Supplementary Information to:

General and species-specific recommendations for minimal requirements for the use of cephalopods in scientific research

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[a] www.cephsinaction.org – see also the agenda of the following meetings:
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Contents

Cephalopods: current scientific interest, diversity of forms and adaptations

At the time of finalizing the writing of this work, nine years are now passed after the entry into force of the Directive 2010/63/EU. During this almost a decade, the EU continued to collect statistical data on the use of live animals in research and testing[b], and information for cephalopods is also included. However, data available in the Statistical Reports should be considered preliminary for these invertebrate organisms, also considering the differences in the time of transposition between Member States of the EU, and the required time for the effective implementation of the Directive to these animals.

In analogy to what already proposed by Smith and coworkers,[1] we analyzed original papers indexed by Clarivate Web of Science and published between January 2015 and the first months of 2021 (time span: 2015-2020) with focus on any aspect of cephalopod biology. We combined the query with all of the EU countries (address field), returning 1929 works (note that a similar analysis - by Smith and colleagues in 2013[1] - provided 1231 papers published over a period of five years). In more than one thousand papers the target species was specified or identifiable. For each record we counted the species of cephalopods target of the study and found more than 250 species (for a three years subset see Supplementary Table 1).

Overall, a large number of species has been studied, with some continuing to result to be the most highly utilized (i.e. *Sepia officinalis*, *Octopus vulgaris*).

[b] https://ec.europa.eu/environment/chemicals/lab_animals/reports_en.htm

Supplementary Table 1. Cephalopod species studied and number of papers indexed in Clarivate Web of Science, over the last three years (time span: 2017-2020) with a focus on EU countries. A number of works identified their target organism by genus and/or not referring to a given species; these are not included in this list. In some cases, more than five species resulted to be included in the same study; for the sake of this analysis, we considered only the first five cephalopod species.

Species	Papers
Octopus vulgaris	176
Sepia officinalis	131
Loligo vulgaris	52
Dosidicus gigas	25
Eledone cirrhosa	15
Illex coindetii	15
Illex argentinus	14
Nautilus pompilius	13
Euprymna scolopes	11
Octopus cyanea	11
Octopus maya	11
Sepia pharaonis	11
Loligo forbesii	9
Todarodes sagittatus	9
Kondakovia longimana	8
Alloteuthis media	7
Architeuthis dux	7
Spirula spirula	7
Doryteuthis gahi	6
Eledone moschata	6
Enteroctopus dofleini	6
Galiteuthis glacialis	6
Gonatus fabricii	6
Loligo reynaudii	6
Sepioteuthis lessoniana	6
Abraliopsis morisii	5
Berryteuthis magister	5
Doryteuthis pealeii	5
Octopus bimaculoides	5
Octopus mimus	5
Pyroteuthis margaritifera	5
Vampyroteuthis infernalis	5
Adelieledone polymorpha	4
Bathyteuthis abyssicola	4
Doryteuthis opalescens	4

Species	Papers
Haliphron atlanticus	4
Histioteuthis reversa	4
Sepia orbignyana	4
Abralia veranyii	3
Alloteuthis subulata	3
Alluroteuthis antarcticus	3
Ancistrocheirus lesueurii	3
Argonauta	3
Doryteuthis ghai	3
Filippovia knipovitchi	3
Gonatus antarcticus	3
Histioteuthis bonnellii	3
Liocranchia reinhardti	3
Mesonychoteuthis hamiltoni	3
Moroteuthopsis longimana	3
Octopus briareus	3
Octopus insularis	3
Ommastrephes bartrami	3
Pareledone charcoti	3
Pareledone turqueti	3
Pteroctopus tetracirrhus	3
Rossia palpebrosa	3
Sepietta oweniana	3
Sthenoteuthis oualaniensis	3
Todarodes angolensis	3
Todarodes pacificus	3
Argonauta argo	2
Bathypolypus sponsalis	2
Bathyteuthis bacidifera	2
Chiroteuthis veranyii	2
Chtenopteryx sicula	2
Doryteuthis pleii	2
Enteroctopus megalocyathus	2
Gonatus madokai	2
Gonatus onyx	2

Species	Papers
Gonatus pyros	2
Heteroteuthis dispar	2
Histioteuthis atlantica	2
Illex illecebrosus	2
Loligo pealeii	2
Megaeledone setebos	2
Moroteuthis ingens	2
Moroteuthopsis ingens	2
Nautilus macromphalus	2
Octopus campbelli	2
Octopus macropus	2
Octopus oliveri	2
Ommastrephes bartramii	2
Opisthoteuthis borealis	2
Pareledone aequipapillae	2
Psychroteuthis glacialis	2
Rondeletiola minor	2
Rossia macrosoma	2
Scaeurgus unicirrhus	2
Sepia esculenta	2
Sepia latimanus	2
Sepiella inermis	2
Sepietta neglecta	2
Sepiola atlantica	2
Slosarczykovia circumantarctica	2
Sthenoteuthis pteropus	2
Todaropsis eblanae	2
Abralia redfieldi	1
Allonautilus scrobiculatus	1
Amphioctopus fangsiao	1
Amphioctopus marginatus	1
Ancistroteuthis lichtensteinii	1
Bathothauma lyromma	1
Bathypolypus arcticus	1
Bathyteuthis berryi	1
Batoteuthis skolops	1
Benthoctopus piscatorum	1
Boreoteuthis borealis	1
Brachioteuthis riisei	1
Callistoctopus furvus	1
Callistoctopus luteus	1
Callistoctopus ornatus	1
Chiroteuthis calyx	1
Chiroteuthis mega	1
Cranchia scabra	1

Species	Papers
Cycloteuthis sirventi	1
Enoploteuthis leptura	1
Euprymna berryi	1
Euprymna brenneri	1
Euprymna morsei	1
Euprymna parva	1
Fillipovia knipovitchi	1
Galiteuthis armata	1
Galiteuthis phyllura	1
Galiteuthis suhmi	1
Gonatopsis octopedatus	1
Gonatus antarticus	1
Gonatus berryi	1
Gonatus kamtschaticus	1
Gonatus phoebetriae	1
Helicocranchia pfefferi	1
Heteroteuthinae	1
Heteroteutis dispar	1
Histioteuthis arcturi	1
Histioteuthis eltaninae	1
Histioteuthis hoylei	1
Idiosepius paradoxus	1
Japetella diaphana	1
Lampadioteuthis megaleia	1
Leachia lemur	1
Lepidoteuthis grimaldii	1
Liguriella podophtalma	1
Loligo bleekeri	1
Loligo duvauceli	1
Loligo edulis	1
Loligo forbesi	1
Loligo opalescens	1
Lolliguncula diomedeae	1
Martialia hyadesi	1
Mastigoteuthis agassizii	1
Moroteuthis knipovitchi	1
Moroteuthis longimana	1
Muusoctopus rigbya	1
Muusoctopus robustus	1
Neorossia caroli	1
Nototodarus gouldi	1
Nototodarus sloanii	1
Octopoteuthis sicula	1
Octopus americanus	1
Octopus bimaculatus	1

Species	Papers
Octopus hummelincki	1
Octopus ocellatus	1
Octopus ornatus	1
Octopus rubescens	1
Octopus salutii	1
Octopus taganga	1
Octopus tayrona	1
Octopus tehuelchus	1
Octopus hubbsorum	1
Ocythoe tubercolata	1
Ocythoe tuberculata	1
Ommastrephes brevimanus	1
Ommastrephes caroli	1
Ommastrephes cylindraceus	1
Onychoteuthis borealijaponica	1
Onykia ingens	1
Onykia robsoni	1
Opisthoteuthis bruuni	1
Pareledone albimaculata	1
Pareledone aurata	1
Pterygioteuthis gemmata	1
Pyroteuthidae	1
Rossia megaptera	1
Rossia moeller	1
Sepia aculeata	1
Sepia andreana	1
Sepia apama	1

Species	Papers
Sepia bandensis	1
Sepia bertheloti	1
Sepia brevimana	1
Sepia dollfusi	1
Sepia elegans	1
Sepia gibba	1
Sepia hierredda	1
Sepia lycidas	1
Sepia recurvirostra	1
Sepia vermiculata	1
Sepiadarium austrinium	1
Sepiella japonica	1
Sepietta obscura	1
Sepiola affinis	1
Sepiola pfefferi	1
Sepiola tridens	1
Sepiolinae	1
Sepioteuthis sepioidea	1
Stoloteuthis leucoptera	1
Taningia danae	1
Taonius notalia	1
Thaumeledone	1
Thaumoctopus mimicus	1
Thysanoteuthis rhombus	1
Vampyroteuthis infernails	1
Vitreledonella richardi	1

It is noteworthy to comment that, in some cases, the interaction of EU-based scientists with those operating abroad is the reason why some species, not necessarily mainly utilized in EU countries, resulted as target of the studies considered in this preliminary analysis (e.g., *Dosidicus gigas*, *Octopus cyanea*; see Supplementary Table 1).

Cephalopod molluscs account for almost 800 marine living species[2-5] and represent about 2% of the populous molluscan phylum they belong to, a Phylum counting more than 46,000 species.[6]

Cephalopods fascinate for their unique biological and cognitive features,[7] but also have an important value for human consumption[8,9,c] thus representing an important area of interest for fisheries[10,11] and aquaculture.[12,13] Furthermore, they provide an interesting case for society and general public as highlighted by their success in public displays, media[14-17] and human culture[18-20].

Cephalopods, in particular coleoids (cuttlefish, squid and octopus), are known for the large set of adaptations characterizing them, likely originated as a consequence of the loss of external protective shells, thus to evolve different strategies and the remarkable cognitive capabilities[21-23] underlying levels of biological plasticity somehow comparable to 'higher vertebrates'.[24-27] The evolution of 'intelligence' in cephalopods has been accompanied by an enlargement and enrichment in brain complexity[28,29] making this subclass (i.e. Coleoidea) as the one endowed with the proportionally largest nervous system among invertebrates, often considered as a traditional example of convergent evolution with vertebrates.[22,26] Recent investigations on nociception in cephalopods[30,31] also contributed to discuss about the possibility of pain perception, sentience and consciousness in these animals.[32-36]

Over the last years, cephalopods utilized in scientific research increased both in number and species diversity (see also Supplementary Table 1), partially because of the renewed interest for this taxon brought by a series of important discoveries, including remarkable RNA editing capabilities[37-39], and those originating from the complete genome sequencing that now accounts for eight species.[40-46]

Beyond the vast interest for the neurobiology and behaviour of cuttlefish, squid and octopus that mainly led cephalopod science since the early twentieth century, an ever-growing number of research groups around the world is becoming interested in their

[c] see FAO GLOBEFISH data about Cephalopods Market Reports: http://www.fao.org/in-action/globefish/market-reports/cephalopods/en/ (last visited, June 2021)

genomic organization and evolution, ecology, physiological and behavioural adaptations. Despite the great popularity and scientific relevance, more than 90% of the extant species are little known for their basic biological characteristics.[47,48]

Cephalopods are stenothermic and stenohaline animals, and therefore possess limited tolerance to rapid or significant changes in temperatures, salinity, quality of the water, as reviewed in numerous studies[7,12,13,49] (see also Appendices A and B). Active predators they are characterized by short life-span (in the greatest number of cases) and known for high metabolic rate, which requires a high feeding rate, and elevated levels of protein consumption.[50,51]

Source and summary of Information

Here we provide a brief description about the sources of information and data regarding different aspects for the rearing conditions adopted for a selected list of species belonging to the class Cephalopoda, Phylum Mollusca. The data included herein (see Appendices A and B) originate from various works that reported information about the care of animals under experimental settings for research and aquaculture purposes. A similar approach has been taken in compiling Appendix C that includes thermal, oxygen and salinity tolerance ranges for the target species.

The information summarized in the two appendices provided the basis for defining recommendations about accommodation, management, and care of the most frequently utilized cephalopod species in Europe (see also Table 2, main text); data relevant to different life stages is also provided for each species, whenever available.
This information constitutes a dataset (Appendix A: Environment; Appendix B: Accommodation and Care, Enrichment) deriving from an accurate text mining of about one hundred works (experimental studies, reviews, recommendations/white

papers, and guidelines), not only focused on laboratory maintenance and rearing, but also on those reviewing cephalopod culture.[12,13,52-56]

Appendices A and B are organized following the scheme of the Annex III 'Requirements for establishments and for the care and accommodation of animals' of the Directive 2010/63/EU and in particular: environment (water supply and quality, lighting, noise and vibration), accommodation and care (housing, stocking density, sex ratio, diet), enrichment (social and environmental).[57]

Finally, additional information regarding species-specific tolerance range for temperature, oxygen and salinity according to experimental studies or observations is summarized in Appendix C whose content should be considered as a support to aspects relevant to temperature, dissolved oxygen and salinity included in Appendix A.

Appendix A – Environment: a short overview

As mentioned above, Appendix A includes data about the essential environmental conditions to which live cephalopods should be accommodated. Water supply and quality, lighting, noise and vibration were used as main 'categories'.

Water supply and quality

Several papers and reports have been considered.

Carlson (1991) provided the main source of information for *Nautilus*.[15] Data for cuttlefish were deduced from Jones and McCarthy (2009)[58] for *Sepia officinalis*, Nabhitabhata (2014)[59] for *S. pharaonis*. Hanlon et al. (1997)[60] provided information for *Euprymna scolopes*, Vidal & Boletzky (2014)[61] for *Loligo vulgaris*, while for *Sepioteuthis lessoniana* we referred to Nahbitabhata & Ikeda (2014)[62]. For octopuses we considered

Hanlon & Forsythe (1985)[63] for *Octopus bimaculoides*, Vidal et al. (2014)[13] for *O. vulgaris* and Rosas et al. (2014)[64] for *O. maya*; these works were complemented by other sources.

Cephalopods are all marine. They need continuous supply of seawater of appropriate salinity (psu), within a range that matches their species-specific physiological requirements, as they are stenohaline.[55] Essential trace elements might be added if present below the optimal threshold for the species, while copper (and zinc) should be avoided because of their strong effects on salinity and possible (to be verified) interference with respiratory pigment.[55,65] A minimum value of 21 psu to a maximum of 40 psu have been set for the rearing of *S. pharaonis* juveniles[66,67] and *O. maya* hatchlings/juveniles[13], showing how salinity can differently affect development from the time of hatching until maturation (see also Appendix C).

Oxygen solubility is inversely related to water temperature and its consumption is affected by metabolism and body weight, as feeding and swimming cause large amount of oxygen consumption and small specimens have a relatively higher mass-specific oxygen consumption than larger ones[13]. A dissolved oxygen (DO) close to saturation (85-100%) is the desirable value to be controlled when rearing animals with due species-specific considerations (e.g., hypoxia sensitivity of squid is far greater than in cuttlefish or octopuses).[68,69] A range of 35-65% is considered suboptimal while values between 35-15% are dangerous; values below 15% are considered lethal[70] even though few species can tolerate hypoxia and low oxygen levels such as *Nautilus* and benthic octopods[53,71,72] (see also Appendix C). Of course, a technical control is not necessary if the water is circulating continuously, as O_2 saturation is always closed to 100% and there is a free gas exchange at the surface supported by proper filter systems (i.e. protein skimmer).

The quality of water supplied to animals needs to be maintained within a range considered acceptable for supporting the normal activity and physiology of a given species, in relation also to its developmental stage[55,56].

Water quality should be monitored daily by considering the most significant parameters able to guarantee the maintenance of healthy specimens. Water flow in tanks and in re-circulatory or filtration systems within tanks has to assure the stability of appropriate levels of seawater quality parameters. As indicated by the Directive 2010/63/EU, seawater «shall be filtered or treated to remove substances harmful» to animals, whenever necessary. In addition, and based on species-specific requirements, seawater flow should be adequate to enable the animal to adopt a locomotor pattern, including swimming, that corresponds to «normal behaviour».[55,56,73] However, some cephalopod species live in environments which are naturally characterized by high water turbidity[74,75] and in this case the choice and use of filtration systems should be attentively pondered, in order to possibly match environmental requirements and seawater quality.
Animals should be periodically monitored and appropriate time for acclimatization and adaptation to any changes in water quality conditions should be warranted.[19,55,65]

As for other marine organisms, seawater systems are based on two main different modalities: open, and closed.[76] Semi-open and recirculating aquaculture systems (RAS) can also be adopted.[15,63,76] The open system provides water directly from the sea with less monitoring effort, therefore the technical infrastructure is considered simpler with lower costs for supply. However, these systems must be located on the coast and can be subjected to the risk of contamination together with rough changes in water quality according to seasonal shifts. On the other hand, the closed seawater systems allow for the full monitoring of water parameters (e.g., temperature, salinity, turbidity) providing conditions which are independent from external factors. Nevertheless, the costs and the technical structures are higher when compared to the open systems. Both of them should be endowed with high quality filtration and aeration and must allow for a simple and rapid water exchange.[55] Strong aeration supplied by airlifts or air stones positioned close to the eggs may guarantee a water flow rate sufficient enough to carry the oxygen needed in order to stimulate a controlled hatchling phase.[58,62,77] As for hatchlings, an aeration system able to generate a directional water flow that allows them to easily hover in the water column should be provided.[62]

In all cases, attention should be drawn to eventual formation of bubbles because they may threaten animal welfare by "attaching" on animals' skin or remaining trapped inside the mantle, thus leading to potential harmful side effects (e.g., altering the mucous layer/pH). Other authors suggest that water flow can be slowed down through the bottom, thus oxygenating water without producing any bubbles.[78]

The rate of water exchange may vary on a wide range from continuous[58,78,79] to a few times per day[80] depending on the species, its developmental stage and the tank size.

Water flow systems can include a mechanical filter (i.e. vortex or swirl/filter socks/brushes) for removing particulate matter, a biological filter for removing ammonia and nitrite, a mechanic filter such as a protein skimmer (foam fractionator) for removing organic molecules (including proteins and amino acids) before they break down into nitrogenous compounds,[76,81] or chemical filters such as phosphate absorber, epoxide - to bind copper and heavy metals - or charcoal. Ozone can be employed too, although it could prove harmful for cephalopods, particularly eggs and paralarvae, and therefore requires a careful monitoring.[13,55] UV filters and sterilization, able to kill bacteria, viruses and micro-organisms is suggested for the different phases of species' cycle.[58,60,61,82] In addition, the efficiency of filters such as drum filter or protein skimmer could be reduced by cephalopods high metabolism and ink production. Therefore for a safety reserve, biofilters with bigger capacity should be included in designs. Furthermore, a safety mechanism against electric blackout should be present to avoid accidental emission of toxic H_2S.

Water quality is pivotal for the well-being of the captive cephalopods, and proper values of temperature (°C), salinity, pH, and nitrate levels (mg L^{-1}) should be constantly monitored according to species and life forms.[55]
Water temperature should be set and regulated according to the natural range for the species and the geographical area in which it was captured (e.g., 10-24°C for *O. vulgaris* caught in Europe,[83] up to 28-30 °C for *O. maya*[13] and *S. lessoniana*[62] inhabiting tropical waters) as well as the life form considered. However, a different approach is adopted when specimens are cultured for food consumption or for experimental purposes.

Cephalopods are poikilotherm, thus by increasing the temperature to the optimal maximum for the species a rapid growth will be favored in the short-term. However, if animals are intended to be utilized for experimental purposes or public display, cooling the seawater has been suggested to help promoting a longer lifespan for the animals[13] (see Appendix C). We have no accurate knowledge of the effects on the physiology of individuals and animal welfare linked to the above-mentioned changes in key environmental conditions for housing the individuals. Of course, we are aware of the marked capacity of adaptation/plasticity of the species here considered, but solicit *ad hoc* studies attempted to assess animal welfare and potential harm (and benefits).

Since cephalopods demand a high protein diet, the buildup of ammonia and nitrogenous waste might accumulate and become potentially toxic to the animals, causing behavioral changes and alteration of the chromatic patterning[55]. A variable range of nitrogenous levels can be observed among species and their life stage (e.g., *S. lessoniana* of the temperate countries[84]).

Cephalopods do not tolerate rough changes in pH levels, as this directly impacts hemocyanin's oxygen-carrying properties. It is related to water solutes and the presence of soluble gases. Monitoring pH levels is crucial in closed-water systems in which water exchange is less efficient; a range from 5.9 (for hatchlings of *S. pharaonis*)[66] to 8.4 (for hatchlings of *S. pharaonis*,[66] and of *S. lessoniana*[66,67], and for *L. vulgaris*[85]) is reported (Appendix A).

Lighting

Main information derived from: Carlson (1991; *Nautilus* spp.)[15], Panetta et al. (2017, *S. officinalis*)[78], Yasumuro & Ikeda (2016, *S. pharaonis*)[86], Hanlon (1997, *E. scolopes*)[60], Vidal et al. (2002, *L. vulgaris*)[87], Ikeda et al. (2009, *S. lessoniana*)[88], Forsythe & Hanlon (1988, *O. bimaculoides*)[89], Vidal et al., (2014, *O. vulgaris*)[13] and Rosas et al., (2014, *O. maya*)[64].

As stated in Part A, Annex III of Directive 2010/63/EU, light intensity and photoperiod shall «satisfy the biological requirements of the animals» and, at the same time «the needs for the performance of husbandry procedures and inspection of the animals».[57] However, there is only limited knowledge about the wavelength optimum for cephalopods,[90] and there is also evidence of adaptation to gradual changes in light/dark regime in some species (see Fiorito et al.[55] and references therein). It is preferable to let cephalopods circadian rhythm be regulated by the natural environment[91-93] to avoid any impairment in their physiological and behavioral patterns (e.g., mating, spawning, hatching).[77] However, in the cases this is not possible a photoperiod (L:D) of 12:12 is usually set especially in aquaculture setups,[58,86,94] although not preferred.

When the natural pattern has to be followed, seasonal changes in day/night length should be respected (e.g., 16:8 in summer and 8:16 in winter[95] or 14:10-10:14[87,93,96]). Under artificial settings (i.e. fluorescent or metal halide lighting), generally very low light intensities are preferable, especially for eggs and hatchlings (around 1-15 lx)[13,87,97,98] or in black-walled and white-bottom tanks (60–250 lx).[96] Higher light intensities are used in black tanks (500–700 lx),[93] reaching values up to 900 lx[99] at the water surface while in outdoor conditions, water-repellent nets can be employed in order to excess lighting.[13,91]

Noise and vibration

Information on noise and/or vibration is not included in Appendix A, as it derives mainly from welfare guidelines.[55] Husbandry facilities must be located in low disturbance areas, and holding rooms «shall where appropriate be provided with noise insulation and absorption materials».[57] Background vibrations should also be minimized such as those related to pumps or ventilation units, and the use of sound-attenuating, sanitizable material and rubber pads for the wall is encouraged, as cephalopods could suffer from low-frequency noise traumas, altering their physiology and behavior. For further considerations on how to reduce noise and disturbances in both housing and experimental areas, see Fiorito et al. (2015)[55] and references therein.

Appendix B - Accommodation and care: a short overview

The appendix includes information collected, from our source of information, about accommodation and care for the animals. Housing, grouping and diet were used as variables describing this category. Furthermore, 'enrichment' has been added in Appendix B; it includes here social or environmental enrichment, considering species-specific biology and needs.

Housing

The majority of information derives from Carlson (1991)[15] for *Nautilus*, Panetta et al. (2017)[78] for *Sepia officinalis*, Yasumuro & Ikeda (2016)[86] for *S. pharaonis*, Hanlon et al. (1997)[60] for *Euprymna scolopes*, Vidal & Boletzky (2014)[82] for *Loligo vulgaris*, Ikeda et al.

(2003)[100] for *Sepioteuthis lessoniana*, Hanlon & Forsythe (1985)[63] for *Octopus bimaculoides*, and includes also Rosas et al. (2014)[64] for *O. maya* and Vidal et al. (2014)[13] for *O. vulgaris*. We also considered the application of a series of care-taking and welfare-promoting methods and techniques, that foster animals' expression of the Five Freedoms.

According to the paragraph 3.3(c) of Part A Annex III of Directive 2010/63/EU animal enclosures «design and construction shall be such that no injury to the animals is caused, […] they shall be made from materials that will withstand cleaning and decontamination techniques. The design of animal enclosure floors shall be adapted to the species and age of the animals and be designed to facilitate the removal of excreta».[57] In the case of cephalopods, the most suitable tank should be selected depending on the size, life stage and biological needs and therefore, shape, volume and minimum water depth should be chosen accordingly (for further details see 'Species-specific recommendations, main text). As emerges from our text-mining, different shapes of aquaria have been adopted over the years for different cephalopod species with baskets or floating net cages and circular- or rectangular-shaped being the most adopted ones, respectively for eggs and adults.[58,78] Much more attention is paid on the color and volume of the tank. In fact, the majority of cephalopods is reared in dark-toned,[60,87,101,102] opaque-walled containers[61,86] to avoid or reduce any potential disturbance and interference with their physiological and biological needs,[101] but tanks completely white,[103] grey-white[86] or with black walls and white bottom[96] have also been adopted.

The size in terms of volume capacity is crucial for the welfare of the animals, in that it must allow for natural behavior (e.g., Nautilidae require tanks higher than wider for displaying their nictemeral vertical migration,[104] while Sepiidae need large bottom areas as a proper spawning substrate[105,106]). A covering for the tanks is generally recommended for preventing both escape of the animals and introduction of foreign elements, such as contaminants, chemicals or other organisms.[55] Materials and position related to water surface should not threaten animal welfare in compliance with recommendations for animal enclosures referred in paragraph 3.3(c) of Part A

Annex III of Directive 2010/63/EU.[57] As mentioned above, minimum water depth should respect the natural lifestyle and properly follow the growth rate of the specimens,[107] starting from few millimeters for early life stages,[61] to >60 cm for adults of large cephalopod species[59,84] or 140 cm for *Nautilus*.[79]

Grouping

Information included in this subsection derives from Carlson[15], for *Nautilus* Sykes et al. (2003)[108] for *Sepia officinalis*, Nabhitabhata (2014)[59] for *S. pharaonis*, Hanlon et al. (1997)[60] for *Euprymna scolopes*, Hanlon (1990)[82] for *Loligo vulgaris,* Nabhitabhata & Ikeda (2014)[62] for *Sepioteuthis lessoniana*, Hanlon & Forsythe (1985)[63] for *Octopus bimaculoides,* and considers Rosas et al. (2014)[64] for *O. maya* and Domingues et al. (2010)[109] for *O. vulgaris.*

Cephalopods are either solitary-living species, occasionally and opportunistically involved in schooling behaviors, and gregarious, therefore their natural social 'inclinations', from hatching to maturation, should be respected when reared in animal enclosures. For the few species tolerating or preferring a 'social' lifestyle, grouping can be adopted considering their natural environment and the maximum stocking density that must guarantee each animal a 'living space' (see 'minimum water surface area for each additional animal in group-holding', Main Text). High stocking densities are acceptable for eggs and hatchling phases[63,108] while for adults the density reduces with the growth of large-sized species[110]. When specifically considering specimens of opposite sex, the most employed solutions adopted in controlled conditions is a sex ratio (M:F) of 1:2 or 1:3 resembling natural settings and avoiding competition,[59,111,112] and more rarely a 1:1 ratio.[60,64,84]

Diet

As briefly mentioned in the Introduction, cephalopods present an enormous range of feeding strategies, including active predation, scavenging and filtering from debris and substrates. All cephalopods are exclusively carnivore, many of them are opportunistic and feed upon a wide range of prey while others are more selective.[113,114,115] Interesting insight into the possibility of feeding cephalopods not only on live and dead animals but also upon artificial diets, comes from the evidence of cephalopod culture or laboratory rearing techniques.[12,115]

The source of information considered here originates for: *Nautilus* from Carlson (1991)[15], *S. officinalis* from Jones & McCarthy (2009)[58], *S. pharaonis* from Nabhitabhata (2014)[59], *E. scolopes* from Hanlon (1997)[60], *L. vulgaris* from Tardent (1962)[116], *S. lessoniana* from Nabhitabhata et al. (1996)[117], *O. bimaculoides* from Forsythe & Hanlon (1988)[89], and also from Vidal et al. (2014)[13] for *O. vulgaris* and Rosas et al. (2008)[118] for *O. maya*.

Cephalopods are carnivores (in the greatest majority of cases) and are well known for several predatory strategies adopted.[115] These animals are usually fed a wide range of organisms among their natural prey, such as crustaceans, molluscs and even small fishes.[13,55] It is recommended to provide cephalopods with a proper feeding regime according to the specific developmental stage and lifestyle, paying attention particularly to the type, frequency and quantity. Paragraph 3.4(a) Part A, Annex III of Directive 2010/63/EU, states that «the form, content and presentation of the diet shall meet the nutritional and behavioral needs of the animal».[57] Live organisms are preferable as food, resembling natural environmental conditions, but under laboratory settings, cephalopods can be trained and adapt to several different types of food, from dead or frozen prey[119,120] to artificial mixtures.[61,88] Depending on the purpose of the study in which they enter (e.g., research, aquaculture, public display), cephalopods are fed *ad libitum* or on a daily base. However, taking into account, the differential duration of digestion among species, some authors feed animals few times a day[87] or even every two days[63] with an amount related to species and developmental stages (e.g., 5% of their body weight per day for adults; 35-50% for juveniles of *O. maya*).[64]

Cleaning

Information on cleaning is not always reported in experimental works, therefore it was not included as part of Appendix B. However, some Authors provided recommendations or methods concerning cleaning, such as Carlson for *Nautilus*[15], Jones & McCarthy (2009)[58] for *Sepia officinalis*, Nabhitabhata (2014)[59] for *S. pharaonis*, Vidal & Boletzky (2014)[61] for *Loligo vulgaris*, Vidal et al. (2014)[13] for *O. vulgaris* and Rosas et al. (2014)[64] for *O. maya*.

High hygienic conditions are minimum standard for every holding facility and establishment dealing with animals. Regular and efficient cleaning schedules have to be respected.[57] As for animal enclosures, cleaning is closely related to the water exchange rate and depends on the seawater and filtration system adopted (see Appendix A). For these reasons a proper daily monitoring of water parameters is necessary to prevent contamination, waste accumulation and any potential health and welfare consequences on the animals. Disinfectants and detergents should be carefully managed to avoid any aversive reaction for the animals or sudden alteration of water composition. The use of natural cleaning substances (e.g., vinegar) maybe considered when cleaning tanks in absence of seawater and of course animals. Siphoning[15,58] and cleaning robots[61] are widely adopted systems used in support to filtration, UV sterilization and water exchange for different species.

Enrichment

Major source of information is Barord & Basil (2014)[104] for *Nautilus*, Sykes et al. (2014)[91] for *S. officinalis* and Yasumuro & Ikeda (2016)[86] for *S. pharaonic;* Hanlon et al. (1997)[60]

and Hanlon (1990)[82] for *E. scolopes* and *L. vulgaris*, respectively. Nabhitabhata & Ikeda (2014)[62] were considered for *S. lessoniana*, Forsythe & Hanlon (1988)[89] for *O. bimaculoides.* Methods usually adopted for aquaculture as included in Iglesias & Fuentes (2014)[102] for *O. vulgaris* and Rosas et al. (2014)[64] for *O. maya* where also considered, whenever pertinent.

According to the principle of Five Freedoms, animals should be free of expressing their natural behavior and free from any source of discomfort. Animal enclosures and captivity, with their unnatural conditions, may induce stress to animals – and in the long-term even harmful (e.g., stereotypies, self-harm, etc.) - thus representing one of the major concerns for welfare in animal facilities. For these reasons, social and environmental enrichment are by now a minimum requirement for experimental animals and should always be adapted to the species but also to the individual needs.[57] The natural behavior of the majority of cephalopod species is still unknown, and this makes the choice of appropriate stimuli difficult. However, cephalopods should be provided with the appropriate and sufficient 'amount' of physical, cognitive and sensory stimuli able to foster a healthy behavioral development and to keep their curiosity/motivation high.

Several enrichment strategies may be adopted that enclose almost all the topics discussed in the previous sections.

Sufficient space should be provided in order to let these animals develop and freely express their wide range of locomotory and behavioral repertoire. Moreover, presenting the subjects with new objects or puzzles might be a good way to promote the display of specific abilities like visual/tactile systems and problem solving.

As mentioned above (see Grouping section), the species-specific social behavior should be respected, therefore solitary-living species should be individually reared while social animals should be housed in groups provided that sufficient individual space is guaranteed. The environment surrounding each species should be properly set by considering both the animal care and welfare – avoiding any potential source of infection, contamination or injuries - and the accessibility for the operators[55]. Whenever possible, natural elements usually found in the geographical area of the species are recommended, resembling the wild conditions in which animals were captured. Proper tank shape and color together with wall textures are considered as attractive features according to the cephalopod species; plants, rocks and shells are all mutually used while gravel or sand may be desirable as spawning substrate for benthic species. Shelters and dens are considered mandatory for cephalopod (depending on the species life-style) while other objects (e.g., corals, traps) can represent a valuable enrichment for these animals.[65] Feeding can also be considered as a fundamental part of the enrichment in that by varying the prey species, feeding modality (i.e. avoiding *ad libitum* feeding) texture and presentation, animal motivation is stimulated.

Moreover, enrichment strategies in establishments shall be regularly reviewed and updated in order to ensure all animals needs are met according to the increasing acquisition of knowledge about these species' biology, with the further aim of easing researchers' current challenges when dealing with cephalopods under laboratory settings.[55,57]

Practices for cephalopod culture

It is noteworthy to report that from the analysis of the literature we carried out for the sake of this work, we identified some information relevant to the housing, care and management of the broodstock and mature females of cephalopod species. Most of the data summarized below are reviewed in Iglesias et al.[12] In brief, suggested values for tank size and shape, water parameters (exchange rate, temperature, salinity, DO), stocking density and sex ratio together with information about environmental setting and parameters (diet, photoperiod, social and environmental enrichment) are provided, when available, in various studies for: *S. officinalis*[58,91,107], *S. pharaonis*[59,111], *E. scolopes*[60], *L. vulgaris*[61,82,121], *S. lessoniana*[62,122], *O. maya*[64,123] and *O. vulgaris.*[83,102,124,125] These data should not be considered as welfare recommendations, but simply as an overview of the common practices for aquaculture of the most relevant cephalopod species and therefore of the conditions that allow to maximize the growth and reproduction of the animals.

Appendix A - Environment

Overview of the essential environmental conditions to which live cephalopods should be accommodated for research purposes. Following the categories considered in Annex III of the Directive 2010/63/EU, information about water supply and quality, and lighting together with reference to the relevant works are listed in this appendix (in some instances we also considered Vidal et al.[13] and Fiorito et al.[55]). Notes about noise and vibration are not included here (see text for details). Cephalopod species are listed in taxonomic order following FAO annotated catalogue of cephalopods species[2,4,126] (for the list of species and rationale for their inclusion, see also "Target species" and Table 1 and Table 2 in main text). Information included in 'Water exchange' have been reported following original values and unit of measurements contained in cited works. Abbreviations utilized – **ACF**: Activated Carbon Filtration; **BF**: Biological filtration; **CF**: Chemical filtration; **CR**: Circulation Rate; **DO**: dissolved oxygen; **DO >95%**: dissolved oxygen (close to saturation); **FL**: Fluorescent light; **ilb**: incandescent bulbs; **MF**: Mechanical filtration; **N/A**: information not available; **Nat**: Natural (environmental) light; **NCR**: Natural Circadian Rhythm; **NS**: Not Specified; **oFl**: Open Flow; **OptR**: Optimal range; **Oz**: Ozone; **PS**: Protein skimmer; **RAS**: Recirculating Aquaculture System; **sFl**: Semi-Open Flow; **UgF**: Undergravel filter; **UV**: Ultraviolet sterilization; **w**: week; **psu** (practical salinity unit): defined as the total amount by weight of dissolved salts in one kilogram of seawater.

	Water supply and quality										Lighting		
	Seawater system	Aeration	Water exchange	Filtration and water treatment	Temperature (°C)	Salinity (psu)	DO (mg L^{-1} or % sat)	NH_3 (mg L^{-1})	NO_2 (mg L^{-1})	NO_3 (mg L^{-1})	pH	Photoperiod (L:D)	Type	Light intensity (lx)
Nautilidae														
Nautilus														
Adults[d]	oFl; RAS[15]	Airlift[e], [15]	25%[15] 40%/w[15] 600L h^{-1} or 900L h^{-1} [15]; 50L min^{-1} [79]	MF, BF, UgF[15] UV[79]	15-27[104]	34-36[54]	>95%[85]	<0.10[129]	<0.10[d]	<20.0[d]	8[79]; 8.2[104]	N/A	Red Spot; FL[15]	75-150W[15]; 200W[79]
Sepiidae														
Sepia officinalis														
Eggs	oFl (flow-through)[130] sFl; RAS[91]	Air stones[f, 58]; slow water flow [d, 131,78]	~1.5 L min^{-1} [58]; ~350 L hr^{-1} [78]	MF, BF[58] NS[g,82] UV, PS[58]	18–25[130]; 12-25[132] OptR: 15–18[133]; 15+0.5[78]; similar to wild[58]	30-32[78]	>95%[58,78,132]	<0.10[58,85]; <0.06[78]	<0.10[58,85]; <0.01[78]	<20.0[85]; <5 mg L^{-1} [78]	>7.5[58]; 8.0-8.2[78]; 7.7-8.2[85]	NCR[91]; 12:12 h[78]	N/A	< 200[130]
Hatchlings	oFl, sFl, RAS[91,58]	N/A	NS[h, 91]	MF, BF[58] NS[f,82] UV, PS[58]	20±2[91]; 21–24, 15±0.5[78]	32-38[85]; 30-32[78]	>90%[91] >95%[58,78]	<0.10[58,85]; <0.06[78]	<0.10[58,85]; <0.01[78]	<20.0[85]; <5.0[78]	>7.5[58]; 8.0–8.2[78]	NCR[i,91]; 12:12 h[78]	FL[91,78]	100[j, 77]

[d] Suitable also for sub-adults and juvenile[82, 83]
[e] Slow water flow through bottom; bubbles should be avoided
[f] Strong aeration close to the eggs or underneath them[11]
[g] Reported as "Scaled filtration" by Authors
[h] Reported as "High water-flow rates" by Authors
[i] Taking into account latitude and longitude of the original geographic origin
[j] Measured at the air–water interface

	Seawater system	Aeration	Water exchange	Filtration and water treatment	Temperature (°C)	Salinity (psu)	DO (mg L^{-1} or % sat)	NH_3 (mg L^{-1})	NO_2 (mg L^{-1})	NO_3 (mg L^{-1})	pH	Photoperiod (L:D)	Type	Light intensity (lx)
	Water supply and quality											**Lighting**		
Sepiidae, *Sepia officinalis* -continued														
Juveniles	oFl, sFl, RAS[13,91]	Air stones[91]	~2 L min^{-1} [58] 80% d^{-1}/55L h^{-1} [134]	MF, BF[58] NS[f,82] UV, PS[58,134]	15±0.5[78] 19[134]	25-38[58]; 30-32[78] 33.5[134]	>95%[58,78]	<0.10[58,85]; <0.06[78] <0.5[134]	<0.10[58,85]; <0.01[78,134]	<20.0[85]; <5.0[78]	>7.5[58]; 8.0–8.2[78,134]	NCR[91]; 12:12 h[58,78,134]	FL[78,91]	200 or less[j,k,58,91] shading nets[l,13,91]
Adults	oFl, sFl, RAS[13,91]	Air stones[91]	~2 L min^{-1} [58]	MF, BF[58] NS[f,82] UV, PS[58]	12-25[132] 15±0.5[78]	25-38[58]; 30-32[78]	>95%[58,78]	<0.10[58,85]; <0.06[78]	<0.10[58,85]; <0.01[78]	<20.0[85] <5.0[78]	>7.5[58]; 8.0–8.2[78]	NCR[91]; 12:12 h[58,78]; Dark >12 h/day[m] [135]	FL[78,91]	200 or less[j,j,13,58,91] shading nets[k,91]
Sepiidae *Sepia pharaonis*														
Eggs	oFl, RAS[59]; sFl[136]	Air blower[136]	1 L min^{-1} [n, 59];	ACF[59]; UV[136]	25–28[o, (cited in 59),137-139]; 27-31[138] 28-32[136]	34-36[138]; 22.5-37.5[67] 21-39[(cited in 59)]	>95%[85]	<0.10[139]	<0.05[139]	<50.0[139]	7.8-8.2[138]	[p,59];	N/A	Shading nets[122]

[k] For Indoor tanks

[l] For Outdoors tanks: to prevent excess lighting as well as pH and salinity descent due to rainfall

[m] Stimulates sexual maturation in the female

[n] In oFl; Add. Info: turbidity should be maintained as low as possible through filtration and/or prior sedimentation

[o] Low hatching rate from cultured broodstocks, reared at 25°C [44]

[p] Artificial and unnatural L:D periods should be avoided

	Water supply and quality											Lighting		
	Seawater system	Aeration	Water exchange	Filtration and water treatment	Temperature (°C)	Salinity (psu)	DO ($mg\ L^{-1}$ or % sat)	NH_3 ($mg\ L^{-1}$)	NO_2 ($mg\ L^{-1}$)	NO_3 ($mg\ L^{-1}$)	pH	Photoperiod (L:D)	Type	Light intensity (lx)
Hatchlings	oFl, RAS[59]; sFl[136]	Air blower through air stone[136]	80% day^{-1} [136]	Indigen filter, UV[136]	27-32[138] 28-32[136]	32-36[138]; 28-36[67] 35[136] 21-39[(cited in 59)]	>95%[85]	<0.10[(cited in 59),139]	<0.10[(cited in 59)]; <0.05[139]	<20.0[(cited in 59)]; <50.0[139]	5.9-8.4[66]; 7.6-8.2[138] 7.7-8.2[136]	Positive phototaxis [q,140]	N/A	N/A
Juveniles	oFl, RAS[59]; sFl[136]	Airlift[59]	50-80% d^{-1} [59] 2 L min^{-1} [r]; 20 L min^{-1} [s]; 60% and 30 % d^{-1} [138]	NS [t] [59]	25–28[59] 28-32[136]	N/A	>95%[85]	<0.10[(cited in 59),139]	<0.10[(cited in 59)]; <0.05[139]	<20.0[(cited in 59)]; <50.0[139]	6.0–8.4[67,66]; 7.6-8.3[139]	12:12[86]	Nat[59]; FL[86]	N/A
Adults	oFl, RAS[59]; sFl[136]	Airlift[59]	N/A	NS[v,59]	25-28[59] 28-32[136]	N/A	>95%[85]	<0.10[139r]	<0.10[(cited in 59)]; <0.05[139]	<20.0[(cited in 59)]; <50.0[139]	7.6-8.3[139]	N/A	N/A	N/A

Sepiolidae

Euprymna scolopes

Eggs	oFl, RAS[60]	N/A	N/A	N/A	21-25[60]	30-37[60]	N/A	<0.10[82,141]	<0.10[82,141]	<20.0[82,141]	>8.0[60]	N/A	N/A	N/A
Hatchlings	oFl, RAS[60]	N/A	~22 L min^{-1}. weekly: ~30%[60]	BF[u], particulate and ACF, UV[60]	21-25[60]	30-37[60]	N/A	<0.10[82,141]	<0.10[82,141]	<20.0[82,141]	>8.0[60]	12:12 h[60]	Nat or FL[60]	[v]

[q] Wild hatchlings had a strong positive phototaxis, but this is different from observations in captivity, where this species has a moderate positive phototaxis
[r] 60 L tanks
[s] 500 or 1,000 L tanks
[t] Reported as "Shared filtration" by Authors
[u] In RAS: 1 mm filter (composed of crushed oyster shell with an UgF)
[v] Lower light enhanced feeding, whereas bright light seemed to retard it[13]

	Water supply and quality											Lighting		
	Seawater system	Aeration	Water exchange	Filtration and water treatment	Temperature (°C)	Salinity (psu)	DO (mg L^{-1} or % sat)	NH_3 (mg L^{-1})	NO_2 (mg L^{-1})	NO_3 (mg L^{-1})	pH	Photoperiod (L:D)	Type	Light intensity (lx)
Juveniles	oFl, RAS[60]	N/A	N/A	N/A	21-25[60]	30-37[60]	N/A	<0.10[82,141]	<0.10[82,141]	<20.0[82,141]	>8.0[60]	N/A	N/A	N/A
Adults	oFl, RAS[60]	N/A	N/A	N/A	21-25[60]	30-37[60]	N/A	<0.10[82,141]	<0.10[82,141]	<20.0[82,141]	>8.0[60]	N/A	N/A	N/A
Loliginidae														
Loligo vulgaris														
Eggs	oFl, RAS[61,82] or flow through[61]	Highly aerated seawater [d,61]	NS[h,w,87,142]	BF, UV[61,82]	14-22[61]	34-38[61,85]; 34-42[143]	5 mg L^{-1} [85]	<0.10[85]	<0.10[85]	<20.0[85]	7.7-8.2[85]; 7.8-8.4[143]	NCR[92,143]; 16L/8D or 8L/16D[x,95]	FL[95]	1–6[87]
Hatchlings	oFl, RAS[61,82]	Artificial aeration [d,y,85,61]	5.7 L m^{-1} (1.0-1.4 cm s^{-1})[87]	BF, UV[61,82]	12-22[61]	34-38[85]; 34-42[143]	5 mg L^{-1} [85]	<0.10[85]	<0.10[85]	<20.0[85]	7.7-8.2[85]; 7.8-8.4[143]	NCR[143]; 10L/14D[87]	FL[z,87]	1–6[87]
Juveniles	oFl, RAS[82]	N/A	2 d^{-1} [aa,80]	BF, UV[61,82]	12-22[61]	34-38[85]	5 mg L^{-1} [85]	<0.10[85]	<0.10[85]	<20.0[85]	7.7-8.2[85]	N/A	N/A	N/A
Adults	oFl, RAS[82]	N/A	N/A	BF, UV[61,82]	12-22[61] 17-21[144]	34-38[85] 33-35[144]	5 mg L^{-1} [85]	<0.10[85]	<0.10[85]	<20.0[85]	7.7-8.2[85]	NCR[144]	N/A	N/A

[w] Eggs should be suspended underneath the water inflow of the tanks and/or maintained through an upwelling flow of water
[x] Summer/Winter photoperiods
[y] Not in the rearing tank; it should be in a separate treatment tank
[z] Covered with red plastic filter to reproduce natural light conditions
[aa] Partially renewed, or in slowly running seawater

	Water supply and quality											Lighting		
	Seawater system	Aeration	Water exchange	Filtration and water treatment	Temperature (°C)	Salinity (psu)	DO (mg L^{-1} or % sat)	NH_3 (mg L^{-1})	NO_2 (mg L^{-1})	NO_3 (mg L^{-1})	pH	Photoperiod (L:D)	Type	Light intensity (lx)
Loliginidae														
Sepioteuthis lessoniana (Tropical countries)														
Eggs	oFl, RAS[62]	NS[bb,62]	1 L min^{-1}(cited in 62) 75-80% d^{-1} [145]	ACF(cited in 62)	~28[122] 28-30[145]	30–33[122]; 25-35[145]	>5.0 mg L^{-1} [145]	<0.06(cited in 62)	<0.08(cited in 62)	N/A	6.0–8.0[122]; 7.0-8.5[145]	NCR[cc,62]	N/A	N/A
Hatchlings	oFl, RAS[62]	N/A	[dd, 62]	N/A	24–30(cited in 62); 20–24(cited in 62);	23–36(cited in 62),[67]	N/A	<0.06(cited in 62)	<0.08(cited in 62)	N/A	6.3–8.4(cited in 62),[67]	N/A	Mixed[84,88,100,146,147] FL , metal halide bulbs(cited in 62)	cited in 62
Juveniles	oFl, RAS[62]	N/A	N/A	N/A	24–30(cited in 62); 20–24(cited in 62)	23–36(cited in 62),[67]	N/A	<0.06(cited in 62)	<0.08(cited in 62)	N/A	N/A	N/A	N/A	5-85[ee,146]
Adults	oFl, RAS[62]	N/A	N/A	N/A	24–30(cited in 62); 20–24(cited in 62)	23–36(cited in 62),67	N/A	<0.06(cited in 62)	<0.08(cited in 62)	N/A	N/A	N/A	N/A	<85[ff] (Ikeda, unpub. obs. in[62])

[bb] Reported as "sufficient water current to carry enough oxygen to the capsules" by Authors
[cc] The use of lighting and unnatural daily L:D periods should be avoided; the use of camouflage net that reduces light efficiency by 80% is recommended
[dd] Aeration has to be managed to generate a directional flow; direct current allows them to hover in the water column
[ee] Juveniles avoid both the brightest part (85 Lx) and darkest part (5 Lx) of the tank
[ff] Adults avoid the brightest (85Lx) areas of tanks

	Water supply and quality											Lighting		
	Seawater system	Aeration	Water exchange	Filtration and water treatment	Temperature (°C)	Salinity (psu)	DO (mg L^{-1} or % sat)	NH_3 (mg L^{-1})	NO_2 (mg L^{-1})	NO_3 (mg L^{-1})	pH	Photoperiod (L:D)	Type	Light intensity (lx)
Loliginidae														
Sepioteuthis lessoniana (Temperate countries)														
Eggs	oFl, RAS[62]	N/A	N/A	Particle filter, ACF, UV[62,110] PS, UgF[110]	20-25[148]	35.8± 1.2[88] 34-36[110] 35[148]	N/A	<0.10[110,148] 0.009–0.044[84]; 4.7 ± 11.7[88]	<0.10[148] <0.02[110]; 0.023–0.069[84]; 0.3[88]	<50[110]; <20[148] 9.6–36.0[84]; 114±73[88]	7.9–8.2[84]; 7.8±0.2[88] >8.0[110]	N/A	N/A	N/A
Hatchlings	oFl, RAS[62]	N/A	N/A	Particle filter, ACF, UV[62,110] PS, UgF[110]	N/A	35.8 ± 1.2[88] 34-36[110] 35[148]	N/A	<0.10[110,148] 0.009–0.044[84]; 4.7 ± 11.7[88]	<0.10[148] <0.02[110] 0.023–0.069[84]; 0.3[88]	<50[110]; <20[148] 9.6–36.0[84]; 114 ± 73[88]	7.9–8.2[84]; 7.8±0.2[88] >8.0[110]	Mixed[gg] (12L:12D)[88]	Nat, FL[88]	10–100[hh,84]
Juveniles	oFl, RAS[62]	N/A	N/A	MF[(cited in 62)]	20–23[62,88,100]; 21–26[84,88,146]	35.8 ± 1.2[88]	N/A	<0.10[148]; 0.009–0.044[84]; 4.7 ± 11.7[88]	<0.10[148]; 0.023–0.069[84]; 0.3[88]	<20[148] <50; 9.6–36.0[84]; 114±73[88]	7.9–8.2[84]; 7.8±0.2[88]	Mixed[gg] (12L:12D)[88]	Nat, FL[88]	10–100[hh,84]
Adults	oFl, RAS[62]	N/A	N/A	MF[ss]	20–23[62,88,100]; 21–26[84,88,146]	35.8 ± 1.2[88]	N/A	<0.10; 0.009–0.044[84]; 4.7 ± 11.7[88]	<0.10; 0.023–0.069[84]; 0.3[88]	<50; 9.6–36.0[84]; 114 ± 73[88]	7.9–8.2[84]; 7.8±0.2[88]	Mixed[gg] (12L:12D)[88]	Nat, FL[88]	110–100[hh,84]

[gg] Natural circadian rhythm and artificial light were mentioned by Authors
[hh] Incident lighting was increased with an overhead 400-W metal halide light

	Water supply and quality											Lighting		
	Seawater system	Aeration	Water exchange	Filtration and water treatment	Temperature (ºC)	Salinity (psu)	DO (mg L^{-1} or % sat)	NH_3 (mg L^{-1})	NO_2 (mg L^{-1})	NO_3 (mg L^{-1})	pH	Photoperiod (L:D)	Type	Light intensity (lx)
Octopodidae														
Octopus bimaculoides														
Eggs	RAS[63]	N/A	N/A	BF, MF[ii], PS, UV[63]	18-25, preferred: 22-25[63]	32-38, preferred: 34-36[63]	N/A	N/A	N/A	N/A	>7.5, preferred: 7.7-8.2[63]	N/A	N/A	N/A
Hatchlings	RAS[63]	N/A	N/A	BF, MF[ii], PS, UV[63]	18-25, preferred: 22-25[63]	32-38, preferred: 34-36[63]	N/A	N/A	N/A	N/A	>7.5, preferred: 7.7-8.2[63]	Mixed[jj, 89]	Nat, FL [89]	N/A
Juveniles	RAS[63]	N/A	N/A	BF, MF[ii], PS, UV[63]	18-25; preferred: 22-25[63]	32-38; preferred: 34-36[63]	N/A	N/A	N/A	N/A	>7.5, preferred: 7.7-8.2[63]	Mixed[jj, 89]	Nat, FL Error! Bookmark not defined.[89]	N/A
Adults	RAS[63]	N/A	N/A	BF, MF[ii], PS, UV[63]	18-25; preferred: 22-25[63]	32-38; preferred: 34-36[63]	N/A	N/A	N/A	N/A	>7.5, preferred: 7.7-8.2[63]	Mixed[jj, 89]	Nat, FL [89]	N/A

[ii] Filter cartridges 35mm
[jj] Natural circadian rhythm and fluorescent light from 08:00 to 17:00

	Water supply and quality											Lighting		
	Seawater system	Aeration	Water exchange	Filtration and water treatment	Temperature (°C)	Salinity (psu)	DO (mg L^{-1} or % sat)	NH_3 (mg L^{-1})	NO_2 (mg L^{-1})	NO_3 (mg L^{-1})	pH	Photoperiod (L:D)	Type	Light intensity (lx)
Octopodidae														
Octopus vulgaris														
Eggs	oFl, RAS [85]; sFl[96]	N/A	N/A	MF[kk], CF, PS, UV[13]	15-18[85]	27–38[85]	>95%[85]	<0.10[13,85]	<0.10[13,85]	<20.0[13,85]	7.8-8.2[13,85]	N/A	N/A	11–15[13]
Paralarvae	oFl, RAS[85]; sFl[96]	By air stones[149,150] or open tube aeration[96,ll]	<u>1st week</u>: green-water 100-150% d^{-1} [96,124,151]	MF, CF, PS, UV[13]	19 -25[124] OptR: 20–22[12]	27–38[85] OptR: 32-35[102]	>95%[85] 6-8 mg L^{-1} [102]	<0.10(cited in 102),[13,85]	<0.10[13,85]	<20.0[13,85]	7.8-8.2[13,85]	NCR or 14:10 h[mm,93,96]	Nat, ilb, FL[102]	500–700[93] 60–250[nn,96]
Juveniles	oFl, RAS[85]	N/A	N/A	MF, CF, PS, UV or Oz[oo,13]	10-20[83] OptR: 16-21[152]	27–38[85] 29–34[153]	>95%[85]	<0.10[13,85]	<0.10[13,85]	<20.0[13,85]	7.8-8.2[13,85]	NCR[13]	N/A	From 60[96] to 900[99]
Adults	oFl, RAS[85]	N/A	N/A	MF, CF, PS, UV or Oz[oo,13]	10-20[83] OptR : 16-21[152]	27–38[85] 29–34[153]	OptR: 65-100%; SubOpt: 35-65%[70]	<0.10[13,85]	<0.10[13,85]	<20.0[13,85]	7.8-8.2[13,85]	12DL/12D[96]	N/A	N/A

[kk] using sand, gravel, floss, filter pads or filter bags
[ll] Water flow: gentle water circulation; Add. info: care should be taken to avoid entrapment of air bubbles inside the mantle cavity. Devices for superficial or bottom water distribution to prevent bubbles[52, 56]
[mm] In black-walled and white-bottom tanks
[nn] Higher light intensities are used in black tanks; lower light intensities in black-walled and white-bottom tanks
[oo] Ozone can harm and kill cephalopods, particularly eggs and paralarvae and requires careful monitoring

	Water supply and quality											Lighting		
	Seawater system	Aeration	Water exchange	Filtration and water treatment	Temperature (°C)	Salinity (psu)	DO (mg L^{-1} or % sat)	NH_3 (mg L^{-1})	NO_2 (mg L^{-1})	NO_3 (mg L^{-1})	pH	Photoperiod (L:D)	Type	Light intensity (lx)
Octopodidae														
Octopus maya														
Eggs	oFl, sFl, RAS[64]	Airlift[e,64]	10% d^{-1} [154]	Anthracite earth filter and 50 µm bag filter, UV and PS[64]	OptR: 22-26[155]	>32[64]; 36[13]	>5 mgL^{-1} [64]	<1[64]	<0.15[13]	<20.0[13,85]	>8[64]	N/A	N/A	N/A
Hatchlings (~0.7 cm ML)	oFl, sFl, RAS[64]	Airlift[e,64]	3% d^{-1} [154]	Vertical filter (15 mm), filter bags (100 mm) and rotational water filter; UV and PS[64]	>28 OptR: 25±1[64]	36-40[13]	>5 mgL^{-1} [64]	<1; 0.2±0.05[64]	<0.15[13]	<20.0[13,85]	>8[64]	12:12 h[94] NCR[64]	N/A	N/A
Juveniles (>5 cm ML)	oFl, sFl, RAS[64]	Airlift[e,64]	3% d^{-1} [154]	vertical filter (15 mm), filter bags (100 mm) and rotational water filter; 50 µm bag filter, UV and PS[64]	22-30[13] OptR: 24-26[64]	36-40[13]	>5 mgL^{-1} [64]; 80%[154]	<1[64]	<0.15[13]	<20.0[13,85]	>8[13,64] 7.8-8.2[85,154]	12:12 h [94] NCR[pp,64]	N/A	Nat [64]; 30 lx cm^{-2} [13]
Adults (>8 cm ML)	oFl, sFl, RAS[64]	Airlift[e,64]	10% d^{-1} [154]	anthracite earth filter and 50 µm bag filter; UV and PS [64]	26-29[64]	>32[64]	>5 mgL^{-1} [13,64]	<1[13,64]	<0.15[13]	<20.0[13,85]	>8[13,64]	Natural [64]	N/A	Nat[64]

[pp] Direct sunlight should be reduced 70% by shading

Appendix B - Accommodation and Care; Enrichment

Overview of the conditions utilized for accommodation and care for live cephalopods for research purposes. Following the Annex III of the Directive 2010/63/EU, information about housing, grouping and diet together with reference to the relevant works are listed in this appendix. The appendix also includes information relevant to the enrichment (considered as social and/or environmental enrichment). The information included in the tabularized overview also refers to general recommendations by Vidal et al.[13] and Fiorito et al.[55] Cephalopod species are listed in taxonomic order following FAO annotated catalogue of cephalopods species[2,4,126] (for the list of species and rationale for their inclusion, see also "Target species" and Table 1 and Table 2 in main text). Abbreviations utilized – **Bk**: Basket; **c**: crab; **cop**: copepod; **Cr**: crustacean; **Crc**: Circular; **Cyl**: Cylindrical; **CylCon**: cylindro-conical; **d.o.**: days old; **f**: fish; **FNc**: Floating Net cage; **FSB**: Fish Spawning Box; **g**: gastropod; **hatch**: hatchling; **Hx**: Hexagonal; **ind**: individual; **l**: lobster; **m**: molt; **mol**: mollusc; **my**: mysid; **N/A**: information not available; **Nb**: Net bag; **Nc**: Net cage; **NS**: Not Specified; **RAS**: Recirculating Aquaculture System; **Rct**: Rectangular; **RctMBk**: Rectangular Mesh Basket; **Rcw**: Raceway; **s**: shrimp; **sCrc**: semi-Circular; **sq**: squid; **Sqr**: Square; **Sv**: Sieve; **zp**: zooplankton. **psu** (practical salinity unit): defined as the total amount by weight of dissolved salts in one kilogram of seawater.

	Accommodation and care										Enrichment	
	Housing					Grouping		Diet				
	Tank shape	Tank color	Tank size / volume	Lid	Minimum water depth (cm)	Sex ratio (M:F)	Stocking density	Type	Frequency	Quantity	Social	Environment
Nautilidae												
Nautilus												
Adults[qq]	Hx[79] sCrc[15]	N/A	340-15,000 L[15]; 160 cm high × 70 cm wide each face[79]	Yes[15]	140[79]	N/A	12 ind. m^{-3} [15]	s, f, c [15], l, m[15,104], fish heads[104]	3-6 days w^{-1} [15]; 1-2 d^{-1} [15]	N/A	Yes[15,79,104]	Walls' texture [104]; gravel [15]; shelters[79]
Sepiidae												
Sepia officinalis												
Eggs	RctMBk[58]; Sv[rr,78]	N/A	22x15x18 cm in ~150 L [58]; 45Øx30cm in 130×75cm[78] FSB (20x10x10 cm)[ss]	Mesh lid[58]	NS[tt,107]	N/A	~50 /Bk[58]; 2000 /250 L [130]; Up to 5 L[ss]	N/A	N/A	N/A	N/A	N/A

qq Suitable also for sub-adults and juvenile[82,83]

rr Embryonic development should be completed in bowl-shaped tanks[85]

ss L. Juergens, personal communication

tt Reported as "Water column should be low. While cuttlefish grows, the water column should be increased to generate more volume" by Authors

	Accommodation and care										Enrichment	
	Housing					Grouping		Diet				
	Tank shape	Tank color	Tank size / volume	Lid	Minimum water depth (cm)	Sex ratio (M:F)	Stocking density	Type	Frequency	Quantity	Social	Environment
Hatchlings	CylBk[134], or Rcw[91,101] 2-4 weeks: CrcSv[78], FSB (20x10x10 cm)[ss]	Preferred: black[91,101]	Hatching Bk 1,500L[91,101]; Sv: 23Ø×5.5cm[78] Cyl: 30Øx10cm, 707 cm^2 [134]	N/A	5.5[78]	N/A	Hatchling Bk: 500 hatch. m^{-2} [108]; Sv: 20 hatch./sieve (~725 hatch. m^{-2})[78] 4 hatch./ tank[134] Net or box min 100cm^2, 40cm^2 per animal[uu,ss]	my, s [13,134,156-161] *P. varians*[162]; Gammarids[78]	2 d^{-1} [58]; 3 d^{-1} [78]	*Ad libitum* [58,78]; Optimum: from 16.2% of BW (10 d.o.) to 10% BW by 40 d.o.[163]	Preferred in groups[91]	shelters, sand (1-2 cm)[58]; Plastic plants; narrow PVC Pipes (min 5x3.5cm; max 11.5x12.5cm)[78]
Juveniles	Preferred: Crc[91], Sqr[13,58] or Rct[13,58,78]	Preferred: black[91]	250L[119,156] to 9,000L tanks[13,106]; 130×75 cm[78] Cyl: 30Øx10cm, 707 cm^2 [134]	N/A	15[58]	N/A	16-33[105]/ 20-33[107] ind. m^{-2} [vv] 120 ind. m^{-2} [uu,108]; 2cm ML: 70 m^{-2} [78] 4 ind./tank[134]; min 500 cm^2, 300 cm^2 per animal[ss]	Cr [119,120,160], c [158,159]; s [106,119]	2 d^{-1} [58]; 3 d^{-1} [78]	*Ad libitum*[58,78]	Preferred in groups[91], once able to catch shrimp single-housing is recommended[ss]	shelters, sand (1-2 cm)[58];
Adults	Preferred: Crc[ww,91] Sqr or Rct[13,58]	Preferred: black[91]	250[119,156] to 9,000 L tanks[13,106]	N/A	18[ss]	1:2[91]	min 1200 cm^2, 500 cm^2 per animal[ss]	N/A	2 d^{-1} [58]	*Ad libitum*[58]	Preferred in groups[91] grouped only for breeding purposes[ss]	shelters, sand (1-2 cm)[58];

[uu] Flow-through seawater systems

[vv] In RAS[58,60]

[ww] to promote mating

	Accommodation and care										Enrichment	
	Housing					Grouping		Diet				
	Tank shape	Tank color	Tank size / volume	Lid	Minimum water depth (cm)	Sex ratio (M:F)	Stocking density	Type	Frequency	Quantity	Social	Environment
Sepiidae												
Sepia pharaonis												
Eggs	Bk (5 mm mesh size) any shape (cited in 59) Crc[86] or Nb[164]	[xx,86]	<u>Crc plastic arenas</u> : 24Ø[86]	N/A	13[86]	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Hatchlings	Crc[86]	Gray-white walls[86]	25Ø×20 cm depth[ss,86]	N/A	30[59]; +50 mm [ss,59]	N/A	500 ind. m^{-2} [59]	my, f[yy,59]; s[139]	2 d^{-1} [136]	*Ad libitum*[59]; 20–28% BW d^{-1} (cited in 59)	Solitary, occasionally gregarious[zz,59]	Sand, small stones, gravel or rocks[86]

[xx] Walls covered using black cloths

[yy] preferably crustaceans to fish; adult *Artemia* spp. as a supplementary or reserve food

[zz] group behaviour is beneficial for the training period during which they feed on dead prey

	Accommodation and care										Enrichment	
	Housing					Grouping		Diet				
	Tank shape	Tank color	Tank size / volume	Lid	Minimum water depth (cm)	Sex ratio (M:F)	Stocking density	Type	Frequency	Quantity	Social	Environment
Juveniles	Cyl[137]; Rct[86]; Steel-framed FNc(cited in 59); RctRcw[139,165]	Gray-white[86]	FNc: 60×80×40 cm(cited in 59); Cyl: 60 L; 500 L; 1,000 L[138]; 3,000 L[137]; RctRcw: 65,000 L[139,165] 27–107 d.o: 54×38×20 cm, 107-117 d.o: 60×43.5×40 cm[86]	N/A	FNc: 30(cited in 59)	N/A	375–400 ind. m^{-2} (10 d.o); 280–300 ind. m^{-2} (20 d.o); 500[137] to 200[59,137] or 100 ind. m^{-2} [138] (30-d.o)	30-50-d.o (~20-30 mm ML): f[aaa,59]; my[137]; s (trained after 70 days)[139]	2 d^{-1} [59]	*Ad libitum*[59]	Solitary, occasionally aggregative[zz]	Sand, small stones, gravel or rocks[86]
Adults	Cyl[137]; Steel-framed FNc(cited in 59); RctRcw[139,165]	Gray-white[86]	FNc: 60×80×40 cm(cited in 59); Cyl: 60 L; 500 L; 1,000 L[138]; 3,000 L[137]; RctRcw: 65,000 L[139,165] 107-117 d.o: 60×43.5×40 cm[86]	N/A	N/A	1:3[59]	10 ind m^{-3} [138] (180 d.o; ~145 mm ML and 325 g):	N/A	N/A	N/A	Solitary, occasionally aggregative[zz,(cited in 59)]	Sand, small stones, gravel or rocks[86]

[aaa] training the animals to feed on dead food

	Accommodation and care										Enrichment	
	Housing					Grouping		Diet				
	Tank shape	Tank color	Tank size / volume	Lid	Minimum water depth (cm)	Sex ratio (M:F)	Stocking density	Type	Frequency	Quantity	Social	Environment
Sepiolidae												
Euprymna scolopes												
Eggs	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Hatchlings	Crc (200 mm-mesh screen bottom in a seawater tray tank of 3-6 cm)[60]	Black[60]	25Ø cm[60]	N/A	N/A	N/A	N/A	zp, Cr, f[bbb,60]	1-5 d^{-1} [60]	N/A	N/A	Sand[60]
Juveniles	Rct[60]	N/A	35×39 cm[60]	N/A	N/A	N/A	N/A	s (*Crangon* and *Palaemonetes* spp.)[60]	N/A	N/A	N/A	Sand (~1-2 cm)[60]
Adults	Rct[60]	N/A	35×39 cm;	N/A	N/A	N/A	2-3 in 35 X 39 cm [60]	s (*Crangon; Palaemonetes* spp.)[60]	N/A	N/A	N/A	Sand (~1-2 cm)[60]
Loliginidae												
Loligo vulgaris												
Eggs	N/A	N/A	220 L (95x40 cm)[87]	N/A	N/A	N/A	five to ten[ccc,61]	N/A	N/A	N/A	N/A	N/A

[bbb] hatchlings are voracious predators that prefer very large prey relative to their own size

[ccc] egg strands suspended with nylon thread in the tanks and/or in an upwelling flow of water; small bunches of eggs allow proper aeration

	Accommodation and care										Enrichment	
	Housing					Grouping		Diet				
	Tank shape	Tank color	Tank size / volume	Lid	Minimum water depth (cm)	Sex ratio (M:F)	Stocking density	Type	Frequency	Quantity	Social	Environment
Hatchlings	Cyl[61]	Black[87]	220 L (95x40 cm)[87]	N/A	N/A	N/A	25 L^{-1} [61]	*Artemia*, c, cop, my, f, zp[61]	4-5 d^{-1} [87]	50–150 L^{-1} [87]	N/A	N/A
Juveniles	Crc[82,144,166] Rcw[82]	Opaque walls[61]	25Ø-40 cm (5-45 L)[80]	N/A	40-100[61]	[ddd,82]	N/A	f, s[82] or artificial diets[61]	N/A	N/A	Groups [82]	N/A
Adults	Crc[82,144,166] , Rcw[82]	Black sides and white bottom[144]	2.0mØ; long-oval Rcw 10×2.0 m: 10,000 L[61,82]	N/A	N/A	[ddd,82] 1:1[61]	10-15 squids/tank (~150-250mm ML) [82]	f[116], s[82,116]; artificial diets[61]	1 d^{-1} [116]	15% BW d^{-1} [82]; *ad libitum*[61]	Groups[82]	N/A
Loliginidae												
Sepioteuthis lessoniana (Tropical countries)												
Eggs	Fiber glass or FNc (2.5mm mesh size)[145]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Hatchlings	Crc[62] or Nc[145]	N/A	<u>Crc</u>: 2.0–3.0 mØ[62]; <u>Nc</u>: 1.5×1.5×1.5 m[145]	N/A	50[eee,62]	N/A	5-10 ind.L^{-1} [62]; <u>Nc</u>: 300-400 [145]	up to 30 d.o: my[(cited in 62)]; prey size ~50–200% of their ML[62]; f[(cited in 62)];	N/A	*Ad libitum*[62]; ~28 % of BW d^{-1} [(cited in 62)]; ~7 (1–22) d^{-1}	Group[62]	N/A

[ddd] It is better to maintain the squids of the same size and sex in the tanks to avoid competition

[eee] As the squid grow, the water depth is increased by 5 cm every day or every second day

	Accommodation and care										Enrichment	
	Housing					Grouping		Diet				
	Tank shape	Tank color	Tank size / volume	Lid	Minimum water depth (cm)	Sex ratio (M:F)	Stocking density	Type	Frequency	Quantity	Social	Environment
Juveniles	FNc (13-mm mesh size net) (cited in 62)	N/A	1.0x1.0x1.0m ; efficient size: 3.0x3.0x3.0 m[145]	N/A	N/A	N/A	5–10 ind·m^{-2} (3-7 ind.·m^{-3})(cited in 62) Nc: 9 m^{-3}; 250 ind/cage[145]	f [62]	2 d^{-1} [62]	*Ad libitum*[62]	N/A	N/A
Adults	FNc (13-mm mesh size net) (cited in 62)	N/A	2.0×2.5×2.0 m; submerged to a depth of 1.5 m (7.5 m^3 seawater) (cited in 62)	N/A	N/A	1:1[122]	N/A	N/A	N/A	N/A	N/A	N/A
Loliginidae												
Sepioteuthis lessoniana (Temperate countries)												
Eggs	Crc[84,146,148,167,168]	Black or dark grey[110]	30Ø×27cm, 20 L[167]; 70Ø×34cm, 120 L[168]; 1.8Ø m (3000L)[84,146,148]	Styrofoam cover[84]	60[84]	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Hatchlings	Crc(cited in 62),88,100,110,146,	Black[84] or dark grey[110]	20, 50[88,100,110,146] or 120 L[62] 1.5 or 2.5 mØ[110]	Styrofoam cover[84]	75-125[110]	N/A	N/A	my[146] (34-38), f[88,100,146]	N/A	*Ad libitum*[62] 60–300 d.o: 20–35 % BW·d^{-1} [fff,146]	N/A	N/A

[fff] Estimated feeding rate in RAS

	Accommodation and care										Enrichment	
	Housing					Grouping		Diet			Social	Environment
	Tank shape	Tank color	Tank size / volume	Lid	Minimum water depth (cm)	Sex ratio (M:F)	Stocking density	Type	Frequency	Quantity		
Juveniles	Rcw or Crc[84,88,100,110,146]	N/A	<u>Rcw</u>: 2.4×6.1×0.9 m, 15,000 L; <u>Crc</u>: 6.5Ø×1.75 m, 50,000L[84,110,146] or 4.0Ø×1.0 m, 10,000 L[88,100]	N/A	N/A	N/A	5-6 ind· m^{-3} (15,000 L Rcw or 50,000 L Crc)[84,110,146]	Dead organisms[62]; mixture of live and dead frozen prey (fish)[88,100]	N/A	N/A	Group [169]	N/A
Adults	Rcw or Crc[84,88,100,110,146]	N/A	N/A	N/A	N/A	N/A	N/A	A variety of prey items, either alive or dead[62]	N/A	*Ad libitum*[ggg,62]; 60–300 d.o: 20–35 % $BW \cdot d^{-1}$ [fff,146]	N/A	N/A

Octopodidae

Octopus bimaculoides

Eggs	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Hatchlings	N/A	N/A	N/A	N/A	4.0-5.0[89]	N/A	300–700 hatch. m^{-2} [63]	Cr, s, c, f, g [89]	Every two days[63]	*Ad libitum*; ~20% BW d^{-1} [63]	N/A	>1 ind^{-1} ceramic cylinders[hhh] (1.0Ø×1.0cm) [89]
Juveniles	Rct[63]	N/A	201 (120×60×28 cm) to 400 (240×60×28 cm) L[63]	N/A	4.0-5.0[89]	N/A	N/A	Cr, s c, f, g [89]	N/A	*Ad libitum*; 20 to 5% BW d^{-1} [63]	N/A	>1 ind^{-1} PVC pipes [89]

[ggg] to avoid starvation and cannibalism

[hhh] fairly evenly distributed throughout the tank

	Accommodation and care										Enrichment	
	Housing					Grouping		Diet				
	Tank shape	Tank color	Tank size / volume	Lid	Minimum water depth (cm)	Sex ratio (M:F)	Stocking density	Type	Frequency	Quantity	Social	Environment
Adults	Rct[63]	N/A	201 (120×60×28 cm) to 400 (240×60×28 cm) L[63]	Yes, 6 mm layer [63]	15-20[iii,63,89]	N/A	N/A	Cr, s, c, f,g [89]	N/A	*Ad libitum*; ~5% octopus BW d^{-1} [63]	N/A	>1 ind^{-1} PVC pipes[89]
Octopodidae												
Octopus vulgaris												
Eggs	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Paralarvae	CylCon[102]	Dark tanks[102] White [103] or black walls and white bottom[96]	CylCon: 500 and 1,000 L[102]	N/A	N/A	N/A	10 ind L^{-1} [102]	s, c, cop, *Artemia* (enriched or not), my [13,102]	N/A	N/A	N/A	N/A
Juveniles	RctFNc[170] Cyl[152]	Opaque-grey[55]	4 m^3 [170] 400 L[152]	N/A	40[55]	N/A	Tanks: 10 kg m^{-3} and 20 kg m^{-3} [112]; FNc: similar-sized 10-14 kg m^{-3} [170,171]; 4, 8 and 15 kg m^{-3} [jjj,109]	Cr, mol, f[13,124,125,172] 5	1 d^{-1} [152]	*Ad libitum*[152]	Group rearing not recommended[55]	Shelters[124] sand [55]

[iii] for octopuses up to 1 kg

[jjj] mortality was significantly lower in low density groups

	Accommodation and care										Enrichment	
	Housing					Grouping		Diet				
	Tank shape	Tank color	Tank size / volume	Lid	Minimum water depth (cm)	Sex ratio (M:F)	Stocking density	Type	Frequency	Quantity	Social	Environment
Adults	Rct[112,150] Cyl[152]	Opaque-grey[55]	5-10 m^3 [112] 2 m^3 (3.6x1.1m)[109] 400 L[152]	Solid mesh cover[109]	50[109] 60-70[150]	Brood stock: 1:3[13,149]; 1:1[112]	Tanks: 10 kg m^{-3} and 20 kg m^{-3} [jjj,112]; FNc: similar-sized 10-14 kg m^{-3} [170,171]; 4, 8 and 15 kg m^{-3} [jjj,109]	Cr, mol f [13,124,125,172 173]	1 d^{-1} [109,152]	*Ad libitum*[152] 5% BW d^{-1} [109]	Group rearing not recommended[55]	Shelters[kkk,124] sand [55]
Octopodidae ***Octopus maya***												
Eggs	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Hatchlings (~0.7 cm ML)	Rct[64,94]	Black[64]	5.0×1.5×0.4 m (7.5 m^3)[64]; 1.4x0.7x0.5 m (500L)[94]	N/A	N/A	N/A	50-60 ind. m^{-2} [64]	No food in the first 5–7 days[174]; *Artemia*, zp, amphipods, isopods, c [175]; squid paste-bound gelatine[118,176]	2 d^{-1} [64]; 3 d^{-1} [13]	90-100 % WW d^{-1} [13,64]	N/A	3 shells ind^{-1} (e.g., *Melongena corona bispinosa* or *Strombus pugilis*) [13,64]

[kkk] especially when males and females are kept together

	Accommodation and care										Enrichment	
	Housing					Grouping		Diet				
	Tank shape	Tank color	Tank size / volume	Lid	Minimum water depth (cm)	Sex ratio (M:F)	Stocking density	Type	Frequency	Quantity	Social	Environment
Juveniles (>5 cm ML)	Crc[64]	Black[64]	6.0Ø m[64]; 12,000 L[123]	N/A	50[64,154]	N/A	10-25 juveniles m^{-2} [64]	c, Cr [175]; squid paste-bound gelatine[118,176] see review in[13]	2 d^{-1} [64]; 3 d^{-1} [13]	50 % WW [64] 90-100% d^{-1} [13]	N/A	3 shells ind^{-1} (e.g., *Melongena corona bispinosa* or *Strombus pugilis* (juveniles up to 10g WW)[13,64] PVC tubes, pots and cages [64]; 3 ind^{-1} [13]
Adults (>8 cm ML)	Crc[64];	Black[64]	6.0Ø m[64]; 12,000 L[123]	N/A	50[64]	1:1[64]	2.9 - 3.8 kg m^{-3} [lll,154]	c, f, sq, s[64]	2 d^{-1} [64]	5% WW d^{-1} [13]	N/A	PVC tubes, pots and cages[64]

[lll] for animals with initial body weight between 300-500 g

Appendix C - Thermal, oxygen and salinity tolerance ranges

Overview of the thermal, oxygen and salinity tolerance ranges for the target species. Data are derived from the analysis of the available literature with focus on studies that rely on physiological measures.

Species listed as indicated in legend to Appendices A and B. Abbreviations utilized - #: recommended values for maximizing growth deriving from aquaculture studies; **ArB**: Arterial Blood; **lSD**: Low Stocking Density; **N/A**: information not available; **oFl**: Open Flow; **OptR**: Optimal Range; **RAS**: Recirculating Aquaculture System; **Rec**: Recommended; **S**: Summer; **VeB**: Venous Blood; **Tol**: Tolerance; **W**: Winter. **psu** (practical salinity unit): defined as the total amount by weight of dissolved salts in one kilogram of seawater.

Order/species	Life Phase	Thermal range (wild)	Thermal tolerance (Lab)	Oxygen tolerance (Lab)	Salinity tolerance (Lab)
Nautilidae *Nautilus*	**Adults**	can thrive with wide changes of temperature within few minutes: 6-24°C or 8-30°C[15]; animals do not approach shallow water at 28°C[15]	15-27°C[104]; animals do not survive at 27 °C for longer than 48h[15]; a rhythmic day/night cycle (18-21°C) is recommended to mimic normal migration patterns[mmm,15]	high tolerance for low O_2 and hypoxia[71,72]; blood O_2 capacity of 2.30±0.57 vol%, P_{50} is 16.9 mmHg at pH 7.45 and 18°C[177] ♦ average ArB-P_{O2}=99.2±31.7 mmHg, fully O_2-saturated ♦ average VeB-P_{O2}=20.4 ±12.9 mmHg, 65% O_2-saturated[177]	34.5-35.6 psu, the lowest both in the surface and at 800 m while the highest at 180 m in Papua Guinea[178] *N. pompilius:* osmolarity is reduced <19% of seawater salinity when shell is half emptied; *N. macromphalus:* change in cameral liquid volume from 50% to 0% with a rise in salinity from 20-30%, to seawater salinity[179]
Sepiidae *Sepia officinalis*	**Eggs**	15-20°C[180]	OptR:15–25°C[130,133] but embryos do not develop <12°C[130#]; successful hatching at 20°C[nnn,181,182]	can survive > 8 mg L^{-1} [182]	28-35 psu[ooo,181,182]; most successful hatching >29.8 psu and no hatching <23 psu[183]; when water salinity < 18psu eggs regressed in size and maturation stopped[182]
	Hatchlings	S: 14-19°C, W: 11.7-18°C[180]	OptR:15–18°C[133]; 25°C[ppp]	can survive 7 mg L^{-1} [182]	Developmental rate decreased at salinity values of 28.7 psu or less at 17°C; below 22.4 psu embryos with morphological malformations were found[183]
	Juveniles	S: 14-19°C, W: 11.7-18°C[180]	15-24°C[180]	Critical P_{O2} at ambient O_2 levels (4-7 mg L^{-1}): ♦ smaller individuals: 40 mmHg ♦ larger animals: ~ 70 mmHg[qqq,184,185]; recovery is still possible at 2 mg L^{-1} for a period of at least 1h[186]	25-38 psu, OptR:34-36 psu[58#]
	Adults	S:14-19°C, W:11.7-18°C[180]	OptR:11-23°C[187]; can survive ranges of 7.0-26.8°C with a gradual increase or decrease of 1°C h^{-1} [rrr,187,188]	O_2-transfer functions nicely between 11-23°C for 15°C-acclimated cuttlefish but further acute warming or cooling leads to progressive internal hypoxia, time-limited survival, and eventually, death[187]	25-38 psu, OptR:34-36 psu[58#]

[mmm] even though a temperature fluctuation regimen opposite to that observed in nature is applied to reduce condensation on windows[10].

[nnn] and salinity of 28-35 psu

[ooo] at 18-25°C

[ppp] L. Juergens personal communication

[qqq] O_2-binding capacity of 3 mM at rest with about 80% release of bound oxygen to tissues at 17°C

[rrr] but accumulation of inorganic phosphate was found below 8°C and above 26.8°C

Order/species	Life Phase	Thermal range (wild)	Thermal tolerance (Lab)	Oxygen tolerance (Lab)	Salinity tolerance (Lab)
Sepiidae *Sepia pharaonis*	**Eggs**	18-24°C at the coasts for hatching[2]	27-31°C[164]	incubated at 8.01 ± 1.23 mg L^{-1} [189]	can survive 27-33 psu; optimum: 30 psu[189]; >80% eggs hatched at 24, 28, 32 and 36 psu and failed to hatch at 16, 20, 40 and 44 psu, OptR:21.8-36.6 psu[67]
	Hatchlings	Tropical distribution 24-28°C[190]	23-28°C (lSD)[165]	8.01 ± 1.23 mg L^{-1} [189]	24-33 psu[189]; >80% survival at 24, 28, 32 and 36 psu OptR:21.4-39.4 psu[67]
	Juveniles	Tropical distribution 24-28°C[190]	23-28°C (lSD)[165]	Critical P_{O2}=1.09 ± 0.12 mg L^{-1} at 28.5°C[191]; cuttlefishes moved rapidly and irregularly when $O_2 < P_{O2}$, O_2 consumption accelerated, and the animals died within 10–15 min[191]	34-39 psu[165#]
	Adults	18-24°C at the coasts for spawning[2]	23-28°C (lSD)[165]; at 21°C little success in culturing consecutive generations[139]	6.24±0.48 - 7.33±0.81 mg L^{-1} [192,193]	34-39 psu[165#]

Order/species	Life Phase	Thermal range in nature	Thermal tolerance in captivity	Oxygen tolerance in captivity	Salinity tolerance in captivity
Sepiolidae *Euprymna scolopes*	**Eggs**	25-29°C[sss,194]	OptR:21-25°C; Tol:18-19°C[60]	N/A	30-37 psu[60]
	Hatchlings	25-29°C[sss,194]	OptR:21-25°C; Tol:18-19°C[60]	N/A	30-37 psu[60]
	Juveniles	25-29°C[sss,194]	OptR:21-25°C; Tol:18-19°C[60]	N/A	30-37 psu[60]
	Adults	25-29°C[sss,194]	OptR:21-25°C; Tol:18-19°C[60]	Survive 7-11 mg L^{-1} after 21h shipment in plastic bags[60]	30-37 psu[60]
Loliginidae *Loligo vulgaris*	**Eggs**	14-19°C (peak at 16.5°C) off North-west Portugal[195]	Normal hatching at 12-24°C[ttt]; deformed or dead embryos at 6, 8, 10, 26, and 28°C[196]	Rec: 5 mg L^{-1} [85]	32-42 psu with the highest hatching success at 38 psu and the lowest at 42 psu[197]
	Hatchlings	14-19°C (peak at 16.5°C) off North-west Portugal[195]	10-22°C[198,199]#	Rec: 5 mg L^{-1} [85]	32-42 psu with the highest hatching success at 38 psu and the lowest at 42 psu[196]
	Juveniles	14-19°C (optimum at 16.5°C)[195]	12-22°C[61]#	Rec: 5 mg L^{-1} [85]	Rec:34-38 psu[85]
	Adults	15-17°C[195]	12-22°C[61]#	Rec: 5 mg L^{-1} [85]; P_{50}= ~30.6 -75.0 mmHg at 10-20°C and pH 7.4[200]	Rec:34-38 psu[85]
Loliginidae *Sepioteuthis lessoniana* (Tropical countries)	**Eggs**	Regularly found at 28°C[62]#	*oFl*: 24–30°C; *RAS*: 20–24°C; Rec: 28°C[62]#	N/A	30–33 psu[122]#; 22–37 psu to achieve a hatching rate of at least 50 %[67,201]
	Hatchlings	20-27°C[148]	*oFl*: 24–30°C; *RAS*: 20–24°C[62]#	N/A	23–36 psu[66,67,201]
	Juveniles	20-27°C[148]	*oFl*: 24–30°C; *RAS*: 20–24°C[62]#	O_2 concentration was not less than 2.5 ml L^{-1} as dyspneic levels are reached at 1.6 ml L^{-1} at 30°C[202]	23–36 psu[66,67,201]
	Adults	20-27°C[148]	*oFl*: 24–30°C; *RAS*: 20–24°C[62]#	N/A	23–36 psu[66,67,201]

[sss] shallow coastal waters of the Central Pacific and Hawaiian islands

[ttt] allow 80 % of hatching success; if temperature should be changed it is recommended to change no more than 1°C per 24h

Order/species	Life Phase	Thermal range in nature	Thermal tolerance in captivity	Oxygen tolerance in captivity	Salinity tolerance in captivity
Loliginidae *Sepioteuthis lessoniana* (Temperate countries)	Eggs	18-24°C[62]	N/A	N/A	>80% eggs hatched at 24, 28, 32 psu and failed to hatch at 12 and 40 psu, OptR:22.5-37.5 psu[67]
	Hatchlings	18-24°C[62]	N/A	N/A	>70% survival at 28 and 32 psu; OptR:23.2-35.5 psu[67]
	Juveniles	18-24°C[62]	20–26°C[62,88,100] # mortality increases when temperature is < 20°C[203]	N/A	Animals stop feeding at 27.5-28 psu and mortality increases at 23.7 psu similarly to *S. sepioidea*[204]. At low salinity animals hyperexcitation and violent reactions to neutral stimuli were observed as well as sudden and rapid mortality, accompanied by uncontrolled swimming motion[67]
	Adults	18-24°C[62]	20–26°C[62,88,100]# mortality increases when temperature is < 20°C[203]	P_{50}= ~20.0 -29.1 mmHg at 15-25°C and pH 7.4[200]	Animals stop feeding at 27.5-28 psu and mortality increases at 23.7 psu similarly to *S. sepioidea*[204]. At low salinity animals hyperexcitation and violent reactions to neutral stimuli were observed as well as sudden and rapid mortality, accompanied by uncontrolled swimming motion[67]
Octopodidae *Octopus bimaculoides*	Eggs	17-24°C[4]	18-25°C; OptR:22-25°C[63]	N/A	32-38 psu, OptR:34-36 psu[63]
	Hatchlings	17-24°C[4]	18-25°C; OptR:22-25°C[63]	N/A	32-38 psu, OptR:34-36 psu[63]
	Juveniles	17-24°C[4]	18-25°C; OptR:22-25°C[63]	N/A	32-38 psu, OptR:34-36 psu[63]
	Adults	17-24°C[4]	18-25°C; OptR:22-25°C[63]	min critical P_{50}: 16 mmHg at 10°C[50]	32-38 psu, OptR:34-36 psu[63]

Order/species	Life Phase	Thermal range in nature	Thermal tolerance in captivity	Oxygen tolerance in captivity	Salinity tolerance in captivity
Octopodidae ***Octopus vulgaris***	**Eggs**	10-20°C (Europe)[83] up to 28°C (Caribbean tropics)[205]	Rec: 15-18°C[85]; Tol:14°C[206]	N/A	Rec: 27–38 psu[85]
	Paralarvae	10-20°C (Europe)[83] up to 28°C (Caribbean tropics)[205]	19 -25°C[124#] OptR:20–22°C[102#]	6-8 mg L^{-1}, should not fall below 4 mg L^{-1} [124#]	Survival ranged from 60 to 83% at 28 psu, 73–100% at 30 psu and 100% between 33–37 psu[207]
	Juveniles	10-20°C (Europe)[83] up to 28°C (Caribbean tropics)[205]	OptR:19.5-23°C (spring-summer) and 23.5-12.3°C (autumn-winter)[153]	If the O_2 concentration in the experimental tank fell below 2.5 mg L^{-1}, the experiment was terminated earlier, since below 2 mg L^{-1} octopuses begin to show signs of distress and O_2 consumption declines[208]	27–38 psu[85,153]
	Adults	10-20°C (Europe)[83] up to 28°C (Caribbean tropics)[205]	OptR:19.5-23°C (spring-summer) and 23.5-12.3°C (autumn-winter)[152,153]	In normoxia: ♦ average ArB-P_{O_2}=78.1±2.9 mmHg, 98% O_2-saturated ♦ average VeB-P_{O_2}=30.0 ±3.2 mmHg, 14% O_2-saturated In hypoxia O_2 consumption declined markedly below an ambient PO_2 of 90 mmHg[209] Critical P_{O_2} is 2.3±0.57 mg L^{-1} at 17-20°C[70]; ♦ optimal range: 65-100%, ♦ suboptimal: 35-65%, ♦ dangerous: below 35%, ♦ lethal: below 15%[70]; if O_2<2.5 mg L^{-1} , the experiment is stopped as <2 mg L^{-1} octopuses begin experience distress and O_2 consumption declines[208]	27–38 psu[85,153]

Order/species	Life Phase	Thermal range in nature	Thermal tolerance in captivity	Oxygen tolerance in captivity	Salinity tolerance in captivity
Octopodidae *Octopus maya*	Eggs	24-27°C[210]	24-27°C[210]; OptR:22-26°C[155]; <27°C embryos experience changes in the oxidative system and do not recover; at 30°C, embryo metabolic rate is 25 and 50% lower than at 26 and 22°C, respectively[64]#	5.0-6.7 mg L^{-1} [64,211]#	32-36 psu[13,64]#
	Hatchlings (~ 0.7 cm ML)	24-27°C[210]	24-28°C[13]# OptR:25±1[64]#	5.0-6.7 mg L^{-1} [64,211]#	36-40 psu to favor growth[13]#
	Juveniles (>5 cm ML)	24-27°C[210]	♦ 8.1-31.8°C after accl. at 18°C ♦ 11.6-32.7°C after accl. at 22°C ♦ 13.7-34.8°C after accl. at 26°C ♦ 19.0-36.5°C after accl. at 30°C[211] OptR:24-26[64]#	5.0-6.7 mg L^{-1} [64,211]	36-40 psu to favor growth[13]#
	Adults (>8 cm ML)	24-27°C, 28-30°C negatively affects reproductive performance[210]	OptR:24-27°C[154,210]; Tol:30[64]#	5.0-6.7 mg L^{-1} [64,211]#	> 32 psu to favor spawning[64]#

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