Supplementary Information to

FELASA Working Group Report

Capture and Transport of live cephalopods: recommendations for scientific purposes

A.V. Sykes (Convenor)[1,a], V. Galligioni[2,b], J. Estefanell[3,c], S. Hetherington[4,d],

M. Brocca[5], J. Correia[6], A. Ferreira[7],

E.M. Pieroni[8,e], G. Fiorito[8,9,*]

[1] CCMAR – Centro de Ciências do Mar do Algarve, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

[2] Comparative Medicine Unit, Trinity College Dublin, Ireland

[3] Ciclo Superior Cultivos Acuicolas, Instituto de Educacion Secundaria les Profesor Cabrera Pérez, Las Palmas, Spain

[4] CEFAS - Centre for Environment, Fisheries and Aquaculture Science

[5] TECNIPLAST S.p.A., via I Maggio, 6, 21020 Buguggiate (VA), Italy

[6] Flying Sharks, Rua do Farrobim do Sul 116, 9900-361 Horta – Portugal

[7] Praceta do sol lote 4 nº57 3ºD, 2775-795 Lisboa, Portugal

[8] Association for Cephalopod Research 'CephRes', Italy

[9] Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Villa Comunale, Napoli, Italy

*Corresponding author: Graziano Fiorito
email: graziano.fiorito@szn.it; rd_innovation@cephalopodresearch.org

Representing FELASA Members:

[a] SPCAL – Sociedade Portuguesa de Ciências em Animais de Laboratório, Portugal

[b] AISAL - Associazione Italiana per le Scienze degli Animali da Laboratorio, Italy

[c] SECAL - Sociedad Española para las Ciencias del Animal de Laboratorio, Spain

[d] LASA - Laboratory Animal Science Association, UK

[e] Corresponding Member, Association for Cephalopod Research 'CephRes' a non-profit organization, Italy

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Reference to the Ancillary work

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Legislative framework

We analyzed the available legislation and recommendations regarding capture and transport of aquatic animals, and identified more than 20 different documents[a]. Here we do not provide a detailed overview about the legislative framework, but refer to the ancillary work (see Pieroni et al., 2021) and suggest the Reader to refer to it for completeness (see also table 1 in Pieroni et al., 2021).

Our analysis revealed that most of the recommendations are aimed at protecting animals during transportation, with limited detail provided for the capture from the wild, at least when aquatic species are considered. Another key- and reiterated element in these norms is the attention to the competence of personnel; i.e., the need for a proper training for those involved in the capture and transport of wild animals. All of them point out that the main source of suffering and distress in animals being

[a] We refer to: 86/609/EEC; 91/67/EEC; ETS No.193; EC No 1/2005; 2006 No. 3260; 2007/526/EC; Directive 2010/63/EU; EAZA Position Statement on Council Regulation 1/2005: Protection of Animals during Transport; NC3Rs' best practice for animal transport; Guidelines for the Transport of Laboratory Animals; Conf. 10.21 (Rev. CoP16); Packer's Guidelines Inv1/Aquatic invertebrates; Aquatic Animal Health Code; Guide for the Care and Use of Laboratory Animals; Australian code for the care and use of animals for scientific purposes 8th Edition 2013; Olfert et al., 1993.

captured, handled, and transported is related to the limited competence of people involved.

Available legislation and guidelines also include information about proper ***i.*** documentation and planning of the journey, ***ii.*** methods and means of transport including design of containers, ***iii.*** health assessment, food, and water supplies for the animals, ***iv.*** the need of acclimatization before and after transport of wild animals.

All the documents we considered have very little information (or any) relevant to cephalopod molluscs.

Competence and attitude of the personnel involved in capture and transportation

The team of collectors or fishers and shippers has to be properly trained for the scope, and it is responsibility of Authorities to verify that this will occur. Depending on the cases, the team should avail of the support and advice of a Designated Veterinarian who shall check the health status of the animals and shall take the proper actions to avoid or end suffering.

Annex IV of the EU Council Regulation No 1/2005[1] states that drivers and attendants (as referred to in Article 6(5) and Article 17(1)) shall have successfully completed the training and have passed an examination approved by the NCA, ensuring that examiners are independent. The courses should include technical and administrative aspects of Community legislation concerning the protection of animals during transport, including animal physiology (e.g., drinking and feeding needs), animal behavior and the concept of stress. Moreover, practical aspects of animal handling and of the emergency care both for the personnel and the animals should be considered in the training program assuring that personnel involved (drivers) should know how the driving behavior might impact on the welfare of the transported animals thus also impacting the quality of meat, if the animals are destined for food consumption (see Annex IV)[1].

Principles for Transport, documentation and planning of the journey

Regardless of the purpose of capture and transport of animals, the main principles included in the guidelines and legislations (review in Pieroni et al., 2021) concerning the planning phases prior collection and transport, may be applied to cephalopods after the due considerations.

Transport, and in particular long journeys, might be detrimental for the health conditions of animals[1, 2] thus, all the planning should be done in advance and the transport should be carried out without delay to the place of destination.

According to the available knowledge that with increased duration of confinement for transport, both consumption of available oxygen and detrimental changes in water chemistry (e.g., accumulation of ammonia, carbon dioxide and depletion of oxygen), we can distinguish[3]:

i. **Short duration journey** (< 2h; e.g., from local capturing site to local establishment). Although this is most likely the less stressful situation, there are important conditions that should be monitored before and during the trip to the final location (e.g., pH, temperature and salinity, saturation of oxygen, light exposure). Vibration, noise, and any other kind of interference should be limited and/or avoided; attention to the infliction of any physical damage should be constantly given.

ii. **Long duration journey** (> 2h; e.g., between towns, countries and/or intercontinental). The same precautions described for the short duration trip applies also to this situation. However, considering that the journey might imply the shipping of the animals by sea or by air, special attention should be given to the containers used for this kind of transportations. Care should be provided to include the documentation concerning management of the welfare state of the animals during and after the journey (see Supplementary Info and Pieroni et al., 2021).

During a short-duration transport, plastic bags may be replaced with large plastic buckets or boxes with a lid containing sufficient pre-oxygenated seawater to allow the animal to be completely immersed[3, 4]. For long-duration transport and similarly to fish[5], considering animals' body size, the cephalopod should be placed with adequate volume of seawater and oxygen-enriched air in double common aquarium aerated bags (see Table 2). For transport periods over 12 hours, aeration and oxygenation may be necessary being careful not to induce 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 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[3]. 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 'Other General Requirements' in Table 2).

In Europe, but also according to other national legislations (see table 1 in Pieroni et al., 2021), every transportation should be preceded by accurate planning of the journey and shall be accompanied by a proper documentation stating: **a)** the origin and their ownership; **b)** the place of departure; **c)** the date and time of departure; **d)** the intended place of destination; **e)** the expected duration of the intended journey (see Chapter II, Art. 4 and Appendix included in EU Council Regulation No 1/2005)[1]. The regulation recommend that a competent scientific committee shall be consulted about the duration and the route plans of animal transport, and according to a harmonized European model, certificate for transporters must be presented. Authorities should shall take the necessary measures to prevent or reduce to a minimum any delay during transport (e.g., programming special arrangements at the place of transfers, exit points and border inspections to give priority to the transport of animals) but resting periods at specific control posts shall be planned if the journey is longer (Art. 22(1))[1]. Furthermore, the consignment shall be done immediately unless the detainment is necessary for the health of the animal or for public safety, such as possible spread of zoonosis if there are some diseased animals (Art. 22(2))[1]. For such a reason, veterinary checks at borders' inspection include the analysis of the welfare conditions in which the animals are transported.

Further documentation is needed if transporting wild, timid or dangerous species, providing instructions about feeding, watering and any special care required for them (see Chapter II, Section 1.3)[1].

The person planning the journey has to bring and compile a journey log, which reports in detail any daily event including animals' health status, any intervention performed and any detour from the original plan (see Annex II)[1]. As for the regulation of the international transport of animals the ETS No.193[6] applies.

On the basis of EU legislation and other documents - such as ETS No.193 and Council Recommendation 2007/526/EC (see Pieroni et al., 2021 for details) - specifically addressed to experimental animals, LASA produced a WG Report 'Guidance on the transport of laboratory animals'[7] which lists all the documentation needed during the planning of the journey (see Paragraph 3.3[7]). Of course, the number and type of documents to be filled up depend on the journey type, species, microbiological status, and route. However, the following information should not be missing:

i. shipment documentation details such as waybill number or IATA Shipper's certificate (for Air transport), import licenses 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 authorizations 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[7] (see also Pieroni et al., 2021 for details).

Different forms, different methods: considerations for Capture and Transport methods of different cephalopods' life stages

It is essential to take into account the life stage of the target cephalopod species.

Live cephalopods or eggs?

Collection and standardized transport of eggs for target cephalopod species have been proposed as an alternative, since these appear easier to manage.

For 'classic' laboratory species (e.g., fish), moving of embryos, sperm or eggs between research facilities has increased and is now well established. This approach is also considered as a way to comply with Refinement since transport appear to be stressful for live animals and could impact their welfare, while for early life stages it seems limited. On the other hand, the use of captive animals (raised from eggs) would allow to fulfill experimental requirements, such as controlling previous experiences. Under similar considerations, the use of animals taken from the wild would be in accordance with the principle of using animals that have been 'primed' by natural environmental stimuli.

When applied to cephalopods, considerations should be taken to offer a standardized method of egg collection to reduce the impact over natural resources (e.g., collecting those stranded on the beach or those captured as by-catch), and to minimize the harm to the egg masses. Though, cephalopods eggs require delicacy in handling, accurate temperature and salinity control during all phases, including transportation[8, 9]. It is noteworthy to remind that eggs collected at advanced stages of development may induce premature hatch during transport and because of handling as they also have higher metabolic requirements[10], and - for some cephalopod species - maternal care is needed in order to develop in a proper and healthy way.

Methods for transport of eggs of several cephalopod species have been developed achieving some standardization. Examples are available for cuttlefish, squid and octopus eggs[9, 11].

The Directive 2010/63/EU considers the protection of this taxon from hatching. However, several studies are based on the collection and culture of egg masses from the wild which - although easier to obtain and then transport in terms of size of containers and water volume - require particular care, being very sensitive to even little changes in temperature, pH and salinity (for example see Iglesias et al., 2007)[12]. Storing conditions during embryonic development should also be monitored because eggs health state will consequently affect the "quality" of the hatchlings. As mentioned, it is also crucial to consider whether maternal care is required, like in incirrate octopods or oceanic squids[13, 14] and attempts to properly simulate are mandatory. Maternal care is a critical factor for the proper embryonic development and to limit the risk of premature hatching[15-19].

From hatchlings to adult

Cephalopod hatchlings are either miniature adults or planktonic paralarvae with relatively short arms and limited swimming ability[20] and represent extremely delicate developing forms, very sensitive to any insult or change in the water parameters from the site of collection to the containers. Nevertheless, the majority of studies (see table 2 in Pieroni et al., 2021) used trawls and bongo nets for collection, with few reporting also that animals which resulted damaged were excluded from the experiments (for example see Otero et al., 2016)[21]. A few notes about capture and transport of paralarval stages of cephalopods is given in the ancillary work to this paper (Pieroni et al., 2021).

The collection of juveniles is a challenging task because these may be selective and with rigid feeding requirements[22]; appropriate cautions should be applied in these circumstances, because a traumatic capture might affect highly feeding and behavioral responses that may translate into fatal conditions.

Adults have also important temperature, salinity and pH requirements as most of the cephalopods are stenotherm and stenohaline. It follows that the inappropriate capture and transport of any stage from the wild might result in high mortality and therefore considerable species-specific care is needed if viable animals have to be returned to the laboratory[3, 11, 23]. Further information and summary of requirements are provided in Pieroni et al. (2021).

Other Recommendations for live cephalopods transport

Prior to transport, and before long-journeys, wild-caught cephalopods should be acclimated to captivity in aquaria and should be monitored during this phase, checking for good appetite, presence of any skin lesions and potential unusual behaviour.

If the journey to the lab is brief, small sepioids and octopuses could be temporarily placed in containers part-filled with seawater. If temperature, pH and oxygen content values change, renewal of seawater is mandatory[4]. For transport lasting more than one or two hours, small numbers of small individuals could be carried 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. Survival for 8-10 h was reported to be easily possible by sealing and keeping the bags cool.

Another factor to consider is food deprivation. It is suggested to prevent animals from feeding for 24 hours before long-term journeys because it helps keeping metabolic rate under baseline, possibly limiting ammonia build-up during transport. However, food deprivation depends on the animal normal feeding frequency, oro-anal transit time and renal ammonium ion excretion for the species[24]. Sedation is not essential and is not recommended for transport of most cephalopods. However, sedation methods have been utilized for the transport of some species, in some cases with controversial results. Interestingly Grimpe[11] suggested that very long duration transportation - i.e. requiring more than two days - should be achieved in steps allowing 'resting' periods in appropriate locations which is what nowadays is indicated by the European and international legislations for the transport of live animals (for details see Pieroni et al., 2021).

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