FELASA Working Group Report

Capture and Transport of live cephalopods: recommendations for scientific purposes

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Abstract

On January 1st 2013 research using cephalopod molluscs, from hatchlings to adults, became regulated within the Directive 2010/63/EU. There are significant difficulties in captive breeding in the great majority of currently utilized species; thus, scientific research relies upon the use of wild-caught animals. Furthermore, live cephalopods are shared and transported between different stakeholders and laboratories across Europe and Continents. Despite the existing European and National legislations, codes, guidelines, and reports from independent organizations, a set of recommendations specifically addressing the requirements for capture and transport of animals belonging to this taxon are missing. In addition, although training and development of competence for all persons involved in the supply chain are essential and aim at ensuring that animals do not suffer from pain, distress or lasting harm, the requirements for those capturing and transporting wild cephalopods have not been considered.

This WG reviewed the current literature to recognize the best practice and scientific evidence, and compiled a set of recommendations to provide guidance on the 'techniques' to be used for the capture and transport of live cephalopods for their use in scientific procedures. In addition, future efforts are proposed in order to ***i.*** develop standardized approaches able to assess recommended methods and objectively quantify the impact of these processes on animals'health and stress response; ***ii.*** design a training program for people attaining the necessary competence for capture and transportation of live cephalopods, as required by Directive 2010/63/EU.

Background

Cephalopods (nautilus, cuttlefish, squid and octopus) are the sole invertebrates listed among the species regulated by the Directive 2010/63/EU for the use in scientific research. The taxon counts about 800 living species, all marine, and constitutes a class belonging to the phylum Mollusca.

The research dimension that makes cephalopod molluscs at the centre of a renown scientific interest expanded over the last decade, and nowadays also a larger number of species is possibly utilized. Furthermore, advances on the study of genomic, physiological, neural and cognitive characteristics,[1-4] have further boosted the interest for these invertebrates all over Europe. At same time, the relevance about their welfare status[5] and its consequences on scientific outcome increased, both in the commercial and scientific fields.

Article 9 of the Directive 2010/63/EU specifies that animals must not be taken from the wild for use in procedures (Article 9.1), unless the relevant National Competent Authority (NCA) grants an exemption (Article 9.2) based on the scientific justification that the purpose cannot be achieved using bred animals.[6] For the great majority of laboratory animals - including vertebrate aquatic model species - production technology has reached a maturity that allows their breeding for use in procedures. On the contrary, cephalopod culture is still at its infancy, facing several bottlenecks at the point that only few cephalopod species are currently cultured in captivity at limited local scale.[7, 8] Culture protocols of cephalopods for scientific purpose are not fully developed yet, and evidence for successful rearing of multiple generations in captivity without altering their welfare and behaviour is still lacking, possibly with a few exceptions. Similarly, doubts and criticisms arises around the

development of a possible industrial aquaculture for these animals[9,a] considering their sentience, sophisticated neural organization and cognitive capabilities.[1, 2, 5, 10]

Despite the increased interest for these organisms in several fields of biology and neuroscience, most of the research performed on these molluscs still relies upon the collection and transport of wild-caught animals.

The Directive also requires that the capture of live wild animals should be accomplished by competent persons using methods which do not cause avoidable pain, suffering, distress or lasting harm (PSDLH; Article 9.3). In addition, adequate care has to be assured to prevent physical injury and stress to animals at all stages in the supply chain, including capture, transportation and acclimatization to laboratory conditions (and quarantine, when required). Thus, the capture and transportation of cephalopods should be well planned, meticulously prepared, and effectively performed.

Here we present the outcomes of the work of the FELASA WG[b] 'Capture and transport of cephalopods' with the aim of developing recommendations about methods to be utilized, and guidance about the required competence of people involved in the capture and transport of these animals for scientific research.

[a] see also: Lara, E. (2020). Welfare and Environmental Challenges of Octopus Aquaculture, https://youtu.be/wS5r0DJgHWA

[b] http://www.felasa.eu/working-groups/working-groups-present/wg-capture-and-transport-of-cephlapods/

Cephalopods as laboratory animals: a legislative framework for capture and transport

Since the entry into force of the Directive 2010/63/EU in 2013, no gold standard method has been proposed to capture wild aquatic animals - including cephalopods – for their use in scientific research. We do not trace any legislative or regulatory document with explicit mention to these species.

A detailed survey of the available legislation is out of the aims of this work, but available in the ancillary work (Pieroni et al., 2021). Here we only briefly overview the main aspects relevant to the legislative framework ecompassing aquatic species' wellbeing in the context of commercial and trade purposes (see also Supplementary Info).

The Council Directive 91/67/EEC[c] and the following European legislations regulating the transport of animals for commercial or experimental purposes, is centred on vertebrate species, mainly terrestrial animals. Interestingly, the United Kingdom legislation extended the concept of animal to which the EC No 1/2005 should be referred including all the "cold-blooded invertebrates".[11] Furthermore, and as relevant to transport of wildlife, both the Office International des Epizooties (OIE) and CITES[d] consider all animals listed in the Live Animal Regulation (LAR), including cephalopods (for details see Pieroni et al., 2021). CITES has also published the "Packer's guidelines"[e] for aquatic invertebrates (therefore, we assume, cephalopods included) with detailed instructions about their general welfare, the

[c] on health conditions of aquaculture animals to be placed on the market, no longer in force

[d] Convention on International Trade in Endangered Species (2020). *Appendices I, II and III* [Online]. Geneva: CITES Secretariat. Available: https://cites.org/eng/app/appendices.php [Accessed March 2021].

[e] https://cites.org/eng/resources/transport/inv1.shtml

arrangements of transport and shipment as well as the design of the container. The US Institute for Laboratory Animal Research (ILAR)[f] published detailed guidelines concerning the capture and transport of laboratory animals (mainly vertebrates) including a mention to cephalopods (see section 'Applicability and Goals'[12]). Finally, it is since 1993 that the Canadian Council of Animal Care refers in its welfare act to any non-human vertebrates and cephalopods.[13] It includes extensive guidelines on procurement and transportation of purpose-bred animals and wildlife (see Pieroni et al., 2021), without specific mention to cephalopod molluscs. Furthermore, Australian legislation for the use of animals for laboratory purposes includes cephalopods and obliges to minimise the risk of injury or stress-induced diseases during their capture and transport. In the Australian law, capture and handling of wildlife (cephalopods included) must comprise: «***i.*** the involvement of a sufficient number of competent people to restrain animals in a quiet environment and prevent injury to animals and handlers; ***ii.*** chemical restraint (e.g., sedatives) where appropriate, if the period of handling is likely to cause harm, including pain and distress to animals; ***iii.*** restraint and handling of animals for the minimum time needed to achieve the aims of the project or activity; ***iv.*** making provisions for captured animals that are ill or injured, including treatment of pain and distress».[14]

Despite the European Directives and various guidelines mentioned above, it is indeed clear that regulations on capture and transport lack specific recommendations about wild cephalopod species, both at general and species-specific level (see table 1 in Pieroni et al.,

[f] www.nationalacademies.org/ilar/institute-for-laboratory-animal-research

2021). However, the most recent available considerations and precautions on capture and transport of cephalopods for research purposes are included in the Guidelines for the Care and Welfare of Cephalopods in Research.[15]

Recommendations for capture and transport of cephalopods in research

For the purpose of this WG, we carried out detailed text-mining of numerous published works. This informed a review of existing capture methods for collecting and transporting live wild cephalopods. The outcome of this analysis is presented in detail in the ancillary work: Pieroni et al. (2021).

The analysis of the literature highlighted important considerations: ***i.*** all the capture methods reported in the studies considered have been taken from fishery and readapted in a small set of cases for scientific purposes; ***ii.*** there are no species-specific procedures but rather several 'protocols' and/or variants for the same method; ***iii.*** no particular attention is given to the different life stages of cephalopods used in the studies and this piece of information is often missing; ***iv.*** very little is provided about the capture and transport procedures adopted, and in most cases only one of the two 'parts of the story' is described; ***v.*** some papers provide a list of recommendations which are mainly anecdotal or deriving from indirect communications, and therefore should further be validated by robust studies.

Maximising welfare during the Capture of live wild cephalopods

According to the General Section 4.2 of the Council Recommendation of 18/06/2007:

a. animals should be captured by «humane methods and by persons competent to apply them», minimizing «the impact of the capturing procedures on the remaining wildlife and habitats»
b. «Any animal found to be injured or in poor health should be examined by a competent person. (…) In case of serious injury, the animal should be killed immediately by a humane method» described in the Directive 2010/63/EU
c. «Appropriate and sufficient transport containers and means of transport should be available at capture sites, in case animals need to be moved for examination or treatment».[16]

How shall we apply all this advice to cephalopods?

There is not a unique reliable method to capture every cephalopod species, but rather a small range of techniques that best fits the species-specific needs, also considering their life-stage, physiology and inter-individual variability. Transport of eggs has been suggested as an alternative to move juveniles and/or adult forms, but this has some limitations (see Supplementary Info).

When considering the following capture methods (Table 1), we recommend associating a severity assessment in order to possibly predict the impact of a given protocol on the welfare of wild cephalopods.

Fishing: what can we learn from it?

Cephalopods are animals of great interest for commercial purposes, currently accounting for a 5% of the marine capture volume worldwide[g], with a significative increase in their demand, though recently slowed down by COVID-19 outbreak[h]. For such reason, a great number of capture methods have been developed by artisanal and small-scale fisheries[17]. On the other hand, many reports on cephalopods biology and fishing have been published (see table 2 in Pieroni et al., 2021), but no comprehensive summary of the numerous cephalopod capture methods is currently available for a given species and life-stage. However, Rathjen[18] stated the need for "more resource-friendly" fishing method for these animals. In his work, line jigging resulted to be the most suitable gear for squids (*Loligo forbesii, Illex argentines, Todarodes pacificus, Nototodarus sloani*); the technique appears selective and adjustable for the size of the specimen, thus limiting the impact on the environment or other fauna. Trawling is also much utilized for fishing cephalopods, but causes by-catch of other animals, not being species- or size-specific.

Traps and pots are utilised in many geographical areas and represent traditional gears for fishing cephalopods. These rely on the natural trend of some species to search for dens and hidden refuges. Spearing, multiple hooks and trolling are still used, although local geographical adaptations, and relatively small variations.

In our opinion, the fundamental question for the scientific community would be whether we can adjust some of the currently available fishing methods to render them suitable for

[g] Globefish (2016). *World Congress on Cephalopods: Markets and Trade* [Online]. FAO. Available: http://www.fao.org/in-action/globefish/news-events/details-news/en/c/449821/ [Accessed January 2021].

[h] for more info see: http://www.fao.org/in-action/globefish/market-reports/cephalopods/en/

collecting cephalopods used for research purposes. The answer is yes, providing that a coordinated, cooperative interaction between different stakeholders is established.

Capture of live cephalopods for research purposes

A tabularized overview of the best, recommended methods for capture of live cephalopods for research purposes is provided in Table 1.

Collectors and scientists unanimously agree upon the use of baited or light traps as the best method for capturing live wild nautiluses. A prototype originally reported by Carlson[19] is still utilized with some variations including modern monitoring systems. From the little information available about the capture methods for cuttlefishes, the most feasible techniques seem to be traps, and in particular basket or cuttlefish traps, very similar to those employed for squid, but larger and lighter. Juvenile and adult cuttlefishes are captured uninjured for both aquaculture and laboratory rearing. The use of these size-selective gears may be accompanied by seabed as an attractive spawning substrate for broodstock captured for aquaculture purposes.[20] Large nets, such as trammel nets, are also suitable for catching both juvenile and adult animals without excessive constraint and without constituting any environmental issue as it is for trawling. Seine nets and dipnets are mostly used for adult forms of sepiolids such as *Euprymna scolopes* and *E. tasmanica* destined to research and are considered the less traumatic method for these little-sized cephalopods.[21] For squids, one of the most frequently employed capture method is the jig lure with barbless hooks operated mechanically or by hand;[22] however, further analyses revealed that its use induces some injury and lasting harm to the animals (see Table 1). Alternatively, several kinds of nets have been utilized for capturing squids for laboratory use: pound nets, bongo nets, seine and dip

225 nets, all proved to be harmless if properly used by trained hands. These nets are large and
226 squids are able to swim before they get caught forming a consistent sample size.[23]
227 Furthermore, these appear suitable for capturing specimens at any life form paying
228 attention to the by-catch of egg masses. From our literature review (see Pieroni et al., 2021)
229 the most recommended capture method could be size-selective box traps and trap nets, with
230 usually a top hole from which the animal can spontaneously enter, but from which cannot
231 escape.
232 Undoubtedly the best existing capture method for octopuses is the pot.[17, 24] Pots, like traps,
233 are generally made of natural, non-toxic materials, with non-abrasive surfaces and exploit
234 the natural tendency of these animals to search for a den. Octopuses spontaneously settle in
235 these gears which are very likely to catch undamaged specimens. From the literature survey
236 we carried out (see also Pieroni et al., 2021) pots should have dark tone, narrow entrance
237 and a large interior that allows the animal to see outside without exposing itself to danger.
238 A series of adjustments have been proposed, such as the insertion of a GPS monitoring
239 system or of a removable lid that might also be useful for transportation. Pots are alluring
240 both for juvenile and adults and very often they can be chosen as substrate for laying eggs,
241 that should be reinserted in nature. A combination between pots and traps are the so called
242 Japanese baited pots (JBPs) combining shelter and bait (Table 1).
243

Transport methods of cephalopods: maximising welfare from sea to the lab and between labs

As for the capture, the best transportation methods should avoid (or at least limit) PSDLH and should help to reduce the stress associated with the capture technique. Again, most likely there is not a unique protocol to transport cephalopod species (see table 3 in Pieroni et al., 2021); inter-individual variability, species-specific features – body size, physiology, biological requirements for every life stage - must be considered when preparing the animal for the journey.

A detailed list of considerations for transportation of live cephalopods for research purposes is provided by Fiorito and colleagues[15] which in turn is mostly based on available guidelines for the transportation of live fishes,[25] in compliance with the codes and regulations for European and international transport of live animals (see Supplementary Info). The work could be therefore considered as the ground for building up the best recommendations for cephalopod transport (see Table 2).

In the first known guidelines about the rearing of cephalopods for scientific use (Grimpe's 'Care, treatment and rearing of cephalopods for zoological and physiological purposes')[26, 27] several insights about the methods of transport and their maintenance during the journey for different species of cephalopods are included. These can be considered for the development of good practice. In his words, a vital point is to always keep cephalopods in well-oxygenated seawater throughout the journey, as these animals have a high metabolic rate that rapidly produces large amount of carbon dioxide and ammonia.[28, 29]

For this reason and to limit distress (e.g., agonistic attacks and aggression, inking), animals should be individually kept in separate bags. Some cephalopods are shipped in Styrofoam fish boxes[i], although a temperature slightly below the optimum has been suggested because considered to reduce the animals' metabolic rate, allowing the shipping water to hold more oxygen and reduce waste production[j]. However, containers should be kept in shade when transporting (e.g., by boats), or using air conditioning when using by car or other vehicles (see: Supplementary Info; 'Other General Requirements' in Table 2).

At the moment there are no specific aerated containers designed for cephalopods and neither there is an open-system for their transport, but these might be obtained by adjusting those available for live fish transportation.[25, 30]

Similarly to the capture methods, we reviewed literature concerning transportation of cephalopods (see Pieroni et al., 2021 and table 3 therein) in order to extrapolate general indications that might turn out useful for building up some species-specific recommendations.

Table 2 offers a tabularized overview of recommended methods for transport of live cephalopods for research purposes.

Boxes or insulated chests with chilled seawater have been recommended for both juvenile and adult of different species of nautiluses and should be preferred to plastic bags which

[i] cooled or heated according to the species

[j] See also Vidal, E.A.G., Villanueva, R., Andrade, J.P., Gleadall, I.G., Iglesias, J., Koueta, N., Rosas, C., Segawa, S., Grasse, B., Franco-Santos, R.M., Albertin, C.B., Caamal-Monsreal, C., Chimal, M.E., Edsinger-Gonzales, E., Gallardo, P., Le Pabic, C., Pascual, C., Roumbedakis, K., and Wood, J. (2014). "Chapter One - Cephalopod Culture: Current Status of Main Biological Models and Research Priorities," in *Advances in Marine Biology*, ed. E.A.G. Vidal. Academic Press), p. 1-98.

284 can be worn out by these animals, risking their welfare. More specimens can be contained
285 in the same box, providing each animal with at least 4 L of seawater.[19]
286 Transportation of cuttlefish can be challenging and suggestions have been made to transport
287 few animals per plastic bag or barrels according to the size[31] with the use of large containers
288 in which storing the bags/boxes.
289 As for the cuttlefishes, sepiolids are mainly transported in plastic bags containing few
290 individuals according to their volume and relative body size. It is recommended to put these
291 bags in larger insulated boxes that ensure no leakage or asphyxiation of the animals.[32]
292 During transport, squids should be individually placed in plastic bags, barrels or buckets
293 filled with seawater and oxygen of appropriate proportions (Table 2) sealed and placed in
294 larger tanks or Styrofoam boxes.
295 The personal experience of Grimpe with *Eledone moschata*, *Octopus vulgaris* and other species
296 made the Author suggest the use of enamel pots placed in Demijohn-baskets with stuffed
297 hay between them in order to reduce potential insults related to the transport method[k].
298 These have cylindrical base and are conically tapered at the top; only the lower part of the
299 pot, containing between 20 and 80 L should be filled with water: the rest must be air, the
300 circulation of which must be assured by multiple holes in the cork.[26]
301 Pots, like those utilized for the capture of octopuses, should be employed for facilitating
302 transportation (see Table 2 and Supplementary Info). These should be placed in a larger
303 container or tank, as in a modern version of Demijohn-baskets.[26, 27] Containers must be filled
304 with seawater (recommended from the collection site) and oxygen in appropriate relative

[k] See Figure 116 of 'Grimpe's 1928 – a translation' in De Sio et al. (2020)

volumes. Our suggestion from the knowledge of the biology of these animals is to keep each in individual in separate bags or pots and not together with other specimens.

Grimpe's description has been readapted in different ways, but his approach in considering cephalopods transport is still valid and has been widely applied.

Future Needs and How to Achieve

Two main actions are suggested for the future: ***i.*** collaborative efforts between fishermen and scientific research to further standardize and implement the best recommended methods for capture and transport of live cephalopods for research purposes; ***ii.*** a training program that may help to increase the acquisition of the required competence for people involved. Hereunder, we will briefly illustrate the two.

What emerges from the analysis of the scientific literature, from various recommendations, technical reports and unpublished tata (see Pieroni et al., 2021) is the need for more in-depth studies on capture and transportation methods able to specifically address the best way to handle these animals and their welfare under such circumstances. Body size and life stages, species biological and physiological needs are fundamental aspects to consider. However, the great majority of methods currently utilized have been based on personal experiences and interactions with local fishermen,[26] but relatively little scientific systematic studies have been carried out for the purpose of assessing the best capture and transport methods for cephalopods.

Approaches to assess stress response (indicator of animal welfare) of aquatic animals to capture and transport methods have been applied to fish and a few crustaceans. These works allowed to identify the most appropriate catching gear and to design containers for different types of transportation journey. As for cephalopods, only recently Araújo and co-workers[33] studied the effects of a simulated long journey transportation at high density on live *O. vulgaris* (see also Pieroni et al., 2021); no mortality was recorded at the end of 48 h simulated transport at the different density of animals considered; no significant changes in the physiological parameters were found. Another study by Barragán-Méndez and colleagues[34] evaluated the wellbeing of *E. moschata*, *E. cirrhosa* and *O. vulgaris* after capture (trawl; Pieroni et al., 2021 for review). Despite these works, studies are still required to facilitate an informed guidance on capture and transport methods (species-specific) supporting measurements and control of stress-induced levels in live cephalopods.

This WG promotes *ad-hoc* studies that will help achieving this goal.

We recommend a set of experiments designed to evaluate the physiological effects of different combinations of capture and transport methods on both sexes of juveniles and adults of the most commonly utilized cephalopod species in scientific research. The idea behind is to adopt a collaborative effort with selected, geographically distributed, fishermen communities using a significant number of individuals for each species (and possibly in two separate seasons) utilizing a couple of capture methods chosen for comparison with those 'claimed' to be the most recommended ones such as: ***i.*** nets vs traps in cuttlefishes, ***ii.*** jigs vs traps in squids, ***iii.*** pots vs traps in octopus. Then, for each, different transport conditions

will be tested (e.g, individual vs multiple 'storing' of animals kept into standard open large, darkened buckets). After transportation (no more than 3-4 hours) the welfare status of the animals will be assessed using different indicators selected among those reported in table 5 of the FELASA guidelines[15]. Monitoring of individual animals should be performed at Day 1 and at Day 4/5 after capture, to measure how much time the animals take to recover and acclimatise to the estimated baseline levels of these physiological indicators. Once preliminary studies will prove effective in guaranteeing survival of the cephalopods, other investigations will follow in close collaboration with fishermen to make a consensus of the benefits and expanding the study to a larger group and conditions (geographical, boats, etc). We aim at finding the best conditions of capture and transport that could be used to improve animal welfare in different circumstances (e.g., capture and transport of different life stages, intercontinental journeys etc.).

Our final goal is to actively cooperate with fishermen and transporters by involving them as scientific suppliers for live cephalopod species once the good practice for the capture and transport of healthy cephalopods will be validated through the pilot studies.

As already mentioned earlier what jeopardises most the welfare status of the animals is the limited training and competence of the people involved (see Supplementary Info). Article 23 of the Directive 2010/63/EU specifies the need for competent personnel when (a) carrying out procedures on animals, (b) designing procedures and projects, (c) taking care of animals, or (d) killing animals - as to limit and/or avoid the induction of PSDLH in the animals.[6] Both capture and transport of cephalopods are part of the phases that relate to the 'life' of live

371 animals for scientific purposes and must be performed by trained and expert personnel. As
372 such, it is in our view the "A working document on the development of a common education
373 and training framework to fulfil the requirements under the Directive" should be addressed
374 also to people specifically involved in capture and transport of living cephalopods. Annex
375 IV of the EU Council Regulation No 1/2005 already provided instructions concerning the
376 training for transporters which shall include notions on animal physiology and their needs,
377 handling and impact on stress and welfare[35].
378 Here, we propose the need for suppliers of live animals to be trained to deal with
379 cephalopods with the aim to assure compliance with species-specific biological,
380 physiological, behavioural needs and welfare requirements. We are convinced that by
381 focusing on the training of fishermen - whose expertise and practical knowledge of the sea
382 are undoubtable - we will be able to develop the best practice for capturing cephalopods in
383 the most humane way. Of course, fisherman's belief and behaviour depend upon the
384 economic and social structure within which he/she is operating (see discussion in Pieroni et
385 al., 2021). We want to firmly rely on the experience of proud lifetime cephalopod artisanal
386 and small-scale fishers, and we are interested in their holistic analysis of the context. What
387 we want to achieve is a joint action of trained personnel that will ensure that wild-caught
388 cephalopods will be properly captured and transferred to their destination without
389 experiencing unacceptable pain or suffering.
390 The most challenging part is to approach fishermen and transporters and persuade them in
391 taking part to a training and education process. The major resistance could be due to the
392 loss of potential work by undertaking the training without having a beneficial personal

393 profit. It is therefore our aim to find a general balance between their needs and the
394 obligations necessary for fulfilling competence- as required by the increasing attention on
395 all aspects of animal care due to the inclusion of live cephalopods in regulations for scientific
396 research purposes. In our view, incentives should be provided for attendees that will
397 partake at becoming suppliers of live wild animals for the laboratories, such as gaining a
398 greater income and the possibility of working through all Europe, and not necessarily in
399 their own country or for the local supply. Moreover, since the successful Trainees will
400 receive a certificate of completion, this may help them to have access more easily to fishing
401 licenses according to the national (and possibly to international) legislations. A coordinated
402 effort between different stakeholders including non-profit organizations, local and national
403 governments will be then required. The training framework should be accessible, affordable
404 – and with joint effort of the Member States, hopefully free -, and flexible so as to meet the
405 working time or shifts of the trainees.
406 Box 1 summarizes the organization of the proposed training program. In our aim fishermen
407 and transporters of live cephalopods should face the challenge of improving the well-being
408 of the animals they work with by achieving awareness about the concept of welfare and
409 ethical approaches when dealing with cephalopods as animals destinated to scientific work.
410 In order to reach the maximum degree of commitment a special edition of a dedicated
411 training course for cephalopods will be designed so as to allow persons coming from
412 different cultural backgrounds to undertake a first induction course that will help them
413 jointly converge to the same level when approaching the objectives of the main training
414 program (Box 1; see also Pieroni et al., 2021)..

Once successful, collectors and shippers may start working under the supervision of an expert for a couple of months to further monitor if the required skills match the animals and stakeholders benefits..

Concluding remarks

In this report the Capture and Transport of Cephalopods FELASA WG wanted to highlight the current limited knowledge about protocols of capture and transport methods for cephalopods destined to scientific research.

The current European legislations and recommendations about the transport conditions of animals are not sufficient and do not directly address cephalopods (review in Pieroni et al., 2021), while regulations concerning the wildlife capture methods are poorer in taxon-specific information (even for vertebrates). Different organisations and few other countries include cephalopods among the animals whose welfare should be protected during the capture and transport and are mainly bounded to international transport and shipping rules. From the text-mining carried out for the aims of this WG (see also ancillary work, Pieroni et al., 2021) some considerations emerged and pointed out the need to never omit fundamental information in the scientific works, as also recommended by PREPARE[36, 37] and ARRIVE[38, 39] guidelines.

We attempted to define the capture and transport conditions likely to be the most suitable for specific taxon (Tables 1, 2) with attention to the target life stages needed for experimental

studies (see details in Pieroni et al., 2021). General considerations that can be drawn from this analysis are as follow:

a. The best capture method is any harmless tool that exploits the natural behavioural tendency of the animal (e.g., octopus' preference for den, seabed substrate for spawning cuttlefish) and that considers its daily-cycle and diet composition – according to the species and its life stage - to catch it more efficiently. A good capture method should be classified as a mild procedure and therefore the target cephalopod should experience only a short-term distress. All the large-scale non-selective methods (e.g., trawl) must be avoided because of the enormous impact on the welfare state of marine animals and on the environment.
b. The best transportation method is the one able to avoid or reduce further stress related to the capture procedure. The key factor in preventing the animals from experiencing PSDLH is planning (duration, resting place, number of health checks) to avoid any delay that may compromise the animal welfare. Environmental requirements (e.g, oxygen, pH, salinity, temperature) must be monitored throughout the journey and they should be fitting the welfare requirements of the different cephalopod taxa. Depending on the duration of the journey, particular attention should be paid on the type, size and equipment of the means of transport as well as the containers that will keep the animals while onboard.

These two processes are not independent from each other and therefore, to better perform transport, the capture procedure should be done properly to avoid any handling and

459 exposure to aversive conditions from the collecting site to the container. Bearing this in
460 mind, we proposed pilot studies that could be carried out in a collaborative fashion with
461 selected, geographically distributed, fishermen communities to compare the effect of
462 different combinations of capture and transport methods upon the survival rate, physical
463 conditions, and physiological milieu of the adult form of the most common cephalopod
464 species.
465 Finally, a pivotal role in the success of both capture and transport methods is played by the
466 competence of the personnel carrying out these activities. For such a reason, we proposed a
467 special edition of the Education and Training programme for cephalopods (CBC)[1] dedicated
468 to fishermen and transporters that should be attended in order to acquire the required
469 competence. The proposed course will be organised according to a modular training
470 structured in theoretical and practical sessions, around learning outcomes based on defined
471 assessment (Box 1).
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[1] https://www.cephalopodresearch.org/training-school/

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The Author(s) declare(s) that there is no conflict of interest.

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Table 1 - Capture Methods. Overview of the most and least recommended capture methods of live cephalopods for research purposes. Data are summarized by method (i.e., Trawl, Traps, Pots, Jigs, Nets) and taxon. For detailed review see ancillary work in Pieroni et al. (2021) and table 2 therein. Recommendations are colour coded – Green: First most recommended method; Yellow: Second most recommended method; Blue: Third most recommended method; Grey: Not Recommended; Light red: Not Generally Adopted method (NGA).

Scientific works that constituted the basis for the compilation of this table are listed in Supplementary Information (see: 'Additional References – Overview of Capture and Transport Methods') and not in the Reference List in this work; for details see also Pieroni et al. (2021).

Abbreviations utilized - 'Welfare issues' (*): we refer to the possible induction of Pain, Suffering, Distress and Lasting Harm (PSDLH); see also Andrews et al.,[40] Fiorito et al.[15, 41] and Ponte et al.[5]; 'Environmental issues' (**): we consider that trawling might cause environmental damage due to the alteration and destruction of sea floors, and also the non-selectivity of this method which results in by-catch; **GPS**: GPS or other monitoring systems have been proposed to be added to the capture gear providing useful data[42, 43].

	Nautilus	Cuttlefish	Sepiolidae	Squid	Octopus
Trawl	Welfare(*) and environmental issues(**)	Welfare(*) and environmental issues(**)	Welfare(*) and environmental issues(**)	Welfare(*) and environmental issues(**). When employed, hand-selection among damaged animals has been reported	Welfare(*) and environmental issues(**)
Traps	**Baited traps** or **light traps**. Suitable for both juvenile and adults. GPS	**Basket traps** or **cuttlefish traps** (larger and lighter than squids'). Size-selective and employed for catching adults. Seabed is often included as substrate. Females and attached eggs[m] should not be taken (for conservation)	Not effective because small body animals	**Light traps** or **baited traps**[26]. Size-selective and employed for catching of adult forms. GPS	Can be used in absence of pots. Can be used for both juvenile and adult. See also **Japanese baited pots**
Pots	NGA	NGA	Very small animals	**Japanese baited pots** could be employed as they are similar to traps and size-selective, adjustable for both juvenile and adult. GPS	Dark with narrow entrance and larger inside[n]. Females and attached eggs should not be taken for conservation issues[a]. **Japanese baited pots** are also recommended because they combine the advantage of both pots (shelter) and traps (bait). GPS.

[m] Unless authorized for research purposes and included in the licensed project

[n] A lid can be added but not needed because of the natural tendency of these animals to search for a den and remaining inside.

	Nautilus	Cuttlefish	Sepiolidae	Squid	Octopus
Jigs	NGA	**Squid jigs** might be adopted but not recommended for welfare issues(*)	NGA	Widely employed with bait or with light lures and barbless hooks Not recommended for welfare issues(*)	Rarely used Not recommended for welfare issues(*)
Nets	NGA	**Trammel nets:** large enough to catch a reasonable number of animals without excessive constraint (both juveniles and adults).	**Dipnets** or **Seine nets** are large enough to catch a reasonable number of animals without excessive constraint (both juveniles and adults).	**Pound** or **seine nets:** large enough to catch a reasonable number of adult animals without excessive constraint. **Bongo nets** suitable for paralarvae.	Mostly adopted for paralarvae, but not generally adopted for adults[o].

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[o] which rarely get trapped in (unless caught with hand-net by trained personnel performing scuba diving [2, 52-54]

Table 2 - Transport Methods. Overview of the most and least recommended transport methods of live cephalopods for research purposes. Data are summarized by method (= container; i.e., Plastic bag, Box, Tank) and taxon. For detailed review see ancillary work in Pieroni et al. (2021) and table 3 therein.
Recommendations are color coded – Green: First most recommended method; Yellow: Second most recommended method; Blue: Third most recommended method; Grey: Not Recommended; Light red: Not Generally Adopted method (NGA).
Scientific works that constituted the basis for the compilation of this table are listed in Supplementary Information (see: 'Additional References – Overview of Capture and Transport Methods') and not in the Reference List in this work; for details see also Pieroni et al. (2021).
Each trip/journey/shipment must be preceded by the presentation of the suitable documentation and planning according to the national or international legislation. Depending on the mean of transport utilized, the correct adaptation should be followed to ensure the welfare of the animals being transported(§). Vibration, noise, and direct light must be kept to a minimum otherwise the stress response might be lethal (increasing ammonia waste in seawater or induction of self-harm)

	Nautilus	Cuttlefish	Sepiolidae	Squid	Octopus
Plastic bag	Animals tend to bite plastic bags	The bag should be aerated, and properly sealed[p] and doubly secured. Few animals per bag can be placed depending on the volume of the bag, the duration of the journey and on the size of the animals. Could survive up to 12h	The bag should be aerated, and properly sealed[p] and doubly secured. More than one animal per bag can be placed because of the small size of these species, depending on the volume of the seawater and the duration of the journey. Reported to survive for up to 21h	Few specimens can be stored depending on the volume of the bag, the duration of the journey and on the size of the animals Individual bags more recommended for increasing the chance of survival (transport up to 20h)	Suitable for every life stages according to the size of the animal. Suggested for paralarvae, even at high densities with high survival chances at 6, 12, 24h. For small octopuses a survival of 8-10h has been reported. If appropriately sealed[p] and doubly secured could survive over 12h

[p] e.g., twisted at the top and folded over

	Nautilus	Cuttlefish	Sepiolidae	Squid	Octopus
Box	Few specimens inside (no more than 4 animals in a 20 L box, suitable for both small and large specimen), in chilled water (15-18°C). Tightly sealed and contained in a larger Styrofoam box. Reported to be suitable for travel up to 4-24 h	Can be used as a container for holding bags (transparent to facilitate inspection if required) to ensure that an appropriate temperature is maintained during transport. Can contain seabed substrate[q]	Can be used as a container for holding bags (transparent to facilitate inspection if required) to ensure that an appropriate temperature is maintained during transport.	Can be kept in similar conditions to bags and can be of different forms (buckets, cooler, barrel) but can be considered more secure in terms of resistance to insults and vibration. Reported to survive up to 8-11h	Can be used with similar modalities to bags and tanks in different forms (tubes, jars, buckets). Can be used as a container for holding bags (transparent to facilitate inspection if required) to ensure that an appropriate temperature is maintained during transport. Can accommodate the pot used for collection. See also[r]

[q] for spawning

[r] Reported to survive up to 24h in PVC tubes of 16 cm in diameter, located in a 200 L tank.

	Nautilus	Cuttlefish	Sepiolidae	Squid	Octopus
Tank	Can be used as a container for holding bags (transparent to facilitate inspection if required) to ensure that an appropriate temperature is maintained during transport.	Can be used as a container for holding bags (transparent to facilitate inspection if required) to ensure that an appropriate temperature is maintained during transport. Can contain seabed as substrate[q]	Can be used as a container for holding bags (transparent to facilitate inspection if required) to ensure that an appropriate temperature is maintained during transport.	Not frequently used but recommended for a large sample size (20 specimens in a 60 x 90 cm fiberglass tanks filled to a depth of 30 cm) or for species of large body size. Can be used also as a container for holding bags or boxes (transparent to facilitate inspection if required) to ensure that an appropriate temperature is maintained during transport	Large tanks for individual rearing are the most recommended during the journey. Can be used also as a container for holding bags or boxes (transparent to facilitate inspection if required) to ensure that an appropriate temperature is maintained during transport. Reported to survive up to 12h

[(§)] **Other General Requirements:** The holding containers in which animals are stored should be composed of 1/3 seawater (preferably at the water temperature at the collection site), and 2/3 oxygen which should not be pumped in to avoid the generation of bubbling in the mantle of the animals. Water collection on site should be promoted to avoid air exposure and dehydration together with sudden temperature changes from the sea to the tank. Animals should be food-deprived prior to the trip/journey/shipment (depending on the duration), in order to prevent ammonia waste upbuilding in the seawater. Sedation (e.g., cold water, $MgSO_4$ or $MgCl_2$) is not essential and is not recommended for the transport of most cephalopods. The welfare state of the animals should be periodically checked during the journey or at the resting place (if the journey is very long) and expert personnel should be able to take the most humane decision in case of harmed specimen.

Box 1. Topics and Learning outcomes of the Education & Training accredited course for collectors and transporters of live wild cephalopods to be used in research. The content of the course follows the modular scheme and organization included in "A working document on the development of a common education and training framework to fulfil the requirements under the Directive" Brussels, 19-20 February 2014 by the National Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes. The education and training will be provided through the attendance of about 20 hours course designed and delivered as part of the Cephalopod Biology and Care (CBC) FELASA accredited Training Program. The course will be structured in theoretical and practical sessions (at least 8 additional hours), around learning outcomes based on defined assessment and pass-fail criteria. For collectors and transporters, the skills that the course should provide are suggested to be considered equivalent to Directive 2010/63/EU functions a), c) and d).

Abbreviations included – AWB: Animal Welfare Body; NCA: National Competent Authority; DV: Designated Veterinary; PSDLH:– Pain, Suffering, Distress and Lasting Harm; TAC: Total Allowable Catch.

General principles: Collectors, transporters and shippers should become familiar with some essential concepts which will be provided through a 20 hours training (spanned in three days).

Topics covered:

i) **sustainability** as an essential trigger for improving animal care and consequently the quality of scientific data, while ameliorating the economic profit of the specialising personnel involved

ii) general considerations about the **Directive 2010/63/EU** and why cephalopods are included as the sole invertebrates

iii) **ethics and culture of welfare** when referring to animals

iv) **PSDLH** and how to assess **stress response** in live cephalopods when performing capture and transport

v) basic knowledge of **general and species-specific biology and behaviour** of cephalopods

vi) **legal aspects**, national and international legislations concerning capture (licenses, TAC and fishing quota regulations) and transport (e.g., regulations for the transport of live aquatic animals as defined by IATA and ATA LAR)

vii) suitable **capture and transport methods** and protocols to ensure the well-being of the live wild-caught cephalopods

viii) principles of **hygiene** and **care** of animals

ix) methods of **handling**, sedation (whenever applicable), stunning and humane killing for cephalopods

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Attention will be given to practical aspects and hands-on-training aimed at getting the trainees more easily involved with the recommended, standardised and validated equipment/protocols to be used during the actual capture and transport. Attention will be given to train them how to properly handle a given cephalopod species.

The practical skills will be the object of evaluation (OSPE) after Trainees being successful in the first theoretical training phase.