Chapter 11
Sepia officinalis

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Abstract This chapter reviews the importance of the European cuttlefish, *Sepia officinalis*, as a potential species for aquaculture and its applications. It provides an overview of cuttlefish culture, its current state of art and future trends. Present cuttlefish culture-related research and recently developed technologies are described. This includes a description of the culture systems for the different life stages, broodstock and egg acclimatization to captivity and management, hatchlings, juvenile and adult-rearing methodologies. Values of fecundity and fertility obtained in different culture conditions (variables include tanks, stocking densities, sex ratios and food); a characterization of different types of cuttlefish egg morphology; growth rates, mortality, feeding rates and food conversions at the hatchling and juvenile stages (including live, frozen and artificial diets); and a comparison between different growout setups are presented. Finally, current bottlenecks are enumerated, prospects for future research are suggested and an overview of whole animal use by the industry is given.

Keywords European cuttlefish · *Sepia officinalis* · Zoo technology · Stocking densities · Diets · Tank and earthen pond culture

11.1 Commercial Value and Capture Methods

The European cuttlefish, *Sepia officinalis* Linnaeus 1758, is mostly found in eastern Atlantic and in the Mediterranean Sea (Boletzky 1983). Cephalopod catches have set a new record in 2008 (FAO 2010) but only contributed to approximately 4% of the world catch from fisheries in 2010 (FAO 2012). Cuttlefish was one of the species attaining high market value in the Mediterranean and Asian markets (FAO 2010);

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countries such as Spain, Italy and Japan being the main consumers (Boucaud-Camou 1990; FAO 2012).

S. officinalis had an average world production of 16,769 t year^{-1} (2000–2010), showing an increasing trend during this period (FAO-FIGIS 2012). The species is primarily caught by otter and beam trawlers, either as a target species or as catch of demersal finfish fisheries. In southern European countries like Portugal, this species is caught by several fishing methods such as iron traps, trammel nets, bottom trawl, gill nets and purse seine (Sendão et al. 2007), similar to the traditional fishing gears used in other European countries. While trawlers operate in inshore and offshore fishing grounds for both juvenile and adult specimens, artisanal gears, like fish traps, are used to catch spawning animals mainly in inshore areas.

The species potential for aquaculture has been recognized by Barnabé (1996) and Boucaud-Camou (1989). This was due to a set of biological and physiological aspects that were described by Forsythe and Van Heukelem (1987) and which are shared by other cephalopod species. *S. officinalis* has been successfully reared in extensive aquaculture experiments at a medium scale in several EU countries such as Italy, France and Portugal.

The commercial culture of cuttlefish will possibly have a high impact on fisheries production in the near future, as it could allow the sale of undersized individuals (approximately 50 g) that may be produced in only 45–60 days.

11.2 State of the Art

Cultured *S. officinalis* is used as an animal model for biological and biomedical research (e.g. physiology, neuroscience, nutritional biochemistry, ageing, molecular biology or immunology), for aquaculture production and also for public exhibition in aquariums. During the past 20–25 years, a great part of the research on cuttlefish has focused on its use as a new species for aquaculture. When viable, economical and logistic cuttlefish culture is attained, this species will become one of the most relevant marine animal models and make an important impact in other fields of research. The use of cephalopods as animal models has already greatly contributed to the scientific advance of humankind (i.e. the Nobel Prize won by Hodgkin and Huxley in 1963 regarding the squid's giant axon). Thus, research on cuttlefish culture will have further application for the development of culture technology of other cephalopod species and will provide a cultured invertebrate model that shares some biological features with vertebrates.

The most recent revision on the status of cuttlefish culture was performed by Sykes et al. (2006b), in which the major bottlenecks limiting its transition into an industrial scale were identified. Currently, the three main factors still delaying the large-scale culture are (1) the dependence on live prey during the first part of the life cycle, (2) the lack of an adequate artificial diet for all life stages of this species and (3) full control of reproduction in captivity. Due to the low number of laboratories involved in the resolution of these problems, small but steady progress was made

since then. Consequently, not only technology has evolved but also cephalopods have been included in the EU animal welfare legislation. Thus, the advances in *S. officinalis* culture technology will be revised also considering a good welfare practice.

11.3 Broodstock Rearing Conditions and Acclimatization to Captivity

Considering that reproduction in captivity is one of the bottlenecks in cuttlefish culture (Sykes et al. 2006b), broodstock maintenance knowledge and conditions have been updated.

11.3.1 Seawater Systems and Conditions

Depending on the location of the husbandry, the three types of seawater systems (open, semi-open or closed) may be applied. Commonly, cuttlefish breeder stocks are obtained from captive cultured populations or through wild captures as described by Sykes et al. (2009). After being checked for illness and skin damage, cuttlefish are conditioned in open seawater systems because of logistics, welfare and economical aspects. Nonetheless, as the technology used in closed and semi-closed systems is becoming cheaper and aquaculture environmental concerns are scaling up, the use of closed seawater systems (RAS), similar to those reported by Hanlon (1990) for cephalopods and recently by Martins et al. (2010) for fish, is expected in the near future. The technology has progressed since 2000, from using 250 L tanks (Correia et al. 2005; Sykes et al. 2006a) to increasing sizes, such as 9,000 L, currently being used after the results of Sykes et al. (2013b, Fig. 11.1).

A typical tank setup may be built indoors or outdoors of the husbandry facilities but it must be located in a low disturbance area (Sykes et al. 2006b). Each tank should have a setup consisting of round-shaped fibreglass tanks comprising enough airlifts, fixed on the tank walls, and air stones in the middle of the tank. These conditions will make water move slowly but like a drain to the outlet pipe, which is located at the centre of the tank. Since cuttlefish is a benthic species (Boletzky 1983), the tank should have large bottom areas (Domingues and Marquez 2010; Sykes et al. 2013b).

If toxic materials (e.g. PVC glue, silicone for marine environments) are used while assembling the tank setup, it should be first filled with tap water for 24 h, followed by a 24 h seawater filling and running, before the animals are placed in the tank. While designing, building and operating this setup, the use of metallic parts, especially copper, should be avoided and kept away from all seawater systems, as it can be toxic in the first stages of development of the cuttlefish (Establier and Pascual 1983) and even in the remaining life stages (Hanlon and Forsythe 1985;

Fig. 11.1 Tanks used at Centro de Ciências do Mar do Algarve (CCMAR) for cuttlefish reproduction. The first experiments (in 2000) were performed in 250 L tanks and the current methods include the use of 9,000 L tanks. (Photos by A Sykes)

Paulij et al. 1990). The use of IP67-electric-certified equipment and material (e.g. illumination) is mandatory if these are installed near the tank.

The use of semi-open or closed systems should comprise a scaled filtration based on the basic setup described by Hanlon (1990) to assure that protein load in the water (resulting from feeding and eventual inking due to reproductive behaviour or stress response) is kept low and spikes or build-up of nitrogenous compounds is avoided. Upgrades on the equipment used in these types of systems are advised to help reduce the area occupied and to attain increased efficiency. The build-up of anoxic areas in the tank, due to low water circulation, should be prevented while designing the system setup.

Airlifts and outflow pipes should be covered with plastic nets of appropriate mesh size to prevent animals and eggs from escaping or being washed out. While designing the seawater system, it should be considered that sharp objects or rough surfaces must not exist inside the tank since they may cause skin damage to the animals. If needed, a wall foam protection system similar to that described by Hanley et al. (1999) should be used. Following a good welfare practice regarding enriched environments and considering the animal's ability to camouflage; this tank should be under low light intensities of 200 Lx or less (measured at the water-air interface of the tank) and under a normal photoperiod that should replicate natural

geographical conditions during spawning in the wild. This may be achieved using a combination of natural or natural-resembling artificial light sources and tank colours. If placed outdoors, the 200 lx light intensity may be obtained by using water-repellent shading nets (Fig. 11.1). The use of these nets will prevent excess lighting as well as pH and salinity descent due to rainfall.

In any of the setups described above, animals, tanks, equipment to maintain specific conditions, water flow and aeration should be checked twice a day, in the morning and late afternoon. Water quality parameters should be monitored every day in the morning. Depending on the seawater system used, the determination of different parameters will apply. Open seawater systems will require the measurement of temperature, salinity and dissolved oxygen. It is not possible to fully evaluate the use of semi-open or closed seawater systems, since there are not enough data regarding reproduction and egg quality under these conditions. Nevertheless, if these systems are the only option, determination of previous parameters, plus pH and nitrogenous compounds (ammonia, nitrites and nitrates), is mandatory. In semi-open or closed systems, these parameters should be kept as similar as possible to natural seawater and according to the highest values reported for the culture of this species by Forsythe et al. (1991) and Oestmann et al. (1997). The technology for closed systems has progressed since the end of the 1980s, and there is an increasing pressure to reduce the aquaculture footprint by using these systems (Martins et al. 2010). However, cephalopods are highly sensitive to nitrogenous compounds and the removal of these, to prevent a system overload which will translate into massive death, still requires highly expensive technology, logistics and operational costs. Some trace elements, in particular strontium and calcium, should be kept close to natural seawater values (Hanlon et al. 1989). The use of natural seawater is suggested. However, if, due to logistics, commercial seawater salts are used to produce artificial seawater, its content should comply with these requirements to avoid malformations and the death of hatchlings (Hanlon 1990).

In any case, temperature, salinity, dissolved oxygen, pH and nitrogenous compounds should be maintained according to values reported by Boletzky (1983) for the species. There are reports of culturing cuttlefish beyond the maximum (Domingues et al. 2001a) and minimum (Sykes et al. 2006a) temperature values reported for the species, but a flow-through system was used in both cases, where changes in water quality occurred slowly due to the high volumes of water utilized. Regardless of the seawater system used, water flows should be high enough to maintain water quality and sustain the best reproduction results.

If closed and semi-open systems are used, the use of UV filtration will be necessary to avoid pathologies, as described by Forsythe et al. (1991). Although the effect of ozone on the species is not reported in the literature, its use is not suggested as a way to attain similar results of salubriousness. Nonetheless, if used, measurements of O_3 concentrations and the redox potential of water entering the tank should be enforced. The application of EU Directive 2010/63/EU from January 2013 onwards will impose the use of alarm systems locally and externally, to the person responsible for animal care, through SMS messages or similar systems. Not only should these alarms be set to report a failure of water circulation in tanks or in the marine

station but also if a digital solution is applied, a report on these abnormal situations will be created by the system, and later used as experimental data and for reporting animal welfare.

Tank cleaning should be avoided as much as possible to prevent stress, by enforcing an ad libitum feeding scheme. Water and tanks should be kept clean from leftovers, faeces and other debris and removed through water siphoning. Therefore, to avoid problems with cleaning, sand substrates should not be used (Forsythe et al. 1994).

11.3.2 Sex Ratio and Stocking Densities

Reports on stocking densities and sex ratios are scattered in the literature, and experiments dealing primarily with this issue are scarce. Sex ratios and stocking densities are of particular importance since they are thought to have a high impact on cuttlefish fecundity and fertility. This assumption is based on our experience of culturing the species and on previous studies which were not directly related to reproduction (Boal et al. 1999; Forsythe et al. 2002). However, researchers do not agree on which are the best densities and sex ratios that will maximize egg quantity and quality obtained in cuttlefish reproduction in captivity. In addition, most of them do not consider the importance of tank bottom areas with regard to a species that is benthic or the individual contribution of parents that may change depending on available area, stocking densities and sex ratios. For instance, Forsythe et al. (1991) mentions that sex ratios of one male for three females should be used to avoid male aggression and aggressive mating behaviour, and Forsythe et al. (1994) suggests densities of two cuttlefish m^{-2} for breeders.

Table 11.1 summarizes the most relevant fecundity and fertility results obtained in different tanks, stocking densities, sex ratios, temperature and food items (Correia et al. 2005; Domingues et al. 2001b, 2002, 2003b; Sykes et al. 2006a, 2009, 2013b). It is suggested that sex ratios should be maintained at two females for one male and stocking densities kept relatively low when setting up a broodstock. For instance, a 9,000 L tank should have 21 animals, 14 of them females and 7 males, which will correspond to a low stocking density of 4 cuttlefish m^{-2}.

11.3.3 Food Supply

Despite Boletzky's (1983) and Nixon's (1985) descriptions that cuttlefish shifts its food preferences from a predominant crustacean diet (crabs, prawns and shrimps) to a mixture of crustaceans and fish while maturing and reproducing, recent manuscripts report the successful culture of the species using only a frozen grass shrimp (*Palaemonetes varians*)-based diet (Sykes et al. 2006a, 2013b, Table 11.1) during those periods.

The use of different kinds of food throughout the different stages of the life cycle of cuttlefish was never studied, but examples of different feeding strategies are

Table 11.1 Fecundity and fertility results obtained in different tanks, stocking densities, sex ratios, temperature and food

Density (cuttlefish. m−2)	Tank	Animals	Sex ratio	Temperature (°C)	Generation	Food	Fecundity (eggs/♀)	Fertility (%)	Reference
38	250 L round	30	1♂: 1♀	27.0 ± 3.0	F1	Frozen *Carcinusmaenas*	144	50.0	Domingues et al. (2001b)
19	250 L round	15	3♂: 1♀	15.0 ± 4.0	F2	Live P. *varians*	225	41.0	Domingues et al. (2002)
19	250 L round	15	1♂: 1♀	≈ 17.0	F3	Live P. *varians*	150	33.0	Domingues et al.
19	250 L round	15	1♂: 1♀			Frozen P. *varians*	411	85.0	(2003b)
16	250 L round	13	3♂: 1♀	24.5 ± 1.4	NK	Mixture of live *P. varians*,	834	35.8 ± 9.4	Correia et al.
76	250 L round	60	3♂: 1♀			*Carcinusmaenas* and fish	290	62.0 ± 16.9	(2005)
9	250 L round	27	1♂: 2♀	17.1 ± 1.7	F2	Live *P. varians*	370	NK	Sykes et al.
9	250 L round	30	3♂: 1♀	23.4 ± 1.4	F3		301	16.0	(2006a)
13	250 L round	35	1♂: 2♀	15.2 ± 3.0	F4		247	47.7	
8	250 L round	50	1♂: 1♀	21.1 ± 2.6	F5		478	67.5	
3	250 L round	60	2♂: 1♀	24.2 ± 1.7	F6		293	0	
15	400 L rectangular	18	1♂: 2♀	19.5 ± 1.1	F2	Frozen *P. varians*	787	n.d.	Sykes et al. (2009)
4	9,000 L round	23	1♂: 2♀	20.5 ± 2.9	F1	Frozen *P. varians*	1,383	72.0	Sykes et al.
15	750 L round	23	1♂: 1♀	19.0 ± 2.2	F1	Frozen *P. varians*	223	66.0	(2013b)
29	250 L round	23	1♂: 1♀	21.2 ± 3.4	F1	Frozen *P. varians*	325	48.0	

Sex ratios are given as observed and not established in the experiments. In a given column, when a given variable is shared by one or more groups, only one value is presented. Fecundity is individual fecundity. Fertility is the percentage of hatching eggs

Values shared by several different groups are presented solely in that column

n.d. not determined, *NK* unknown

found within the literature. For instance, Domingues et al. (2001b) tested the effects of feeding either *Artemia* sp. or *Paramysis nouvelli* during the first 20 days after hatching (DAH), followed by a diet of *P. varians* until 70 DAH and frozen *Carcinus maenas* for the remaining life cycle with similar results of fecundity (Table 11.1). Domingues et al. (2002) used *P. nouvelli* for the first 20 DAH and afterwards live grass shrimp to close the cycle, achieving narrow increased results in individual fecundity but lower fertility (Table 11.1) when compared with the 2001 F1 generation. The effects of using either live or frozen grass shrimp, after being fed during the first 15 DAH, were tested by Domingues et al. (2003b) attaining increased individual fecundity and fertility when using the frozen diet (Table 11.1). A mixture of live *P. varians, C. maenas* and fish used by Correia et al. (2005) attained one of the highest individual fecundity ever in 250 L tanks (834 eggs per female, Table 11.1). We believe that this result is related more to temperature, densities and sex ratio than type of food, but further studies are needed.

Sykes et al. (2006a) studied five consecutive generations of cuttlefish in captivity using a diet of live *P. varians* in 250 L tanks and obtained a maximum individual fecundity of 478 eggs per female and fertility of 67.5% at the F5 generation (Table 11.1). On the other hand, Sykes et al. (2009) confirmed an increased fecundity in a F2 generation (787 eggs per female; Table 11.1), being fed on live grass shrimp for the first 20–30 DAH and frozen grass shrimp onwards (in accordance with the methods described by Sykes et al. 2006b). However, the sex ratio was completely inversed when compared with that obtained by Correia et al. (2005).

Recently, Sykes et al. (2013b) obtained the best values of individual and overall fecundity (1,383 and 16,593 eggs, respectively) and an acceptable individual fertility (72%, Table 11.1) following a similar feed methodology but increasing the bottom area of the tanks.

Based on these results, it seems that the diet per se will not have a direct effect on egg number and quality. However, we do not discard the fact that the use of frozen grass shrimp may be influencing the higher individual and overall fecundity and fertility by promoting lower energy expenditure associated with feeding.

11.4 Spawning Process

11.4.1 Female Conditions

S. officinalis attains sexual maturity at very different sizes/weight (Sykes et al. 2006a) and has an estimated potential fecundity of a maximum of 8,000 eggs in nature (Laptikhovsky et al. 2003). The species displays a very typical behaviour of courtship, mating and agonistic male-to-male interaction, briefly described by Boletzky (1983) and Hanlon and Messenger (1996), where males mature earlier than females. Females receive the spermatophores in the paired seminal receptacles, under the buccal mass, where it may remain and used for as much as 2–5 months

(Hanlon et al. 1999). This is probably due to the biology of the species, with males maturing precociously (Forsythe et al. 1994). Males, before inserting their sperm into this pouch, jet large amounts of seawater inside the seminal receptacles to flush out the sperm from previous males and, in this way, assure that only its genetic contribution will be used (Hanlon et al. 1999).

Females mate repeatedly (Hanlon et al. 1999), display intermittent or chronic spawning (Boletzky 1987) depending on captive conditions and usually will die shortly after laying the eggs. Nevertheless, we have observed an extension of this intermittent spawning when using increased tank bottom areas (based on comparisons between data from Sykes et al. 2006a, 2013b). However, this does not happen repeatedly and in all tanks tested. Therefore, we suspect that the optimal conditions to obtain more and better eggs are related not only to bottom areas but also to other variables, which are being investigated in current projects.

According to Boal (1997), females prefer males that have mated recently instead of choosing males based on a dominance hierarchy, which is promoted by captive conditions (Boal et al. 1999). If this is related to social recognition (Boal 2006), chemoreception (Boal and Marsh 1998; Boal and Golden 1999) or specific pheromones (Zatylny et al. 2000, 2002) remains to be unveiled and are objectives of future studies.

After the copula, it is not advisable to separate females from males since this will not promote a reproduction resembling wild conditions and might have a negative influence on egg quantity and quality. According to Boletzky (1983), bigger females will lay bigger eggs; which does not completely agree with more recent data from Sykes et al. (2013b). By having extended intermittent spawning due to different culture conditions, the amount and quality of eggs should be better. However, what is a quality egg in this species and how can we say that bigger eggs are better eggs, since no results on this subject have been presented?

In the species, no parental care of eggs was ever observed, and senescent females die after spawning. As a senescent species, reproduction will absolutely drain females, which incorporate their little reserves in the eggs. The ability of cuttlefish females to have more than one maturation cycle or continuous maturation during this intermittent spawning is something that will need to be further investigated in the near future, since not all the females or males will die after mating and eggs are laid.

11.4.2 Egg Capture and Handling

Eggs may be obtained from nature or from captive-bred populations, but the latter is recommended. The use of eggs from a known source not only reduces the impact on the environment but also assures that eggs will have a very low probability of introducing contaminants and/or pathologies into the culture facilities. Within the EU, these Animal Resource Centres are regulated by law and a wide network exists (for details, check European Marine Biological Resource Centre—EMBRC—http://www.embrc.eu/).

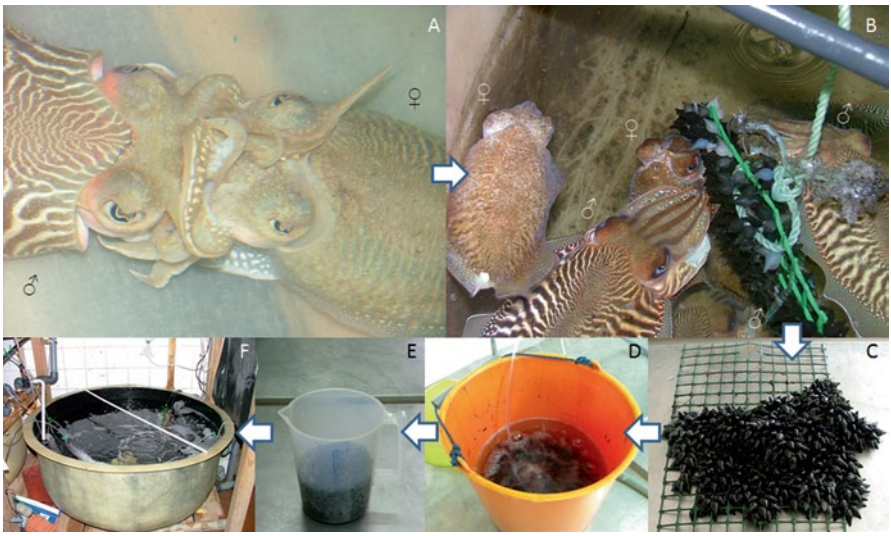

Fig. 11.2 From copula to incubation in captivity. Copula (**a**), egg laying (**b**), extraction, separation and assessment of eggs (**c, d, e**) and incubation of eggs in 250 L tanks (**f**). (Photos by A Sykes)

In nature, cuttlefish females lay their eggs on hydrodynamic locations, attached to wild flora and fauna or to human-related structures (Boletzky 1983). Egg masses are commonly found on sea grass, seaweeds, polychaetes, buoys, fishnets, traps, boat ropes, etc. Based on this knowledge, Sequi and Palmegiano (1984) and Blanc and Daguzan (1998) described man-made egg collectors that use floating ropes to collect eggs on spawning grounds. However, it is recommended to obtain eggs in the wild directly from the beaches, when they are stranded by storms or other hydrodynamic conditions. The impact of man on wild populations is therefore reduced, considering that these correspond to a percentage of eggs that normally would not hatch because of being exposed to dry conditions.

In captivity and after copulating (Fig. 11.2a), females attach their eggs on ropes, airlines and nets (Fig. 11.2b, c). To make the process of removing the eggs from the tanks and egg collectors easier, it is recommended to use plastic nets. Each breeding tank should have at least one of these collectors that should be checked for eggs every day, preferably in the morning and before feeding. It is very important to avoid disturbing the animals while spawning and proceed accordingly. Afterwards, the egg collector should be removed from the tank and kept humid while eggs are separated and individualized. We recommend the use of a plastic net, with 1 cm × 1 cm net holes (Sykes et al. 2006b), to facilitate egg removal (Fig. 11.2c). The easiest way to perform this operation is using a small knife or scalpel, which will cut the egg lace connecting to the plastic, preventing any harm to the corion. Freshly laid eggs are very soft and gelatinous and should be removed carefully, while those not from that day will be firmer and easy to manipulate. Irrespective of the egg source (nature or captivity) and depending on their numbers, eggs being removed from the

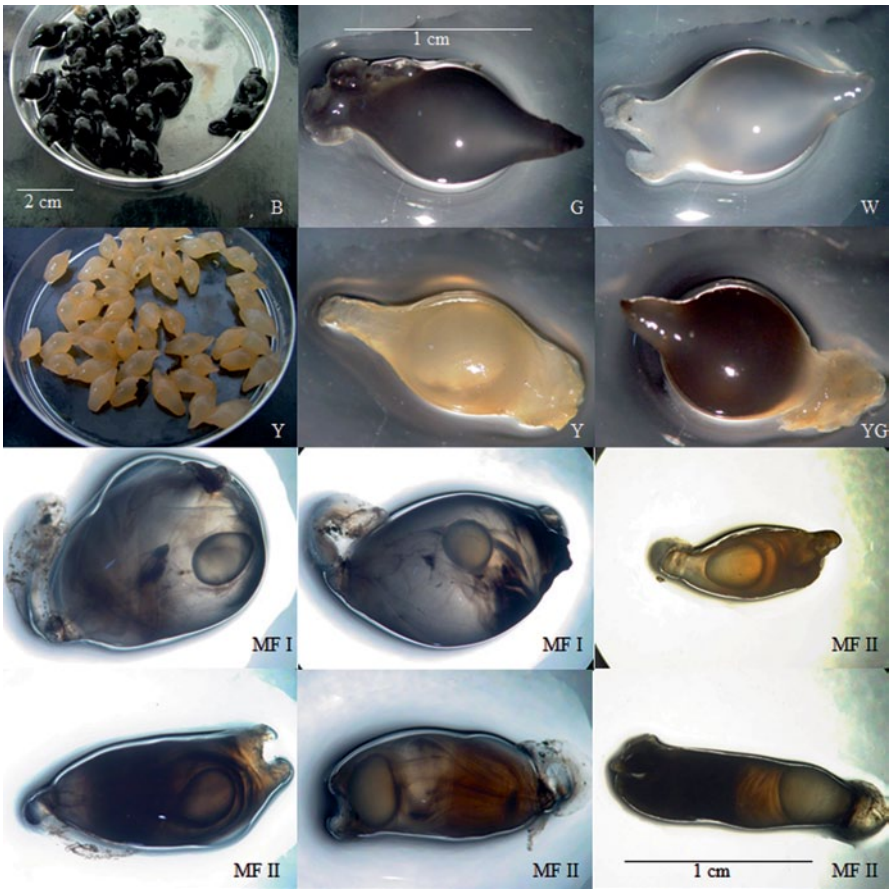

Fig. 11.3 Morphology of different types of cuttlefish eggs obtained in captivity (characterization and identification presented in Table 11.3). (Photos by A Sykes)

natural or man-made collectors should be individually sorted and placed in a bucket with high aeration that will make them move gently (Fig. 11.2d). During egg extraction from both collectors in captivity and egg collection at the beach, eggs should be kept moist and the use of a spray with seawater to accomplish this is recommended. At this time, the eggs should be separated according to their morphology and colour (Figs. 11.2e and 11.3).

Eggs collected from the wild are usually black, present a flask shape and are attached in grape-like clusters to different substrata fixed on the bottom (Boletzky 1983; Boletzky et al. 2006). Eggs will display weights ranging from 0.1 g up to 2.5 g (Sykes et al. 2006a) and a diameter ranging from 1.2 to 1.4 cm. Only black (ink-stained) eggs with a flask-shape morphology should be chosen, while those with a different colour and morphology (Fig. 11.3) should be discarded properly (Sykes et al. unpublished data).

Table 11.2 Characterization of different types of cuttlefish egg morphology

Egg type	ID	Shape	Colour	Transparency
Normal	N	Flask	Black	No
Grey	G	Flask	Grey	No
White	W	Flask	White	No
Orange	O	Flask	Orange	Semi-
Yellow-grey	YG	Flask	Yellow-grey	No
Malformation type I	MF I	Globular	No	Yes
Malformations type II	MF II	Elongated	Dark brown	Semi-

ID—identifies a given egg type in Fig. 11.3

Table 11.2 discriminates and classifies the different egg types ever obtained in captivity.

Despite the recent attempts by Sykes et al. (2013b), there is currently no methodology to assess the quality of eggs at this time and before animals are born. However, in captive conditions, both percentage of rejected eggs and individual egg weight might be indicative of the quality of the egg masses.

After the egg quality assessment and sorting, the eggs should be placed in round-shaped tanks of a flow-through or semi-open system (Fig. 11.2f). If a closed seawater system is used, then the recommendations of Hanlon et al. (1989) regarding strontium should be considered. The tank setup should have airlifts on the walls and air stones in the middle which promote a gentle elliptical movement of the eggs (Fig. 11.2f). This also assures an oxygen-enriched environment, which has proven to prevent necrosis. The amount of eggs should not exceed the tank's carrying capacity, considering that water parameters should remain relatively constant and, therefore, water flows will be small. Water parameters should be set according to the conditions of eggs' geographical location described in the literature (Sykes et al. 2009). So, if needed, seawater should be heated or refrigerated. Nonetheless, according to Palmegiano and Sequi (1984), salinity will increase an egg's viability above 90 % if its values are within 28–50 psu.

Detailed information regarding the embryonic development of the species was reported by Naef (1928), Lemaire (1971) and Boletzky (2003) and was reviewed recently by Boletzky et al. (2006). However, the duration of this stage is dependent on temperature (Koueta et al. 2006), without any linear correlation (Richard 1971) and geographically conditioned (e.g. temperature vs. duration varies considerably between Faro, Portugal and Caen, France (Sykes et al. 2009)). It may range from 40 to 45 days at 20 °C to 80–90 days at 15 °C in the English Channel populations (Boletzky et al. 2006) and from 25 days at 25 °C to 60 days at 15 °C in southern regions, such as Portugal (Sykes et al. 2006b).

It is common that eggs laid during a week by one or more females will display a synchronized hatching on the same day. Whether this synchronized hatching is related to the action of ILME (a waterborne pheromonal peptide released by eggs; Zatylny et al. 2000) or to any other unknown peptide or process remains to be determined.

11.5 Hatchlings Culture

As this stage is considered as one of the bottlenecks in cuttlefish culture (Sykes et al. 2006b), zoo-technology and nutritional knowledge have been updated in this chapter.

11.5.1 Hatchlings Collection and Transfer

According to Paulij et al. (1991), cuttlefish embryos hatch during periods of darkness, so it is important to check the egg-hatching tank in the morning to eventually collect and separate the new offspring.

After hatching, and depending on the purpose, hatchlings may be kept in the same tank or carefully removed to a different one. According to the new animal welfare legislation and cephalopod proposed guidelines, this tank or group of animals will be considered a captive population and assigned an identification, which should include information regarding the generation and source of eggs.

If removed to a new tank, the physical and chemical conditions of the new tank should be similar to those of the hatching tank, although these might be changed, gradually, afterwards. Normally, the procedure of transfer, if the animals remain in the hatchery, is to collect them individually using small movements with an aquarist net. This reduces stress and abrasion against either tank or net. Afterwards, hatchlings are placed in a plastic container of small volume (preferably black) and then submerged in the new tank. The latter will allow cuttlefish to swim freely out of this container into the water of the new tank. If a transfer of the newly hatched animals to other culture facilities is intended, then specific information for the species may be found in the recent animal welfare review by Sykes et al. (2012).

11.5.2 Culture Conditions

11.5.2.1 Seawater Systems

The seawater systems used for the hatchling stage are similar to those used in other life stages of the species and detailed for breeders (see Sect. 11.3.1). Nonetheless, the type of tanks used should be adapted according to the most recently published literature, where factors such as the culture densities of 500 hatchlings m^{-2}, minimum bottom areas of 0.06 m^2 and the avoidance of sand and shelters (Sykes et al. 2003) should be considered. The use of flow-through seawater systems with UV filtration is recommended to avoid problems derived from pathogens present in the water. This stage of the life cycle is the most problematic and where most of the mortality in cuttlefish culture usually occurs.

Fig. 11.4 Different hatchling culture systems. **a** In the hatching tank. **b** In a white basket within a 1,500 L tank. **c** In a black basket within a 1,500 L tank. **d** In raceway tanks. (Photos by A Sykes)

Significant progress has been obtained in the optimization of culture conditions at this life stage. Sykes et al. (2006b) previously recommended to rear cuttlefish in the hatching tank (Fig. 11.4a) but currently the use of hatching baskets (Fig. 11.4b), preferably in black (Fig. 11.4c; Sykes et al. 2011), or raceway tanks (Fig. 11.4d) is suggested. The use of low light intensities during this stage is also suggested (Sykes et al. 2013c). These conditions of tank colour, light intensity and similar seawater physical conditions with respect to embryonic development should be maintained to promote appropriate welfare conditions, lower mortality rates and normal growth and development.

11.5.2.2 Physicochemical Conditions

Seawater physicochemical conditions should resemble natural conditions as much as possible, as long as this does not interfere either directly or indirectly with cuttlefish

growth and survival. Depending on the seawater system used during this stage, different water parameters should be set and checked (see Sect. 11.3.1 for the list and procedures). Normally, hatchlings' rearing is performed at 20±2 °C, in high dissolved oxygen conditions (>90 %) and high water-flow rates to avoid nitrogenous waste-derived problems in low-volume seawater systems.

11.5.2.3 Light

There is no known minimal amount of light necessary for this stage but recent results recommended 100 lx at the air–water interface of the tank (Sykes et al. 2013c), provided by daylight (450–650 nm) fluorescent bulbs. From published data, only Koueta and Boucaud-Camou (2003) studied the effect of photoperiods in hatchlings and suggested that cuttlefish hunts more during daylight. Photoperiodicity should match habitat and lifestyle at given geographical locations (Sykes et al. 2006b), and daylight should be set with a 12–16 h light cycle. Nonetheless, little is known regarding the daily activity and daily feeding rhythms at this life stage or even for the species (Quintela and Andrade 2002) and this should be researched in the near future.

Up till now, the use of dusk-and-dawn light simulations in the photoperiod or the use of polarization (light or filters) to enhance growth and survival was never reported. Cuttlefish has polarized vision, which it uses to hunt in the natural environment (Shashar et al. 2000, 2002). This characteristic should be further explored to determine if it could enhance conditions in captivity.

11.5.2.4 Diet

Cuttlefish may hatch prematurely, mature or 'over-mature', the differences being characterized by the respective amount of inner yolk reserves remaining. These reserves will determine how long the animal will endure without food and commonly last between 3 and 5 days (Sykes et al. 2004). During this period, the animal must be given the opportunity to feed on an external diet or there will come a point of no return and the cuttlefish will die.

Diets for hatchlings have been one of the most investigated themes in the past 12 years and where research has attained some of the most relevant results. It is known that in nature, cuttlefish hatchlings prey preferably on live crustacean species (Boletzky 1983), and a series of different alternative live diets were tested (DeRusha et al. 1989). For several years, the rearing of cuttlefish during the hatchling stage was obtained using a diet of live mysids followed by live shrimps, when the animals grew bigger (Correia et al. 2005, 2008a; Domingues et al. 2001a, b, 2002, 2003a; Koueta and Boucaud-Camou 1999). Domingues et al. (2004) proposed the use of live *P. varians* as an alternative prey for the rearing of cuttlefish at this life stage, while Sykes et al. (2006a) extended this further by proposing the use of grass shrimp for the culture throughout the life cycle of *S. officinalis*. Irrespective of the prey used, its degree of availability and the use of freshly caught or starved live food affect cuttlefish growth (Correia et al. 2008a, b).

After a series of zoo-technology optimizations for this life stage, Sykes et al. (2013a) managed to rear cuttlefish throughout the hatchling stage performing an early weaning on the first day after hatching with some success (Fig. 11.4d on the right—cuttlefish hatchlings eating frozen grass shrimp). These results are encouraging but further research regarding this issue in terms of nutrition, digestion, etc. is needed.

For the time being, the use of live grass shrimp of appropriate size is still recommended during the hatchling stage and the weaning to frozen grass shrimp should be performed after 20–30 DAH depending on the temperature.

11.5.3 Growth and Survival

Information regarding cuttlefish hatchlings' behaviour, growth and morphological changes during this stage was reported by Nixon and Mangold (1998). Growth rates vary directly with temperature, inversely with size, and play a major role in determining the species' life span (Domingues et al. 2006). Maintaining animals of different age groups together should be avoided at any stage but especially at this stage. It is acceptable, for rearing purposes, to join hatchlings with an age difference of up to 10 DAH. Joining animals with larger age differences will generate increased competition for food, promote the establishment of food hierarchies and, therefore, increase mortality. This stage of cuttlefish life is characterized by the highest growth rates but, at the same time, where mortalities may be higher. Table 11.3 resumes the hatchlings growth rates, mortality, feeding rates and food conversions (during the first 30 days of life, according to type of tank and food) published since 1999.

Normal growth, development and survival depend on the egg content (this content reflects food given to females and the same female may lay eggs with different amounts of yolk depending on the number of spawns and age), food quality, the best quality of seawater and tank setup conditions. Food consumption and feeding rates during this life stage are also extremely high, when compared with the values registered in the subsequent stages.

11.6 Ongrowing of Juveniles and Adults

11.6.1 Tank and Earthen Pond Conditions

Despite the first attempts of rearing cuttlefish in earthen ponds which were performed by obtaining eggs in the wild with egg collectors and placed in these ponds and collecting the juveniles 3 months later (Palmegiano and Sequi 1981), this type of procedure is considered extensive culture. This was, and still is, performed in countries such as Italy, Portugal (Coelho et al. 1989; Gonçalves 1989) and Tunisia (Rodger and Davies 2000). Nevertheless, some authors believe that the potential of cuttlefish culture is able to sustain semi-intensive or intensive aquaculture.

Table 11.3 Growth rates, mortality, feeding rates and food conversions during the first 30 days of life of *Sepia officinalis*

Tank/System	Gen.	T (ºC)	S (PSU)	Food/Light Intensity	IGR (% BW/d)	Mortality (%)	FR (% BW/d)	FC	Reference
70 L cylinder tanks/ Semi-open	F1	19.0	33.5	Live mysids (*Mesopodopsis slabberi* and *Schistomysis* sp.), *Gammarus* sp., and young shrimps	7.3	3.0	4.6	3.2	Koueta and Boucaud-Camou (1999)
				Live mysids (*Mesopodopsis slabberi* and *Schistomysis* sp.) and frozen mysids (30% BW/d)	4.6	3.0	4.2	2.3	
250 L/Open	F1	27.0 ± 3.0	37.0 ± 3.0	*Artemia* sp. (20 DAH) + *P. varians* afterwards	5.2	33.0	n.d.	n.d.	Domingues et al. (2001b)
				Paramysisnouveli (20 DAH) + *P. varians* afterwards	10.2	10.0	n.d.	n.d.	
				Artemia sp. (10 DAH) + *P. varians* afterwards	8.8	None	n.d.	n.d.	
				P. nouveli (10 DAH) + *P. varians* afterwards	10.0	None	n.d.	n.d.	
Individual baskets /250 L/Open	F1	27.0 ± 3.0	37.0 ± 3.0	*Artemia* sp. (10 DAH) + *P. varians* afterwards	9.9	None	24.3	3.0	Domingues et al. (2001a)
250 L/Open	F2	≈18.0	36.0	*Artemia* sp. (20 DAH) + *P. variansa*fterwards	8.7*	None	n.d.	n.d.	Domingues et al. (2002)
Individual baskets /1,000 L/Semi-open	F1	20.0 ± 1.0	35.0	*Crangon crangon* (5 DAH) + live shrimp	8.9	None	n.d.	n.d.	Domingues et al. (2003)
				Crangon crangon (5 DAH) + live fish fry	5.8	None	n.d.	n.d.	
10 L plastic rectangular tanks /Open	F4	20.0 ± 2.0	36.0 ± 1.0	*P. nouvelli*	6.2	9.9	n.d.	n.d.	Domingues et al. (2004)
				P. varians	7.5	26.7	n.d.	n.d.	
				Atherina sp.	2.9	None	n.d.	n.d.	
250 L/Open	F2	17.4 ± 1.8	35.0 ± 2.0	*P. varians*	6.4	14.8	n.d.	n.d.	Sykes et al. (2006a)
	F3	23.7 ± 1.1	37.0 ± 2.0		19.3	20.0	n.d.	n.d.	
	F4	13.3 ± 1.1	35.0 ± 2.0		2.7	14.0	n.d.	n.d.	
	F5	24.0 ± 1.8	35.0 ± 2.0		10.4	None	n.d.	n.d.	
10 L blue plastic rectangular tanks /Open	NK	18.5 ± 0.5	36.0 ± 1.0	*P. nouvelli* (15% BW/d)/535 Lx	7.0	None	13.3	n.d.	Correia et al. (2008a)
				P. nouvelli (30% BW/d)/535 Lx	7.7	None	14.8	n.d.	
45 L raceway/Open/ Black	F2	19.3 ± 0.5	37.0 ± 1.0	Mix of *Mesopodopsis slabberi* and *Artemia* sp. (20 DAH) + *P. varians* afterwards/320 Lx	4.6	21.7	n.d.	n.d.	Sykes et al. (2011)
45 L raceway/Open/ Sand					4.1	30.0	n.d.	n.d.	
45 L raceway/Open/ White					4.3	36.7	n.d.	n.d.	
10 L raceway/Open/ Black	F4	24.4 ± 1.3	37.0 ± 2.0	Live *P. varians*	10.4	2.9	n.d.	n.d.	Sykes et al. 2013
				Live *P. varians* (5 DAH) + frozen *P. varians*	5.8	14.3	n.d.	n.d.	
				Frozen *P. varians*	6.2	16.2	n.d.	n.d.	
10 L raceway/Open	F1	23.8 ± 1.1	37.0 ± 1.0	Live *P. varians*/100 Lx	8.0	11.1	104.5	2.0	Sykes et al. 2013c
				Live *P. varians*/350 Lx	7.9	8.9	104.4	2.0	
				Live *P. varians*/1,200 Lx	8.9	38.9	124.4	4.6	

IGR values were estimated when no values were specifically presented. Values shared by several different groups are presented solely in that column
Gen generation, *T* temperature, *S* salinity, *IGR* instantaneous growth rate (% BW/d), *FR* feeding rate (% BW/d), *FC* food conversion, *n.d.* not determined, *NK* unknown. *DAH* days after hatching, *BW* body weight
[a] Represent estimation from data on graphics

Fig. 11.5 Juvenile and adult grow out in tanks. **a** 1,500 L not painted. **b** 1,500 L painted in black and earthen ponds. **c** Pond at Necton, S.A. and cuttlefish juveniles. (Photos by A Sykes)

The conditions presented in this section are based on pilot projects performed in Portugal and Spain with the objective of determining the viability and conditions of a commercial operation. These studies were performed both in aquaculture research stations and in earthen ponds belonging to aquaculture companies, which have expressed interest in cuttlefish culture as an alternative species for diversification.

After going through the hatchling stage in the hatchery, cuttlefish should have attained full development of the digestive system and a mean weight of 5 g. At this point, depending on the facilities and the rearing objectives, cuttlefish may be relocated to tanks of different types (either in fibreglass or in concrete with increased size) or to earthen ponds. If kept in tanks (Fig. 11.5a, b), the seawater systems and conditions used should be similar to those detailed for breeders (see Sect. 11.3.1). Similar to the hatchling stage, the use of black tanks and low light intensities are recommended.

In order to increase the probability of cuttlefish finding the prey and spend the least energy possible while hunting, the water column of the tanks should be low. While cuttlefish grows, the water column should be increased to generate more volume (Forsythe et al. 1994).

If animals are relocated to earthen ponds (Fig. 11.5c), it should be taken care not to place them in a pond with a very different temperature, salinity, pH and dissolved oxygen below 80%. Big differences in these parameters (e.g. 1 °C, 1 psu) will promote immediate mass mortality. Transportation should be performed considering the aspects discussed in Sykes et al. (2012), such as duration and eventual use of anaesthesia. Acclimation after transportation similar to that achieved for fish in an

Table 11.4 Pros and cons of tanks versus earthen ponds

Variables	Tanks	Earthen ponds
Temperature	Only controlled in semi-open and closed seawater systems	Not controlled
Salinity	Only controlled in semi-open and closed seawater systems	Not controlled or hard and expensive to control
Dissolved oxygen	Fully controlled	Controlled through the use of aerators but primary productivity may generate spikes of lower oxygen during the night
Nitrogenous compounds	Controlled in open and semi-open systems	Controlled in ponds with water renewal
Density/biomass	Fully controlled	Hard to control due to water turbidity
Mortality/cannibalism	Fully controlled	Hard to control due to water turbidity
Food/feeding hierarchies	Controlled	Controlled
Predators	None	Require bird nets, filters of appropriate size in inlet water supply, if water renewal is used
System setup and maintenance	Logistics, time consuming and expensive in setup Maintenance is time consuming	Expensive in pond setup. Low expenses during maintenance
Filters inlet/outlet	Inlet filters in water of the facilities are expensive and require logistics and maintenance. Outlet filters used to prevent escape of animals	Inlet filters to prevent entrance of natural predators. Outlet filters to prevent escape of animals. Both are time consuming
Type of culture	Intensive and semi-intensive	Intensive, semi-intensive and extensive
Type of integrated aquaculture	Water used for growth of micro or macroalgae after leaving the tank	Pond used for bivalves and water used for micro or macroalgae after leaving the pond

aquarium should be enforced to obtain the best results. While culturing in ponds, water inlet and outlet filters of appropriate sizes should be used to prevent predator inputs and cuttlefish outputs. In addition, net barriers should be used for preventing cuttlefish capture by birds. Since these ponds are dynamic ecological systems, with proper fauna and flora, the use of oxygenators to efficiently supersaturate the rearing water is recommended. This might be performed either by mechanical movement (paddles) or by oxygen injection and will be particularly important at high water temperatures, in very productive waters and especially during nighttime, when microalgae also consume oxygen.

The pros and cons of using either tanks or ponds are presented in Table 11.4.

11.6.2 Density

Published information provides data regarding density in either open, semi-open and closed systems. As in other life stages, considering not only densities but also the

available bottom area is of maximum importance. Forsythe et al. (1994) suggested a density of 20 cuttlefish m^{-2} in closed seawater systems at this ongrowing stage but, despite recognizing that bottom areas are important, no value was provided. A density of 400 cuttlefish m^{-2} is suggested by Forsythe et al. (2002) in 1,800 L circular tanks and closed seawater system. From this study, performed at rearing temperatures of 25 °C, this very high density is on the verge of impacting growth and survival, due to the increase of biomass present in the tanks.

As for densities in flow-through seawater systems, Sykes et al. (2003) suggested the use of 120 cuttlefish m^{-2} and minimum area of about 1,083 cm^2 (in 10 L raceway tanks), when starting a new juvenile tank with individuals of approximately 5 g. According to these authors, these density and bottom area values are valid for animals up to 25 g. Likewise, Domingues and Marquez (2010) studied the effects of both density and bottom areas in open seawater systems (in concrete raceway tanks) and obtained results that support the use of high-density and large bottom areas (33 cuttlefish m^{-2} with an average weight of 9.5 g), registering similar feeding rates (≈ 10 % body weight d^{-1}) but different food conversions. In fact, mortality and growth were similar between high- and low-density tanks using similar large bottom areas, which indicate that the bottom area seems to be more important than the density itself.

Independently from the rearing seawater system, density must be decreased and bottom areas increased while cuttlefish grows. It is suggested that, from 30 DAH to 10 g, the cited values of Sykes et al. (2003) should be used and from this weight and to maturation (which is temperature dependable) the findings of Domingues and Marquez (2010) should be considered.

If cuttlefish is reared in earthen ponds, special attention should be paid to the density and bottom areas, considering the fast growth rates that the species display and the inability to correctly observe what is happening within the ponds. If the pond's carrying capacity and biological limits are reached, cuttlefish mass mortality and loss of total biomass produced will occur. This will be due to the inability to clean the pond which will result in a spike in nitrogenous compounds and a drop in dissolved oxygen. A common observation to detect that this limit is being reached is to find eaten cuttlefish or cuttlebones in the pond as well as cuttlefish floating or swimming in the pond's surface.

11.6.3 Food

According to Warnke (1994), when cuttlefish are fed in a group, individuals hunt three times faster than when isolated, more food is ingested and feeding hierarchies are established.

Currently, growout of cuttlefish juveniles is performed with crustaceans as diet, mainly the grass shrimp—*P. varians* (Sykes et al. 2006a). This is due to the easiness of collection (logistics) and results obtained with this diet (Sykes et al. 2006a). Nonetheless, several different food items have been tested throughout the years, either solely or as mixed diets. Domingues et al. (2001a, b) used the crab *C. maenas*; Domingues et al. (2002, 2003b) and Sykes et al. (2006a) used live or frozen *P.*

varians with success. DeRusha et al. (1989); Domingues et al. (2004) and Almansa et al. (2006) tested the effect of exclusively using fish and reported lower growth than when using grass shrimp.

Despite all this effort, it is not economically viable to produce cuttlefish in large numbers with a growout based on any of those foods. Not only is the amount of food biomass too high but also the availability of these food resources is scarce. Therefore, further research has been performed with the objective of developing alternative diets to cephalopods. Several works regarding trials on artificial diets were performed first by Lee et al. (1991), Castro and colleagues (Castro 1991; Castro et al. 1993; Castro and Lee 1994) and, more recently, by Domingues et al. (2005, 2008) and Ferreira et al. (2010). However, all these attempts to feed cuttlefish on a prepared diet failed to achieve proper growth and survival (for details, see Table 11.5). Fish pellets have also been tried and accepted by juvenile cuttlefish. However, after only 2 days, individuals started to reject this food and cannibalism was observed.

The lack of proper knowledge regarding the physiology and metabolism of the species, at different geographical locations, has hampered the existence of a successful design. Only by having a well-designed, inexpensive and storable artificial diet, cuttlefish aquaculture will reach its maturity and industrial stage (Sykes et al. 2006b). In this way, Domingues et al. (2009) tested the effects of thermal treatment of food given to cuttlefish and obtained interesting growth and survival data that suggest this process affects diet quality, by provoking protein denaturation, washing of proteins and amino acids and lipid oxidation. Current research in Portugal is focused on developing new diets based on the most recent knowledge regarding cuttlefish physiology, metabolism and raw materials. Up to now, similar results to those obtained by Hanlon et al. (1991) and Lee et al. (1991) were registered. Nonetheless, the lack of knowledge regarding cuttlefish physiology and metabolism still remains as the bottleneck to overcome.

11.6.4 Cleaning Techniques

In ponds, periodical cleaning is only performed at the inlet and outlet filters but several cleaning routines should be performed in tanks.

According to Forsythe et al. (1994), no substratum is needed for normal growth and survival, even in cultures performed at high densities. This practice not only facilitates cleaning itself but also prevents pathologies and promotes good welfare under culture conditions (Sykes et al. 2012). Tanks should be siphoned daily with a hose and purged (total time spent will depend on the water quality and volume of the tank). On a weekly basis, airlifts, air stones and water outlet filters should be cleaned with a scrubber with tap water. Afterwards, these elements should be kept for 24 h in Atlantol 914 (Atlantol, Belgium), rinsed with tap water and then placed in VirkonS (DuPont Animal Health Solutions, Europe) for 15 min. Finally, all these materials should once again be abundantly rinsed with tap water. The nets or any material used for animals handling should follow a similar procedure to avoid contaminations, pathologies and spread of diseases.

Table 11.5 Cuttlefish juveniles' growth rates, mortality, feeding rates and food conversions of animals fed live, frozen and artificial diets

Rearing system	Days	T (°C)	S (PSU)	Food	MWWi (g)	GR (% BW/d)	Mortality (%)	FR (% BW/d)	FC	Reference
Closed; 72 L round	57	UK	35.0	Penaeid pellet	13.9±2.6	1.5	35.0	n.d.	n.d.	Lee et al. (1991)
Closed; 500 L round	45	22.0±1.7	35.0	*Palaemon setiferus*	74.5±12.4	2.7	5.0	6.0–8.0	39.0–45.0	Castro et al. (1993)
				P. setiferus pellet		0.3	32.5	<1.0–3.0	0.0–20.0	
				Catfish surimi		0.5	77.5	8.0–<4.0	0.0–20.0	
Closed; 500 L round	14	21.0±1.0	35.0	*P. setiferus*	106.0±17.6	2.8	None	7.9	34.6	Castro and Lee (1994)
				Surimi		0.1	None	5.3	0.8	
				Surimi—menhaden oil+egg albumin		−0.2	None	3.2	−8.3	
				Surimi+egg albumin		−0.1	None	3.4	0.1	
				Surimi+casein		0.3	None	4.2	8.6	
				Surimi+whole egg		−0.3	None	2.5	−13.5	
Open; 10 L plastic rectangular tanks	29	24.7	36.5	Live *P. varians*	5.5±0.8	4.4[a]	22.2	11.9[a]	31.0–46.0	Domingues et al. (2003b)
				Frozen *P. varians*	5.6±0.9	4.4[a]	2.2	10.9[a]	34.0–49.0	
Open; 10 L plastic rectangular tanks	28	20.0±2.0	36.0±1.0	Live *P. varians*	0.6±0.1	5.7	None	n.d.	n.d.	Domingues et al. (2004)
				Live *Atherina* sp.		2.4	8.3	n.d.	n.d.	
	28			Live *P. varians*	5.7±0.1	5.8	None	n.d.	n.d.	
				Live *Atherina* sp.		3.5	None	n.d.	n.d.	
	35			Frozen *P. varians*	2.2±0.4	5.1	20.0	12.4	0.4	
				Frozen *Atherina* sp.		3.3	8.1	11.7	0.3	
Closed; 8 L aquaria in 150 L tank	35	19.0	NK	*C. crangon*	2.0–2.8	3.7	None	9.3	2.5[a]	Grigoriou and Richardson (2004)

Table 11.5 (continued)

Rearing system	Days	T (°C)	S (PSU)	Food	MWWi (g)	GR (% BW/d)	Mortality (%)	FR (% BW/d)	FC	Reference
Closed; 1.5 L aquaria in 150 L tank	40	11.0		*C. crangon*	3.0–8.2	0.7	None	2.8	4.0[a]	
		19.0		*C. crangon*		3.5	27.0	8.3	2.4[a]	
Closed; 500 L	30	23±1.5	35.0±2.0	Surimi lysine diet 1	321.8±57.9	−0.02	12.5	2.7	−40.8	Domingues et al. (2005)
				Surimi lysine diet 2		0.2	20.8	2.7	26.7	
				Surimi lysine diet 3		0.1	20.8	2.8	80.9	
				Surimi lysine diet 4		0.3	20.8	2.8	10.7	
Closed; 9 L	21			Surimi lysine diet 1	451.5±103.7	−0.3	NK	1.1[a]	−3.1[a]	
				Surimi lysine diet 2		0.1	NK	2.7[a]	−1.4[a]	
				Surimi lysine diet 3		0.1	NK	1.8[a]	22.1[a]	
				Surimi lysine diet 4		0.4	NK	2.3[a]	6.3[a]	
Open; 250 L	60	21.5±1.5	36.0±1.0	Live *P. varians*	13.3±3.4	3.1	NK	n.d.	n.d.	Almansa et al. (2006)
				Live *Atherina* sp. and *Gobius* sp.	12.9±2.8	1.7	NK	n.d.	n.d.	
				Live *P. varians*	85.6±11.7	2.1	NK	n.d.	n.d.	
				Live *Atherina* sp. and *Gobius* sp.	86.2±8.6	1.0	NK	n.d.	n.d.	
Open; 27 L		21.0±1.0	37.0±1.0	Frozen *Palaemonetes* sp.	44.4±0.5	1.5	None	7.8	18.6	Domingues et al. (2008)
				Frozen *Procambarus clarkii*		1.1	None	8.4	13.7	
	29			Frozen *Sardina pilchardus*		0.3	None	4.4	5.8	
				Artificial diet 1		−0.7	None	2.0	<1.0	
				Artificial diet 2		−0.5	None	2.4	<1.0	

Table 11.5 (continued)

Rearing system	Days	T (°C)	S (PSU)	Food	MWWi (g)	GR (% BW/d)	Mortality (%)	FR (% BW/d)	FC	Reference
Open; 40 L	20	20.5±1.0	37.0±1.0	Frozen *P. varians*	12.5±1.0	2.0	None	7.4[a]	23.1	Domingues et al. (2009)
				100 °C boiled *P. varians*	12.4±0.6	0.5[a]	3.3	8.5[a]	3.0	
				60 °C dried *P. varians*	12.6±0.6	−0.2[a]	None	7.7[a]	1.4	
	40	21.5±1.0		Frozen *P. varians*	5.5±0.6	2.1	None	8.6	25.7	
				60 °C dried *P. varians*	5.9±0.3	0.5	8.3	8.7	6.6	
				Freeze-dried *P. varians*	6.0±0.4	1.8	8.3	8.7	20.4	
Open; 40 L	29	21.0±1.0	37.0±1.0	Frozen *Palaemonetes* sp.	13.8±2.3	3.8	3.0	8.8	42.6	Ferreira et al. (2010)
				Frozen *S. pilchardus*		0.8	20.0	6.6	13.6	
				Frozen *P. clarkii*		2.3	13.0	10.5	20.9	
				Artificial diet 1 (fish powder)		−1.8	47.0	4.5	<1.0	
				Artificial diet 2 (shrimp powder)		−0.3	47.0	2.4	<1.0	

Values were estimated when no values were specifically presented. Values shared by several different groups are presented solely in that column
Days days of rearing, *T* temperature, *S* salinity, *MWWi* mean wet weight at the beginning of experiment, *GR* growth rate (% BW/d), *FR* feeding rate (% BW/d), *FC* food conversion, *n.d.* not determined, *NK* unknown

[a] Represent estimation from data on manuscript

11.6.5 Growth, Survival and Sampling

Time to marketable size will greatly depend on the requested product itself. For instance, in some Mediterranean countries, such as Portugal and Italy, undersized cuttlefish individuals (5–25 g) are extremely appreciated and their commercial value is higher than that of animals surpassing 100 g. Time to market will also be dependent on culture conditions, especially temperature and food.

S. officinalis is cultured extensively in Portugal, Italy and Tunisia. By definition, this type of culture does not involve human action at the food level, so eggs are caught and left in earthen ponds with naturally occurring prey, where animals are grown and collected a few months later when they reach the marketable size. According to Palmegiano and Sequi (1981), in this type of culture, 0.15–0.30 kg of eggs will produce 800–1,200 kg of cuttlefish with a mean weight of 0.04–0.08 g. The semi-intensive experiments performed in Italy in the 1980s obtained fast growth rates (14.2 g in 60 days, at 21–24 °C in ponds (Palmegiano and Sequi 1981); 25 g in 40 days and 80 g in 100 days, at 21–24 °C in concrete tanks (Palmegiano and Sequi 1984); 80 kg ha^{-1} in 90 days, at 21–24 °C in net cages in ponds (Sequi and Palmegiano 1984)).

Similar high growth rates were obtained in 2004, in earthen ponds of a commercial company at Algarve (Portugal; unpublished data). From an initial biomass of 600 g of cuttlefish juveniles (with mean wet weight of 1.65 ± 0.62 g), 5,000 g of cuttlefish (30.75 ± 11.25 g) were produced in 52 days, with a mean water temperature of 21.3 ± 2.0 °C. Higher growth rates were not obtained due to problems related to initial adaptation of cuttlefish to unfavourable conditions, such as oxygen depletion, lack of food during the fattening and bird predation observed during fishing at the end of the experiment. These factors promoted an overall mortality of 35 %.

Summarizing and comparing data in tanks from Sykes et al. (2006a) with those obtained in earthen ponds, it is possible to see that it takes longer (70–90 days in total) to achieve a similar mean weight in tanks than in ponds, when cuttlefish is reared at a similar temperature. When food is available and other identified conditions are met, cuttlefish will grow faster outdoors than indoors, which is in accordance to the findings of Domingues et al. (2006). So depending on the weight request, it is possible to produce cuttlefish to meet market demands within 2–3 months or even less. To maintain a high-quality product for human consumption and enforce the best welfare practice, cuttlefish should be killed by thermal shock in ice slurry water.

11.7 Trends in Research and Industrial Level

The bottlenecks in the cuttlefish culture identified by Sykes et al. (2006b)—reproduction, feeding and nutrition—are still under research. They need to be solved to improve the current methodology and thereby apply to an industrial scale.

Currently, Centro de Ciências do Mar do Algarve (CCMAR) is the only research centre performing the culture of European cuttlefish in large numbers and they are able to supply cultured animals to research centres and public aquaria. In terms of

application, there is potential beyond aquaculture for human consumption, development of refined guidelines and best practice methods for cuttlefish culture. For instance, it is expected that cuttlefish will become the marine laboratory rat, as animals are needed for formation in aquaria or universities and as marine animal models for research. As a matter of fact, cuttlefish is included as one of the model species available at *EMBRC*, a platform created by the EU to facilitate research with animal models, such as specific inbred animals only obtained under culture conditions.

Regarding aquaculture, while commercial retailers are eager to get cultured cuttlefish, with the most appreciated sizes (e.g. cuttlefish under the allowed limit for being captured), the aquaculture industry sees the development of this technology as one with a very low potential, such as something that will never be reached.

At present, gaps in the knowledge of cuttlefish biology, the time expended to develop the culture methods (due to the low number of researchers and research laboratories involved) and the fact that most of the aquaculture investors who are quite unadventurous with regard to business planning are still hampering the excellent opportunity for the diversification of marine aquaculture. The worldwide market prices for the species are high and the short-life cycles will support an easy and short payback time of the investment made. In fact, the payback can be shortened and profit increased if cuttlefish culture is performed in integrated aquaculture. The water of the tanks is extremely rich in nitrogenous compounds which may be used for micro- and macroalgae production. In addition, while performing the experiments in ponds, cuttlefish can be reared at the same time with bivalve species, which will lower the microalgae content in the water and can be sold at the time of harvesting, promoting increased income.

It is also important to acknowledge that, if resources are used efficiently, the whole animal may be sold to be used by different transformation industries. For instance, cuttlefish mantle and ink not only will be used for human consumption but also may be enforced for recycling of by-products for feeds (e.g. cuttlefish viscera is naturally rich in n-3 and n-6 fatty acids and amino acids) and natural products (e.g. cuttlefish bone is made of aragonite and it could be used in the pharmacological industry). For further details, see Chap. 9 ‘Applications, Uses and By-products from Cephalopods’ of this volume.

The species potential is there and it needs to be grasped in short-term future.

11.8 Conclusions

Steady progress in the development of cuttlefish culture technology has been attained since the 1980s, when this species was suggested to have a potential for aquaculture due to the short life cycle and high market prices which translates into short payback time of the investment. This chapter of the book includes a very thorough description of the methods to culture cuttlefish. However, the existing knowledge is still not sufficient to sustain the culture of the species at the industrial level for human consumption. This is due to the existing bottlenecks regarding reproduction, feeding and nutrition that are currently being researched.

The species has an incredible potential regarding its use in integrated aquaculture with bivalves and regarding the use of the whole animal as a resource for different transformation industries, such as recycling of by-products for feeds and other pharmacological products. The species is also seen as a very interesting animal model for several fields of research that include physiology, genetics, etc. For instance, the opportunity to have this animal model culture in the laboratory will allow for inbred samples for upcoming research on the *Sepia* genome. The existing culture protocols allow for small-scale culture for the supply of research centres and public aquaria.

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