of the issues.



## 7. Notes

We are committed to constantly improving our modules to ensure they effectively meet our users' needs. Your valuable insights and suggestions are vital to testing the modules to achieve this goal.

### a. Course Organisers' Feedback Form

We highly value your input as a course organiser. Please take a few moments to complete the form linked below. Your feedback will provide invaluable insights to help us refine and improve our modules, ensuring they meet your needs and those of your learners.

[https://forms.uu.nl/universiteitutrecht/TestReview\\_LASlearning\\_CO](https://forms.uu.nl/universiteitutrecht/TestReview_LASlearning_CO)

### b. Course Participants' Feedback Form

As you deliver the courses to your participants, please share the link below with them. This will allow us to gather their perspectives and insights, helping us enhance the learning experience for future users.

[https://forms.uu.nl/universiteitutrecht/TestReview\\_LASlearning\\_CO](https://forms.uu.nl/universiteitutrecht/TestReview_LASlearning_CO)

### c. Instructions 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)