Review on the methods of capture and transport of cephalopods for scientific purposes

Outcomes of FELASA Working Group 'Capture and Transport of Cephalopods'[1]

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Since January 2013 cephalopod molluscs, including hatched juveniles and adult forms, were listed in the Directive 2010/63/EU among other laboratory animals regulated for their use in scientific research.
According to the Directive, animals should be bred in purpose and the use of wild animals must be authorized by NCAs. To date, cephalopods captive breeding is still facing some bottlenecks thus making scientific studies relying upon the use of wild-caught animals. In addition, in several instances animals are transferred across European and between extra-European research facilities. The lack of specific regulation for this taxon, and the requirements of current legislations, guidelines, and codes referred to other organisms, pushed for the need for the development of recommendations for the capture and transport of cephalopods for scientific purposes. FELASA established a Working Group aimed at contributing to the development of recommendations for capture and transport of cephalopod molluscs for their use in scientific research. Here we review available knowledge – based on a systematic literature analysis of the current available knowledge on this topic. Our aim was to identify the most suitable 'protocol' to be adopted for the capture and transport of cephalopods as experimental animals. Based on the scientific literature, we also indicate a potential experimental pipeline aimed at evaluating the impact of the suggested capture and transport methods on animal welfare.

Training and Education for the personnel involved in the supply chain is a crucial requirement to spare animals any avoidable pain, distress, suffering or lasting harm during capture and transport. The development of a training program focussed on the achievement of competence for those in charge of capture and transportation of live cephalopods (as required by Article 23, Directive 2010/63/EU) is also suggested.

Keywords: Capture; Transport; Cephalopods; Directive 2010/63/EU; Animal Welfare

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Background

Cephalopods are the first among invertebrates introduced since January 2013 in the Directive 2010/63/EU for their use in scientific procedures. Annexes on the Directive provide general guidance on several requirements, but specific details are lacking for cephalopod molluscs. This has led to the preparation and publication of the 'Guidelines for the Care and Welfare of Cephalopods in Research' (Fiorito et al., 2015) endorsed by FELASA, contributed by members of the international cephalopod research community, and supported through the non-profit organization CephRes and the COST Action FA1301 Cephs*In*Action.

Despite all the advances made over the last years regarding cephalopods welfare (see for example Moltschaniwskyj et al., 2007; Holden-Dye et al., 2019 and works cited therein; Ponte et al., 2019; Birch et al., 2021) most of the research performed on these molluscs still relies upon the collection and transport of eggs and wild-caught animals between research facilities.

Article 9.1 of the Directive states that animals must not be taken from the wild when they have to be used in procedures, unless the National Competent Authority (NCA) accord a permission (Article 9.2) following the provision of sufficient justification the scientific goals could only be achieved through the use of wildlife (European Parliament and Council of the European Union, 2010). In most vertebrate aquatic laboratory animals, breeding for the use in procedures is based on well-advanced techniques whereas cephalopod culture, whether it is for research or aquaculture purposes, is still facing several issues at the point that this is feasible for a very limited number of species (Iglesias et al., 2014; but see Jacquet et al., 2019; and also Franks et al., 2021)[2]. Therefore, until culture protocols are not fully developed for cephalopods, and adequate safeguards of their welfare set in place in culture systems, there is sufficient justification for collecting specimens belonging to this taxon from the wild.

It is without doubt that capture and transport of live wild species should be performed only by competent persons using methods which must prevent animals from experiencing physical, physiological and any other pain, suffering, distress or lasting harm (PSDLH; Article 9.3 of European Parliament and Council of the European Union, 2010) regardless of the life stage and the level [7]in the supply chain in which they are, including acclimatization or quarantine – whenever appropriate - to laboratory conditions. Article 23 of the Directive states that personnel carrying out and designing procedures and projects, taking care of and killing animals must be competent. Each Member States is asked to establish the way training and competence assessment will be carried out. This means that also capture and transportation of cephalopods should follow the same overarching principles (European Parliament and Council of the European Union, 2010).

FELASA established a Working Group[3] aimed at developing recommendations about methods to be utilized for capture and transport of live cephalopods for research purposes. The WG also explored the possibility of developing scientific-based improvements for the available methods, and guidance for training of people involved in the capture and transport of these animals, whenever necessary.

The present review is ancillary to Sykes et al. (2022) – i.e. the FELASA WG Report -, and summarizes the background information and an overview of the in-depth analysis of the available knowledge on the topic carried out by the Working Group. As way of doing, the FELASA WG Capture and Transport of cephalopods carried out a text-mining of the current available literature, prepared a summary of evidences and best practice (included in this review), and then elaborated a synthesis and recommendation (Sykes et al., 2022).

Here, we first survey the legislation, codes, guidelines and rules concerning the capture and transport of aquatic animals – with emphasis to the lack of specific norms for the taxon of interest. We then review available works, listing and/or commenting existing capture methods for collecting live cephalopods (i.e. nautilus, cuttlefish, sepiolid, squid and octopus) in the wild, and of those focusing on methods for transporting these organisms from the wild. As mentioned above, and as presented in the Report of this WG (Sykes et al., 2022), the preparatory analysis of available literature served to inform the identification of taxon-specific recommendations for capture and transport of cephalopods in research.

As mentioned, this review is a companion to the 'FELASA Working Group Report - Capture and Transport of live cephalopods: recommendations for scientific purposes' (Sykes et al., 2022).

2 see also recent debate: https://www.ciwf.org.uk/research/species-aquatic-animals/octopus-factory-farming-a-recipe-for-disaster/?id=161421; https://www.ciwf.org.uk/news/2021/10/octopus-farming-a-recipe-for-disaster; last visited July 2022.

3 FELASA WG Capture and Transport of cephalopods; see https://felasa.eu/working-groups/present/id/6; last visited July 2022.

1. Legislation, Codes, Guidelines and Rules concerning the Capture and Transport of Aquatic Animals

We found no specific legislation or any rule concerning capture and transport of live cephalopods. Therefore, in the following pages we provide information about the general framework regulating aquatic species in the context of commercial and trade purposes.

To the best of our knowledge, the Council Directive 91/67/EEC[4] was the first attempt at protecting the health conditions governing the placing on the market of aquaculture animals and products. Interestingly, in the document the term 'aquaculture animals' is used to refer to live fishes, but also to crustaceans and molluscs originating from a farm, and also to those from the wild intended to be farmed (Art. 2). Article 4 stated «Aquaculture animals must be dispatched in the shortest possible period to the place of destination, using means of transport that have been cleaned and, if necessary, disinfected in advance with a disinfectant that is officially authorized in the Member State of dispatch», and also that «Transport shall be carried out in such a ways to safeguard effectively the health of the animals, in particular by changing the water» (Council of the European Union and Council of the European Communities, 1991b; see also Annex D). In the following Council Directive 98/58/EC, rules for the protection of animals for farming purpose, including fish and amphibians, have been put in place. However invertebrate species, animals caught from the wild, laboratory animals and animals intended for competition were explicitly excluded (see Art.1 of the Council of the European Union, 1998).

Furthermore, the same Council Directive provides no recommendation and/or regulation concerning the time of capture and transport for the animals listed, but rather gives general considerations about the housing and accommodation of organisms intended for aquaculture and farming activities including supply of food, water and other substances or the breeding procedures (see Annex of the Council of the European Union, 1998).

The experimental use of live animals and the question of how to handle them by limiting PSDLH, started to be addressed by the Council Directive 86/609/EEC[5] and the following Council Recommendation 2007/526/EC of June 18th 2007. Both documents provided a first set of rules and suggestions regarding the capture from the wild, stressing out that only competent trained persons should acquire animals, by adopting humane methods in order to preserve their wellbeing while limiting interference with the remaining wildlife and environment to a minimum (see Annex II, Section 3.2 of the Council of the European Union and Council of the European Communities, 1986; see General Section 4.2.1 of the Commission of the European Communities, 2007). According to the Council Recommendation 2007/526/EC, once the animal has been caught it must be examined by a Designated Veterinarian in order to check its health state and eventually promptly intervene with treatment of injuries or, if the animals has severe harms, by adopting a humane killing method (Commission of the European Communities, 2007)[6].

The question of transportation of animals, whether they are destined for commercial use or scientific research, or whether they are terrestrial or aquatic species, has been later analysed much more in depth by the European legislation and more information is available when compared to capture. The European Convention for the Protection of Animals during International Transport (ETS No.193) applies only to vertebrate species to be traded from one country to another, excluding journeys of less than 50 km and 'movements' between Member States of the European Community (Council of Europe, 2003).

The Council Regulation (EC) No 1/2005 of 22/12/2004 includes detailed information about the welfare status during the transport of vertebrate animals being used for research (Council of the European

4 No longer in force, date of end of validity: 31/07/2008; ELI: http://data.europa.eu/eli/dir/1991/67/oj. Repealed by Council Directive 2006/88/EC (Document: 32006L0088), that is also repealed and replaced by Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016.

5 No longer in force, date of end of validity: 09/05/2013; Latest consolidated version: 01/01/2013; see ELI: http://data.europa.eu/eli/dir/1986/609/oj. Repealed by the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

6 In line with the principles set out in the 'European Commission Recommendations for the euthanasia of experimental animals' (Part 1: doi: 10.1258/002367796780739871; Part 2: doi: 10.1258/002367797780600297).

Union, 2004), ranging from general condition of transportation (Art. 3), the documentation (Art. 4), planning needed (Art. 5), and to the technical rules concerning the means of transport (Chapter II), including species-specific biological needs of the animals (Chapter V). Moreover, despite at the time of the Council Recommendation 2007/526/EC publication no reference to cephalopods existed, it already provided recommendation and general sections on capture (4.2), transportation (4.3), quarantine, acclimatization and isolation (4.4) for the animals, and a species-specific (section K) guidelines for fish (Commission of the European Communities, 2007).

Both EC No 1/2005 and Council Recommendation 2007/526/EC establish that people involved in dealing with animals - at all levels - should be competent on the set of tasks involving the capture and transportation of live specimens. Interestingly, the Welfare of Animals (Transport) (England) Order 2006 - and the parallel legislations in Scotland, Wales and Northern Ireland - provides an ethical-based statement concerning the transport of animals, extending the protection of the welfare state from vertebrates to cold-blooded invertebrates (UK Statutory Instruments and the Secretary of State, 2006). This UK legislation should be considered as a supplement to the EC No 1/2005 applied to the 'movement' of live animals within the EU by different means of transport, each one specifically designed and managed in order to be suitable for the animal and its needs and to face a certain type of travel (UK Statutory Instruments and the Secretary of State, 2006).

Since the transport of live animals is not exclusively performed within the EU, here we also consider international Codes and Guidelines, developed by Independent Organizations which refer to the rules of the transport and shipping companies throughout the world.

The 'Office International des Epizooties' (OIE), also known as 'The World Organisation for Animal Health' was created on January 25th 1924, because of the need to fight the spread of animal diseases at global level. It is an intergovernmental organization responsible for improving animal health worldwide. In 1994, OIE published a review concerning the welfare of the animals transported between nations for, as there stated, «social, cultural, economic and emotional reasons» including food, companionship, sport and scientific studies (Adams, 1994). The work paid particular attention to the explanation of animal welfare as matter of both "science and morality".
The document focuses on major stressors related to transport that could impair the animals' health state (e.g. separation from a familiar environment, overcrowding in confined spaces, vibration, temperature, journey duration etc.). It was thus highlighted that acclimatisation to the conditions prior transportation of animals should be of paramount importance to complete national or international transport without inflicting any harm to the specimens. The last part of the review reports the different means of transport also for specific categories of animals - mainly farm ones – are provided.
The 2019 edition of the annual 'Aquatic Animal Health Code' by OIE includes some considerations for aquatic safe transport (e.g., containers, means of transport, sanitary measures etc.) (Aquatic Animals Commission, 2019). The Code promotes the welfare status of the animals by including detailed instructions on transport conditions and how they properly can meet the species-specific requirements. The OIE codes were created considering the United Nations Environment Program (UNEP) that, through the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)[7], seeks to ensure that international trade in the listed species is sustainable, legal and traceable.

1.1 Topics covered by Legislation and Recommendations

Table 1 provides a tabularized overview on the reference documents, rules, legislative acts that include guidance and represent a framework for transport (and capture) of live animals. The table also includes information about the countries, where they apply or that have promoted, their purposes and the taxa to which they are addressed to.

The European Association of Zoos and Aquaria (EAZA) in 2010 published the 'EAZA guidelines on animal transport'. The EAZA guidelines highlight how the Council Regulation (EC) No 1/2005 refers only to the agricultural industry and not to animals to be held in zoos and aquariums which should

7 CITES includes a total of 183 Parties (182 countries, plus the European Union), and entered into force on the 1st July 1975. In the sixteenth meeting of the Conference of the Parties to CITES (CoP16), significant decisions were taken to bring many new species of precious wild fauna and flora, including commercially valuable marine species, under the Convention (Conf. 10.21 (Rev. CoP16)* Transport of live specimens).

not be intended for trade or commercial purposes. As a consequence different EU Member States 'interpret' in a variable way the Council Regulation, causing a lack of consistency, thus negatively affecting animal welfare (European Association of Zoos and Aquaria, 2010). In order to ameliorate the target of the EU regulation, EAZA promoted a revision of Council Regulation No 1/2005 with the purpose of recognising the species-specific welfare needs of the animals transported between zoos and aquaria, *de facto* combining the existing CITES guidelines with the Live Animal Regulation (LAR) for International Air Transport Association (IATA) and Animal Transportation Association (ATA), by air and by road, rails or sea respectively[8].

Apart from European and national legislations (see Table 1), several recommendations on animal transport have been formulated for various species from organizations such as the NC3Rs (www.nc3rs.org.uk/) or the Laboratory Animal Science Association (LASA; www.lasa.co.uk/). However, only the US Institute for Laboratory Animal Research (ILAR)[9] developed guidelines considering humane transportations of research animals (traditional, agricultural, wildlife and aquatic laboratory vertebrate species), that may be applicable to «wildlife and aquatic species studied in natural settings, or invertebrate animals (e.g., cephalopods) used in research» (National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011). Nevertheless, both legislations and recommendations agree that, transport of animals intended to be utilized in scientific research should respect their welfare state and their biological needs, and attention should be given for proper planning of transport process (see Table 1).

1.1.1 Competence and attitude of the personnel involved in the transportation

A constant in every guidance document and legislation available is the need of proper training for those involved in the capture and transport of wild animals. All of these documents pointed out that the main source of suffering and distress in animals being captured, handled and transported is the limited competence of people involved. In sum, regulatory bodies should ensure that collectors, fishers, transporters and shippers are well-trained for the scope; the team should avail of the support and supervision of a designated veterinarian who has the responsibility to check animals' health status and shall undertake the proper actions to spare or terminate pain. Annex IV of the EU Council Regulation No 1/2005 states that the NCA has to assure that all the attendees (as referred to in Article 6(5) and Article 17(1)) are independent after a successful training and examination (Council of the European Union, 2004). All the technical and administrative aspects of Community legislation concerning the protection of animals during transport, such as animal physiology (e.g., drinking and feeding needs), the behaviour of animals and information around the concept of stress should be included in the training course. Moreover, the specialising personnel shall learn how to handle animals and how to professionally manage emergency situations for both sides. Additionally, transporters should learn how the driving behaviour affects animal welfare, with consequences also on meat quality, if the animals are destined for food consumption (Annex IV of the Council of the European Union, 2004).

1.1.2 Documentation and planning of the journey

As summarized in Table 1 and according to European and national legislations, every shipment requires a meticulous planning of the journey. In addition, it should be accompanied by the proper documentation stating: ***a.*** the origin and the ownership; ***b.*** the place of departure; ***c.*** the date and time of departure; ***d.*** the place of destination; ***e.*** the expected duration of the intended journey (see Chapter II, Art. 4 and documents in Appendix of the Council of the European Union, 2004). Furthermore, competent personnel or scientific committee shall check if the certificate for transporters, the journey duration and the route plans of animal transport follow the European model. The NCA shall take the necessary measures to prevent or reduce any delay during transport to a minimum by programming special arrangements at the place of transfers, exit points and border inspections to give priority to the transport of animals. Resting periods at specific "control posts" are mandatory if the journey is longer (Art. 22(1) of the Council of the European Union, 2004). Furthermore, delivery should be immediate even though sometimes, detainment is required for preserving animal health or public safety, as with potential spread of zoonosis in the case of diseased specimens (Art. 22(2) of the Council of the European Union, 2004).

8 See https://www.iata.org/en/publications/store/live-animals-regulations/ and https://www.animaltransportationassociation.org/

9 www.nationalacademies.org/ilar/institute-for-laboratory-animal-research

Table 1. **Legislation and Recommendations available for Capture and Transport of Animals**. Text in bold highlight the inclusion of Cephalopods among the animal target. For each document, a green tick or a red cross mark, respectively, the presence or lack of information for any specific capture and transport topic. Abbreviations: S - Scientific/Research; C - Commercial/ Trading; P - Public display; NS – Not specified; Lgs – Legislation; Rcm – Recommendation; Gdl – Guidelines.

Document	Country Organisation	Description	Purpose	Animal Target	Capture instructions		Transport instructions							Type of Document
					Competence	Intervention	Competence	Documentation	Planning	Means	Container	Care	Health check	
/609/EEC[(a)20]	EU	Council Directive on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes	S	Any live non-human vertebrate, including free-living larval and/or reproducing larval forms, but excluding foetal or embryonic forms	✓	✓	✓	✓	✗	✗	✗	✓	✓	Lgs
/67/EEC[(b)20]	EU	Council Directive concerning the animal health conditions governing the placing on the market of aquaculture animals and products	C	Any live fish, crustacean or **mollusc** coming from a farm, including those from the wild intended for a farm	✗	✗	✗	✗	✗	✓	✓	✓	✓	Lgs

[20] No longer in force

Table 1. Continued

Document	Country Organisation	Description	Purpose	Animal Target	Capture instructions		Transport instructions							Type of Document
					Competence	Intervention	Competence	Documentation	Planning	Means	Container	Care	Health check	
ETS No.193[c]	EU	European Convention for the Protection of Animals during International Transport (Revised)	C	All vertebrate animals	✗	✗	✓	✓	✓	✓	✓	✓	✓	Lgs
EC No 1/2005[d]	EU	Council Regulation on the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No 1255/97	C	Live vertebrate animals	✗	✗	✓	✓	✓	✓	✓	✓	✓	Lgs
2006 No. 3260[e]	England (similar application in Scotland and North Ireland)	The Welfare of Animals (Transport) (England) Order 2006	C	All vertebrate animals and **cold-blooded invertebrate** animals	✗	✗	✓	✓	✓	✓	✓	✓	✓	Lgs
2007/526/EC[f]	EU	Commission Recommendation on guidelines for the accommodation and care of animals used for experimental and other scientific purposes	S	Rodents, rabbits, dogs, cats, ferrets, non-human primates, farm species, mini-pigs, birds, amphibians, reptiles and fish	✓	✓	✓	✗	✓	✗	✓	✓	✓	Rcm

Table 1. Continued

Document	Country Organisation	Description	Purpose	Animal Target	Capture instructions		Transport instructions							Type of Document
					Competence	Intervention	Competence	Documentation	Planning	Means	Container	Care	Health check	
Directive 2010/63/EU[(g)]	EU	Directive of the EU Parliament and of the Council on the protection of animals used for scientific purposes	S	a) Live non-human vertebrate including independently feeding larval forms and foetal forms of mammals as from the last third of their normal development; b) live cephalopods (from hatching)	✓	✓	✓	✗	✗	✗	✗	✗	✗	Lgs
EAZA Position Statement on Council Regulation 1/2005: Protection of Animals during Transport[(h)]	EAZA	EAZA guidelines on animal transport	P	**All species intended to be accommodated in a zoo or aquarium**	✗	✗	✓	✓	✓	✓	✓	✓	✓	Gdl
NC3Rs' best practice for animal transport[(i)]	NC3Rs (UK)	Best practice for animal transport	S	Rodents, rabbits, ferrets, dogs, cats, nonhuman primates, minipigs and amphibians including Xenopus	✗	✗	✓	✓	✓	✓	✓	✓	✓	Rcm
Guidelines for the Transport of Laboratory Animals[(j)]	LASA (UK)	Report of the Transport Working Group established by the LASA	S	Rodents, rabbits, ferrets, dogs, cats, nonhuman primates, minipigs and amphibians including Xenopus	✗	✗	✓	✓	✓	✓	✓	✓	✓	Gdl

Table 1. Continued

Document	Country Organisation	Description	Purpose	Animal Target	Capture instructions		Transport instructions							Type of Document
					Competence	Intervention	Competence	Documentation	Planning	Means	Container	Care	Health check	
Conf. 10.21 (Rev. CoP16)[(k)]	CITES	CITES guidelines for transport of live wild animals and plants	NS	All the animals listed Appendices I, II & III (28/08/2020) - **including cephalopods -**	✗	✗	✓	✓	✓	✓	✓	✓	✓	Gd
Packer's Guidelines Inv1/Aquatic invertebrates[(l)]	CITES	CITES guidelines for aquatic invertebrates	NS	**Aquatic invertebrates**	✗	✗	✓	✓	✓	✓	✓	✓	✓	Gdl
Aquatic Animal Health Code[(m)]	OIE	Control of aquatic animal health risk associated with transport of aquatic animals	S, C	Fish, **Molluscs** and Crustaceans listed in LAR	✗	✗	✓	✓	✓	✓	✓	✓	✓	Rcm
Guide for the Care and Use of Laboratory Animals[(n)]	ILAR (US)	Guide for the Care and Use of Laboratory Animals	S	All the traditional, agricultural, wildlife and aquatic laboratory vertebrate species. **Could be applied to invertebrate animals (e.g., cephalopods)**	✗	✗	✓	✓	✓	✗	✗	✓	✓	Ggl
Australian code for the care and use of animals for scientific purposes 8th Edition 2013[(o)]	Australia	Australian code for the care and use of animals for scientific purposes	S	Any live non-human vertebrate (including purpose-bred animals, livestock, wildlife) and **cephalopods**	✓	✓	✓	✓	✓	✗	✓	✓	✓	Lgs
Olfert et al., 1993[(p)]	CCAC (Canada)	Guide to the care and use of experimental animals	S	Any non-human vertebrate or a **cephalopod** used for scientific research	✓	✓	✓	✓	✓	✗	✓	✓	✓	Gdl

Table 1. Notes

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l. Convention on International Trade in Endangered Species (1979). *Packer's Guidelines Inv/1 – Aquatic invertebrates*. https://cites.org/eng/resources/transport/inv1.shtml.

m. Aquatic Animals Commission (2019). "Control of aquatic animal health risks associated with transport of aquatic animals," in Aquatic Animal Health Code, ed. Aquatic Animals Commission. (Paris: OIE, World Organization for Animal Health), 4.

n. National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals (2011). "The National Academies Collection: Reports funded by National Institutes of Health," in Guide for the Care and Use of Laboratory Animals. (Washington (DC): National Academies Press (US) Copyright © 2011, National Academy of Sciences.).

o. National Health and Medical Research Council (2013). Australian code for the care and use of animals for scientific purposes 8th Edition. Canberra: National Health and Medical Research Council.

p. Olfert, E.D., Cross, B.M., and McWilliam, A.A. (1993). Guide to the care and use of experimental animals. Canadian Council on Animal Care Ottawa, 1-298.

For this reason, checks at border inspection by veterinarians shall include the assessment of the welfare conditions throughout the transportation period. Additional documentation providing instructions about feeding and any special care may be required when transporting wild, timid or dangerous species (see Chapter II, Section 1.3 of the Council of the European Union, 2004).
A journey log is recommended as part of the planning strategy to report relevant details such as animals' health status, any intervention performed and any eventual detour from the original plan (see Annex II of the Council of the European Union, 2004). As for the regulation of the international transport of animals, the aforementioned ETS No.193 (Council of Europe, 2003) applies.

EU legislation and other documents (i.e. ETS No.193 and Council Recommendation 2007/526/EC) specifically address experimental animals and inspired LASA to produce a WG Report ('Guidance on the transport of laboratory animals') listing all the documentation required prior transportation (see Paragrah 3.3 of Swallow et al., 2005). The proper documents to fill in are related to the journey type, species, health status and route.

The following information should never be missing:

i. shipment documentation details such as waybill number or IATA Shipper's certificate (for Air transport), import licences issued by the State Veterinary Service, CITES permits where necessary (for intra-European and Third-country shipping), invoices for Customs purposes, health certificate of the animal transported signed by the Designated Veterinarian, journey log or transfer authorisations from specific bodies that regulate laboratory animals' use;

ii. animal details such as species, strain, scientific name, number, sex, age, weight, identification numbers or any special requirements resulting from phenotype;

iii. personnel details such as contact information of sender, intermediaries, consignee, shipper/carrier, veterinarian;

iv. crates with date and times the animals were packed loaded, and departed with clear 'Live animals' and orientation arrows labels;

v. expected events, such as proposed and actual rest periods, pre-journey review of plan by consignor and post-journey review of plan by new owner (Swallow et al., 2005).

1.1.3 Means of transport and container design

As detailed in Chapter II of the EC No 1/2005 (see also other legislations and recommendations, Table 1), provisions for all[10] «means of transport, containers and their fittings shall be designed, constructed, maintained and operated so as to: (a) avoid injury and suffering and to ensure the safety of the animals; (b) protect the animals from inclement weather, extreme temperatures and adverse changes in climatic conditions; (c) be cleaned and disinfected; (d) prevent the animals from escaping or falling out and be able to withstand the stresses of movements [e.g., for aquatic species it shall be avoided water spilling; *Note of Authors*]; (e) ensure that air quality and quantity appropriate to the species transported can be maintained; (f) provide access to the animals to allow them to be inspected and cared for; (g) *present a flooring surface that is anti-slip*; (h) *present a flooring surface that minimises the leakage of urine or faeces*; (i) provide a means of lighting sufficient for inspection and care of the animals during transport» and in addition, allow enough space inside the compartment to allow animals to adopt adequate posture « without on any account hindering their natural movement for movements» (Annex I: Technical Rules - Chapter II, Council of the European Union, 2004).

Furthermore, it is recommended that any animal that is not fitting for the container or the means of transport should not be transported under those conditions. Containers in which animals are transported «shall be clearly and visibly marked, indicating the presence of live animals and with a sign indicating the top of the container» and «shall be secured so as to prevent displacement due to the movement of the means of transport» (ibid., Council of the European Union, 2004).

Sections 2-5 of the Annex I (Chapter II) of the Council Regulation (EC) No 1/2005 provide more specific instructions for a chosen mean of transport, including transportation by road, rail, roll-on-roll-off vessel or by air (which has to be performed in accordance to the international agreements; see IATA and ATA LAR codes) and to the legislation of the country of destination (Council of the European Union, 2004).

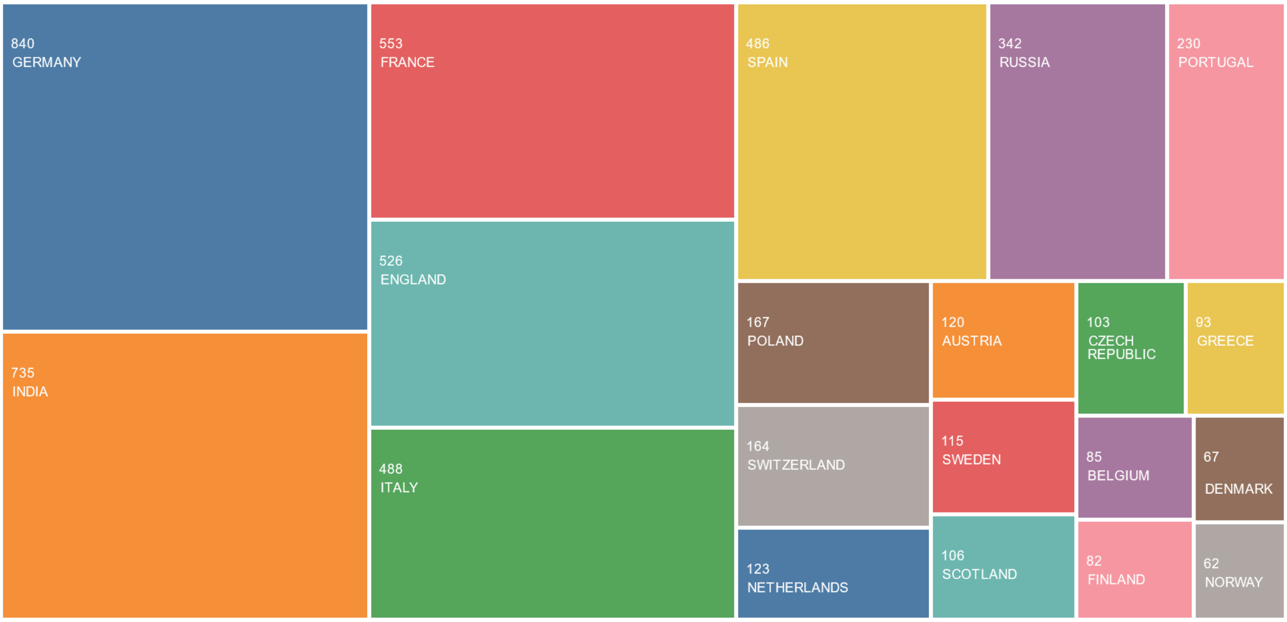
10 In italics those non strictly applicable to aquatic animals.

1.1.4 Health check, food and water supplies

Recommendations include the checking of animals transported at suitable intervals, and provided with food and rest according to the species, size and conditions of the specimen. The welfare status should be assessed by a veterinarian before starting (in order to assess if the animals are suitable for the transportation; see Fitness for transport in Annex I - Chapter I,Council of the European Union, 2004), and during the journey to check if they have been injured, harmed and stressed during the unloading phase. In the cases of need a rapid intervention should be carried out by competent and trained personnel (Art. 3 of the Council of the European Union, 2004).

1.1.5 Acclimatisation before and after transport of wild animals

Especially in the case of wild animals, legislative and other documents of reference include recommendations of allowing individual animals to become acclimatised to the mode of transport prior to the proposed journey. Capture and handling of wild and feral animals has to be humane and performed exclusively by experienced and well-trained persons (Art. 9.3 of the European Parliament and Council of the European Union, 2010). Special considerations should be given to the acclimatisation, quarantine, housing, husbandry and care of wild caught animals. In addition, particular attention should be given in order to establish in advance the actions to be undertaken on wild caught animals after the end of the scientific procedures (i.e. before these begin), in order to ensure that the practical difficulties and welfare issues associated with any subsequent release to



the wild can be satisfactorily addressed (see Annex III, Section 3.2 of the European Parliament and Council of the European Union, 2010).

1.2 Capture and Transport of Cephalopods – a Legislative framework

Since implementation of the Directive 2010/63/EU in 2013, no gold standard method has been suggested to capture wild cephalopods destined for scientific research. Nevertheless, the remarkable cognitive abilities and complex behavioural repertoire exhibited by these animals (for review see e.g., Edelman and Seth, 2009; Amodio and Fiorito, 2013; Tricarico et al., 2014; Marini et al., 2017; Amodio et al., 2019; Ponte et al., 2019; Birch et al., 2020; Birch et al., 2021; Schnell et al., 2021), have recently increased the interest in studying these invertebrates all over Europe (Figure 1). Concurrently, the focus on the welfare of cephalopods has increased both in the commercial and scientific field with all its implications on the quality of the scientific data produced.

Figure 1. Cephalopods as Laboratory Animals: current dimension in Europe. Number of scientific papers regarding cephalopods (any field) with affiliations in European Institutions (n = 4,511); time span: 2015-2021 (Source: Clarivate-Web of Science, last update August 2022).

As seen above, very little information is available about standardised methods of capture and transport of aquatic animals and of cephalopods.

The Council Directive 91/67/EEC (on health conditions of aquaculture animals to be placed on the market, *no longer in force*) generally stated the need for aquaculture animals to be dispatched in the shortest possible period to the place of destination, using means of transport that have been cleaned and, if necessary, disinfected in advance (Council of the European Union and Council of the European Communities, 1991b). Other recommendations include ***i.*** design of vehicles to avoid any leakage from the container during the journey (since water is used in overland transport), ***ii.*** safeguarding the health of the animals, also considering renewing the water (in regulated facilities) in a manner that respects the its hygienic properties thus not affecting the health of the species transported (contaminations and infections; Annex D of the Council of the European Union and Council of the European Communities, 1991a). However, following European legislations regulating the transport of animals for commercial or experimental purposes, focused only on vertebrate species, mainly terrestrial animals. By introducing the Welfare of Animals (Transport) (England) Order 2006, the United Kingdom included all the "cold-blooded invertebrates" among animals the EC No 1/2005 statements should be extended to (UK Statutory Instruments and the Secretary of State, 2006).

Regarding transport of wildlife, both OIE and CITES consider all animals listed in the LAR, including cephalopods (Convention on International Trade in Endangered Species, 2020). In addition, CITES "Packer's guidelines"[11] for aquatic invertebrates includes cephalopods, with detailed instructions about their general welfare, the arrangements of transport and shipment as well as the design of the container. Furthermore, other countries have produced guidelines for the procurement, care, and use of animals for scientific purposes.

ILAR provided exhaustive guidelines on capture and transport mainly conceived for vertebrates to be employed under laboratory settings, but these might be further applied with the proper adjustments to cephalopods as well (see "Applicability and Goals" of National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011). The Canadian Council of Animal Care (CCAC), since 1993 refers in its welfare acts to any non-human vertebrates and cephalopods (Olfert et al., 1993) including guidelines on procurement and transportation of purpose-bred animals and wildlife (Canadian Council on Animal Care, 2003; 2007); in this case no specific mention to cephalopod molluscs is found. The Australian legislation imposes great attention in avoiding/minimise the risk of injury or stress-induced diseases during the capture and transport of animals, including cephalopods for scientific scopes. According to the Australian law, capture and handling of wildlife (cephalopods included) must concern: «***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 purpose and aims of the project or activity; ***iv.*** making provisions for captured animals that are ill or injured, including treatment of pain and distress» (National Health and Medical Research Council, 2013).

Despite the European Directives and various guidelines mentioned above, the lack of standardisation regarding capture and transport of wild cephalopod species, both at general and species-specific level is undisputable. One possible reason is the limited availability of scientific data on species-specific biological requirements (i.e. need, *sensu* Bracke et al., 1999a; b; Bracke et al., 1999c) for these molluscs. An attempt to develop a species-specific description and indicators of the state of need has been promoted by the COST Action FA1301 and initiated in a series of contributions (Cooke et al., 2019; De Sio et al., 2020)[12]. Finally, it is worth to remind that general considerations on capture and transport of cephalopod species for research purposes are included in the FELASA Guidelines for the Care and Welfare of Cephalopods in Research (Fiorito et al., 2015) which will be therefore used by this WG as an additional basis of analysis.

11 https://cites.org/eng/resources/transport/inv1.shtml

12 See Cephs*In*Action Working Group 4 - Cephalopod Welfare and CWI database: http://www.cephsinaction.org/working-groups/working-group-4/wg4-cwi-database/

2. Maximising welfare during the Capture of live wild cephalopods

The majority of cephalopods are not bred on purpose, but they need to be captured directly from the wild.
According to the General Section 4.2 of the Commission Recommendation 2007/526/EC:

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 (Commission of the European Communities, 2007). Furthermore, in the case of serious injury, «the animal should be killed immediately by a humane method» considering those described in the Directive 2010/63/EU (European Parliament and Council of the European Union, 2010); and
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» (Commission of the European Communities, 2007).

How shall we comply with these statements when dealing with cephalopods?

There is not an exclusive method to capture all cephalopods but rather there should be a defined set of suitable techniques that best fits species-specific needs by also taking into account their physiology and inter-individual variability. Therefore, in the following pages we will illustrate capture "protocols" that should be accompanied, when adopted, by the associated severity assessment in order to possibly classify how well they address the question of animal welfare when performed on wild cephalopods.

2.1 Live cephalopods or eggs?

A solution to the use of wild animals in research and for the standardisation of capture and transport conditions might be the use and sharing of cephalopod eggs as these appear easier to manage during both collection and transportation (see also ancillary work, Sykes et al., 2022).

Moving embryos, sperm or eggs of other laboratory vertebrate species between research facilities has become a quite common practice and it can be considered part of the refinement principle, since transport could impact mature animal welfare, while early life forms might result unaffected. Besides, the use of captive animals raised from eggs would fulfil some experimental requirements, such as the need for subjects without previous experiences. Conversely, the use of wildlife would comply with the demand of animals that have been 'primed' by natural environmental stimuli.

Indeed, consideration should be taken to offer a method of cephalopod egg collection that should minimise the impact and exploitation over natural resources (e.g., collecting those stranded on the beach or those captured by the fishing gears) and should prevent damaging egg masses as these require care during handling, temperature and salinity control throughout the transportation. Additionally, eggs collected in their latest developmental stages may undergo premature hatching during transport and handling due to their higher metabolism (Boletzky, 1989; Villanueva et al., 2016), and for some cephalopod species, maternal care is needed to develop in a healthy way.

Despite the great challenge it represents, approaches for egg transport of some cuttlefish, squid and octopus species have been proposed and methods or protocols tested, achieving few degrees of standardization (Grimpe, 1928; Jones et al., 2009; Deryckere et al., 2020)[13]; for example see the section 'Rearing cephalopods from the egg' by Grimpe (1928) [see the English translation in the Supplementary Info by De Sio et al. (2020)].

2.2 Fishing: what can we learn from it?

The significant interest in cephalopods as a food resource is underlined by the fishing commercial statistic on landings, which currently report cephalopods as animals accounting for about 5% of the marine capture volume worldwide (GLOBEFISH, 2016), despite the reduction in demand caused by the COVID-19 outbreak[14]. Hence, given their remarkable economic value, the majority of capture

13 for English translation of Grimpe see De Sio et al. (2020).
14 for more info: http://www.fao.org/in-action/globefish/market-reports/cephalopods/en/

methods known and mentioned for research purposes were conceived and implemented mainly by artisanal and small-scale fishers (Santarelli, 1932). Many reports on cephalopods fishing have been published, such as those by FAO (Food and Agriculture Organisation), but no comprehensive summary of the numerous cephalopod capture methods (but see Boyle and Rodhouse, 2005) is currently available for specific target species and at different time of their development (Jereb and Roper, 2006; Jereb and Roper, 2010; Roper et al., 2016).

Rathjen (1991) in his overview about the most used existing capture methods highlighted the need for valid species-specific cephalopod fishing techniques. Although he referred to commercial and trade purposes, he pointed out the need for what he defined a 'more resource-friendly' fishing method. For instance, in his publication Rathjen reported trawling as a widely employed technique for fishing cephalopods but the risk of by-catch is very high and is not species-specific, failing to take into account size, physiology and needs of its cephalopod target. The Author also reported line jigging as likely the most suitable gear for squids (*Loligo forbesii, Illex argentines, Todarodes pacificus, Nototodarus sloani*). This technique is adjustable for the size of the animals and is selective, thus avoiding the destruction of the environment or other fauna.

Traps and pots for cephalopods are utilized in many geographical areas and most often represent traditional gears for fishing these animals. Such methods are based on the knowledge that coleoid species, like octopuses, display a natural tendency to lurk in dens and hidden refuges (e.g., Kayes, 1973; Mather, 1988). Other methods involve spearing, multiple hooks and trolling of these animals. Up to date, these methods are still used, with some geographical adaptations (Pierce et al., 2010; Pascual-Fernández et al., 2020; Pita et al., 2021) and have been accompanied by relatively small innovation, with the exception of aquaculture-like on growing by collecting cephalopods in proper container located by the shore (Vidal et al., 2014).

We believe the central question to which the scientific community should answer is whether we can borrow and adjust some of the currently available fishing methods and render them suitable for collecting live wild cephalopods employed in research.

2.3 Review of existing Capture methods for collecting live cephalopods in the wild

In this review the capture methods utilized for wild-caught cephalopods is deduced from the analysis of research papers as summarised in Table 2. A list of taxon-specific recommended capture methods is available in the WG Report ancillary to this work (see table 1 in Sykes et al., 2022).

From our analysis considerable aspects emerged:

- ***i.*** all the capture methods included in the original studies have been borrowed from fishery and eventually readapted to suit scientific purposes;
- ***ii.*** there is no species-specific technique, but rather a bunch of approaches/variations for the same method;
- ***iii.*** no particular attention is given to the cephalopods life stage under study, and this piece of information is partially or completely missing;
- ***iv.*** very little is provided by the Authors about the capture and transport procedures utilized, and in most cases only one of the two is briefly mentioned;
- ***v.*** publications often provide a list of recommendations mainly based on personal experiences or indirect communications and as such it should further be addressed by properly designed experiments.

A very common and spread method for catching live cephalopods is trawl; this is a commercial gear readily available from fisheries and able to provide great supply for many laboratories. Many studies on aquarium-maintained octopuses (e.g., Mangold and Boletzky, 1973; Boyle, 1981), sepioids (von Boletzky et al., 1971) and squid (Hulet et al., 1979; Lipiński, 1985; Hanlon, 1990) have been based on trawl-caught specimens. However, if we take into account how trawling works, it is clear this might not be a suitable method for research purposes.

Cephalopods are delicate, soft-bodied animals, easily damaged by the mechanical abrasion and insult potentially induced by trawls. Moreover, it is a non-selective method; thus, representing a serious concern for both the welfare of the by-catch organisms and the marine environmental context.

Line jigs or hand jigs are also used but depending on material, structure and position, their efficacy in terms of uninjured animals caught change.

The same applies to nets. These are of many kinds and respond to different fishing needs, but only a few have been borrowed by researchers - such as seine nets and dip nets, which seem to be less traumatic for the target cephalopods (Boyle, 1981; Boletzky and Hanlon, 1983; Gonçalves et al., 2009). Some studies have reported the combined use of hand nets and scuba diving (Table 2; see Budelmann, 2010; Zúñiga et al., 2011; Kawashima et al., 2019).

This method is preferable if the collector has great competence and knowledge about the anatomy and physiology of the animals. Though, if the required number and size of the experimental subjects to collect is large, this might not be the best procedure to procure animals.

Thus, it is clear that to promote cephalopods welfare and consequently to produce excellent science, a reasonable trade-off between the most rapid and efficient method should be reached. It is therefore our aim, in accordance to the scientific evidence acquired so far, to set the stage and the boundaries of this essential compromise.

Below we overview the most used capture methods for *Nautilus*, cuttlefish, squid and octopus, in an attempt to figure out which are the most suitable and atraumatic techniques for collecting cephalopods while protecting their welfare state.

2.3.1 Capture methods for nautiluses

Nautilus macromphalus and *N. pompilius* are species considered of commercial value as food mainly in Indonesia, Fiji, New Caledonia, and the Philippines (Jereb and Roper, 2006). However, unlike coleoid cephalopod species, nautiluses are not frequently sought-after for food consumption, but rather for the collection of their shells or, more frequently, for rearing them in aquaria.

N. macromphalus was the first (in 1958) living nautilus to be maintained in a public aquarium (Catala, 1964), but since then many aquaria around the world started to rear these species so that a proper catching protocol without inflicting damage or distress was soon needed. Several decades afterwards, Bruce A. Carlson published a guide for the collection and aquarium maintenance of *Nautilus* in which detailed description of the capture method for this taxon is included (Carlson, 1991). All the recommended traps represent a variant of the traditional fishing bamboo traps used in the Philippines - first described by Dean (1901) - and consisting of four hoops (1 m in diameter), made from six fencing wire spaced covered with chicken wire so as to make a cylindrical trap 2 m in length (Carlson, 1991). At each end there is a funnel with tips pointing inward while a 0.5 m screen baffle is placed midway to avoid nautiluses escape. A steel reinforcing bar on the top of the trap provides rigidity and some weights are secured at the bottom so that the traps remain set. At the centre of the trap there is a suspended bait trapped in chicken wire in order to avoid its consumption and so the production of toxic waste material (Carlson, 1991). As an effective variant to the bait light as an attractant for these animals has been suggested by Muntz (1994). The location and secure of the trapping technique depend on the water depth and on the means used.

As summarized in Table 2, collectors and researchers collectively agree baited traps are the appropriate method for capturing live wild nautiluses and besides the possibility of introducing slight variations - including modern ways for monitoring the trap position or the use of materials that are able to last more (see table 1 in Sykes et al., 2022) - the prototype provided by Carlson appears suitable for the purpose and is still applied (e.g., Dunstan et al., 2011).

2.3.1 Capture methods for cuttlefish and sepiolids

Information about the capture methods of cuttlefishes for scientific research is missing; in the great majority of cases the available literature is based on the culture of these animals from egg collection (Anil et al., 2005; Chacko and Patterson, 2010; Ferreira et al., 2010; Yasumuro and Ikeda, 2016).

Cuttlefishes are considered highly adaptable to life in captivity because of their large eggs and their high hatching survival, when reared and maintained in aquaria in both Europe and North America (Richard, 1971; Pascual, 1978; Boletzky and Hanlon, 1983; Forsythe et al., 1994; Lee et al., 1998; Domingues et al., 2001).

The methods reported in the studies that used collecting live wild animals (Table 2), live cuttlefishes and sepiolids resulted caught by bottom trawl with net retrieval and by careful sorting of catch, identifying animals with minimal skin damage (Boletzky and Hanlon, 1983; Nabhitabhata et al., 2005). As pointed out before, this method - frequently used by industrial fisheries - has to be avoided considering animal welfare and environmental concerns that it raises.

More feasible and recommended are traps and nets that, similarly to what occurs for squids, seem to be able to allow a correct capture of uninjured juvenile and adult cuttlefishes (Jones and McCarthy, 2009; Vidal et al., 2014). Very often, squid traps are employed but cuttlefish traps have also been produced, as larger and lighter than the squid ones.

As described by Pereira et al. (2019), cuttlefish traps are generally rectangular shaped with a steel frame covered with a plastic mesh. A funnel entrance is located in the smaller side and an opening lid is present on the top, allowing the removal of the catches. Cuttlefish traps are individually set with a line connected with a buoy at the sea surface, while they are anchored with flat stones that reduce the movement on the seafloor (Pereira et al., 2019). Usually, as in the artisanal fisheries, the use of much more selective gears may be accompanied by light or seabed as an attractive spawning substrate for adult females, mainly captured as broodstock for aquaculture purposes (Watanuki and Kawamura, 1999; Watanuki et al., 2000). Fishing harpoons, spears, and line jigs are not recommended for such delicate animals.

Very little information is available from the fishing methods of sepiolidae which are caught mainly as trawl by-catch (Jereb and Roper, 2006). Nets, and in particular, seine nets are mainly employed for adult sepiolids, selected for laboratory studies. These are considered as the less traumatic methods for these little-sized cephalopods (Montgomery and McFall-Ngai, 1993; Nabhitabhata and Nishiguchi, 2014).

2.3.2 Capture methods for squid

Bottom and pelagic trawls are widely spread in squid fisheries in different parts of the world (Rathjen, 1991; Jereb and Roper, 2010; Pierce et al., 2010; Lishchenko et al., 2021). These techniques have been reported for wild-caught squids intended to be maintained in aquarium or laboratory conditions (Walker et al., 1970; LaRoe, 1971; Balch et al., 1985; Lipiński, 1985; DeRusha et al., 1987; Gonçalves et al., 2009; Promboon et al., 2011). Nevertheless, as often self-reported by the Authors, there is a subsequent hand-selection of the animals remained uninjured among the capture volume (Ford et al., 1986; Durholtz and Lipinski, 2000; Nabhitabhata et al., 2005; Buresch et al., 2009; Sakai et al., 2011; Kaplan et al., 2013; Zakroff et al., 2018), highlighting again the unsustainability of this method.

Several kinds of nets are employed for capturing squids for laboratory use, such as pound nets, seine, dip nets and hand nets. All have been demonstrated to be harmless for these animals if properly used by trained hands. Many researchers considered these tools as efficient means for capturing wild squids, since most of the time are free to swim until they are collected and hence report minimal damage (Hanlon et al., 1983; Chabala et al., 1986; Boyle, 1991). In any case, attention must be paid to the by-catch of egg masses that, if not damaged by the impact on the net, should be reinserted in nature.

The method considered as the most suitable for capturing the squids without causing them skin or fin damages is undoubtedly the jig (Boletzky and Hanlon, 1983; Moltschaniwskyj et al., 2007; Gonçalves et al., 2009; Vidal et al., 2014). According to Boyle, the best tool is the jigged lure with barbless hooks operated mechanically or by hand (Matsumoto, 1976; Boyle, 1991). Some studies report the use of light lures attached to jig-lines to be effective in attracting these cephalopods

(Toyofuku and Wada, 2018). Guidelines for the Care and Welfare of Cephalopods in Research - and based on published evidences - recommended jigs as methods for capturing the majority of squids (Fiorito et al., 2015). However, as can be seen from Table 2, the target species are heterogeneous, therefore attention should be paid to eventually adapt the method to a given species. Therefore, as pointed out by Fiorito et al. (2015), there is the need for further assessment before extending their use to all squid species.

For example, studies conducted on *Doryteuthis opalescens* (Perretti et al., 2016) and *Todarodes pacificus* (Flores et al., 1976) caught by jigging reported some injury to the animals. Furthermore, Cabanellas-Reboredo and colleagues proved - by a simulation on captive squids - some detrimental effects of handline jigging on the health and predatory behaviour of *Loligo vulgaris* and discussed about the possibility that this capture method may result in considerable damage and lasting harm in the animals (Cabanellas-Reboredo et al., 2011).

There are also studies reporting the use of size-selective box traps and trap nets that consider the size, age and genus of the target squids (O'Dor et al., 1977; Balch et al., 1985; Dawe et al., 1985; Puneeta et al., 2015); these might turn out to be more effective and atraumatic for catching unharmed squids (see table 1 in Sykes et al., 2022). Traps slightly vary in size, shape and composition, according to the geographical position or to the needs but, are generally made of natural materials (e.g., bamboo fibres) or non-toxic plastic. Traps can be very similar to octopus pots, they provide a shelter that looks alluring, especially for spawning females (O'Dor et al., 1977), with usually a top hole from which the animal can spontaneously enter. The main difference with the pots is that once inside, the cephalopods get trapped in. To maximise the welfare state of the animals during transportation, it is recommended to use baits wrapped in non-palatable non-toxic material, as to prevent the food consumption that could induce a build-up of toxic ammonia wastes in the containers during the trip to the laboratory (see Section 3 below). Light attractant might be used in substitution to food instead (Thorrold, 1992).

2.3.3 Capture methods for octopuses

A paper published in the Bulletin of the Oceanographic Institute by Santarelli and dated back to 1932 reported a series of fishing methods specifically for *Octopus vulgaris*, used by the local fishermen in the Gulf of Naples (Mediterranean Sea). In general, apart from general consideration about the 'quality' of live speciments, little consideration is taken for potential impacts on animal welfare. Among the methods Santarelli listed the "pesca a fuoco" or "a cacatrapene"[15] – both characterised by the additional use of harpoons or hooked gears. Most of the capture methods listed therein seem adequate for their applicability in scientific research. Here we list the "lancelle" - positioned at 3-5 m deep in which the octopus got caught -, the pots or similarly the "nasse", i.e. cane pots filled with rocks and baits in order to attract and catch the animals (Santarelli, 1932).

Octopuses are still a popular food source for humans around the world, and they are harvested in a range of fisheries from subsistence catches through to valuable, large-scale commercial fisheries. As mentioned in Section 2.1, diverse techniques are used to capture octopuses, ranging from small-scale subsistence and artisanal harvests to large-scale commercial fisheries. According to FAO, the primary techniques employed are: ***1.*** direct capture by hand, hook, or spear; ***2.*** line capture (using lures and/or baits); ***3.*** use of weighted pots (unbaited or baited); and ***4.*** use of nets, including trawls (e.g. otter, seine, beam), cast, and static nets (e.g. fyke). In many regions of the world, hand, line, and cast net capture can include the use of lights at night to harvest nocturnally active species (Roper et al., 2016).

One very recent review of the fishing methods for *O. vulgaris* in Europe reports traps and pots as the most used techniques (Pita et al., 2021). This is due to the fact that, although in Europe cephalopods fisheries are not included in the total allowable catch and quota regulations under the scope of the common fisheries policy, the antiquity of the exploitation and the economic importance of these animals brought a series of legislations in Mediterranean countries setting gear and license limitations in order to avoid overfishing (Pita et al., 2021).

15 '***Pesca a fuoco***' (fishing with fire) required the use of lamplight to catch octopuses at night. With the help of a feather, fishermen spread the water surface with oil making it more transparent and by using an acetylene lamp they were able to see clearer octopuses and hit them with a harpoon. '***Pesca a cacatrapene***' employed 'cacatrapene', i.e. a piece of white rock tied with a 40 m-canapé rope with 4-5 crustaceans secured on it. Once baited, the octopuses were caught with hand nets or hooks.

Traps and more effectively pots, are very successful methods for octopuses and do not seem to be dangerous for the animal as they are generally made of non-toxic materials or with non-abrasive surfaces. Because of the natural tendency for octopuses to search for a den they spontaneously settle in the pots, which, when lifted every 2-3 days, are very likely to catch undamaged specimens. For such reasons, many published works choose and/or recommend these two capture methods also for wild octopuses intended to be used for research purposes (e.g., Boyle, 1991; Moltschaniwskyj et al., 2007; Vidal et al., 2014; Fiorito et al., 2015). Carreira and Gonçalves tested for the first time in the Atlantic Sea the so called Japanese baited pots (JBPs) as a capture tool for *O. vulgaris*. These traps combine two alluring features for octopuses, a shelter and a bait. The trap (see figure 1 of Carreira and Gonçalves, 2009) is a box, inside which a crab is tied to a string; when the animal enters, the box closes and it remains inside. The Authors proved JBPs to be very specific for the octopus and also this method resulted sustainable in that about 25% of the total catch was of legal marketable size and the animals not suited can be brought back to the sea in a good status while preventing by-catches (Carreira and Gonçalves, 2009).

Once proved to be the less traumatic method for catching these animals, it is important to improve the quality of pots to efficiently obtain a healthy sample size. Borges and colleagues assessed octopuses' preference by comparing the traditional Portuguese amphora-shaped clay pots used by local fishermen (the 'alcatruz') with two other types of pots: a plastic cylindrical pot and a newly designed amphora-shaped plastic pot (see figure 1 of Borges et al., 2015). No preference for the material was observed, confirming the ability of these animals to adapt to a natural environment contaminated by human waste in the sea (e.g., Pedà et al., 2022).

A particular preference for the amphora-shaped pots over the cylindrical one was observed instead (Borges et al., 2015). The reason could be found in the narrower entrance and the wider inner volume of the newly designed pots, together with the settling angle (8.6L volume and 30° settling angle for the new pot *versus* 7L volume and approximately 0° settling angle for the cylindrical pot) that possibly allows the animal to observe the external surroundings while remaining sheltered in a bigger space (Borges et al., 2015). For what concerns the colour preference there was a clear choice for the black pots when compared with the redbrick or the white ones, a finding in line with previous literature reporting octopuses' preference for more elusive darker tones (Messenger and Sanders, 1972; Fiorito and Scotto, 1992; Okamoto et al., 2001; Borrelli et al., 2020).

Also baited traps are among the most used type of capture techniques that seem to be quite atraumatic for octopuses which - again spontaneously - enter the structure and remains unharmed but trapped inside. As shown in Table 2 the species caught by this method are quite heterogeneous, making it suitable - with the right refinements - for more target laboratory organisms.

An interesting number of studies uses scuba diving for catching octopus often combined with attractive lights, hand net or less frequently with anaesthetics (Smale and Buchan, 1981; Kawashima et al., 2019). Nevertheless, as previously mentioned, scuba diving requires much more time and effort for obtaining the desired sample size, and it appears suitable for small size specimens and limited ad hoc sampling. In any case, narcosis and consequent welfare impairment should be avoided, unless verified that no additional harm and stress are provided to the animals.

Summing up, pots seem to be the best method for maximising octopus welfare by atraumatic catch (see table 1 in Sykes et al., 2022). A series of adjustments are currently being studied in order to make them more alluring as shelter in order to procure experimental animals and possibly to ease the subsequent transport procedure[16]. The application of a removable door or a sock could be implemented to prevent the animals from escaping during the collection with the possibility of opening it allowing inspection of water level and welfare monitoring while transporting the animals. However, since these dens might be a suitable place for female benthic octopuses to attach and grow egg masses (Garci et al., 2016), the pots containing brooding females and eggs, should be avoided (unless required from the specific experiment) for ethical and conservation issues.

A consistent capture of cephalopods should be possible by setting a number of pots and traps that might be identified and thus monitored via GPS sensors.

Any other physical damage associated with pots or traps are not yet been investigated as for example, the effect of the difference in pressure that might arise from moving the cephalopod from a depth of more than 10 m up to the surface, but it seems to be related to the speed of collection (e.g., Rudershausen, 2013).

16 see https://www.occotech.com.au/ - pots designed exclusively for octopus and very similar to the aforementioned JBPs described in Carreira and Gonçalves (2009).

Table 2. **Capture Methods of cephalopods for Scientific Purposes**. Species are listed following FAO indications for Order and WoRMS for Genus. List of abbreviations utilized - Life stages: Ad – Adults; sAd - sub-adults; Hatch - Hatchlings; Juv - Juvenile; Other: NA – Not available; ND – Not determinable; NS - Not specified; Sources: Exp - Experimental; Gdl - Guidelines; Bk - Book Chapter; hBk - Handbook; Rev - Review; M – Male; F - Female; BW - Body Weight.
BW - Body Weight.

Taxon	Methods	Species	Life stage	Additional information	References	Type of article
Nautilus	Traps	*Nautilus spp*	NS	Cylindrical and baited traps	Carlson (1991)	Gdl
		Nautilus pompilius	0.002% Juv, 42% sAd, 58% Ad	NA	Dunstan et al. (2011)	Exp
			NS	NA	O'Dor et al. (1990)	Exp
			NS	Baited trap	Oba et al. (1992)	Exp
			10-15 cm (ventral shell ø) assumed Ad	V- or Z-shaped baited traps with light	Muntz (1994)	Exp
			NS	Baited trap	Linzmeier et al. (2016)	Exp
		Nautilus macromphalus	Ad	Baited trap	Uchiyama and Tanabe (1999)	Exp
Cuttlefish	Trawl	*Sepia officinalis*	Ad	NA	Koueta and Boucaud-Camou (2003)	Exp
	Traps	*Sepia officinalis*	Ad	Cuttlefish traps	O'Brien et al. (2018)	Exp
			Ad	Cuttlefish traps	Jones and McCarthy (2009)	Bk
			Ad	Basket traps	Solé et al. (2019)	Exp

Table 2. Continued

Taxon	Methods	Species	Life stage	Additional information	References	Type of article
Cuttlefish	Traps		Ad	Basket traps	Watanuki and Kawamura (1999)	Rev
			Ad	Cuttlefish traps. Rectangular with a steel frame covered with 35 mm square plastic mesh, funnel entrance on a side and opening "door" on top to remove the catches. Bushes as spawning substrata. Individually set with a line connected to a buoy and anchored with flat stones for sinking the gear and reduce its movements	Pereira et al. (2019)	Exp
		Sepia esculenta	Ad	Basket traps	Watanuki et al. (2000)	Rev
		Sepia pharaonis	Ad	Basket traps	Watanuki and Kawamura (1999)	Rev
			Ad	Squid traps	Nabhitabhata and Nilaphat (1999)	Exp
		Sepia spp.	NS	Basket traps	Moltschaniwskyj et al. (2007)	Rev
	Jig	*Sepia pharaonis*	Ad	Squid jigs	Deepak and Patterson (2011)	Exp
	Nets	*Sepia officinalis*	Ad	Trammel nets	Şen (2013)	Exp
			Ad	NA	Carere et al. (2015)	Exp
Sepiolids	Trawl	*Euprymna hyllebergi*	Ad	Otter trawl and beam trawl	Nabhitabhata et al. (2005)	Exp
			Ad	Board trawlers and beam trawlers	Nabhitabhata and Nishiguchi (2014)	Bk

Table 2. Continued

Taxon	Methods	Species	Life stage	Additional information	References	Type of article
Sepiolids		*Sepiolidae*	Ad	Bottom trawl	Boletzky and Hanlon (1983)	Rev
	Scuba diving	*Euprymna parva*	Juv; Ad	Hand net. At 0.5-0.8 m depth during the night	Drerup et al. (2020)	Exp
		Euprymna tasmanica	Ad	At depth less than 5 m	Squires et al. (2013)	Exp
			NS	Hand net	Swift et al. (2005)	Exp
	Nets	*Euprymna scolopes*	sAd; Ad	Dipnets	Wei and Young (1989)	Exp
			Ad	Dipnets	Montgomery and McFall-Ngai (1993)	Exp
		Euprymna tasmanica	Ad	Seine nets	Nabhitabhata and Nishiguchi (2014)	Bk
		Sepiola atlantica	Ad	Beach seine net	Jones et al. (2009)	Exp
			NS	Hand nets or beach seine nets	Jones and McCarthy (2009)	Bk
Squid	Trawl	*Loligo pealeii*[21]	Ad	NA	Buresch et al. (2009)	Exp
			NS	Otter trawl	Summers and McMahon (1974)	Exp
		Doryteuthis pealeii	Ad	NA	Kaplan et al. (2013)	Exp
			Ad	NA	Zakroff et al. (2018)	Exp
		Lolliguncula brevis	NS	Bottom trawl	Ford et al. (1986)	Exp

[21] Accepted name *Doryteuthis (Amerigo) pealeii* or *Doryteuthis pealeii;* source: WoRMS

Table 2. Continued

Taxon	Methods	Species	Life stage	Additional information	References	Type of article
Squid	Trawl		NS	Bottom trawl	Durholtz and Lipinski (2000)	Exp
		Sepioteuthis lessoniana	Juv	*Payang* (traditional Indonesian mini-trawl)	Ahmad (1997)	Exp
		Brachioteuthis spp.	Hatch	High-speed trawl	Carrasco et al. (2021)	Exp
		Lycoteuthis spp.				
		Octopoteuthidae				
		Illex argentinus	Ad	Bottom trawl	Sakai et al. (2011)	Exp
		Dosidicus gigas	Hatch	Zooplankton trawls	Ruvalcaba-Aroche et al. (2020)	Exp
		Sthenoteuthis oualaniensis	Hatch	Zooplankton trawls		
		Onykia spp.	Hatch	High-speed trawl	Carrasco et al. (2021)	Exp
	Scuba diving	*Squid*	Ad	NA	Fiorito et al. (2015)	Gdl
	Traps	*Loligo pealeii*[21]	NS	Floating fishtraps	Summers and McMahon (1974)	Exp
		Sepioteuthis lessoniana	Juv	*Sero* (set trap)	Ahmad (1997)	Exp
			Juv; Ad	Squid traps	Vidal et al. (2014)	Rev
		Illex illecebrosus	Juv	NA	O'Dor et al. (1977)	Exp
			Ad	Box trap	Balch et al. (1985)	Exp

Table 2. Continued

Taxon	Methods	Species	Life stage	Additional information	References	Type of article
Squid	Traps		Ad	Trap net	Dawe et al. (1985)	Exp
		Todarodes pacificus	Ad	Trap net	Puneeta et al. (2015)	Exp
		Squid	Ad	NA	Fiorito et al. (2015)	Gdl
	Jig	*Loligo vulgaris*	Ad	NA	Mladineo et al. (2003)	Exp
			Ad	Hand jigging	Cabanellas-Reboredo et al. (2011)	Exp
			NS	Line jigs	Carreno Castilla et al. (2020)	Exp
		Loligo forbesi[22]	Ad	NA	Porteiro et al. (1990)	Exp
			Ad	Squid jigs	Gonçalves et al. (2009)	Exp
		Loligo pealeii[21]	Ad	Squid jigs	Buresch et al. (2009)	Exp
			Ad	Hand-jigging. Single line with barbless hooks	Matsumoto (1976)	Exp
		Doryteuthis opalescens	Ad	NA	Perretti et al. (2016)	Exp
		Sepioteuthis lessoniana	Juv	NA	Ahmad (1997)	Exp
			Juv; Ad	NA	Vidal et al. (2014)	Rev
		Uroteuthis (Photololigo) edulis	Ad	Light-lure	Toyofuku and Wada (2018)	Exp
		Ommastrephes bartramii	Ad	Hand-line jigging	Vijai et al. (2015)	Exp

[22] Accepted name *Loligo forbesii*; source: WoRMS

Table 2. Continued

Taxon	Methods	Species	Life stage	Additional information	References	Type of article
Squid	Jig	*Todarodes pacificus*	Ad	Hand jigging	Puneeta et al. (2015)	Exp
			250–500 g (BW); ND	Jigs with hand reels	Bower et al. (1999)	Exp
			NS	Squid headline with multiple jigs	Flores et al. (1976)	Exp
		Squid	NS	Jigs with barbless hooks	Boyle (1991)	hBk
					Budelmann (2010)	hBk
			Ad	Squid jigs	Boletzky and Hanlon (1983)	Rev
				Hand jigging	Fiorito et al. (2015)	Gdl
	Nets	*Loligo vulgaris*	Hatch	Bongo nets	Otero et al. (2016)	Exp
					Olmos-Pérez et al. (2018)	
					García-Mayoral et al. (2020)	
		Loligo forbesi[22]	Ad	Dipnets, lampara, seine	Gonçalves et al. (2009)	Exp
		Loligo opalescens[23]	Hatch	Paired nets or bongo nets	Zeidberg and Hamner (2002)	Exp
		Loligo pealeii[21]	Juv	Fine-meshed shallow dip nets	Hatfield et al. (2001)	Exp
			Ad	Pound Nets	Chabala et al. (1986)	Exp
		Alloteuthis media	Hatch	Bongo nets	Otero et al. (2016)	Exp
					Olmos-Pérez et al. (2018)	

[23] Accepted name *Doryteuthis (Amerigo) opalescens* or *Doryteuthis opalescens*; source: WoRMS

Table 2. Continued

Taxon	Methods	Species	Life stage	Additional information	References	Type of article
Squid	Nets				García-Mayoral et al. (2020)	
		Alloteuthis subulata	Hatch	Bongo nets	Otero et al. (2016)	Exp
					Olmos-Pérez et al. (2018)	
					García-Mayoral et al. (2020)	
		Doryteuthis pleii	Juv; Ad	Dip net	LaRoe (1971)	Exp
		Sepioteuthis lessoniana	Juv; sAd	Net sets	Ikeda et al. (2004)	Exp
			Juv; Ad	Set nets and purse nets	Vidal et al. (2014)	Rev
		Sepioteuthis sepioidea	Juv; Ad	Dip net	LaRoe (1971)	Exp
			26.7-60.4 g (BW); ND	Seine and hand nets	Wood et al. (2008)	Exp
		Todarodes pacificus	Juv	Net sets	Takahara et al. (2017)	Exp
			Ad	Set net	Sakurai et al. (1993)	Exp
		Watasenia scintillans	NS	Hand nets	Gleadall (2013b)	Exp
		Squids	NS	Hand nets	Fiorito et al. (2015)	Gdl
	On-growing floating cage	*Sepioteuthis lessoniana*	Juv	Cage sides were planked to modify the influence of current and wave action	Saso (1979) cited in Nabhitabhata and Ikeda (2014)	Exp
				A 5 m^3 net cage was used with a shielding net to reduce light intensity	Sugita (2012) cited in Nabhitabhata and Ikeda (2014)	Exp

Table 2. Continued

Taxon	Methods	Species	Life stage	Additional information	References	Type of article
Octopus	Trawl	*Octopus vulgaris*	Hatch	Double oblique trawl	Roura et al. (2012)	Exp
			Juv	Cylindrical trawls	Estefanell et al. (2013)	Exp
			Juv; Ad	Bottom trawl	Barragán-Méndez et al. (2019)	Exp
			sAd	NA	Rodríguez-González et al. (2015)	Exp
			Ad	Trawl nets	Iglesias and Fuentes (2014)	Bk
			0.18–2.20 kg (BW); ND	NA	Valverde and García (2005)	Exp
		Octopus californicus	76-330g (BW); ND	Benthic otter trawl	Seibel and Childress (2000)	Exp
		Octopus mimus	Hatch	High-speed trawl	Carrasco et al. (2021)	Exp
		Bathypolypus arcticus	NS	Scallop trawl	Wood et al. (1998)	Exp
		Eledone cirrhosa	Juv; Ad	Bottom trawl	Barragán-Méndez et al. (2019)	Exp
		Eledone moschata	Juv; Ad	Bottom trawl	Barragán-Méndez et al. (2019)	Exp
			Ad	Otter trawl	Mather (1985)	Exp
		Tremoctopus spp.	Hatch	High-speed trawl	Carrasco et al. (2021)	Exp
	Scuba diving	*Octopus vulgaris*	Juv; Ad	Capture was facilitated by introducing a suspension of chloroform in seawater to the lair from a plastic squirt bottle	Smale and Buchan (1981)	Exp

Table 2. Continued

Taxon	Methods	Species	Life stage	Additional information	References	Type of article
Octopus	Trawl		Ad	NA	Castellano et al. (2018)	Exp
			60–663 g (BW); ND	NA	Miliou et al. (2006)	Exp
		Octopus bimaculoides	76-940 g (BW); ND	NA	Seibel and Childress (2000)	Exp
		Octopus insularis	Ad	At a depth of 7–9 m	Lenz et al. (2015)	Exp
		Octopus mimus	Juv	Hand collection	Zúñiga et al. (2011)	Exp
			Juv	NA	Zúñiga et al. (2011)	Exp
			Ad	Gear hook	Olivares et al. (2019)	Exp
		Octopus micropyrsus	4.65 g (BW); ND	NA	Seibel and Childress (2000)	Exp
		Amphioctopus marginatus	Smaller animal (authors presumed the youngest)	At a depth of 2–8 m	Shepherd et al. (2014)	Exp
		Eledone cirrhosa	NS	NA	Boyle (1991)	hBk
		Hapalochlaena lunulata	Juv	Animals were attracted by light and caught with hand nets	Kawashima et al. (2019)	Exp
		LPSO (*Octopus nomen nudum*)[24]	Ad	Hand collection	Caldwell et al. (2015)	Exp

[24] Larger Pacific Striped Octopus is a species occurring from Panama to Baja referring to be very close to *O. chierchiae* (R.L. Caldwell, personal communication)

Table 2. Continued

Taxon	Methods	Species	Life stage	Additional information	References	Type of article
Octopus	Trawl	*Octopoda*	Ad	Not deeper than 40-60 m	Boletzky and Hanlon (1983)	Rev
			Ad	Hand net	Fiorito et al. (2015)	Gdl
			NS	Hand collection	Budelmann (2010)	hBk
	Traps	*Octopus vulgaris*	Ad	Fish traps	Meisel et al. (2006)	Exp
			Ad	Individual traps	Iglesias and Fuentes (2014)	Bk
			Ad	Artisanal octopus traps	Tur et al. (2018)	Exp
			935 g (average BW); ND	Artisanal traps	Castellanos-Martínez and Gestal (2018)	Exp
			524.71-703.97 g (BW); ND	Net traps	Prato et al. (2010)	Exp
			NS	Baited traps	Gleadall (2013a)	Exp
		Octopus bimaculoides	Ad	NA	Lee et al. (1991)	Exp
		Octopus macropus[25]	Ad	Fish traps	Meisel et al. (2006)	Exp
		Octopus sinensis	Ad	Octopus traps	Dan et al. (2018)	Exp
		Octopus tehuelchus	Ad	At a depth of 10 m	Braga et al. (2021)	Exp

[25] Accepted name *Callistoctopus macropus;* source: WoRMS

Table 2. Continued

Taxon	Methods	Species	Life stage	Additional information	References	Type of article
Octopus	Traps	*Amphioctopus aegina*	Ad	Octopus traps	Promboon et al. (2011)	Exp
		Amphioctopus fangsiao	NS	Baited traps	Gleadall (2013a)	Exp
		Enteroctopus dofleini	Juv	Baited traps	Gleadall (2013a)	Exp
		Hapalochlaena maculosa	Authors selected animals of 5 g (BW); ND	False-shelter traps made of a 20 cm PVC pipe (20-25 mm ø) with a central cement plug. Cable ties connected to a 20 m of rope and hold down with cement blocks. Trap were marked and weekly checked by GPS	Morse et al. (2018)	Exp
		Opisthoteuthis californiana	NS	Prawn traps	Matsuzaki (2017)	Exp
		Octopoda	NS	NA	Boyle (1991)	hBk
			NS	NA	Fiorito et al. (2015)	Gdl
	Pots	*Octopus vulgaris*	Hatch	1L-pots in which females were found	Fuentes et al. (2011)	Exp
			Ad	Creels	Iglesias and Fuentes (2014)	Bk
			Ad	Brazilian pot fishery technique	Bastos et al. (2020)	Exp
			43.4-2603.9 g (BW); ND	Line strings with about 21 plastic Japanese baited pots	Carreira and Gonçalves (2009)	Exp

Table 2. Continued

Taxon	Methods	Species	Life stage	Additional information	References	Type of article
Octopus	Pots		1.2 kg to 1.9 kg (BW); ND	Traditional pots: amphora shaped, redbrick colour, 33 cm height, 13 cm ø, 9 L and 38° settling angle Other shelter pot: plastic, cylindrical, black, 35 cm height, 11 cm ø, 7 L and approx. 0° settling angle New shelter pot: plastic, amphora shaped, 31 cm height, 12 cm ø, 8.6 L and 30° settling angle	Borges et al. (2015)	Exp
			NS	Empty pots	Gleadall (2013a)	Exp
		Octopus maya	NS	Unbaited earthenware pots	Walker et al. (1970)	Rev
		Octopus pallidus	Juv; Ad M: 142-589 d old F: 110-475 d old	Research line with pots of variable volumes made from PVC pipe, and 3000 mL commercial fishery pots made from moulded plastic	Leporati et al. (2008)	Exp
		Eledone cirrhosa	355–950 g (BW); ND	Creels	Regueira et al. (2018)	Exp
			NS	Creels	Boyle (1981; 1991)	Rev, hBk
		Enteroctopus dofleini	Juv	Empty pots	Gleadall (2013a)	Exp
		Octopoda	Ad	NA	Fiorito et al. (2015)	Gdl
			NS	Empty pots	Boletzky and Hanlon (1983)	Rev
			NS	NA	Moltschaniwskyj et al. (2007)	Rev

Table 2. Continued

Taxon	Methods	Species	Life stage	Additional information	References	Type of article
Octopus	Pots		NS	NA	Budelmann (2010)	Bk
			NS	NA	Vidal et al. (2014)	Rev
	By hand on low tide	*Octopus vulgaris*	Juv; Ad	Capture was facilitated by introducing a suspension of chloroform in seawater to the lair from a plastic squirt bottle	Smale and Buchan (1981)	Exp
	On-growing floating cage	*Octopus vulgaris*	Different ages	Twelve cylindrical cages of 10 m^3 each one and 250 PVC dens harbouring 200 animals per cage were used	Castellanos-Martínez and Gestal (2018)	Exp
		Octopus spp.	Juv	NA	Vidal et al. (2014)	Rev
	'Polpara'	*Octopus vulgaris*	sAd	Non-invasive gear	Casalini et al. (2020)	Exp
	Jig	*Octopus vulgaris*	784-1447 g (BW); ND	Jigging line	Cooke et al. (2019)	Exp
		Octopus maya	Ad	Baited with no hooks	Vidal et al. (2014)	Rev
		Amphioctopus fangsiao	NS	Unbaited rod and line	Gleadall (2013a)	Exp
	Nets	*Octopus vulgaris*	Hatch	Bongo nets	Castellano et al. (2018)	Exp
					Otero et al. (2016)	Exp

3. Transport methods of cephalopods: maximising welfare from sea to lab

Similarly to capture, the ideal transportation methods should be able to avoid any form of PSDLH and should be the best at reducing the stress associated with the severity of the capture technique (Vidal et al., 2014; Fiorito et al., 2015; De Sio et al., 2020).

There is not a single protocol to transport cephalopod species but rather inter-individual variability and species-specific features (e.g., body size, physiology, biological requirements for every life stage) must be considered when preparing the specimen for the journey. The planning phases preceding collection and transport of animals should comply with the main principles enclosed in the guidelines and legislations analysed in Section 1, and - with the due adjustments - may be applied also to cephalopods.

It has been widely recognised that transportation might be detrimental for animal health conditions (Council of the European Union, 2004; Commission of the European Communities, 2007). Therefore, all phases of the journey should be planned in advance to better address their welfare and in a way that avoids delay to the place of destination. A very detailed description of the transportation precautions and recommendations in order to meet the animal welfare is provided by Fiorito and colleagues (2015), which in turn refers to the FAO guidelines for the transportation of live fishes (Berka, 1986) in compliance with the above mentioned codes and regulations for European and international transport of live animals (i.e. 'Packer's Guidelines Inv1/Aquatic invertebrates' from CITES; OIE's 'Control of aquatic animal health risks associated with transport of aquatic animals '; IATA and ATA LAR regulations) and could be therefore considered as the ground for building up the best recommendations for cephalopod transport. As reviewed by Fiorito et al. (2015), we can identify two kinds of 'transport' considering that, by increasing the duration of confinement during transport, both oxygen and water parameters will be subjected to detrimental effects (e.g., accumulation of ammonia and carbon dioxide and depletion of oxygen; Fiorito et al., 2015):

i. **Short duration journey** (< 2h; e.g., from local capturing site to local establishment). This is most likely the less stressful situation for animals, but some vital conditions should be constantly monitored before and during the trip. Crucial is to control water parameters such as pH, temperature and salinity, and of course reduce air and light exposure to prevent dehydration. Any kind of mechanical interference (e.g., vibration, noise) should be avoided or minimised and a proper attention should be paid to avoid inflicting potential physical damage.

ii. **Long duration journey** (> 2h; e.g., between towns, countries and/or intercontinental). The precautions described for the short duration trip are valid but few additional aspects should be considered as this kind of journey might include live animal shipment by sea or by air. Thus, the type and size of the containers utilized are of paramount importance and the full documentation concerning the management of the animal welfare state should be provided.

3.1 Review of existing transport methods of live cephalopods in the wild

The first known work reviewing best methods and recommendations for the rearing of cephalopods for scientific use is Grimpe's "Care, treatment and rearing of cephalopods for zoological and physiological purposes" dating back to 1928 (Grimpe, 1928). This 'guideline' has been recently translated from German (De Sio et al., 2020) is based also on the Author's direct experiences at several aquaria and scientific institutes around the world – but mainly at Stazione Zoologica di Napoli (Italy) -, previous studies and personal communications he collected (De Sio et al., 2020). It is interesting to note that Grimpe considered the methods reported almost outdated by the time of publication (1928!) and some of the approaches he suggested have been overridden by recent advances and ways-of-doing. However, his work provided several insights on the methods of transport and maintenance of different species of cephalopods in aquaria, and therefore it should be taken into account as a starting point for setting a series of rules for good practice.

Of crucial importance is to ensure cephalopods are reared in well-oxygenated seawater during the entire journey, as their high metabolic rate leads to the release of a considerable amount of carbon dioxide and ammonia (e.g., Boyle, 1983; Katsanevakis et al., 2005). Additionally, it is recommended to individually store animals in separate bags to prevent any form of distress (e.g., inking, agonistic attacks and aggression; see Fiorito et al., 2015). Whenever this condition will not be possible, enough space and seawater – thus to attempt to limit spontaneous interaction - should be applied. It is also recommended to contain animals in double bags (to prevent accidental leakage) placed within a sealed container. When juvenile and adult are transported, the selection of the container (and total volume of bags) should be done in order to maintain the appropriate volume of seawater, again avoiding any leakage.

Depending on the species, water temperature should be maintained as close as possible to the norm for the species, and water collection on site should be promoted; also, air exposure and dehydration should be avoided and all efforts made to avoid sudden temperature changes from the sea to the bag/tank (Vidal et al., 2014; Fiorito et al., 2015).

Some cephalopods are shipped in Styrofoam fish boxes or coolers with ice or heating packs according to the species (Chabala et al., 1986; Wood et al., 1998; Seibel and Childress, 2000; Zakroff et al., 2018). Although a temperature slightly below the optimum might be suggested because of its action in lowering animals' metabolic rate, allowing the shipping water to hold more oxygen and reduce waste production (Vidal et al., 2014), it is in general to avoid – unless animals proven to acclimate to the new condition (i.e. seawater temperature) and that the difference with the original seawater temperature is not of marked significant change.
In any case, temperature fluctuations during transport might be minimized, by keeping containers in shade and occasionally using air conditioning according to the vehicle adopted. Also, when using a commercial freight company for long-distance shipping, consider overnight delivery.

Specimens might require simple plastic bags during a short-duration transport, instead of large plastic buckets or boxes provided sufficient pre-oxygenated seawater is present to completely cover the animal (Budelmann, 2010; Fiorito et al., 2015). For long-duration transport - as for fish (Berka, 1986) - and according to the body size, cephalopods should be placed with 1/3 pre-oxygenated seawater and 2/3 oxygen-enriched air in double common aquarium aerated bags (Carlson, 1991; Budelmann, 2010; Fiorito et al., 2015). A proper sealing (e.g., twisted at the top and folded over) and a doubly secured closing (e.g., two rubber bands or cable grips) will be necessary. For journeys lasting more than 12 hours, aeration and oxygenation may be necessary being careful not to induce adverse conditions that may cause distress to the animals (i.e., water turbulence and bubbling can cause air entrapment in the mantle cavity or produce microbubbles affecting the integrity of the mucus layer on the skin). Sealed holding bags (transparent to facilitate inspection if required) containing oxygenated seawater should be placed into insulated boxes (e.g., Styrofoam) to ensure that a temperature appropriate to the species is maintained during transport (Fiorito et al., 2015). Bags should be packed with cushioning material (e.g., paper, Styrofoam pellets) to ensure they do not move during transport and the external shipping box should report the labels: "this side up" and "live animals" (see: 'Packer's Guidelines Inv1/Aquatic invertebrates' from CITES; OIE: 'Control of aquatic animal health risks associated with transport of aquatic animals'; IATA and ATA LAR regulations; LASA: 'Guidelines for the Transport of Laboratory Animals').

No specifically designed aerated containers or open-system are available at present for cephalopod transport although these might be obtained by adjusting those already used for live fish transportation (Berka, 1986; Lekang, 2019).

3.1.1 Further considerations

It is suggested not to feed animals 24 hours before long-term journeys, thus limiting ammonia build-up during transport. However, species-specific and individuals' normal feeding frequency, oro-anal transit time and renal ammonium ion excretion should be considered as they will affect the period of food deprivation (Vidal et al., 2014; Sykes et al., 2017) and the kind of food suitable for maintaining animal wellbeing over long-journeys. In addition, feeding the animals properly discourages the onset of agonistic interactions when more specimens are kept in the same container (group 'housing').

Sedation is not recommended for the transport of most cephalopods although methods such as cold water (Bower et al., 1999) or magnesium sulphate (Gleadall, 2013b) have been employed for the transport of some species with controversial results.

Grimpe (1928) suggested that transportation requiring more than two (2) days, should include 'resting' periods in appropriate locations (De Sio et al., 2020) which is exactly what nowadays is indicated by the European and international legislations for the transport of live animals.

As part of the documentation needed for the safe transportation of live animals during long journeys, the shipping should be tracked and the package's progress should be always accessible in order to check the status and any potential delays (Vidal et al., 2014).

Some studies reported also the need for an acclimatisation period for wild-caught cephalopods prior transport and shipping, as a method for assessing the health conditions and therefore the fitness to transport of the animals (Seibel and Childress, 2000; Ikeda et al., 2004; Shepherd et al., 2014; Matsuzaki, 2017). After transport and unloading, if some animals appear to be in an unhealthy state, quarantine could be indicated in order to assess if any unnoticed infection or contamination happened during transport and to take the most appropriate measures after consultation with veterinary and expert caretakers.

Similarly to the capture methods, we reviewed literature concerning transportation of cephalopods and tabularized in a detailed overview (Table 3) in order to extrapolate general indications that might turn out useful for building up some species-specific recommendations.

A list of recommended transport methods for each taxon is available in the WG FELASA Report (see table 2 in Sykes et al., 2022).

3.1.2 Transport methods for nautiluses

As we highlighted before, nautiluses are regularly collected from the wild mainly for rearing them into aquaria. Very often the site of capture and the site of destination are quite distant and this requires a proper transport protocol to be adopted. Carlson's guide for the trapping and rearing of live specimens provides useful observations and depicts the best conditions to be fulfilled in order to properly transport nautiluses (Carlson, 1991).

According to the Author, animals have to be immersed in seawater of reduced temperature as soon as the capture is performed; individuals must be kept at 18°C in a Styrofoam-insulated chest containing one animal per 4 L of seawater. Cooler water is provided if the journey lasts more than one hour with no aeration needed to avoid the warming of the water. Agitation and mechanical insults must be limited and the animals should be checked for signs of harm or distress (e.g., the release of copious faecal material in the water) – in such cases seawater must be replaced with new one at the same temperature. Carlson reported that nautiluses tend to bite plastic bags which therefore are not suitable for the scope. Insulated, non-toxic, waterproof boxes of 20L filled with 2/3 chilled water should be used instead and work particularly well for large specimens like *Nautilus belauensis*. The remaining space between the water and the lid should be filled with oxygen and then the package should be securely sealed and wrapped in a plastic bag placed in a cardboard to prevent any leakage.

Smaller species such as *Nautilus pompilius* can be packed in the same manner or could be placed in 1/3 filled seawater individual plastic containers (20 cm^2 and 12 cm deep) placed in a larger container with holes that permit the circulation of water. Finally, the larger container should be wrapped in a heavy-gauge plastic bag (Carlson, 1991).

Although Carlson did not cite any source, but rather referred to the experience lived during his time working at the Waikiki aquarium, other experimental works used similar approach to face the challenge of successfully transporting nautiluses to their laboratory (e.g., O'Dor et al., 1990; Uchiyama and Tanabe, 1999).

3.1.3 Transport methods for cuttlefish and sepiolids

As for the capture methods, also detailed recommendations for the transportation of live juvenile or adult cuttlefish and sepiolids is lacking and the only works that specified protocols adopted refer of plastic bags or barrels (Hanlon et al., 1997; Jones and McCarthy, 2009; Jones et al., 2009; Carere et al., 2015).

Transportation of cuttlefish can be challenging as these cephalopods can release ink that may impact their wellbeing. Suggestions have been provided to transport a maximum of 20 cuttlefishes (30–40 mm dorsal mantle length) in 6L of seawater (Hanlon, 1990). Similarly to what reported for squids, it is recommended to use large containers in which storing plastic bags with one or few specimens (Bower et al., 1999; Ikeda et al., 2004; Jones and McCarthy, 2009; Vidal et al., 2014). When using tanks for their transport, patches of sea grass can provide a refuge zone for wild-caught cuttlefishes and these have been suggested to be suitable as a spawning substrate for brooding females (Vidal et al., 2014). Although it was reported that the use of $MgCl_2$ proved itself helpful for the safe transport of juvenile (> 4 weeks old) and adult cuttlefishes when they begin to jetting excessive amount of ink (James, 1992; Jones and McCarthy, 2009), the CCMAR (Portugal) has a record of 100% successful long-distance shipping of cuttlefish with no anaesthesia; any physical wounds or death have been recorded during or after transportation by the Portuguese institution records (Sykes et al., 2012). In any case the use of sedation has to be avoided for the transportation of these cephalopods.

It is mandatory to verify accurately conditions related to the shipping procedure, such as the volume and size of the containers and the control of the quality of seawater parameters (see also table 2 in Sykes et al., 2022).

As for sepiolids, Jones and colleagues (2009) recommend to use polythene bags (3L; 31x39 cm) containing ambient temperature seawater and a 3 to 4 cm bed of fine sand taken from the collection site for transporting about five specimens of *Sepiola atlantica*. The bags were then placed in insulated boxes of appropriate size (in the study: 18x12x17 cm) to reduce temperature fluctuations during transport (Jones et al., 2009).

3.1.4 Transport methods for squid

Different tanks, according to the size and species of squids have been utilized; many of these are endowed with pumps that are able to induce a continuous water flow that prevent the accumulation of ink and waste products (Cabanellas-Reboredo et al., 2011; Toyofuku and Wada, 2018; Carreno Castilla et al., 2020). Other Authors reported the use of plastic bags, buckets or barrels, containing chilled aerated seawater, connected to a pump in the aperture as efficient methods for transportation of squids (from 90-100% survival rate; Chabala et al., 1986; Gonçalves et al., 2009).

Although some studies applied conditions that seem not recommended - i.e. housing of more squids in the same bag, continuous bubbling of oxygen in the tank, the lack of control of the water temperature (e.g., Matsumoto, 1976; O'Dor et al., 1977; Hatfield et al., 2001) - the majority of the works we accessed readapted the regulations for the transport of live fishes to these animals, such as the filling of containers (bags) with 30-50% seawater with the remaining space filled with oxygen (e.g., Matsumoto, 1976; review in Budelmann, 2010) using double sealing in larger, secured containers (Flores et al., 1976; Porteiro et al., 1990; Gonçalves et al., 2009; Gleadall, 2013b). Providing the tank bottom with sand or seagrass collected during the capture of the animals has been suggested as a factor able to reduce the stress of capture (Vidal et al., 2014), depending on the species.

According to Boyle, squids can be held in the same conditions described for octopus providing sufficient tank space for more than one animal, but some Authors (Flores et al., 1976; Lipiński, 1985) store them individually or in small groups within perforated plastic bags floating in the main tank (Boyle, 1991).

Some works reported sedation for the transport of squids by using cold water (0-1°C seawater; Bower et al., 1999) or anaesthetics (such as magnesium sulphate; Gleadall, 2013b). Three concentrations of magnesium sulphate (10, 20 or 30 mmol/L in 2L; approx. 0.3–0.7% w/v), dissolved in a small amount of cold seawater, were used compared to two controls (2 or 4 L of plain, aerated, cooled seawater) for the transport of *Watasenia scintillans* (Gleadall, 2013b). The results showed that a dose of 30 mmol/L magnesium sulphate produced the earliest squid deaths at all stages, probably through chronic effects on the respiratory centres. The only survivors at the end of the experiment were those that had received 20 mmol/L magnesium sulphate, presumably a light enough dose that the respiratory centres remain active throughout, but sufficient sedation to reduce oxygen consumption and nitrogen excretion. However, these animals, after more than 80 h in 'transport' conditions, were placed in fresh cold, aerated seawater and recovered within a minute or two after some orientation and behavioural re-adjustment to normal status (Gleadall, 2013b). A following attempt was made shipping the animals with a 500g-jar of magnesium sulphate heptahydrate per 100 L of clean, cold seawater but only verbal reports claimed the success in the survival rate of the squids hold in these conditions.

3.1.5 Transport methods for octopus

Prior to transport it is recommended to acclimate wild-caught octopus (and preferably all cephalopods) to captivity in aquaria (Bower et al., 1999; Ikeda et al., 2004). It is also suggested to monitor animals during this phase, checking for good predatory response, the presence of any skin lesions and potential unusual behaviour. In addition, animals should not be fed prior shipping to avoid compromising water quality while confined (Hanlon, 1990; Ikeda et al., 2004). Checking on captive animals regularly is fundamental to ensure they are not injured or worn out by the stress of confinement. The same applies during the trasport.

Individual refuges, such as suitably sized pots – possibly the same used for the capture (as recommended in Bastos et al., 2020) –, should be a minimum requirement for the comfort and security of octopuses. The personal experience of Grimpe (1928) at the Stazione Zoologica di Napoli (Italy) with *O. vulgaris* and *Eledone moschata* made him suggest the use of enamel pots, placed in Demijohn-baskets with stuffed hay between them in order to reduce potential insults related to the transport method. Pots have cylindrical base and are conically tapered at the top; only the lower part of the pot, containing between 20 and 80 L should be filled with water: the rest must be air, the circulation of which must be assured by multiple holes in the cork (Grimpe, 1928; De Sio et al., 2020).

Grimpe's description has been readapted in different ways, but his approach in considering cephalopods transport is still valid and has been widely applied. In the 'UFAW handbook on the care and management of cephalopods in the laboratory' Boyle suggested that small octopuses and sepioids could be placed temporarily in containers part-filled with seawater if the journey to the lab is brief. However, if temperature, pH and oxygen content values change, renewal of seawater is mandatory (Boyle, 1991). For transport lasting more than one or two hours, Boyle recommend a reduced number of small-sized individuals to be transported in cooled boxes with only sufficient water to cover the animals, each one contained in a polythene bag about 1/3 filled with seawater with oxygen filling the remaining space. The bags could be sealed and kept cool. Survival for 8-10 h was reported to be easily possible in these conditions (Boyle, 1991).

By looking at the information included in Table 3, it appears evident that in many instances the use of PVC tubes, rigid plastic jar with secure lid or individual double bags placed in large Styrofoam aquarium boxes is reported as a valid method to reduce mortality (and injuries) during transport for octopuses (Shepherd et al., 2014; Vidal et al., 2014). Many authors utilized chilled, filtered and aerated seawater inside differently-sized buckets, tanks or cold boxes with success both for juvenile and adult of different species of octopuses (Smale and Buchan, 1981; Wood et al., 1998; Seibel and Childress, 2000; Valverde and García, 2005; Budelmann, 2010; Uriarte et al., 2010; Zúñiga et al., 2011; Estefanell et al., 2013; Matsuzaki, 2017; Kawashima et al., 2019; Maldonado et al., 2019; Bastos et al., 2020; Braga et al., 2021). For us the same cautions about seawater temperature, and its changes when compared to the original temperature where animals are accommodated, still remain mandatory.

Fuentes and co-workers attempted at reproducing and ameliorating the transport method of live adult common octopus in a 24 h journey (Fuentes et al., 2011). Tanks installed onboard of the trucks had the same conditions used for fish and crustaceans, i.e. with constantly monitored water aeration and temperature. The specimens were placed in PVC tubes of 16 cm diameter, coated with mesh net and positioned in a 200 L-tank. The simulated transport conditions ranged between 50 and 165 kg/m^3 density and the temperature was maintained between 13.3-19.5 °C. Even though the stress response of the animals was not evaluated in this study, interesting outcomes on the mortality rate were observed. In fact, survival rate was higher than 95% for all density conditions and the few deaths recorded were due to the escaping attempts of the specimens. This work provided some insights into the possibility of successfully transporting live octopuses with more economic standards and less effort (due to the possibility of keeping more animals together), as may required for aquaculture. However and as already mentioned, no hints on the stress levels potentially experienced by the octopuses is reported and therefore this study is not considered here sufficient for validating the described protocols (Fuentes et al., 2011).

Table 3. **Transport Methods of cephalopods for Scientific Purposes**. Species are listed following FAO indications for Order and WoRMS for Genus. List of abbreviations utilized - Life stages: Ad – Adults; Hatch - Hatchlings; Juv - Juvenile; Other: ND – Not determinable; NS - Not specified; Sources: Exp - Experimental; Gdl - Guidelines; hBk - Handbook; Rev - Review; Animals: M – Male; F - Female; BW - Body Weight.

Taxon	Methods	Species	Life Stage	Additional information	Means and Duration of Transport	Reference	Type of article
Nautilus	Box	*Nautilus pompilius*	NS	Cold box	NS	O'Dor et al. (1990)	Exp
		Nautilus belauensis	NS	No more than 4 animals in a 20L-box at 15-18°C with pumped O_2 to fill air space, all wrapped in plastic bag and in cardboard box for shipping	The trip occurred by boat. Animals have been reported to survive more than 4 h	Carlson (1991)	Gdl
	Plastic bag	*Nautilus pompilius*	NS	Plastic containers (20x12 cm) 1/3 filled with seawater and 2/3 O_2 then placed in a larger container. Holes were practiced for circulating water and containers were wrapped in a heavy gauge plastic bag tightly sealed with rubber band. Such bags were then placed in Styrofoam boxes	The journey occurred by air cargo with a survival for up to 24 h if properly packed	Carlson (1991)	Gdl
	Insulated chest	*Nautilus spp*	NS	Animals were placed in 4L-chilled seawater (18°C). No aeration, no vibrations	The trip occurred by boat. Animals have been reported to survive more than 4 h	Carlson (1991)	Gdl
Cuttlefish	Plastic bags	*Sepia spp*	Ad	Animals can be placed in double common aquarium bags with 1/3 pre-oxygenated seawater and 2/3 O_2-enriched air. The bag should be aerated, and properly sealed (e.g. twisted at the top and folded over) and doubly secured. Sealed holding bags (transparent to facilitate inspection if required) containing oxygenated	Animals can survive over 12 h	Fiorito et al. (2015)	Gdl

Table 3. Continued

Taxon	Methods	Species	Life Stage	Additional information	Means and Duration of Transport	Reference	Type of article
Cuttlefish	Plastic bags			seawater should be placed into insulated boxes (e.g. Styrofoam) to ensure that an appropriate temperature for the species is maintained during transport. The transport box should be darkened with a secure lid to prevent stress to the animals.			
		Sepia officinalis	Juv	Max 15 cuttlefish should be transported in every 5 L of seawater, placed in a 10 L polythene bag with the addition of O_2. Polyethylene bags are then placed in insulated boxes to reduce temperature fluctuations	NS	Jones and McCarthy (2009)	Bk
			Ad	Animals can be placed in plastic bags filled with transport water (30L) and O_2. Can be sedated by adding $MgCl_2$ (75 g to 1L of distilled water) to the transport water. Trizma® buffer (pH 8.3) can be added to prevent fluctuation of water pH which in turn reduces the toxicity of ammonia.	Cuttlefish may die if left for too long in $MgCl_2$ solution	Jones and McCarthy (2009)	Bk
	Barrell	*Sepia officinalis*	Ad	Animals were placed in a 36 cm Ø, 36 cm height barrel	The trip occurred by car and lasted 30 min	Carere et al. (2015)	Exp
Sepiolids	Plastic bag	*Sepiola atlantica*	Ad	Animals were placed in 3 L-polythene bags (31x39cm) containing ambient temperature seawater and a 3 to 4-cm bed of fine sand from the collection site. Placed in insulated boxes (18x12x17cm) to reduce temperature fluctuations during transport	The trip lasted up to 2 h	Jones et al. (2009)	Exp

Table 3. Continued

Taxon	Methods	Species	Life Stage	Additional information	Means and Duration of Transport	Reference	Type of article
Sepiolids	Plastic bags		NS	Journeys < 2 h: ~5 *Sepiola* placed in 3L polythene bags (31 x 39 cm) filled with seawater and 3-4 cm bed of fine sand from the collection site. Then placed in dark in small (18 x 12 x 17 cm) insulated boxes to reduce temperature fluctuations. The presence of sand reduces stress. Journeys > 2h: transport water should be aerated using a battery operated air pump or adding O_2 to polythene bags. If the animals are to be transported long distances over several hours the bags can be placed in insulated boxes containing ice packs in order to keep the transport water cool.	NS	Jones and McCarthy (2009)	Bk
		Euprymna scolopes	Ad	Animals were placed in 3L-plastic bags containing 1.5 L of natural seawater. The seawater was filtered to 10 μm, heavily aerated and spiked with 1 g/L tris buffer; the remaining 1.5 L of each bag was filled with O_2 and the bags were fitted in insulated shipping boxes	The trip occurred by air and lasted 21 h	Hanlon et al. (1997)	Exp
		Euprymna tasmanica	NS	Animals were individually stored	NS	Swift et al. (2005)	Exp

Table 3. Continued

Taxon	Methods	Species	Life Stage	Additional information	Means and Duration of Transport	Reference	Type of article
Squid	Tank	*Loligo vulgaris*	Ad	Animals were placed in a 100-L tank with a bilge pump renewing 750 L/h of seawater ensuring that O_2 and temperature levels were safe and prevented ink from accumulating	The trip occurred by car and lasted 5-7 min	Cabanellas-Reboredo et al. (2011)	Exp
			NS	Animals were placed in a 100 L-tank aerated and filtered using a bilge pump, circulating fresh seawater	NS	Carreno Castilla et al. (2020)	Exp
		Lolliguncula brevis	NS	Animals were placed in holding tanks	NS	Durholtz and Lipinski (2000)	Exp
		Illex illecebrosus	Juv	A maximum of 20 squid were placed in 60 x 90 cm fiberglass tanks filled to a depth of 30 cm with continuous water flowing	A 1h trip of 25 km to a dock near the Aquatron was performed. Then the animals were transferred to a truck and supplied with air from a battery-driven compressor during the 10-15 min needed to move them to the Aquatron pool.	O'Dor et al. (1977)	Exp
	Cooler	*Loligo pealeii*[26]	Ad	Animals were placed in 60L-cylindrical containers cooled at 12°C with ice bags and 12 V with a piston-type air compressor provided aeration	The trip lasted approx. 4-5 h lapsed from the time the squids were placed in the tubs until they arrived at the holding facility. Then transported by truck. A 90% survival rate was reported	Chabala et al. (1986)	Exp

[26] Accepted name *Doryteuthis (Amerigo) pealeii* or *Doryteuthis pealeii*; source: WoRMS

Table 3. Continued

Taxon	**Methods**	**Species**	**Life Stage**	**Additional information**	**Means and Duration of Transport**	**Reference**	**Type of article**
Squid	Cooler	*Doryteuthis pealeii*	Ad	Seawater cooler	Transported by car to the facility immediately after the ship's return (< 6 h post-capture)	Zakroff et al. (2018)	Exp
	Plastic bag	*Loligo forbesi*[27]	Ad	Animals were individually placed in 50 L-bags and introduced into a 500 L-seawater box. A tube with seawater, connected to a pump, was inserted in the bag aperture. At other the end, the bag was perforated for outflow of seawater	The trip lasted 15-45 min with a reported 100% survival	Gonçalves et al. (2009)	Exp
		Loligo pealeii[26]	Ad	Animals were placed in double walled, sealed polyurethane plastic containers (122 cm x 45 cm and 50 cm deep) with a 20 cm opening on top. A piston air pumped for aeration. Kept cool at 10 ± 1 C° for the duration of the trip	The trip lasted approx. 4-5 h from the time the squid were placed in the tubs until they arrived at the holding facility. Then transported by truck	Chabala et al. (1986)	Exp

[27] Accepted name *Loligo forbesii*; source: WoRMS

Table 3. Continued

Taxon	Methods	Species	Life Stage	Additional information	Means and Duration of Transport	Reference	Type of article
Squid	Plastic bag		Juv	Four-five squids per plastic bag with water were placed in a covered shipboard seawater transport tank (152 × 92 × 30 cm deep) so that the air-to-water transfer would last only a few seconds. Afterwards they were transferred to the laboratory by slowly filling a large thick clear plastic bag with water and herding a small number (4–5) of individuals into the bag. Animals were released into the experimental tanks from the water-filled bags as carefully as possible; they were allowed to swim slowly from the bag into the tank	NS	Hatfield et al. (2001)	Exp
		Doryteuthis bleekeri[28]	Ad	Two-three squids were placed together in 40 cm diameter vinyl chloride bags, half-filled with filtered seawater. Inflated with high pressure O_2 gas and put into a cardboard box	The trip lasted 5 h	Matsumoto (1976)	Exp

[28] Accepted name *Heterololigo bleekeri*; source: WoRMS

Table 3. Continued

Taxon	Methods	Species	Life Stage	Additional information	Means and Duration of Transport	Reference	Type of article
Squid	Plastic bag	*Todarodes pacificus*	250–500 g (BW); ND	Animals were placed in separate bags containing about 5 L of 0–1°C seawater. Each bag was then filled with about 10–15 L of O_2 gas, sealed with rubber bands, placed in a shipboard tank (15°C) with cold anaesthesia and packed in a Styrofoam box (27x39x70 cm; 2 bags per box). Before closing each box, the bags were surrounded with pieces of ice (100–1500 cm^3)	Twenty-four squids were kept for up to 11 h to examine how long they could survive the procedure. Transport by car (2 h) to Tokyo airport, by air (1.25 h) to Hakodate airport, and then by car (1 h) to the Usujiri Fisheries Laboratory of Hokkaido University. After 6.5 h, 13/14 squids survived with no signs of distress or shock. Two-four squids maintained for 10 h in 0–1°C seawater survived. None of four squids maintained for 11 h in 0–1°C seawater survived.	Bower et al. (1999)	Exp
		Watasenia scintillans	NS	Animals were placed in plastic bags put in 15 L- containers of expanded polystyrene with shut tight-fitting. Air above the seawater was replaced with pure O_2. The animals were sedated with $MgSO_4$	Animals receiving 20 mmol/L $MgSO_4$ survived more than 80 h; Animals receiving $MgSO_4$ survived but only verbal reports claimed it	Gleadall (2013b)	Exp

Table 3. Continued

Taxon	Methods	Species	Life Stage	Additional information	Means and Duration of Transport	Reference	Type of article
Squid	Plastic bag	*Squids*	Ad	Animals were individually collected with 1/3 seawater and O_2 for the remaining part. The bags were then sealed and kept cool	The journey lasted about 8-10h. The animals survived up to 20 h	Budelmann (2010)	hBk
			Ad	Animals are placed with 1/3 pre-oxygenated seawater and 2/3 O_2-enriched air in double common aquarium bags. The bags should be aerated, properly sealed (e.g., twisted at the top and folded over) and doubly secured. Sealed holding bags (transparent to facilitate inspection if required) containing oxygenated seawater should be placed into insulated boxes (e.g. Styrofoam) to ensure that a temperature appropriate to the species is maintained during transport. The transport box should be darkened with a secure lid to prevent stress to the animals.	Animals can survive over 12 h	Fiorito et al. (2015)	Gdl
	Barrell	*Doryteuthis opalescens*	Ad	Animals were placed in a 121 L-aerated barrel	NS	Perretti et al. (2016)	Exp
		Sepioteuthis lessoniana	Juv; Ad	Plastic barrel	Animals were first maintained in a tank with running seawater at a fishing port for 4.5 h and then transported by car for less than 30 min	Ikeda et al. (2004)	Exp

Table 3. Continued

Taxon	Methods	Species	Life Stage	Additional information	Means and Duration of Transport	Reference	Type of article
Squid	Buckets	*Doryteuthis pealeii*	Ad	The healthy squids were hand-selected after captured by trawl	The trip lasted 1 h	Kaplan et al. (2013)	Exp
		Illex illecebrosus	Ad	After collecting them with buckets they were placed in 60 x 90 cm fiberglass tanks filled to a depth of 30 cm. A max of 20 squid were held in each tank aboard the tending vessel with a continuous, copious flow of water	About 1h trip for 25 km to the dock near the Aquatron. Tanks were then transferred to a truck and supplied with air from a battery driven compressor during the 10 to 15 min needed to move them to the Aquatron pool.	O'Dor et al. (1977)	Exp
		Todarodes pacificus	NS	Two squids per 10L-plastic buckets were inserted in polyethylene transport pails of a 50 L capacity with 40 L of seawater each. Aeration was provided by an ordinary aquarium air pump	The trip occurred by van and lasted 15 min	Flores et al. (1976)	Exp
	Box	*Loligo forbesi*[27]	Ad	Animals were placed in a insulated box (1.5 x 0.5 x 0.5 m) and kept in a circular tank with a 3 m Ø and water level of 0.65 m (ca 4,600 L) in a closed-circuit system at a temperature of approx. 16°C	NS	Porteiro et al. (1990)	Exp

Table 3. Continued

Taxon	Methods	Species	Life Stage	Additional information	Means and Duration of Transport	Reference	Type of article
Squid	Box	*Doryteuthis bleekeri*[28]	Ad	About 10-12 squids were placed in a 1 m x 1 m skeleton box with dark green vinyl cloth with seawater. The boxes were placed in a tank filled to the top with O_2 continuously bubbled into the tank. The temperature was about 17-19° C (not controlled during the trip)	The trip occurred by truck and lasted 3-5 h	Matsumoto (1976)	Exp
		Doryteuthis pleii	Juv; Ad	Animals were placed in an insulated Styrofoam container, aerated with a portable air pump. If neither insulation nor aeration were available, water was agitated frequently and changed whenever there was a noticeable increase in temperature. Water was also changed if there was a copious discharge of ink into the container	The max duration of the journey was 11 h in occasion of a delay	LaRoe (1971)	Exp
		Sepioteuthis lessoniana	Juv	Boxes were then placed in a net cage set	The trip occurred by canoe and lasted 30-60 min. A survival rate of 80-90% was reported	Ahmad (1997)	Exp
		Sepioteuthis sepioidea	Juv; Ad	Animals were placed in an insulated Styrofoam container, aerated with a portable air pump. If neither insulation nor aeration were available, the water was agitated frequently and changed whenever there was a noticeable increase in temperature. The water was also changed if there was a copious discharge of ink into the container	The max duration of the journey was 11 h in occasion of a delay	LaRoe (1971)	Exp

Table 3. Continued

Taxon	Methods	Species	Life Stage	Additional information	Means and Duration of Transport	Reference	Type of article
Squid	Box	*Uroteuthis (Photololigo) edulis*	Ad	Animals were placed in 4 plastic containers (616 × 924 × 210-mm depth) with sandy bottoms contained in a rectangular tank (3 × 12 × 1.4-m depth), constantly supplied with natural seawater (3.5 m^3/h).	NS	Toyofuku and Wada (2018)	Exp
		Small squids and sepioids	NS	Cool boxes with chilled filtered seawater to cover the animals	The trip lasted 2-3 h with a reported survival up to 8-10 h	Budelmann (2010)	hBk
Octopus	Chilled filtered sea water	*Octopus californicus*	76-330g (BW); ND	Type of container not specified. Kept at 5°C	Animals were maintained approx. 3 d at the University of California, Santa Barbara until transport. There they were maintained in flowing seawater at 6°C without feeding for additional 7 d	Seibel and Childress (2000)	Exp
	Cooler	*Octopus insularis*	NS	Plastic cooler with aerated seawater	NS	Maldonado et al. (2019)	Exp
		Bathypolypus arcticus	NS	Type of container not specified. Kept below 10°C	The trip occurred by boat and lasted more than a week	Wood et al. (1998)	Exp
	Cotton bag	*Octopus vulgaris*	Juv; Ad	Animals were placed in numbered cotton bags. Food debris was collected and placed with the occupant in the bags	NS	Smale and Buchan (1981)	Exp
	Aerated seawater	*Octopus tehuelchus*	Ad	NA	NS	Braga et al. (2021)	Exp

Table 3. Continued

Taxon	Methods	Species	Life Stage	Additional information	Means and Duration of Transport	Reference	Type of article
Octopus	Plastic bag	*Octopus vulgaris*	Hatch	Paralarvae were put inside transparent 30L-plastic bags. The tubes were introduced in 10L of sea water supplemented with O_2 and the rest of the bag (2/3 of its volume) was completed with pure O_2. The bags were sealed hermetically, closing their extreme open by means of plastic straps. Then placed in cylindrical tanks of 100L	Paralarvae survival was tested at 6, 12, 24h	Fuentes et al. (2011)	Exp
		Octopus digueti[29]	Ad	Animals were placed in 3L-separate bags of seawater with an equal volume of pure O_2	NS	DeRusha et al. (1987)	Exp
		Octopus joubini	Ad	NA	The trip occurred by air, from Florida to London	Bradley (1974)	Exp
		Amphioctopus fangsiao	NS	Four animals were put in each bag then placed in a large cooler box containing sufficient seawater under an atmosphere of pure oxygen	The trip occurred by train, from Ushimado to Tohoku University in Sendai	Gleadall (2013a)	Exp
		Amphioctopus marginatus	Smallest animals (authors presumed the youngest)	Animals were collected with their den	The return to the field station occurred by *banka* (approx. 30 min transit time) where the animals were staged in holding containers. The next morning, they were removed from the ocean and	Shepherd et al. (2014)	Exp

[29] Accepted name *Paroctopus digueti*; source: WoRMS

Table 3. Continued

Taxon	Methods	Species	Life Stage	Additional information	Means and Duration of Transport	Reference	Type of article
Octopus	Plastic bag				transported to Manila by car (approx. 120 min) where they were prepared and exported to the Steinhart Aquarium.		
		Small octopuses	NS	Animals were then placed in cool boxes with only sufficient water to cover them. To promote individual survival, particularly in the case of larger specimens, each animal was contained in a polythene bag about 1/3 full of seawater and still O_2 filling the remaining space. Animals captured at sea are best held in deck tanks of seawater continuously pumped from the ocean	Survival for 8 – 10 h was reported as possible	Budelmann (2010)	hBk
		Octopoda	Ad	Animals were placed in double common aquarium bags with 1/3 pre-oxygenated seawater and 2/3 O_2-enriched air. The bag should be aerated, and properly sealed (e.g. twisted at the top and folded over) and doubly secured. Sealed holding bags (transparent to facilitate inspection if required) containing oxygenated seawater should be placed into insulated boxes (e.g. Styrofoam) to ensure that an appropriate temperature for the species is maintained during transport. The transport box should be darkened with a secure lid to prevent stress to the animals.	Survival rate is possible for over 12 h	Fiorito et al. (2015)	Gdl

Table 3. Continued

Taxon	Methods	Species	Life Stage	Additional information	Means and Duration of Transport	Reference	Type of article
Octopus	Bucket	*Hapalochlaena lunulata*	Juv	Animals were placed in a cube-shaped bucket (27 × 27 × 27 cm) filled with seawater and aerated with a bubbler.	NS	Kawashima et al. (2019)	Exp
	Jar	*Octopus vulgaris*	Ad	Animals were placed in PVC tubes of 16 cm in diameter, lined with red mallet and located in a 200 L tank. For the supply of air conditioning, a rectangular structure formed by porous tubes is provided in the tank bottom, connected by means of a plastic pipe to a conduit that provides 100 L of compressed air per min. The O_2 concentration was measured every hour over the course of a full day, except for 2 h intervals at night.	24 h	Fuentes et al. (2011)	Exp
		Amphioctopus marginatus	Smallest animal (authors presumed the youngest)	Animals were placed in rigid plastic jars packed in Styrofoam aquarium boxes	The trip back to the field station lasted 30 min by boat and then a ride to the shipping facility occurred by car, lasting 2–3 h	Shepherd et al. (2014)	Exp
	Box	*Opisthoteuthis californiana*	NS	Boxes were kept at 3°C	Animals were kept in a deep seawater tank for 2 d in Rausu Town. They were again transported to the exhibit tank at Aquamarine Fukushima both by air and land taking 12 h	Matsuzaki (2017)	Exp
	Enamel pot	*Octopus spp*	NS	Animals were placed in Demijohn-baskets with water only in the lower	The journey lasted about 3-12 h	Grimpe (1928)	Rev

Table 3. Continued

Taxon	Methods	Species	Life Stage	Additional information	Means and Duration of Transport	Reference	Type of article
Octopus	Enamel pot	*Eledone spp*		part, the rest must be air, the circulation of which must be assured by multiple holes in the cork			
	Tank	*Octopus vulgaris*	Ad	Animals were placed in a 1000 L tank with seawater	NS	Bastos et al. (2020)	Exp
			870 g (average BW); ND	The tank was provided with pure O_2	NS	Estefanell et al. (2013)	Exp
			0.18–2.20 kg (BW); ND	The 100 L tank was filled with aerated seawater	NS	Valverde and García (2005)	Exp
			784-1447 g (BW); ND	Animals were kept in a 40 L tank together with water being renewed every 20 min; the temperature was 16-18°C and O_2 above 6.5 mg/L	NS	Cooke et al. (2019)	Exp
		Octopus mimus	Juv	Tank volume of 60 L	NS	Zúñiga et al. (2011)	Exp
		Eledone cirrhosa	NS	Animals can be kept in plastic tanks of seawater through which fresh seawater was pumped continuously. A net covering to the tank is advisable to prevent escapes	The lapsed time between capture and reaching the aquarium can be 3-12 h.	Boyle (1981)	Rev
		Enteroctopus dofleini	Juv	Animals were individually hold in large, cylindrical aquaria covered with attached netting to prevent escapes	Animals were caught locally at Onagawa and transported by road to Sendai	Gleadall (2013a)	Exp
		Robsonella fontaniana	Ad	Tank volume of 70 L	NS	Uriarte et al. (2010)	Exp

4. Different forms, different methods: considerations for Capture and Transport of diverse cephalopod life stages

A further essential point that should be taken into account when designing experiments and choosing the capture and transport methods to be used, is the life stage of the target cephalopod species needed. The Directive 2010/63/EU only protects cephalopods from the hatching moment, but several studies may require the collection and culture of egg masses from the wild. As mentioned previously, although eggs demand less effort in terms of size of containers and water volume during transport, these are extremely susceptible to temperature, pH and salinity changes (Iglesias et al., 2007). Storing conditions during embryonic development should also be monitored as the egg state will consequently affect the health of the deriving hatchlings. The display of maternal care, as in incirrate octopods or oceanic squid is crucial during this phase (Seibel et al., 2005; Bush et al., 2012), for determining whether the embryo will properly develop or will prematurely hatch (Vidal et al., 2014). Newly hatchlings might appear either as miniature adults or as planktonic paralarvae with relatively short arms and limited swimming ability (Villanueva, 1995) and represent extremely delicate developing forms, very sensitive to any insults or change in the water parameters from the site of collection to the containers. Nevertheless, the majority of studies (Table 2) used trawls and bongo nets for collection, with few reporting also that animals which resulted damaged were excluded from the experiments (Otero et al., 2016). Usually, for the use of paralarvae in laboratory, spawning females are captured and reared until the egg masses hatch (Uriarte et al., 2010; Lenz et al., 2015; Tur et al., 2018; Deryckere et al., 2020; Braga et al., 2021) while the collection of paralarvae from the wild is mainly focussed on samples to be subjected to molecular analysis (Otero et al., 2016; Olmos-Pérez et al., 2018; García-Mayoral et al., 2020). Therefore, the transport protocol is almost always missing with the exception of a work from Fuentes and colleagues which provided the first protocol for a six, 12 and 24 h transportation of *Octopus vulgaris* paralarvae stored at different densities (Fuentes et al., 2011). After collection, these were put inside transparent 30L-plastic bags placed in tubes filled with 10L of seawater supplemented with oxygen and the rest of the bag (2/3 of its volume) was completed with pure oxygen. The bags were sealed hermetically, closing their extreme open by means of plastic straps and then placed in cylindrical tanks of 100L. Fuentes and colleagues demonstrated a mortality below 0,20% when storing 100-500 paralarvae per litre regardless of the transport duration. Notable mortality begins to be observed when transporting for 12 and 14 h plastic bags containing 4000-6000 paralarvae per litre (mortality ranging from 3% to 59-60%). Thus, by using plastic bags kept at 14°C with a stocking density not higher than 3000 paralarvae per litre it is possible to obtain a survival near 100% for a up to 24h-journey. While hatchlings use a combination of yolk and prey food sources - that in some cases might be very different from the diet of adults (Villanueva, 1995) - the collection of juveniles is very difficult because of their selective and rigid feeding needs (Boletzky and Hanlon, 1983). This might be relevant because a traumatic capture might affect highly specific feeding responses that may translate into fatal conditions.

Adults (see previous sections), have also important temperature, salinity and pH requirements as most cephalopods are stenotherm and stenohaline. Therefore, inappropriate capture and transport from the wild of any kind might result in high mortality rates. Considerable species-specific care, as pointed out in the previous sections, is needed if viable animals have to be delivered to the laboratory (Grimpe, 1928; Boyle, 1991; Fiorito et al., 2015).

A reference to the life stages, where available and appropriate, is reported in Table 1 and 2 of the shortened version to this work (Sykes et al., 2022).

5. From the sea to the bench: *ad-hoc* pilot studies to assess best practice for capture and transport of live cephalopods for scientific purposes

Our survey of the scientific works, guidelines or grey literature and technical reports (see Tables 2, 3) is the urge for deeper investigations on which capture and transportation methods are able to specifically address the welfare of different cephalopods species. Biological and physiological needs are crucial but size and life stages should also be considered.

As discussed above (section 2), a trade-off should be found in order to meet the well-being of the animals at the same time meeting experimental requirements and expectations. Anyhow, the first available accounts addressing this issue have been based on personal experiences or interactions with local fishermen, with relatively little scientific data (Grimpe, 1928; Boyle, 1981; review in Boyle, 1991; Carlson, 1991) and therefore we can only see them as a fertile ground for growing more solid experimental approaches.

5.1 Some reference studies

Successful attempts to evaluate stress response and, therefore, animal welfare in captured and transported aquatic animals have been applied to fishes (e.g., Haux et al., 1985; Barton and Iwama, 1991; Sampaio and Freire, 2016; Cook et al., 2018) and few crustacean species (e.g., *Homarus americanus* and *Cancer pagurus*, Lorenzon et al., 2007; 2008; Barrento et al., 2010; *Farfantepenaeus brasiliensis*, Souza et al., 2013). These works allowed for the identification of appropriate catching gear and containers, designed for different types of journey (e.g., Berka, 1986; Froese, 1998; Lim et al., 2003; Watson et al., 2010).

To the best of our knowledge no such studies have been carried out on cephalopods. Exceptions are represented by a few recent investigations on capture and transport methods (but not on the combination of the two).

A recent study from Araújo and co-workers (2020) focussed on the effects of a simulated long journey transportation at high density on live *O. vulgaris* reared in conditions very similar to those described by Fuentes et al. (2011). The simulation lasted 48 hours at two densities (50 kg/m^3 and 100 kg/m^3) and consisted of 220-L tanks provided with cooling and aeration equipments. The octopuses were placed in PVC tubes (25 cm length and 14–16 cm diameter) covered and closed by a purse seine net (20 mm mesh). Water temperature was maintained at 9-10°C, in order to promote the decrease of octopus's metabolism and to reduce the consumption of oxygen and the excretion of metabolites. The stress response was evaluated through the analysis of dopamine levels in the haemolymph, examination of protein concentration and Hsp70 levels in muscles, and concentration of ammonia in brain tissues (Araújo et al., 2020). No mortality was recorded at the end of the 48 h simulation for both density conditions, and all the animals shown any impariment in their feeding capacity. Water parameters remained stable with the exception of an increase in the ammonia and nitrate levels in the 100 kg/m^3 density tanks. Ammonia levels in the brain resulted with no measurable variations. Dopamine concentration in the haemolymph and Hsp70 levels in the muscles, resulted only slightly increased at the end of the experiment, but not significantly. Similarly to what was already shown by Fuentes and colleagues (2011), the transport method proved itself successful and suitable for *O. vulgaris*.

Barragán-Méndez and colleagues (2019) investigated the inter-specific effect of bottom-trawl on the survival and physiological state of live *Eledone moschata*, *E. cirrhosa* and *O. vulgaris* after a recovery time (48 hours for *Eledone* species; 24 hours for *O. vulgaris*) in separated aquaculture systems. Survival rates of individuals captured and various physiological parameters were measured: plasma pH, total CO_2, peroxidase activity, lysozyme, hemocyanin concentration/status, proteases, pro-phenoloxidase, anti-proteases, free amino acids, lactate and glucose levels (Barragán-Méndez et al., 2019). The results showed a similar survival rate between the three octopus species with a reduced percentage, though not statistically significant, only in *O. vulgaris* (75% survival vs more than 93% in *Eledone* spp.) that might be attributed to the lower tolerance of *O. vulgaris* to hypoxia, as shown by the lower pH level measured when compared to *Eledone* spp. Plasma pH and CO_2 concentration resulted altered as a consequence of the trawling and the anaerobic conditions to which the animals were exposed to - although the baseline levels were reached in less than 24 h for every surviving octopus. The immune response resulted impaired as well; higher activity level in plasma lysozyme (restored after 6 h in *O. vulgaris* and after 24 h in *E. moschata*; no changes in *E. cirrhosa*) and by the higher proteases and antiproteases levels after capture. Also, increased peroxidase activity was observed up to 24 h post-recovery, with an enhanced response immediately after capture in *O. vulgaris* and *E. cirrhosa*. The activation of the carbohydrate and even more of the amino acid metabolism, highlights the activation of the stress-response after the capture and rearing conditions utilized in this study, and restored the homeostasis after 6–24 h recovery in onboard tanks (Barragán-Méndez et al., 2019). Thus, unreliability of the capture method utilized in this work, mimicking the ones adopted in fisheries, was found; this supports the requirement of a standardised setting of humane capture and transport protocols.

Together with the above mentioned works, other attempts have been made and a growing number of studies is accumulating concerning the capture and transport of live wild cephalopods (see the tabularized overview in Tables 2 and 3), possibly with a bias towards squids species (Matsumoto, 1976; Chabala et al., 1986; Bower et al., 1999; Ikeda et al., 2004; Gonçalves et al., 2009; Jones et al., 2009; Gleadall, 2013b).

5.2 Pilot studies and welfare assessment

Based on the available knowledge (see also Sections 2, 3 and previous paragraphs) accurate, solid and replicable studies are still required to facilitate an informed guidance on capture and transport methods for cephalopods (species-specific) for research purposes, thus providing adequate control – and limitation – of stress-induced levels in animals. The purpose of this section is to provide complementary information to facilitate the outline of *ad-hoc* studies that will help to achieve our overarching goal (see also Sykes et al., 2022 for complementary information).

We aim at suggesting experiments focussed on comparing the physiological effects of different combinations of capture and transport methods on both sexes of juveniles and adults of the most utilized cephalopod species in scientific research, namely: *Sepia officinalis*, *Sepioteuthis sepioidea* (or other congeneric species), *Euprymna scolopes, E. berryi* (and/or Mediterranean and Atlantic analogous species), *Loligo vulgaris* (or other squids of relevance in scientific research), *Octopus vulgaris*, *Octopus maya*. This list should be read as not limited to the mentioned species, but taken as an example of organisms with biological and life-style differences from each other, thus potentially informing a growing number of possible cases.

The idea behind is to allow for a collaborative effort with selected, geographically distributed, fishermen communities that will affort a systematic study on a given number of individuals for each species – based on appropriate experimental design in compliance with 3Rs principle and best practice for laboratory animals (e.g., Festing and Altman, 2002; Flecknell, 2002). The idea is to test also for confounding variables such as temperature and season at time of capture. Exloring differences in two separate seasons - for example - originate from studies on crustaceans and bivalve molluscs where increased mortality rates occur in months with higher sea water temperatures (Raicevich et al., 2014; Méhault et al., 2016; Clements et al., 2018).

In our plan, a couple of capture methods will be selected for comparison with those ‘claimed’ to be the most recommended ones for each species, such as: ***i.*** nets vs traps in cuttlefishes, ***ii.*** nets vs traps in squids, ***iii.*** pots vs traps in octopus. In addition and for each situation, different transport conditions will be tested: e.g., individual vs multiple ‘storing’ of animals. During transport seawater quality parameters (pH, salinity, temperature) will be maintained alike to those of the capture site and will be monitored constantly. The comparison of conditions during transport (group vs individual - keeping of animals from capture and during whole transport to the first possible land facility) is based on the fact that some studies report good results in transporting more than one animal per ‘bag’ (Flores et al., 1976; Matsumoto, 1976; O’Dor et al., 1977; Hatfield et al., 2001), but other Authors suggested that, in order to promote survival, the individuals should be placed into separated containers (Gonçalves et al., 2009; review in Budelmann, 2010).

We suggest that cephalopods will be kept into standard containers usually employed by fishermen (e.g., open large darkened buckets), and will be food deprived during the whole trip (to limit the production of ammonia waste and the consumption of dissolved oxygen induced by increasing metabolism) as in the fishermen habits. In a first study a simulated local journey lasting no more than 3-4 hours will be considered.

After the arrival in laboratory, the welfare state of the animals will be assessed adopting different indicators - selected among those included in table 5 of the FELASA guidelines for the care and welfare of cephalopods in research (Fiorito et al., 2015). In particular, physical, behavioural and physiological indicators will be identified and the corresponding parameters taken as those considered to be easy, replicable and valid biomarkers of animal welfare. We aim to consider:

- ***i.*** skin integrity and colour,
- ***ii.*** locomotor and inking behaviour[17],
- ***iii.*** predatory response (tested after two hours after being placed in the holding tank; see Amodio et al., 2014) if applicable to the context,
- ***iv.*** analysis of faeces and mucus samples for assessing the levels of corticosterone (Larson and Anderson, 2010; see also Chancellor et al., 2021) and hormonal status (see Baldascino et al., 2017)[18],
- ***v.*** cardiovascular and respiratory performance *in vivo* (assessed as soon as possible after being placed in the holding tank; see Pugliese, 2017).

17 intramantle inking after transport have been reported by Bennet & Toll (2011).

18 http://www.cephsinaction.org/activities/stsm/approved-stsm/approved-stsm-2016/#toggle-id-7

Furthermore, in a subsample of individuals we suggest to analyse haemolymph samples to assess hormonal levels (corticosterone, testosterone, oestradiol) and the amount and composition of haemocytes. Haemocytes in cephalopods are considered a potential indicator of organism's health (Ellis et al., 2011), since variations in their number and morphology has been related to stress (Malham et al., 1997; 1998a; 1998b; 2002), and parasitic infections (e.g., da Silva et al., 2008). These observations will 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 (see for example Baldascino et al., 2017). Survival at 2, 10 and 30 days after capture will be also considered. In addition, this will be compared with survival in animals that have been randomly assigned to a relatively higher invasive protocol for the assessment of animal welfare (i.e. haemolymph sampling). Data collected will be used also to estimate a welfare index[19] and to identify a specific level of severity to every combination of methods proposed, thus to explore for the most suitable capture and transport methods that could be reliable, replicable and feasible for each species.

Once preliminary studies will be accomplished, these will support subsequent steps aimed at designing a better experimental plan and investigations – again with close collaboration with fishermen - aimed at explore the best approach (capture and/or transport for each species) via consensus for the benefits (economical, social, scientific), possibly expanding the study to a larger sample and conditions (e.g., geographical areas, boats, gears).

We aim at exploring novel data for identifying 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 cooperate with fishermen, by involving them as scientific suppliers for live cephalopod species and further expand their competence achieving the good practice required for the capture and transport of healthy cephalopods for research purposes (see also ancillary work: Sykes et al., 2022).

6. Education and Training Programme for collectors, shippers and transporters

What jeopardises most the welfare status of the animals is the limited training and competence of the people involved in the supply chain (see Section 1.1.1, legislations, codes and regulations). 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 (European Parliament and Council of the European Union, 2010). To facilitate training of people involved in the four functions, the European Commission established an Expert WG whose efforts produced a working document in 2014 that set common education and training framework when dealing with animals for scientific purposes (National Competent Authorities for the implementation of Directive and EU Expert Working Group to develop a common education and training framework, 2014). It is therefore mandatory, that cephalopod capture and transport for scientific purposes must be performed by trained and expert personnel.

Annex IV of the EU Council Regulation No 1/2005 already provided instructions concerning the training for transporters which shall include notions on: «***a.*** Articles 3 and 4 and Annexes I and II; ***b.*** animal physiology and in particular drinking and feeding needs, animal behaviour and the concept of stress; ***c.*** practical aspects of handling of animals; ***d.*** impact of driving behaviour on the welfare of the transported animals and on the quality of meat; ***e.*** emergency care for animals; ***f.*** safety considerations for personnel handling animals» (Council of the European Union, 2004). Moreover, the regulation available for aquatic animals from OIE (Aquatic Animals Commission, 2019) provides information on the types of containers and procedures to be performed when transporting or shipping live animals for short or long journeys; these should be considered for application to live cephalopods.

19 http://www.cephsinaction.org/working-groups/working-group-4/

Suppliers of live animals should be trained in order to comply with species-specific biological, physiological, behavioural needs and welfare requirements of cephalopods.

We believe fishermen, whose expertise and practical knowledge of the sea are undoubtable, are equipped with the best practice for capturing live cephalopods. However, they need to be trained in order to fulfil the requirements of matching best-practice, efficiency and sustainability (economical and environmental) with animal welfare.
Fishermen's values and attitude rely upon the economic and social structure, and community which they belong to. Indeed, fishing serve for subsistence, market or a combination of both; it may be subject to quota regulations, licensing or other measures. It is distinguished in small-, medium- or large-scale, and is settled as inshore, mid-water or offshore; it may be seasonal or year-round, may be practised full time or part-time, is performed by owners, crewmen hired by land based shipowners, or vertically integrated (Van Ginkel, 2001). Factors like boat size, number of crew members, variability in target species, technology and gear used all contribute in creating differences in cultural rules, practices, styles, and aims (Van Ginkel, 2001).
We aim at firmly relying on experienced cephalopod artisanal and small-, large-scale fishers and we are interested in their holistic vision of the marine framework which is fundamental to trigger a broad common sense and a respectful view when it comes to natural environment and thus the target animals inhabiting it (Uskul et al., 2008). Furthermore, we would like to preserve, for example, the historically based-family business whose effort used to be the outcome of an interdependent cooperation with other local families of fishers, in order to refuel that sense of community which might have been weakened due to the more strict legislations and conditions (e.g., on the number of licenses available or the presence of large-scale fisheries) which boosted competition among fishermen (Jentoft, 2017). We would like to discourage any individualism and rivalry to pursue an 'ethos' of sharing among these workers that will help set the stage for a greater awareness toward animal welfare. Moreover, the commitment to the job - now intended in a broader sense that includes environmental and animal sensibility - should be translated also in shared actions with other suppliers such as transporters, in that their work should result in cooperation at the collection site to properly manage a freshly caught animal so to avoid it any distress or harm (e.g., exposing it to air).
Hence, our goal is to promote a concerted action of trained personnel that will ensure wild-caught cephalopods will be properly captured and transferred to destination sparing them unacceptable pain or suffering.

6.1 Incentive and Benefits

Perhaps, the biggest challenge would be approaching fishermen and transporters and persuade them participating in the training and education process. We expect the major resistance would be their concern about losing potential working hours by undertaking the training without having a beneficial profitable return. It is thus our duty to find the proper balance between these workers' needs and the requirements of competence to care about animal welfare after the inclusion of live cephalopods as experimental models.

Incentives should be provided for attendees interested to become suppliers of wildlife for research aims. Greater incomes and permission to operate all around Europe might be possible outcomes, widening the range of supplied areas. Moreover, an accredited course would allow successful Trainees to easily obtain fishing licenses in compliance with national and European legislations.

Furthermore, it is understandable that after the validation of solid and standardised methods and protocols through dedicated pilot studies (see Section 4), many of the artisanal or traditional fishing techniques may become "obsolete" (in the sense of their applicability for the supply of live animals for scientific purposes) representing a potential issue for fishermen. To overcome this problem, subsidy and economic aids should be available to help them facing any renewal or improvement of their capture equipment. A coordinated effort between different stakeholders including local and national government will then be crucial.
In addition, as also indicated by the same report from the European WG (National Competent Authorities for the implementation of Directive and EU Expert Working Group to develop a common education and training framework, 2014), the training framework should be accessible, affordable – and with joint effort of the Member States, hopefully free - and flexible so as to meet trainees'

working time or shift schedules.

6.2 Training plan and course content

The education and training (E&T) will be provided through the attendance of a 14-18 hours course designed and delivered as part of the Cephalopod Biology and Care (CBC) FELASA accredited Training Program (that is the sole-running with FELASA accreditation exclusively designed for E&T on cephalopods in compliance with Directive 2010/63/EU. The course will be organised according to a modular training structured in theoretical and practical sessions, around learning outcomes based on defined assessment and pass-fail criteria. A detailed description of the topics and the expected Learning Outcomes included in the training program for both collectors and transporters is included in Box 1 of the Supplementary Material of the WG Report (Sykes et al., 2022).
For collectors and transporters, the skills that the course should provide would be considered equivalent to Directive 2010/63/EU functions a), c) and d).

In our aim fishermen and transporters of live animals should face the challenge of improving the well-being of the animals they work with by achieving awareness about the concept of welfare and ethical approaches when dealing with cephalopods as animals destinated to scientific work. A variant of the standard CBC course edition will be designed to allow overcoming any possible educational issue deriving from attendees' different backgrounds and eventually helping them to achieve commitment to the animal cause. By undertaking a simple induction course, fishermen and transporters will converge to the same level of knowledge that will guarantee a proper understanding when approaching the main training objectives.
The course will be designed including legislative aspects (see Section 1.1.1), including the technical and administrative aspects of EU legislation concerning the protection of animals during transport, animal physiology (e.g., respiratory and feeding needs), animal behaviour and providing adequate information and training around the concept of stress and welfare. It will be also focussed on practical aspects of animal handling and of the emergency care both for the personnel and the animals. Attention will be given to inform on how the driving behaviour might impact the welfare of the transported animals and incidentally of the quality of meat, if the animals are destined for food consumption.
The assessment of acquired competence will consist of a multiple-choice test followed by a hands-on evaluation (OSPE) of the practical skills, performed under the supervision of an expert examiner. The successful suppliers will have the permission to start operating under the guidance of a competent supervisor for the next couple of months to become more skilful. A Designated Veterinarian will always be available, until full autonomy is reached. In addition, the program will include a free app for consultation to be used on most common devices and smartphones.
Guidelines for continuing education for all the persons involved in the care and use of animals for scientific purposes are available and these might be similarly addressed for the sake of training and CPD of collectors and transporters. A sort of two years-update program should be included based on surveys to be submitted to collectors and transporters in order to receive feedback that could be useful to improve the teaching and also to fine-tune the protocols and techniques developed and disseminated.

Collectors, transporters and shippers should become familiar with some essential concepts which will be provided through a 14-18 hour training (spanned in two or three days; see also Sykes et al., 2021). In particular, the following topics will be covered:

i. sustainability as an essential trigger for improving both animal care and quality of scientific data, at the same time 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 wildlife;

iv. PSDLH and harm-benefit assessment during capture and transport of live cephalopods;

v. basic knowledge of general and species-specific biology and behaviour of cephalopods;

vi. national and international legislations and legal aspects regarding 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 techniques and protocols to ensure the well-being of the live wild-caught cephalopods;

viii. principles of hygiene, care and health check of animals at every stage of the supply chain;

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

Focal points are practical aspects and hands-on-training aimed at encouraging trainees to deepen their knowledge about standardised and validated equipment/protocols to be used during the actual capture and transport and also to train them how to properly handle a given cephalopod species. Practical skills will be the evaluated (OSPE) after the acknowledgement of successful results in the theoretical training phase (see Box 1 in Supplementary Information, Sykes et al., 2022).

Taxon-specific recommendations for capture and transport of cephalopods in research

Our recommendations are largely illustrated in the ancillary work (Sykes et al., 2021). Here we will summarize what already reported in taxon-specific sections of this paper, for both capture and transport of live cephalopods.

Collectors and researchers unanimously agree upon the use of baited or light traps, traditionally produced by artisanal Philippine fishermen, as the best method for capturing live wild nautiluses. This prototype reported by Carlson (1991) can still be applied (Dunstan et al., 2011) with modern variations concerning the use of modern monitoring systems.

As for transport, boxes or insulated chests with cool seawater (15-18°C) are suitable for both juvenile and adult or for different species of nautiluses and should be preferred to plastic bags which can be worn out by these animals, risking their welfare. More specimens can be contained in the same box, providing each animal with 4 L of seawater. Every box should be filled with 1/3 of seawater and 2/3 of oxygen and then should be securely sealed and wrapped in a plastic bag placed in a cardboard to prevent any leakage (Carlson, 1991).

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 which are similar to those employed for squid but larger and lighter. These allow the capture of uninjured juvenile and adult cuttlefishes for both aquaculture and laboratory rearing (Vidal et al., 2014). The use of these size-selective gears may be accompanied by light or seabed as an attractive spawning substrate for adult females mainly captured as broodstock for aquaculture purposes (Watanuki and Kawamura, 1999; Watanuki et al., 2000). Also large nets, such as trammel nets are suitable for catching a reasonable number of both juvenile and adult animals without excessive constraint without constituting any environmental issues as it is for trawling.

Transportation of cuttlefish can be challenging and suggestions have been made to transport few species per plastic bag or barrels according to the size (a maximum of 20 cuttlefishes of 30–40 mm dorsal mantle length in 6L of seawater has been reported; Hanlon, 1990) with the use of large containers in which storing the bags/boxes (Bower et al., 1999; Ikeda et al., 2004; Vidal et al., 2014). When using tanks for their transport, patches of sea grass can provide a refuge zone for wild-caught cuttlefishes and are suggested to be suitable as a spawning substrate for brooding females (Vidal et al., 2014). Animals kept in this conditions could face a 12 h journey (review in Fiorito et al., 2015).

Nets and in particular, 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 methods for these little-sized cephalopods (Montgomery and McFall-Ngai, 1993; Nabhitabhata and Nishiguchi, 2014). As for the cuttlefishes, sepiolidae are mainly transported in plastic bags containing few species according to their volume. It is recommended to put these bags in larger insulated boxes that ensure no leakage or asphyxiation of the animals. Previous studies reported a 21 h trip by air was performed by these animals in these conditions (Hanlon et al., 1997).

One of the most frequently employed capture method for squid is the jig lure with barbless hooks operated mechanically or by hand (Matsumoto, 1976; Boyle, 1991), but further analyses reveal that its use might induce some injury and lasting harm to the animals (Flores et al., 1976; Cabanellas-Reboredo et al., 2011; Perretti et al., 2016).

Several kinds of nets are employed for capturing squids for laboratory use, such as pound nets, bongo nets, seine and dip nets all proved to be harmless for these animals if properly used by trained hands. These are larger and squids are able to swim before they get caught forming a consistent sample size (Hanlon et al., 1983; Chabala et al., 1986; Boyle, 1991). Furthermore, these kinds of nets are suitable for capturing any specimens at any life form paying attention to the by-catch of egg masses. From our literature review the most recommended capture methods could be the size-selective box traps and trap nets that consider the size, age and genus of the target squids (O'Dor et al., 1977; Balch et al., 1985; Dawe et al., 1985; Puneeta et al., 2015); these might turn out to be more effective and atraumatic for catching unharmed squids. Traps slightly vary in size, shape and composition, according to the geographical position or to the needs but, are generally made of natural materials (e.g., bamboo fibres) or non-toxic plastic. Traps can be very similar to octopus pots in that they provide a shelter that looks alluring, especially for spawning females (O'Dor et al., 1977), with usually a top hole from which the animal can spontaneously enter but from which it cannot escape. Bait or better, light attractant might be used to attract the animals.

During transport squids should be individually placed in plastic bags, barrels or buckets filled with 1/3 seawater and 2/3 oxygen and sealed away in larger tanks or Styrofoam boxes. Providing the tank with a few bottom sand or seagrass collected during the capture of the animals might be indicated as a factor able to reduce the stress of being captured (Vidal et al., 2014), depending on the species.

Undoubtedly the best existing capture method for octopuses is the pot. Pots, are generally made of non-toxic materials and non-abrasive surfaces and exploit the natural tendency of these animals to search for a den. Octopuses spontaneously settle in these gears which are very likely to catch undamaged specimens. From the literature survey it is clear that the most suitable pots should have dark tone, narrow entrance and a large interior that allows the animal to see outside without exposing itself to danger (Borges et al., 2015). A series of adjustments can be made in order to keep ameliorating pots such as the insertion of a GPS monitoring system or of a removable lid that might be useful also for transportation. Pots are alluring both for juvenile and adult form of octopuses and very often they can be chosen as substrate for eggs laying that should be reinserted in nature.

A combination between pots and traps are the so called Japanese baited pots (JBPs) combining two alluring features for octopuses, a shelter and a bait. This trap (see figure 1 of Carreira and Gonçalves, 2009) is a box, inside which a bait is tied to a string attracting the animal inside and getting it trapped. The Authors proved JBPs to be very specific for the octopus and also this method resulted sustainable in that about 25% of the total catch was of legal marketable size and the animals not suited can be brought back to the sea in a good status while preventing by-catches (Carreira and Gonçalves, 2009).

Pots, possibly the same used for the capture (Shepherd et al., 2014; Bastos et al., 2020) – could be employed for facilitating the process of transportation at least until the mean of transport. These should be placed in a larger container or tank, as in a modern version of Demijohn-baskets (Grimpe, 1928). These must be filled with 1/3 seawater from the collection site and with 2/3 oxygen. Boyle suggested that if temperature, pH and oxygen content values change, renewal of seawater is mandatory (Boyle, 1991). Our suggestion from the knowledge of the biology of this animal is to keep the collected specimen in individual bags or pots and not together with other specimens.

Concluding remarks

In this report our FELASA Working Group showed how knowledge and commitment towards cephalopod capture and transport methods is currently limited for what concerns scientific studies.

European legislations and international guidelines are still at their infancy when dealing with wild animal transport conditions and are absolutely lacking information about cephalopods and regulations concerning wildlife capture protocols are missing essential taxon-specific indications (even for vertebrates). Only recently, some organisations and few countries have decided to include cephalopods as experimental animals deserving mong the same welfare attention as vertebrates during the capture and transport and are now becoming bounded to international transport and shipping rules.

We carried out an extensive text-mining of the literature available on the subject, that allowed us to detect the virtues and flaws concerning the current capture and transport methods and pointed out the responsibility science has towards specifying useful information such as:

- ***i.*** species, age and sex of the animals;
- ***ii.*** justification for adopting a specific capture/transport method for that particular species and life form;
- ***iii.*** details of the capture device and transport containers (e.g., shape, dimension, material);
- ***iv.*** health conditions following wildlife capture, including the number of dead and/or injured animals during the process;
- ***v.*** any handling precaution taken to avoid touching or exposing the animal to air (or any kind of life-threatening condition);
- ***vi.*** details about rearing prior and during transport (e.g., water parameters, safety considerations);
- ***vii.*** location of the collection site and of the place of destination;
- ***viii.*** means of transport and duration of the journey;
- ***ix.*** details about animals welfare assessment;
- ***x.*** animal health conditions after transport, including the number of dead and/or injured animals.

This information will help improve both science and animal welfare as it is in the aims of the PREPARE (Smith et al., 2018) and ARRIVE (Kilkenny et al., 2010; Percie du Sert et al., 2020) guidelines that we advise to followed when working with laboratory animals.

We attempted at defining the most suitable capture and transport conditions for a specific cephalopod taxon (nautilus, cuttlefish, sepiolids, squid, octopus) paying attention to its life stage.

From this survey we can draw some general conclusions:

- ***a.*** The best capture method is any atraumatic gear which takes advantage of well-known animals' behavioural tendencies (e.g., octopus preference for den, seabed substrate for spawning cuttlefish), their chronotype and food habits – according to the species and life stage - improving the chance of catching them. Target cephalopods should experience only a short-term distress, without any injury or long-term suffering, classifying the method as no higher than mild procedure. Large-scale non-selective methods (i.e. trawl) must be avoided as they are likely to affect marine animals welfare and the environment.

- ***b.*** The best transportation method is any set of protocols able to prevent or minimise further stress deriving from the capture procedure. Planning of the journey (duration, resting place, number of health checks) plays a pivotal role in avoiding animals from experiencing PSDLH. Physiological requirements of oxygen, pH, salinity, temperature must be monitored throughout the journey to meet the welfare requirements of a specific cephalopod taxon. The duration of the journey influences the characteristics of the means and containers used for transporting specimens (type, size and equipment onboard). Any insult and vibration during the transport should be avoided together with limiting light exposure. Wild-caught cephalopods should be acclimatised prior transportation and kept in accordance to the available regulations on live aquatic marine animals' transport.

Capture and transport are tightly related and both contribute to the main purpose of preserving animal welfare by properly avoiding any exposure to aversive conditions from the site of collection to the container (e.g., air exposure).

We thus propose pilot studies aiming at comparing different combinations of capture and transport methods – among those reviewed in Section 2 and 3 – and their effects on the survival rate, physical conditions, and physiological milieu of the adult form of the most common cephalopod species (see Section 5) in collaboration with selected, geographically distributed, fishermen communities. Ultimately, of paramount importance is the competence of the personnel carrying out these activities.

Hence, we suggest the implementation of a special edition of accredited course for the Education and Training of fishermen and transporters to help them acquiring expertise. Lectures and seminars will be arranged around a modular training structured in theoretical and practical sessions, with learning outcomes based on defined assessment and pass-fail criteria. Once successful, suppliers enter a two year-update program. Through surveys and questionnaires fishermen and shippers will be able to submit useful feedbacks that will definitely help us improve the teaching and also fine-tune reliable standardised capture and transport protocols developed during pilot and further studies addressing this matter.

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