



**European Cooperation
in the field of Scientific
and Technical Research
- COST -**

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COST 015/13

MEMORANDUM OF UNDERSTANDING

Subject : Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action FA1301: A network for improvement of cephalopod welfare and husbandry in research, aquaculture and fisheries (CephsInAction)

Delegations will find attached the Memorandum of Understanding for COST Action FA1301 as approved by the COST Committee of Senior Officials (CSO) at its 187th meeting on 15-16 May 2013.

MEMORANDUM OF UNDERSTANDING
For the implementation of a European Concerted Research Action designated as

COST Action FA1301
A NETWORK FOR IMPROVEMENT OF CEPHALOPOD WELFARE AND HUSBANDRY
IN RESEARCH, AQUACULTURE AND FISHERIES (CEPHSINACTION)

The Parties to this Memorandum of Understanding, declaring their common intention to participate in the concerted Action referred to above and described in the technical Annex to the Memorandum, have reached the following understanding:

1. The Action will be carried out in accordance with the provisions of document COST 4154/11 “Rules and Procedures for Implementing COST Actions”, or in any new document amending or replacing it, the contents of which the Parties are fully aware of.
2. The main objective of the Action is to provide the scientific basis for advancing knowledge about cephalopod welfare in research, aquaculture and public displays and promote their conservation.
3. The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 40 million in 2013 prices.
4. The Memorandum of Understanding will take effect on being accepted by at least five Parties.
5. The Memorandum of Understanding will remain in force for a period of 4 years, calculated from the date of the first meeting of the Management Committee, unless the duration of the Action is modified according to the provisions of Chapter IV of the document referred to in Point 1 above.

A. ABSTRACT AND KEYWORDS

New scientific knowledge and animal welfare concerns led to including cephalopods in the new European Directive on animal protection (Directive 2010/63/EU) making them the first invertebrates obtaining protection. Knowledge of the welfare and best experimental practices for these species has been scarce, scattered among research groups and lacking consensus. Dedicated recent efforts will shortly produce Code of Practice and general Guidelines for care and management of cephalopods in research and education. However, practical implementation and dedicated species-specific procedures to comply with the Directive are required. CephsInAction will establish an interdisciplinary network of experts who will promote training, dissemination, sharing of tools and knowledge to improve such procedures, and it will foster multi-disciplinary and inter-species scientific exchanges to integrate knowledge on welfare practices. A network of experts and users will collect, refine, distribute and improve knowledge that is currently distributed between research groups and available only to them. This will allow development of new experimental practices, actions for reduction, refinement and reuse principles when applied to cephalopods. This COST Action is also aimed to support emerging scientific demands, and to promote cephalopod research, conservation and public awareness.

Keywords: Cephalopod Biology and Welfare; Animal Care; Public Awareness; Training and Dissemination; Three Rs Principles

B. BACKGROUND**B.1 General background**

From January 2013 scientific projects involving cephalopods carried out in European countries and in collaboration with European scientists are regulated by Directive 2010/63/EU. According to Article 61 of the Directive, Member States have to transpose and adopt into national regulations the necessary procedures to comply with it

(http://ec.europa.eu/environment/chemicals/lab_animals/transposition_en.htm).

With its recent revision, special attention is given to strengthen legislation and improve the welfare of animals needed to be used for experimental practices, as well as to firmly anchor the Directive to the principle of the Three R's in the European Union.

The 2010/63/EU Directive includes, for the first time, an entire class of invertebrate species in the list of animals, i.e. live cephalopods. The inclusion of all living cephalopods (larval and adult

forms) has a number of practical implications for those undertaking research, including: supply of animals, transport, housing and handling, anaesthesia, criteria for recognizing pain, suffering and distress, application of humane end-points, and euthanasia. Such implications have been only marginally explored till nowadays.

While ‘Guidelines’ for the use of animals in experimental procedures are available for a large number of laboratory animals (for example mammals and fishes, see also CCAC guidelines: http://www.ccac.ca/en/_standards/guidelines), at present there is little specific guidance for cephalopods, and the current version of the Directive does not provide any information for these species. However, a coordinated effort of the cephalopod community and laboratory animal science associations is ongoing (<http://www.felasa.eu/announcements/felasa-collaboration-on-cephalopods>). It is expected that the first edition of ‘Guidelines for the care and welfare of Cephalopods in experiments and testing’ will be available by fall 2013. This document will be also presented during an *ad hoc* session of the FELASA-SECAL 2013 international congress (10-13 June 2013, Spain; “Cephalopods and Directive 2010/63: challenges and opportunities”, <http://www.felasa2013.eu/index.php/programme>).

A recent survey of published research involving cephalopods identified and mapped institutions and researchers all over the world (www.cephalopodresearch.org/community) and recognized more than one thousand papers published between 2006 and nowadays. European researchers represent a large quota. From this query it resulted that in the European Union the most studied species are *Sepia officinalis* and *Octopus vulgaris* (23% and 16% of the sample respectively). Overall, a very wide range of cephalopod species has been utilized for publications, including some which do not inhabit EU waters (e.g. genera *Nautilus* and *Idiosepius*).

The characteristics of cephalopod species such as richness in the behavioral repertoire, diversity of the adaptations to the marine realm, and the possibility they experience pain and suffering resulted in the inclusion of all living forms of cephalopods in the Directive 2010/63/EU. It is noteworthy to remember that this is the first time that invertebrates are considered at the same “level” of ‘classic’ laboratory animals, i.e. vertebrates.

In addition, due to the elevated commercial interests and together with fishes, the interests for cephalopods welfare assume importance also for fishing methods and aquaculture.

Knowledge about cephalopods welfare is limited and scattered among laboratories, and there are clear gaps of scientific knowledge regarding several aspects of animal well-being (e.g. recognition of pain, suffering and distress, human end-points, anaesthesia and analgesia) which are key requirements of the new Directive. In addition, there is increasing need to develop techniques for captive breeding of cephalopods in order to stop the usage of wild caught animals, now strictly

regulated by the new Directive. These issues are complex and beyond the scope of individual research efforts.

The aim of this COST Action is to collect, distribute and review existing knowledge on cephalopod welfare, refine and improve available procedures by filling knowledge gaps and by assisting in developing more detailed and widespread species-specific guidelines. Coordination and integration of national research efforts is essential to overcome the challenges provided by these scientific issues to eventually develop a comprehensive knowledge of cephalopod welfare. COST provides the perfect framework to start cooperation and reduce fragmentation of European cephalopod research. A dedicated COST Action will also offer valuable opportunities for specialized training and interaction among researchers with expertise on welfare issues in different species, taxa and fields.

B.2 Current state of knowledge

Cephalopod research, i.e. the study of octopuses, cuttlefishes, squids, and of the chambered *Nautilus* is growing in Europe. It currently spans from bioengineering and commercial aquaculture to molecular biology, physiology, evolutionary-developmental biology, animal cognition, neuroscience, and behaviour.

Cephalopod molluscs are cognate far beyond all other invertebrates. Their anatomy and physiology exhibit many similarities to the Vertebrata in terms of complexity. Possessing large brains, cephalopods have extremely flexible behaviour and highly developed attention and memory capacities resembling those of some vertebrates, including discrimination and generalization, social learning and spatial awareness. Indeed, they have been regarded as ‘honorary vertebrates’ due to some of their brain functions.

The challenges provided by the new Directive increased the attention to cephalopod welfare in the cephalopod community. Several organizations dedicated efforts to create a coherent common ground to indicate adequate, standardized procedures for treatment, care, and management of these animals. As a result, several contributions have been produced and ‘Guidelines’ are under development. Still, these guidelines need practical evaluation and extension.

Despite the long standing tradition to use cephalopods as ‘laboratory animals’, there has been very little research on the complex behavioural, neural and hormonal control of physiological responses to stress in captivity and experimental manipulation in cephalopods. Objective indicators that an animal could be experiencing pain, distress or other suffering are essential for welfare assessment. A number of cephalopod species have been successfully kept in both open seawater and re-

circulating water systems but there is a need to clarify optimal rearing conditions. These species tend to be coastal cephalopods, and include benthic octopods and sepioids, whereas pelagic cephalopod species and squids in particular, are very difficult to maintain in captivity. Typical distress behaviours include directional squirting, aggression, repeated self-cleaning, the sloughing of ‘ghost suckers’, food avoidance, inking, and autophagy. Measures to reduce this stress need to be investigated. Physiological parameters, like skin paling, altered respiration or inking, could be useful to detect incipient suffering before behavioural responses arise and thus allow prevention of further pain. Some of these signs have been reported following a potential stressful stimulus, e.g. bradycardia, but no study has evaluated these parameters in response to stress or pain.

Action activities will further characterize physiological and behavioural signs of potential suffering and develop non-invasive and/or automated techniques for their measurement as well as innovative approaches that integrate all influencing factors for a comprehensive assessment of cephalopod welfare.

Knowledge about the capacity of cephalopods to experience pain and the molecules mediating pain perception is scarce as well. Indeed, the mechanism of action of the most frequently used anaesthetics, ethanol, magnesium chloride and clove oil, and whether they truly cause unconsciousness is still unknown. Magnesium chloride has been recently reported to act as a muscle relaxing agent rather than an anaesthetic, and if confirmed would not be any longer acceptable for use. In addition, recent research revealed that different species react unpredictably to various anaesthetics and that ethanol may in fact cause adverse reactions. This Action will support interrogation of, among other issues, the anaesthetics currently used, and assess them to determine species-specific protocols that minimize ‘stress’ to the animals.

Culturing of cephalopods in captivity is not straightforward. Some progress has been made in recent years on the culturing of *Sepia officinalis*, but breeding of *Octopus vulgaris* and most other species is still a challenge, and supply of wild individuals is constantly needed. Attempts at rearing and culturing some octopus species have been successful, but restricted to few regions of the world. Further insights into cephalopods welfare and development of culturing and husbandry techniques will also be beneficial for aquaculture, considering the elevated commercial interests for these species, especially *Sepia officinalis* and *Octopus vulgaris*.

B.3 Reasons for the Action

The call for this Action comes from a significant gap between the requirements of the new law and the available knowledge. Reasons for the Action include:

- Establish linkages among European researchers working on all aspects of cephalopod biology, including welfare, health, management and aquaculture, to promote a concerted action to advance cephalopod welfare and best practices, integrating knowledge from other species;
- Newly developed general guidelines for cephalopods husbandry and best experimental practices need to be diffused and implemented, as well as improved through feedback from the whole community of European cephalopod researchers;
- Knowledge gaps about cephalopod welfare need to be filled through a concerted action;
- Further species-specific guidelines on additional cephalopods species used in research but not currently included in the general guidelines need to be developed;
- It is fundamental to explore practices inspired to the 3R principles to be applied in cephalopods;
- Specialized training for qualified veterinarians and technicians with specific expertise on cephalopods as well as people for their capture from the wild is required, especially in light of the new Directive;
- Need to promote a humane treatment of cephalopods and a reduction of their capture from the wild in aquaculture as in research.

B.4 Complementarity with other research programmes

CephsInAction aims at using its unique platform joining stakeholders and the scientific community of researchers working on cephalopods and other taxa, to discuss welfare guidelines and practices. This platform will tie in with present and past European supported programs such as COPEWELL and WELLFISH, which focus on fish welfare in European aquaculture species. It is worth stressing that, at the moment, there is no structure in place at a European level providing such a network between cephalopod researchers and other stakeholders.

This need is emphasized by the lack of research activities on cephalopod aquaculture development funded by FP5, FP6 and so far by FP7, restricting cephalopod research to national funding, with poor international cooperation. However, the COST framework will serve as a basis for such cooperation, will inject international know-how into projects funded on a national level, and will

promote joint projects via site specific programs including forthcoming Horizon 2020 calls, and existing European Research Networks ASSEMBLE.

C. OBJECTIVES AND BENEFITS

C.1 Aim

The aim of the Action is to provide the scientific basis for advancing and distributing knowledge about cephalopod welfare in research, aquaculture and public displays and promote their conservation. The main objective of this program is to establish an interdisciplinary network of professionals including researchers, veterinarians, NGOs, authorities and others, joined in the common effort to integrate, increase and disseminate knowledge about cephalopod welfare and experimentation and to promote cephalopod research and conservation. This will include experts from non-COST countries and European researchers working with cephalopods and other marine species who will complement each other in the scientific area of expertise, experience with different species and scientific-technical perspectives.

C.2 Objectives

The overall objectives of the Action are:

- 1) Provide proper training on newly established cephalopod husbandry guidelines and experimental practices
- 2) Improve and implement existing general guidelines and experimental practices
- 3) Identify gaps of knowledge in cephalopod welfare, so that these can be addressed through the coordination of on-going national research projects
- 4) Review existing knowledge on species-specific differences in husbandry and treatment and develop species-specific guidelines
- 3) Further develop and establish new experimental practices for emerging scientific demands in cephalopod research
- 4) Promote cephalopod research and conservation
- 5) Promote information exchange and international cooperation
- 6) Advance the use of the 3 R's (replace, reduce, refine) principle in cephalopod research

The specific objectives are:

- a) review the best practices of anaesthetics currently used with cephalopods and systematically

- investigate dose- and species-specific responses, mode of action, depth and length of anaesthesia and recovery time and cardio-circulatory performance;
- b) test potential analgesics and evaluate their efficacy;
 - c) assess long-term effects of anaesthetics and different environmental conditions on brain development;
 - d) characterize behavioural responses to noxious stimuli and define behavioural indices of pain;
 - e) characterize physiological parameters linked to stress, suffering or pain, including biomarkers of immune response linked to disease;
 - f) review optimal and species-specific rearing conditions, including environmental and nutritional factors;
 - g) develop non-invasive and if possible automated techniques for assessing physiological parameters, for monitoring brain changes and effects of treatments;
 - h) develop a model for assessing cephalopod welfare through integration of all influencing factors and available indices.

The following deliverables will be produced:

- A dedicated website, with an interactive database including procedures, protocols, tools, and media, where information will be shared and opinions discussed
- Literature reviews on cephalopod biology, animal welfare and needed research
- Specific guideline publications for the most used European cephalopods
- Two Technical/Scientific workshops
- Training Schools of a specific accreditation program for young researchers
- Accredited courses for cephalopod researchers, veterinarians and technicians

C.3 How networking within the Action will yield the objectives?

This COST Action brings together a very diverse group, both in technical and geographical terms, which includes most of the European internationally recognized researchers and technical experts in the field of cephalopod research. These researchers complement each another in the scientific area of expertise, experience with different cephalopod species, and scientific/technical perspectives. Networking will allow overcoming the challenges provided by cephalopod welfare issues that cannot be solved by the single research groups working independently from each other. The objectives of the Action will be achieved through monthly video conference meetings, to debate and distribute tasks, and semi-annual meetings to reach consensus and present results to the rest of the community, making accessible the facilities, equipment and know-how of each research centre involved in the Action to the other partners, interaction with aquaria holding cephalopods and collaboration with caretakers, workshops and training courses on specific topics and new techniques developed in the Action, writing authoritative review articles in the international literature. Also an interactive web-based database will be constructed, including procedures, protocols, tools, and media, where information will be shared and opinions discussed, and with a section for the general public. Short-Term Scientific Missions (STSMs) will be organized with a particular emphasis on Early Stage Researchers to build up capacity. Research priorities that are identified will be the subject of joint research proposal at national or EU level with the specific objective to provide doctoral or post-doctoral research opportunities. Final guidelines and a book on “Cephalopod welfare in research and aquaculture” will be written.

C.4 Potential impact of the Action

The COST Action will have specific concrete benefits. It will facilitate the dissemination and uniform use of the best cephalopod practices among researchers in Europe, allowing compliance with Directive 2010/63/EU, through Training Schools, Workshops, guidelines and reviews, and a website. It will increase cephalopod welfare in research laboratories, aquariums and aquaculture facilities, by providing improved husbandry guidelines and updated experimental practices. It will help to facilitate the spread of these species in multidisciplinary research (i.e. regeneration studies), by providing proper training and Short Term Scientific Missions. Also, this Action will favour cephalopod conservation by increasing public awareness of cephalopod biology, behaviour and research, including proper and humane cephalopod fishery procedures, through dedicated educational programs for schools, aquariums, fishermen and the general public. It will foster European and international collaboration among cephalopod workers and biologists involved with other marine species by developing a common platform for sharing protocols, tools and

information, sharing facilities and research exchanges. It will create awareness for cephalopod research and attract further researchers to join work on cephalopods.

This Action will contribute strongly to European society ethics (application of 3R's policy) and animal care. It will benefit scientists, holding facilities, universities and schools, policy institutions, private companies dealing with cephalopods and cephalopods themselves.

This will be the first example of a scientific community taking charge to develop husbandry guidelines for such a large group of animals, and can serve as model for the well-informed treatment of other animals.

C.5 Target groups/end users

- Scientists studying biology, growth, behaviour and physiology of cephalopods under laboratory conditions who will gain advantage from the coordination of research efforts and the identification of gaps in knowledge.
- The life science community as well as researchers from other fields like engineering interested in broad interdisciplinary research (e.g. EU FP7 funded Projects: Octopus, Stiff Flop).
- Early career scientists who want to follow a career in cephalopod biology.
- Veterinarians, aquaculture specialists and aquariologists who will now have sources of information as to how to examine evaluate and improve the welfare of these animals.
- Students and the general public who, via the website and dedicated educational programs, will gain an understanding about the welfare of these animals.
- Fishermen who will be informed of the best practices for capture, transport and handling of cephalopods.
- Policy makers and ethics committee who will be informed of the value of such information in support of decision making.

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

Knowledge will be gained by 1) reviewing existing literature, 2) consulting world renown cephalopod experts, 3) gathering unpublished information and data from ongoing research, 4) coordination of national research projects to address gaps in knowledge and perform focused experiments when a specific need arises, e.g., examination of the effects of a specific anaesthesia. These research activities will be supported by the national or European research funding held by the single participants. The Action activities are structured in the following tasks:

TASK 1- Guidelines and improvement of experimental practices

General guidelines and practices for husbandry and research procedures will be reviewed, implemented and improved according to researcher feedback. A key aspect of this task will be interaction with fish researchers to identify success factors in welfare assessment in these species that could be tested in cephalopods. Another important aspect will be to develop and apply new technologies like transcriptomics and proteomics, for assessment of cephalopod welfare and distress, through short term research exchanges between laboratories. This approach will be fundamental to identify and characterize new biomarkers of stress and suffering as well as molecules mediating pain perception in cephalopods. Of fundamental importance is the coordination of national research projects to address gaps in knowledge, such as properties of anaesthetics currently used and potential analgesics, disease, diagnosis and nature of immune response, characterization of physiological and behavioural parameters which could indicate stress, suffering or pain. All these activities fall within the research interests of the single participants and will be addressed thanks to pre-existing national or European research funding.

This will allow knowledge gaps in the following areas to be addressed:

1. Culture protocols for the most common available species (e.g. *Sepia officinalis* and *Octopus vulgaris*) adults and paralarvae in tanks and sea cages: culture technologies, feeding and nutrition, sampling techniques, etc., trials with different environmental parameters of culture (e.g. light, volume of the tank, aeration, etc.) in order to reduce stress; proteomic study of paralarvae to evaluate and optimize paralarvae nutritional state and survival; investigate the potential use of pre- and probiotics for improving animals' health.
2. Anaesthesia and analgesia: experimentation of different anaesthetics at various developmental

stages (e.g. clove oil, magnesium chloride, hypothermia, ethanol, etc.). Several agents have been described over the years for cephalopods. However, little is known about the physiological reaction of cephalopods to these agents because most studies only determine time of induction, sedation and recovery plus some behavioural description. New tools will be developed and those existing for fishes will be applied in order to ameliorate assessment of the effects of given agents.

3. Welfare assessment

The ability to experience welfare is a part of the animal's biological control systems that have developed in the course of evolution to deal with a variable environment and fulfil basic needs. To assess animal welfare, observable and measurable indicators of degree of fulfilment of their needs, i.e. the "state of need", are essential. Biological organisms are regulated by homeostatic control mechanisms that serve proximate goals, and discrepancies in the current state from these goals cause activation of physiological and behavioural responses with the aim of reducing the discrepancies and restoring homeostasis. Animals with advanced CNS monitor the effectiveness of their response by changes in the emotional states, where improvements of current situation are rewarded (positive emotions: satiation, sexual arousal, comfort, safety) while a worsened situation is punished (negative emotions: discomfort, distress, fear, frustration, etc.). In this way, they are guided to fulfil their needs, and here learning and memory are essential for efficient, plastic, goal-directed behaviour. Biological needs include the need for food, water, rest, social contact, reproduction related needs (such as the need for a mate and maternal behaviour), movement, exploration (including foraging and play), body care, elimination (voiding of faeces and urine), thermoregulation, respiration, health and safety. These needs can, somewhat loosely, be categorized as behavioural and physiological needs. All needs, however, have not only an environment-based aspect (food, water, space) but also a behavioural dimension (e.g. searching for food and consuming food), a (stress- and patho-) physiological dimension (e.g. fat deposition, emaciation, disease) and, most important for welfare, also an emotional dimension (e.g. feeling hungry, afraid,..). To assess the overall welfare, all needs are to be assessed with respect to the dimensions of intensity, duration and incidence of both welfare performance criteria and underlying welfare relevant emotions. A legitimate assumption should then be that the "state of welfare" is negatively correlated to the "state of need", i.e. the larger the discrepancy between current state and animals "goal state" the worse is the animal's overall state of welfare.

Welfare indicators are measurable attributes correlated with welfare. Various observable and measurable behaviours or environmental conditions are often associated with the different states of needs. Activation of different motivational systems leading to for example food anticipatory

behaviour indicates high hunger level, while fright behaviour indicates activation of the “fear system” and the animal’s subjective interpretation of the situation as dangerous. These behaviours, or biochemical correlates, can therefore be used as animal-based indicators of the state of need. In other cases, it can be easier to measure the availability of the resources needed to fulfil their needs (e.g. oxygen instead of breathing behaviour). Various long or short lasting environmental conditions may hinder or reduce the animal’s ability to fulfil its needs. These “welfare risk factors” and the observation of their risk of occurrence and severity is a part of a risk or hazard analysis, but is also linked to an overall welfare assessment (OWA).

Although indicators based on environmental conditions, management level and housing systems are important for assessment of welfare and form a good basis for finding possible improvements they are inherently only indirect measures of welfare. Animal-based (direct) attributes such as health, behaviour and physiology are more effect/performance related and directly related to welfare. Animal-based measures are especially valuable to verify formulated ‘hypotheses’ or general impressions based on indirect measures and, to specify individual differences among (groups of) individuals living under generally the same conditions but responding differently due to ‘biological variation’.

Relevant animal-based “non invasive” welfare indicators are locomotion and feeding behaviour, colour changes, appetite, growth and body conditions (condition factor), skin and fin condition, parasite infestation, and mortality/clinical history. In addition samples of blood, brain and organs can give valuable information about physiological functioning and neuro-endocrine states. However, frequent sampling for brain neurochemistry and hormonal measures is usually not feasible, and needs special expertise and expensive analysis (and disturbance and sacrificing of animals). Non-invasive animal-based indicators that are easily applied are therefore needed, especially indicators that are functionally linked to the animals cumulative welfare.

Health: disease diagnosis and identification of pathogens in cephalopods. This will provide an unique atlas of the available knowledge. Particular attention will be provided to evaluate conditions of animals health by non invasive methods and establishing 'evaluation of signs' including behavioural ones.

Deliverables:

- At least 1 literature review on fundamental knowledge relevant to guidelines for welfare and husbandry for the most commonly used cephalopods
- Draft of behavioural indices that allow scoring severity of distress, with detailed descriptions of behaviours displayed together with supplemental photographic and video libraries where possible.

Task 2 - Species-specific research procedures

European cephalopod researchers are investigating over 10 different species out of the existing 700, but husbandry guidelines and experimental practices are in the process of being published only for the two most studied species, *Octopus vulgaris* and *Sepia officinalis*.

By producing an easy to follow classification of the needs and requirements of other cephalopod species based on their ecology and habitat we will create a base for further research on these species. This can be done by not only collecting data from cephalopod researchers around the world but also by reviewing related literature on different taxa (e.g. fishes). Additional data can be collected by distributing questionnaires to fishermen and fisheries monitoring bodies. Interaction will be facilitated with researchers working on these species, like *Loligo vulgaris* or *Nautilus*, in Europe and abroad as well as Aquaria which may be holding them, to understand their specific needs, measures and procedures to be adopted due to biological differences. This process of exchange can boost innovation in the comparative analysis of related and unrelated species.

Deliverables for Working Group 2:

- At least 1 literature review on fundamental knowledge relevant to guidelines for welfare and husbandry for the non-common used cephalopods.
- A database with a classification of cephalopod species based on basic, physiological and behavioural needs.

Task 3 - New technologies and experimental approaches to promote the 3Rs principle

The Action will review the available technology for non-invasive experimental practices and will

test possible applications and procedures for the reduction of animals use and provide opportunities for reuse. To achieve this goal the Action will involve specialist companies who will provide high frequency ultrasound technologies, a non-invasive real time in vivo imaging technique already applied in other species (e.g. zebrafish). Ultrasound is clinically approved and completely painless and would hence not cause discomfort for the animal. In addition, imaging reduces the number of animals used and allows monitoring the same animal over time opening new research possibilities. Exploration and implementation of such a technique for various applications related to welfare, such as wound healing and arm regeneration, assessment of respiratory and haemodynamic parameters – i.e. heart rate and circulatory performance of anaesthetics, disease diagnosis i.e. presence of parasites, cuttlebone and brain morphology and changes in response to different treatments.

Further non-invasive techniques which will be tested are: endoscopic techniques to characterize the sex in immature octopus, and micro computer tomography which could be applied to the study of small sized species and larvae.

In addition, statistical and image based methods will be established for analyzing cephalopod behaviour and cognition; Both by applying existing methods to new species, such as *Octopus vulgaris*, and by developing new methods such as Independent Components Analysis. These approaches provide a way to refine experimental methods and greatly reduce or eliminate negative effects of experiments.

A Cephalopod Welfare Index Model, an overall assessment of cephalopod welfare by semantic modelling, a systematic standardized method to assess animal welfare based on observations of the animals, their biological and physical environment and available scientific knowledge will be developed. This approach considers welfare as the quality of life perceived by the animal and thus accounts for both negative and positive aspects of welfare. It has previously been applied to sows, poultry and to the Atlantic salmon. To implement this method for cephalopods, an internet based database of welfare relevant scientific statements related to cephalopods will be created, including a list of cephalopods basic needs, and a list of measurable or observable welfare indicators that can be linked to at least one need and can be scored into levels, each linked to at least one scientific statement. By using semantic modelling, a science-based model for assessing cephalopod welfare in aquaculture and research will be created and will also identify deficiencies in available knowledge. Finally, as last action for reduction, inspired by NORINA (A Norwegian Inventory of Alternatives, available at: http://oslovet.norecopa.no/fag.aspx?fag=57&mnu=databases_1), CephInAction will

create a database of audiovisual training and teaching alternatives to the use of animals which will be distributed to schools, universities and relevant institutions.

Deliverables:

- At least one report relevant to the new methods.
- A training school on the non-invasive techniques developed.
- Deliverables from the Cephalopod Welfare Index Model design:
Operational, internet based decision support system (semantic model) for monitoring and assessment of cephalopod welfare;
Database of scientific statements and references and their link to cephalopod welfare needs and indicators/attributes, including scores and weighting factors;
Review paper on cephalopod needs and indicators and description of the CWIM model.

Task 4 - Dissemination and Education

The Action will make sure the knowledge gained and information gathered is transferred and used throughout Europe and possibly beyond. To achieve this an interactive database web-based where knowledge can be shared will be created. Part of the website will be accessible only to the participants, to communicate among them and share opinions, and the other part will be available for the general public, to disseminate knowledge about cephalopods and research conducted on them. In addition, as mentioned above, a database of audiovisual alternatives to the animal use in teaching and training will be created, including dissection alternatives, which will be available for schools, universities and research institutes. Via collaboration with aquariums and public displays the Action will foster cephalopod husbandry awareness and promote their conservation.

Deliverables:

Two Training Schools on i) best practice in husbandry, maintenance and care of cephalopods, and ii) on best practices in experimental procedures with and without anaesthesia.

D.2 Scientific work plan methods and means

The tasks of the Action will be achieved through the coordinated work of five Working Groups:

Working Group 1 – Research Needs in Cephalopods Welfare:

This Work Group will integrate the following tasks:

- Task 1 - Guidelines and experimental practices improvement
- Task 2 – Species-specific research procedures
- Task 3 – New technologies and experimental approaches to promote the 3R' principle

The main objectives of this Working Group are:

To determine best laboratory practice and implement the 3R principle, it is essential to fill the gaps of knowledge on cephalopod welfare. Hence this group will address the following research needs:

1. Environmental needs of different cephalopod species: tank size and space requirements, water quality, light conditions; trial with different parameters to reduce stress and increase welfare
2. Nutritional requirements of different cephalopods species at various developmental stages: proteomic study of cephalopods paralarvae, trial of pre and probiotics to improve health

Working Group 2 - Stress & Disease

The main objectives of this Working Group are:

1. Stress, pain and suffering & Immune Response:

This Working Group will evaluate signs and indicators of stress, pain and suffering. Stress has effects on behaviour (locomotion, play, fear of novelty), endocrine system and brain development (network plasticity and neurogenesis). These robust indicators of stress consequences can be measured in cephalopods. It will determine methods for the assessment of health and well-being of cephalopods kept under laboratory conditions such as behavioural, hormonal or immunological methods of assessment. Literature and possible targeted experiments will also be considered to assess potential stress response genes. Evaluation of techniques for assessing immuno-competence in other taxa for use with cephalopods.

Identification of behavioural patterns associated with positive and negative emotions, and scoring of these pattern, to allow recognition of stress severity of the animal and mitigation measures suggested Distinction of 'states' originating from natural processes (e.g. senescence) from those induced by poor welfare and health conditions during handling and husbandry.

2. Disease:

Disease (e.g. bacterial infection and fungal) can cause senescence where left untreated and under poor water quality and high temperature conditions. Actions will be taken to classify, diagnose and prevent diseases in cephalopods. Lesions and ulcers are common on octopuses which appear to be

distressed or ‘anxious’ and can rapidly spread unless treated. Treatments, like cold water, antibiotic and or antifungal treatments by immersion, will be assessed by reviewing literature and unpublished data to help establish best-practice guidelines.

3. Non-invasive methods:

Cephalopods commonly harbour heavy parasite loads in their kidneys and digestive track. Parasite burdens can be assessed following anaesthesia and mantle inversion. This technique is highly invasive. New non-invasive techniques (e.g. sonographic examination) will be explored to assess health conditions and parasite load in cephalopods.

Working Group3: Neurophysiology, Anaesthesia and Humane-end-points

This Working Group will integrate the following tasks:

- Task 1 – “Guidelines and experimental practices improvement”
- Task 2 – “Species-specific research procedures”
- Task 3 – “New technologies and experimental approaches to promote the 3R’ principle”

The main objectives of this WG are:

1. Review and evaluate protocols used up to now in neurophysiology and anaesthesia, analgesia and euthanasia in cephalopods, the efficiency of those in different species and possible similarities with vertebrates in effect, verify their applicability and refine them to minimise ‘stress’ to the animals, The focus will be on the most used cephalopods for research purposes and those which are cultivated in Europe (common octopus, European cuttlefish, squid), but knowledge from other species will also be used.
2. Promote research on the effects of anaesthetics currently used, their mode of action and the physiological and behavioural consequences of these agents and how they are tolerated by different cephalopods and developmental stages. Explore alternatives.
3. Evaluate humane killing methods (e.g. overdosing on anaesthetics or freezing), humane-end-points and criteria for death confirmation
4. Explore non-invasive techniques to be applied in neurophysiology and anaesthesia

Working Group 4 Development of a Cephalopod Welfare Index (CWI)

The main objectives of this WG are:

1. Characterize basic and behavioural needs of cephalopods to define the best physiological markers of pain, suffering and disease.
2. Develop an overall assessment of welfare (OWA) - a systematic attempt to assess the welfare

status of animals based on observations of the animals, their biological and physical environments, and available scientific knowledge. This will be achieved by semantic modelling (SM), in which welfare is defined as the quality of life as perceived by the animals themselves; Both positive and negative aspects of welfare are considered and is therefore a risk benefit analysis. This will lead to the development of the CWI.

Working Group 5: Education and training

Although each WG will be responsible of dissemination of its products (according to the Tasks), it is important to devote a WG for education and training. This WG will focus on producing educational material and training settings to enable dissemination of the knowledge gained among end users and especially veterinarians, young scientists and technicians. This WG will include education personnel and award winning film makers to produce best possible materials.

This WG will be responsible for the organization or development of:

- 3 Training Schools
- 2 Workshops
- Educational material on cephalopods husbandry and physiology that could be integrated into veterinary schools.
- Manuals based on delivered guidelines which could be readily used by holding facilities managers and technicians, including drawing and worksheets.
- Interactive computer videos and software to allow for improved teaching of cephalopods, and for experimental planning, without sacrificing animals.

E. ORGANISATION

E.1 Coordination and organisation

The overall management of the Action will be carried out by the Management Committee (MC) led by the MC Chair and Vice-Chair. The MC Chair will act as a contact person between the Action, COST Office and Domain Committee. The MC Vice-Chair substitutes the MC Chair when unavailable. These will be designated at the first meeting of the Action together with the representatives of each country to the MC.

A leader for each Working Group will be nominated to coordinate the development of WG specific tasks. The WG leaders, MC Chair and Vice-Chair will form the Core Group (CG) of the MC.

The CG will prepare a concerted and detailed work program to be approved by the MC taking into account the research needs and objectives of Sections C and D. The program will also consider

expertise, facilities, and interests of the participating institutions.

The research facilities available within the consortium and on-going research projects on cephalopod biology (*sensu lato*) will be evaluated and taken into account when elaborating and implementing the detailed work program.

An underlying endeavour will be the coordination of future research project applications, to both national and European funding institutions, in the ambit of the objectives and goals of this Action. The MC will meet annually and the CG as often as required for the performance of the work program.

One manager of both Short Time Scientific Missions (STSM) and Training Schools (TS) will be appointed during in the first meeting. A STSM and TS program will be prepared to allow the early start of these financial programming. These are important parts of the Action. STSMs will strengthen collaborations among partners and will allow the development of task-related research projects. In particular, they will promote the involvement of early-stage and promising scientists and mobility for advanced training. TSs will make new equipment and methods familiar to the Action members, and will allow the standardization of experimental and analytical approaches so that large-scale tasks can be effectively done.

In order to successfully disseminate the outcomes of the Action, a Dissemination Coordinator will be selected among MC members. The Dissemination Coordinator will guide the dissemination activities and will be responsible for the development and maintenance of the Action website. This website will disseminate the Action objectives and achievements to interested scientists, and the general public. It will contribute to ensuring public knowledge on cephalopods, their conservation and welfare; it will also contribute to increase the ‘diffusion’ of cephalopods among students and researchers to explore new ideas and achieve significant increased scientific interests in the diversity of these animals, by educating the public on the functions and role of cephalopods and the impacts of human activity and benefits (as food, for example). An important achievement will be the establishment of an online geographical data base of European cephalopod diversity to which scientists and general public may contribute.

Within the first year of the Action, a Workshop will be held to analyze and synthesize relevant information that will enable evaluation of the types of changes that cephalopods are experiencing due to increased fishing pressure and the impact of Directive 2010/63/EU in their use as experimental model. Existing qualitative and quantitative data on cephalopod biology and on the environmental drivers on these animals will be compiled from published studies, reports, websites, online databases, and unpublished sources. This assignment will take advantage of other data base available to the scientific community and the general public.

In the final year of the Action a high-profile, widely advertised Conference will be organized under the general theme of this Action: “*Improvement of cephalopod welfare, husbandry and the promotion of cephalopods research*”. The scientific achievements of the Action will be communicated and future prospects will be considered. An Editorial Board will be created to publish the proceedings of this Conference.

The following list of Milestones are suggested to be considered for this Action:

Milestones	Approx date
1 – First meeting of the Action	Month 1
2 – MC approval of the detailed work program of the Action: coordination of ongoing national research including collaborative research exchanges, short term scientific meetings, application for research funding, etc.	Month 4
3 – Launch of the Website	Month 4
4 – Training School to familiarize European Scientists (including early career) and other potential users with best practice in husbandry, maintenance and care of cephalopods	Month 8
5 – Workshop to analyze and synthesize the available knowledge on cephalopod welfare	Month 12
6 - Training School to familiarize European Scientists (including early career) and other potential users with best practices in experimental procedures requiring anesthesia	Month 18
7 – Establishment of on line database of Alternatives for Cephalopod Use in Research and Training	Month 24
8 – Training School for the implementation of the Cephalopod Welfare Index	Month 28
9 – Workshop to assess cephalopod welfare, husbandry, culturing and experimental and training guidelines for its improvement	Month 36
10 – Final Conference on “ <i>Improvement of cephalopod welfare, husbandry and the promotion of cephalopods research</i> ”	Month 48

E.2 Working Groups

WG1. Research needs in cephalopods welfare

WG1 deals with the requirements (basic, physiological, behavioural, etc.) of cephalopods species (and the research behind) to fit in compliance with the aims and principles stated in Directive 2010/63/EU. Many members of the MC will be part of this WG. The implementation of TS will be particularly important in WG1, to let users at different levels to practice and experience with the procedures compliant with Guidelines and Directive (Task 1), and to test and compare devices, methods and protocols that will help the application of the 3R principle to the cephalopods (Task 3). STSM are also central in WG1, to strengthen the collaborations among partners and to allow the development of task-related research projects, in particular to evaluate the possible extension and relative requirements of other cephalopod species to the Guidelines available, thus to facilitate the spread (Task 2) of the portfolio of species to be utilized in research and training and the possible actions for the establishment of alternatives (Task 3). The biological diversity of cephalopods (including the level of behavioural repertoire), reflected in different physiological adaptations to varying conditions is not consistent with a “universal” welfare guidelines concept. The establishment of acceptable welfare guidelines specific for cephalopod species is a prerequisite for the implementation of the 3R principle in cephalopod research. The publication of the achievements of STSM and TS in peer-reviewed publications will be an important component of WG1 dissemination.

WG2. Stress and diseases and WG4. Development of a Cephalopod Welfare Index

WG2 and WG4 have similar organization and will share information in close interaction with WG1. Interactions between them will be very strong. Integrated information and experimental work between Tasks 1, 2 and 3 will be coordinated by the three WG (WG1,2,4) leaders when appropriate, to assess the interaction between basic and physiological needs of species considered in the Action, evaluation of stress and disease with the aim to improve welfare of cephalopod species and the implementation of a Cephalopod Welfare Index based on a semantic model. Particular attention will be given to facilitate interaction with experts and researchers involved in experiments and training based on non-cephalopod species. STSM, TS and specific meetings will be used as instruments to attain WG specific objectives.

WG3. Neurophysiology, Anaesthesia and Humane end-points

WG3 deals with the requirements of experimental procedures considered to potentially induce distress and painful experience to animals to fit with principles stated in Directive 2010/63/EU. This WG will incorporate several members of the MC in close cooperation and interaction with experts from other disciplines and working on a variety of different taxa. The implementation of TS will be

particularly important in WG3, to let users at different levels to practice and experience with the procedures compliant with Guidelines and Directive (Task 1), and to test methods and protocols that will help the application of the 3R principle to the cephalopods (Task 3). STSM are also central in WG3, to strengthen the collaborations among partners and to allow the development of task-related research projects. The publication of the achievements of STSM and TS in peer-reviewed publications will be an important component of WG3 dissemination.

WG5. Education and training

A key role of this Action is to train people at different levels of expertise in the use of cephalopods for research, training, education and display. Web platforms and databases will be one of the products of this WG. In addition editorial processes will be applied to improve the quality of the education delivered in TS and in events for the general public.

This WG will integrate the achievements of all tasks developed during this action.

E.3 Liaison and interaction with other research programmes

Liaisons and information exchanges have been recently established among cephalopod researchers with the establishment of international activities (see above for ‘Guidelines’, and Cephalopod Sequencing Consortium at: <http://cephseq.org/index.html>).

The activities of this Action will fit into work undertaken under other national and European research programmes, such as:

- a study on sustainable seafood and adaptation to climate change funded by EU-INTERREG and the UK Natural Environment Research Council;
 - a project on cephalopod immune system and disease, combining transcriptomics/metagenomics, and comparison to vertebrate immune and stress response-systems, which is currently being drafted for the next round of funding in the UK and links directly to an European Fisheries Fund project
- In addition, CephInAction will actively promote the sharing of information, and where possible promote joint activities, such as meetings, seminars and workshops, with other national and European research initiatives, such as:

COPEWELL – A new integrative framework for the study of fish welfare based on the concepts of allostasis, appraisal and coping styles (FP7-KBBE-2010-4);

ASSEMBLE - an EU FP7 research infrastructure initiative comprising a network of marine research stations;

SEPIABREED – Improving the reproduction of the European cuttlefish, *Sepia officinalis* in

captivity: a multidisciplinary approach (Portuguese funded national project –

<http://www.ccmар.ualg.pt/fbh/documents/sepiabreed.html>);

SEPIATECH – Development of cuttlefish, *Sepia officinalis*, aquaculture production technology – feed and husbandry refinements (Portuguese funded national project –

<http://www.ccmар.ualg.pt/fbh/documents/sepiatech.html>);

SEPIAWELL – Determining stress-responsiveness of anaesthesia and euthanasia agents through refined animal welfare methodologies in European cuttlefish, *Sepia officinalis* (Portuguese funded national project). Project devoted to the establishment of a common methodology to correctly assess anaesthesia and euthanasia agents in marine animals, using cuttlefish as animal model and non-invasive methods;

EMBRC – European Marine Biological Resource Centre (part of the ESFRI process, FP7 Capacities project).

E.4 Gender balance and involvement of early-stage researchers

This COST Action will respect an appropriate gender balance in all its activities and the Management Committee will place this as a standard item on all its MC agendas. The Action will also be committed to considerably involve early-stage researchers. This item will also be placed as a standard item on all MC agendas.

The gender balance of this Action is about 30% women and 70% men, which reflects the actual gender balance of scientists across the EU. Following a gender Action plan this Action will implement: 1) short-term, exchange fellowships for young-women scientists to visit other laboratories and 2) develop a mentoring education program for both men and women.

General meetings, involving all of the workgroups, will include an educational module for junior and senior researchers informing them about the realities of gender issues in a European context. It has also been learned that men and women PhDs and Post-docs very much benefit from break out sessions “Talk with the Profs” that allow them to discuss career options, family issues, “the 2-body problem” and discriminatory issues for women that differ by country on an individual or small group basis.

Early-Stage Researchers will play an important role in meeting the Action’s scientific goals. Their involvement will be important in all WGs using the STSM financing instruments. There will also be significant opportunity for training of Early Stage Researchers within the program, particularly under the application of new tools (Task 3).

F. TIMETABLE

The Action will last for four years.

At the kick-off meeting, the Management Committee (MC) Chairperson and Vice-Chair persons, the WG leaders, the STSM and TS Manager, and the Dissemination Coordinator will be selected and nominated. This Core Group will prepare a detailed program within 3 months after the kick-off meeting to be presented at the first MC/WG meetings. The MC will meet annually.

The WG meetings for all five WGs will be held together in order to maximize the interactions between them.

In the first year of the Action a Workshop will be organized to analyze and synthesize the available information on cephalopod welfare (see above, Section E1). Years 2 and 3 will involve actual advancements. Towards the end of the 4 year period, a widely advertised Conference will be organized under the general theme of the Action: Improvement of cephalopod welfare, husbandry and the promotion of cephalopod research. The time scale of the Action Tasks is presented in Table 1.

Table 1. Timetable for the Action (including milestones). See Task definition in Section D1 and milestones in Section E1.

Tasks	Year 1	Year 2	Year 3	Year 4
1. Guidelines and experimental practices improvement	xxxxxxxxxxxxx	xxxxxxxxxxxxx		
2. Species-specific research procedures		xxxxxxxxxxxxx	xxxxxxxxxxxxx	
3. New technologies and experimental approaches to promote the 3Rs principle		xxxxxxxxxxxxx	xxxxxxxxxxxxx	xxxxx
4. Dissemination and Education	xxxxxxxxxxxxx	xxxxxxxxxxxxx	xxxxxxxxxxxxx	xxxxxxxxxxxxx

Milestones				
1 – First meeting of the Action	x			

2 – MC approval of the work program	xxxx			
3 – Launch of the Website	xxxx			
4 – TS to familiarize on best practice	xxxxxxxx			
5 – Workshop cephalopods welfare	xxxxxxxxxxxx			
6 - TS on experimental procedures	xxxxxxxxxxxx	xxxxxx		
7 – On line database of Alternatives	xxxxxxxxxxxx	xxxxxxxxxxxx		
8 – TS implementation of Cephalopod Welfare Index	xxxxxxxxxxxx	xxxxxxxxxxxx	xxxx	
9 – Workshop experimental training guidelines for its improvement	xxxxxxxxxxxx	xxxxxxxxxxxx	xxxxxxxxxxxx	
10 – Final Conference	xxxxxxxxxxxx	xxxxxxxxxxxx	xxxxxxxxxxxx	xxxxxxxxxxxx

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: DE, EL, ES, FR, IE, IL, IT, NL, NO, UK. On the basis of national estimates, the economic dimension of the activities to be carried out under the Action has been estimated at 40 Million € for the total duration of the Action. This estimate is valid under the assumption that all the countries mentioned above but no other countries will participate in the Action. Any departure from this will change the total cost accordingly.

H. DISSEMINATION PLAN

H.1 Who?

Target audiences for dissemination of the Action results are:

- European and international researchers working or interested in working with cephalopods, including students and early stage researchers
- Universities interested in providing education about cephalopod biology and research;
- Aquaculture specialists and fishermen,
- Aquaria holding cephalopods and interested in conservation projects
- Veterinarians and caretakers
- General public
- Government policy makers

H.2 What?

1. A CephInAction website will be designed and will be continuously populated. It will contain an ‘open’ section with information for the general public, educational material for schools and universities, including media. In this section there will be also a database with audiovisual aids that could be used as alternatives to animal use in training and teaching, including for example dissection alternatives (as inspired to NORINA, A Norwegian Inventory of Alternatives). This will strongly contribute to promote cephalopod conservation and reduction of animal use. The database will have also a section accessible only to the participants of this Action providing tools, protocols and a forum for discussion. The database will be continued and maintained at least for five years after the conclusion of this Action.

2. Educational programmes for specific University Degrees will be prepared, promoted and offered to any academic institution interested.

3. Accredited courses for veterinarians and technicians will be organized.

4. Training for fishermen on appropriate capture methods, transportation measures and humane killing methods.

5. Training Schools and Workshops for early stage researchers on cephalopod research biology and behaviour.

6. A revision of general ‘Guidelines’ on cephalopod husbandry and experimental practices, including species-specific procedures and recommendations for all people involved with cephalopods will be produced.

7. Reviews, Reports and publications addressed to the scientific community.

8. “Cephalopods Day” at Aquaria or in close connection with public display areas, for the general

public.

9. Conferences dedicated to distributing the knowledge gained through the Action course to European cephalopod researchers and interested scientists, which will bring some of the most international re-known experts on cephalopod biology and animal welfare.

10. A book with the tentative title “Cephalopod welfare in research, training, education and aquaculture”, will be published at the end of the Action. This book will acknowledge the essential role of the COST Action in providing the impetus for its publication.

H.3 How?

1. Participation at international or European conferences on animal welfare or alternative methods for reduction, refinement and reuse of animals, will be organized to distribute the knowledge and experience gained through the Action and to promote the results of the Action. The target audience of these conferences will be the industry, Governmental agencies, Universities and other research institutions.

2. Invitation of the press and other media to our meetings, workshops and conferences.

3. An e-mail forum with a newsletter to which cephalopod and other researchers can subscribe.

4. Short-Term Scientific Missions addressed to early stage researchers to establish collaborations that will go beyond the duration of the Action.