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The cuttlefish *Sepia officinalis*
(N. Koueta, J.P. Andrade, S. v. Boletzky, eds)

CUTTLEFISH CULTURE – STATE OF THE ART AND FUTURE TRENDS

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COMMON CUTTLEFISH
CULTURE TECHNOLOGY
SEPIA OFFICINALIS
REVIEW

ABSTRACT. – The present article provides an overview of cuttlefish culture, its current state of art, and future trends. Present cuttlefish culture related research, recently developed technologies (like culture systems, maternity/nursery and juvenile and adult proceedings) are described. Finally, current problems and prospects for future research are discussed.

GENERAL OVERVIEW AND PAST RESEARCH

Fisheries and human consumption

The increase of the human population has led to a greater demand for fishery products, and therefore intensified diversification of fish catches. Although there has been a decline in fish consumption and production in developed countries, global fish consumption has doubled since the beginning of the 1970's (Delgado *et al.* 2003). During this period, aquaculture production followed this increase and has risen from 6 to 30% of total fishery production (Delgado *et al.* 2003). Landings from worldwide aquaculture increased rapidly in the last decade, to approximately 10–15% per year. According to FAO (2002), total aquaculture in 1996 was 26.7 million tons, and in 2001 increased to 37.5 million tons. Rapid growth was due to the combined effects of an increasing world population, decreasing catches from traditional fisheries (Caddy & Griffiths 1995), and changing consumer preferences in developed countries (Lem & Shehadeh 1997, Tacon 1997). Ultimately, this was reflected in world fish catches, including cephalopods. Between 1950 and 1970, cephalopod landings increased from 20 million tons (Mt) to 70 Mt (Amaratunga 1983). At present, cephalopod species represent an important seafood supply for human consumption worldwide. According to FAO (FAO 2002, 2004), cephalopods contribute approximately 14% of the world fisheries. The fast decline of the worldwide fish stocks, as well as the technological advances over the last 15 years, and decreased prices of commonly cultured species, makes the development of technology for rearing and culture of new species profitable, indeed necessary (FAO 2004). High commercial value of the cephalopods, particularly in the Asian and Mediterranean markets, and some aspects of cephalopod biology and physiology make them good candidates for aquaculture (Kunisaki 2000, Ruíz-Cappillas *et al.* 2002). It is also known that cephalopod consumption is increasing in the American market. Thus, an overall increase in world cephalopod consumption is predicted, since an open market with high growing potential is foreseen in the future. These facts indicate that increasing efforts to start semi-intensive and intensive culture of cephalopod species like the European cuttlefish (*Sepia officinalis*) and the common octopus (*Octopus vulgaris*) should be made.

The top five countries in fish consumption (above 40 kg/head/year; Iceland, Japan, Portugal, Norway and Spain) (European Communities 2004) are also those searching for greater diversification of edible fish species. In these countries, there is an existing market for cephalopod catches. In fact, Japan is the principal consumer of cephalopods, with cuttlefish being the most appreciated and valued species (Boucaud-Camou 1990). Therefore, cephalopod production for human consumption in these countries is advantageous, since their short life cycles and fast growth rates imply lower production periods and associated costs. Also, the non-edible parts of cephalopods, which make up approximately 30% of the animal, can be used for fish meal or bait. According to Kreuzer (1984), the conversion of non-edible parts into products of higher value would also be economically beneficial.

In the case of cuttlefish, there is potential for further exploitation, particularly with regard to the production of undersized individuals allowed by DGPA (Portuguese Fisheries and Agriculture Department), which would reduce the impact of illegal catches on this species from the natural environment. For example, the smallest individuals are considered a delicacy and have the highest commercial value in Portugal. The main reason for this increasing demand is that cephalopods in general and cuttlefish in particular, are a good source of protein and essential lipids (Sinanoglou & Miniadis-Meimaroglou 2000). According to Boucaud-Camou (1990), cuttlefish is composed mainly of water (81%) and protein (16.1%); it has no carbohydrates and less than 1% lipids. It is also a source of mineral salts and vitamins, and is highly digestible. Its nutritional profile, as a high protein and EPA/DHA lipid source, makes it one of the most suitable and healthy forms of human food. Cephalopods can be consumed in a variety of forms: eaten raw as *sashimi* or *sushi*, cooked as *tempura* or deep-fried, boiled as *nimono*, or processed into delicacies like *surume* (dried squid), smoked squid, and *saki-ika* (shredded dried squid) (Kunisaki 2000).

The European cuttlefish have been reared in extensive aquaculture for a number of years. Eggs are collected and left in ponds for 3 months during spring-summer, after which grown animals are captured and sold for human consumption. According to DGPA data, until 1996, this production did not exceed 1 ton.year^{-1} of cuttlefish biomass produced. According to Rodger & Davies (2000), Tunisia has also been producing *S. officinalis* since 1990. Nevertheless, it must be noted that there is a lack of accurate data on this type of production, since most of these captures are not declared.

Culture related research until present

The introduction of a new species in aquaculture requires a series of preliminary studies related to the biology and the ecology of the species. These studies can be performed in the wild or in the laboratory. For the cuttlefish, both approaches were conducted simultaneously and allowed not only the culture technology to be developed but also the knowledge related to local stock dynamics.

The first reports on experimental culture of cephalopods are dated from the 1960's, by the Korean and Japanese on species of the Sepiidae family. These pioneer studies by Choe & Ohshima (1963) and Choe (1966) were then followed and complemented by Richard (1971) at the end of the 1960's.

Richard (1971), Pascual (1978) and Boletzky (1979) were among the first researchers who succeeded in culturing European cuttlefish in the laboratory for one or more consecutive generations. From these works, essential information on growth and feeding under different culture conditions was made available. Richard (1971) extended data regarding various aspects of culture, but also included data on the population inhabiting the English Channel. Boucaud-Camou (1973) and Boucaud-Camou & Péquignat (1973) studied the digestive apparatus and its biochemical processes, and Yim (1978) addressed the development of the digestive gland in the post-embryonic phase.

From 1980 onwards, research concerning the possibility of using cuttlefish as a candidate species for aquaculture rapidly expanded. Initial experiments utilizing this species in coastal lagoons were employed by Italians (see Palmegiano & Sequi 1984, for a review) and Portuguese (Gonçalves 1989, Coelho & Martins 1991).

Other contributions on feeding and digestion were made by Boucaud-Camou *et al.* (1985), Nixon (1985), and Guerra *et al.* (1988); on fecundity by Boletzky (1987); and alternative prey items by DeRusha *et al.* (1989).

A review on the laboratory maintenance, rearing, and culture of cephalopod molluscs was presented by Boletzy & Hanlon (1983). Forsythe *et al.* (1987) published the first synopsis of cephalopod pathology in captivity. Boletzky (1983) also addressed the biology and ecology of *S. officinalis*, while a review on the early stages, life cycle processes, trophic relations, and exploitation of cephalopods in general can be found in Boyle (1987). In the 1990's, further information was published on rearing, culture and production of the European cuttlefish (Forsythe *et al.* 1991, 1994, Loi & Tublitz 1998, Warnke 1994).

The first reports on the use of pellet diets and surimi in cephalopods (Lee *et al.* 1991) and especially cuttlefish (Castro 1991, Castro & Lee 1994, Castro *et al.* 1993) demonstrated poor results in growth and survival (e.g. 67.5% and 22.5% survival rates for pellets and surimi, respectively). Use of artificial diets promoted cannibalism. An approach to the nutritional requirements of cephalopods by Lee (1994) revealed high amino acid (AA) metabolism and the importance of protein/energy ratio. Complementary data were published regarding nutritional characterization of free l-amino acids (D'Aniello *et al.* 1995) and lipid changes during starvation (Castro *et al.* 1992).

Several articles regarding social and sexual behaviour (Adamo & Hanlon 1996, Boal 1996, 1997, Boal & Marsh 1998, Hanlon *et al.* 1999) and crowding (Boal *et al.* 1999) under culture conditions contributed to increased knowledge of the sexual behaviour of the cuttlefish in captivity and spawning methodologies. Furthermore, Hanlon & Forsythe (1990a, 1990b) described cephalopod diseases, while Forsythe *et al.* (1990) published a formulary for disease treatment.

Contributions to development of artificial surfaces for egg deposition and collection (Blanc & Daguzan 1998) and the influence of temperature (Bouchaud & Daguzan 1990, Bouchaud & Galois 1990), photoperiodicity (Paulij *et al.* 1991), and energy consumption (Bouchaud 1991) during embryonic development were published. A report on the postembryonic predatory behaviour and its relation to the maturation of the brain was published by Dickel *et al.* (1997).

During recent years, the amount of published information on cephalopod and especially cuttlefish culture steadily increased. Growth (IGR) and feeding rates (FR) in hatchlings were studied by Koueta & Boucaud-Camou (2001), while Domingues *et al.* (2001a) extended this knowledge by culturing hatchlings at the upper end of the biological temperature (30ºC) and salinity range (37±3‰) with good results. Grigoriou & Richardson (2004) provided new data on IGR and FR from cultured populations from the North Atlantic. Domingues *et al.* (2002) also studied the effects of temperature in the life cycle of cuttlefish with respect to growth, extension of the cycle, and several reproductive aspects, while Boyle *et al.* (2001) described a model system for partitioning environmental and genetic effects on development and hatching of cephalopod eggs. All these reports support the hypothesis of the existence of different populations, thus enhancing the probability that sub-species exist.

Several papers were also published regarding the use of live and dead feeds for hatchlings (Domingues *et al*. 2001b, Fuentes & Iglesias 2001, Koueta *et al*. 2002; Perrin *et al*. 2004) and juveniles (Domingues *et al*. 2003b, 2004). Moreover, Perrin *et al.* (2004) continued the work on digestive enzymes, but related to alternative feeding of hatchlings. Culture densities/crowding in hatchlings, juveniles, and adults and throughout the life cycle was studied by Correia *et al.* (2005), Domingues *et al.* (2003a), Forsythe *et al.* (2002) and Sykes *et al.* (2003). The latter authors also studied the effects of using sand in hatchling tanks on growth and mortality of newly hatched individuals of cuttlefish. Hunting behaviour and prey preference were addressed by Cole & Adamo (2005) and Darmaillac *et al.* (2004), respectively.

STATE OF THE ART OF CUTTLEFISH CULTURE AND ASSOCIATED TECHNOLOGY

All technology described below has allowed cuttlefish culture at the University of the Algarve for the last 6 years and its use in a first RTD contract with an aquaculture enterprise. Currently, facilities have been utilized as hatchery and grow out locations, while the company is conducting grow out experiments to determine the viability of introducing the species as a new product.

Hatchery technology

Spawners

Spawners should be captured from the wild and/or selected from the most fit juvenile individuals cultured, which will be those displaying no disease or skin damage, and achieving the best growth. After capture and/or selection, animals should be conditioned in 2000-10000 l round shaped tanks of a flow-through system. These tanks should have a bumper system (Hanley *et al.* 1999) or large soft-plastic wall pools should be used. Tanks should also be placed in a low disturbance zone inside the hatchery. Large bottom areas should be used, and density should be low (Correia *et al.* 2005, Sykes *et al.* unpublished results).

Eggs

Eggs can be obtained either by collection from the wild, possibly using artificial surfaces for eggs designed by Blanc & Daguzan (1998), or by reproduction in captivity. Preferably, eggs should be collected in the laboratory rather than from the wild, since there will be an advantage in knowing spawn date and establishing correct incubation conditions. Collection in the laboratory should be made using a plastic rectangular supportive net (1 cm^2 holes), suspended inside the spawners tank. This net should be checked daily, and if eggs are present, they should be carefully removed (since freshly laid eggs are very soft and gelatinous) and individualized. Subsequently, eggs should be counted and weighed (50%, if n<100 or 30% if n>100). Also, eggs should be separated according to shape and colour. Black oval eggs are considered to be viable while eggs of any other shape should be discarded.

Embryonic development should be completed in bowl-shaped tanks with clean natural seawater. At this phase, type of seawater system (open, closed or semi-closed) can be used as long as it provides clean water (with low concentrations of ammonia, nitrites, and nitrates). Nevertheless, the use of flow-through (open) seawater systems is advisable. Tank setup should be as follows: 2 airlifts on the side walls of the tank, and also medium to strong aeration from the middle of the tank produced by a wood air bubbler. This procedure ensures a maximization of hatching efficiency (creating an oxygen-enriched environment), to allow hatchlings to have a bottom where they may settle before being removed for experiments or hatchling culture tanks. It also keeps eggs moving in an elliptical fashion, thus preventing necrosis by either lack of oxygen or fungal/bacterial growth (originated by no movement at all). To ensure normal hatching, all physical factors should be kept at a constant level, including temperature, salinity, pH, nitrogenous compounds, daily photoperiod and light intensity (Hanlon 1990, Forsythe *et al.* 1991). According to Boletzky (1983), water maintenance limits for the species are as follows: between 9-30ºC of temperature, 25-38‰ salinity, ammonia, nitrites below 0.1mg/l and nitrates below 80mg/l.

Light intensity should be lower than 200 lux at the top of the water column of the hatching tank, and photoperiod should resemble natural conditions in spring. A maximum number of 2000 eggs, collected during 5-7 days, can be incubated in a 250 l tank using this setup. For tanks using higher volumes, the number of eggs still must be determined. After being placed in the tank, disturbance of the eggs should be avoided in order to prevent premature hatching that may result in mass mortality. Embryonic development is temperature dependant, but without any linear correlation (Richard 1971, Sykes *et al.* unpubl results). Due to the fact that temperature varies in different geographical areas, it is suggested that temperature should resemble local spring to summer conditions in the culture facility. This means that cuttlefish eggs from the Mediterranean and South Portugal should be incubated at temperatures between 18-25ºC (at these temperatures hatching will occur 30-25 days after being laid, respectively). Length of time to hatching can double when temperatures fall to 15ºC (60 days) and hatching will not occur below 12ºC. Cuttlefish eggs from the North Atlantic are usually big (they are laid by bigger females) and take longer to hatch at the same temperatures (15ºC = 87 days)(see Richard 1971 or Boletzky, 1983 for hatching table and graphics).

Hatchlings

After hatching in bowl-shaped tanks, hatchlings will settle on the bottom of the tank. In this phase, floating egg cases should be removed daily. Eggs that do not hatch in the following 10 days should be removed and considered non viable. The 250 l tank used for egg incubation will now function as a grow-out tank for the hatchling stage during the next 30 DAH. Depending on the size of the eggs, hatchling mean weight can vary between 0.233±0.050g (English Channel) or 0.090±0.030g (South Portugal). Densities (500 hatchlings) and minimal bottom areas (600 cm^2) should be taken into account (Sykes *et al.* 2003). For instance, a 250 l tank will only suffice for 2000 hatchlings for about 15 days (T= 22.5±2.5ºC). After that, hatchlings are separated into 2 similar 250 l tanks (1000 hatchlings each).

Live food should be supplied *ad libitum* and water quality must be similar to that of the egg stage. At this phase, growth and feeding rates are the highest of the whole life cycle, but they are still temperature dependant.

Until now, high growth rates and low mortality have been obtained by feeding live mysid shrimp during the first 10 to 20 days of the life cycle (Domingues *et al.* 2001b, 2001c, Koueta *et al.* 2002). Nevertheless, culture thorugh all phases of the cuttlefish life cycle has been accomplished by Sykes (2003) using a diet exclusively based on live grass shrimp (*P. varians*). The effects of a single prey diet are similar to those of using more than one prey species (Sykes *et al.* unpubl results). The use of a sole prey species for cuttlefish culture drastically reduces costs associated with mysid shrimp production or catching them from the wild, and solves the problem of low mysid fecundity (Domingues *et al.* 1998, Domingues *et al.* 2000), which is considered a bottleneck in the first stage culture of the species.

Grow out of juveniles and adult stages

After being reared for the first 20-30 DAH in the 250 l tanks, cuttlefish hatchlings should have attained a mean weight of 5g; they are now juveniles, ready for a transfer to a larger bottom area tank. This new tank may be a saline pond, research fiber or plastic tank. At this time, new spawners should be selected from fast growers. Spawners will now remain in the hatchery facilities, and those that are grown and sold should be conditioned to outside tanks. Nevertheless, densities (120 juveniles) and minimal bottom areas (1100 cm^2) should be taken into account (Sykes *et al.* 2003). Since there is no surimi diet or a dry pellet known to generate similar growth and survival, live or dead shrimp should be used to accomplish this stage. If the individuals are to be cultured in ponds, then extensive culture should be used. In this case, live prey should be naturally present or deliberately introduced. Depending on the final size needed and the culture temperature, it can take approximately 50-60 days to obtain individuals of 50g (T= 25.0±5.0ºC).

CURRENT PROBLEMS AND FUTURE TRENDS

Sepia officinalis has several characteristics that make it one of the most promising species for future commercial aquaculture. Among them are: large eggs, which are easily transported and maintained; hatchlings resembling miniature adults in shape; many habits and behaviour being understood; ability to handle relatively large prey (sometimes twice as big as themselves); high survival rates of hatchlings, compared to other species of cephalopods; resistance to crowding, disease and handling, so they can be easily shipped; fast growth and short life cycle, in some geographical regions, allowing more than one generation every year.

However, *S. officinalis* culture shows several problematic factors keeping it out of commercial culture, so they represent bottlenecks. Those are: lower fertility and fecundity under culture conditions; semelparous life history, therefore requiring a new group of breeders for each cycle; hatchlings requiring live food and juveniles and adult stages refusing dry pellets; the species is cannibalistic; production of the live food required is not yet developed, so the cost of food supply is high; and a basic immunological system (Forsythe *et al.* 1987 1990) which may generate problems in intensive culture.

In order to establish correct methodologies for cuttlefish culture, the determination of Mediterranean and NE Atlantic subspecies is of extreme importance. Published and unpublished data regarding life cycles, reproduction, and initial hatchling weight seem to indicate that there are, at least, 2 different subspecies. Nevertheless, more comprehensive studies elaborating on Pérez-Losada *et al.* (1999) will solve this problem. Otherwise, culture methodologies and their results will inevitably differ, thus varying in different geographical locations.

The existence of appropriate and inexpensive artificial diets is a vital requirement for the viability of commercial aquaculture. The inability to grow cephalopods on an inexpensive and storable artificial diet has inhibited cephalopod mariculture on a commercial basis (Lee *et al.* 1991). Therefore, formulation of such a diet is one of the primary and achievable goals for a successful large-scale culture of cephalopods (Lee 1994). Furthermore, an artificial diet with known composition can be of extreme importance to understanding the physiological and especially absorption processes of the species in question, which contributes to the knowledge and understanding of the nutrition physiological processes in cephalopods.

Artificial diets for cephalopods have been tested over the past few years in order to lower the costs of mariculture (Lee *et al.* 1991, Domingues 1999). The majority of the artificial diets so far used have been based on shrimp paste (Lee *et al.* 1991). In the last few years, feeding experiments have been conducted with either moist or dry pellets (Lee *et al.* 1991, Castro *et al.* 1993) or surimi (fish myofibrillar protein concentrate; Castro *et al.* 1993, Castro & Lee 1994, Domingues 1999), demonstrating that cuttlefish readily accept prepared diets. *S. officinalis* has been maintained with artificial diets (Castro 1991, Hanlon *et al.* 1991, Castro *et al.* 1993, Castro & Lee 1994, Domingues 1999), yielding very low growth rates (frequently with negative growth rates). Recently, juvenile *Octopus maya* have been maintained with artificial diets during periods of up to 2 months with significant growth, although it was lower than growth obtained with natural diets (Domingues *et al.* unpublished data). Until now, feeding, growth, and survival of cephalopods fed with artificial diets are comparable to fish larvae being weaned from natural to artificial diets (Lindberg & Doroshov 1986). Despite the moderate acceptance of artificial diets by cephalopods, the highest growth rates obtained ($<$0.5% BW d^{-1}) were at least 7 times lower than growth rates recorded during normal laboratory maintenance of this species ($>$3.5% BW d^{-1}) (Forsythe *et al.* 1994, Domingues *et al.* 2003, 2004). Growth rates higher than 5% BW d^{-1} for similar size cuttlefish were obtained by Domingues *et al.* (2002) when using live shrimp as food. Even higher growth rates ($>$ 20% BW d^{-1}) were reported by Domingues *et al.* (2001a) for juveniles.

Future research efforts should now be directed to the knowledge of nutritional profiles of prey, hatchlings, and eggs. Additionally, studies should explore whether the production of grass shrimp as first feed is economically viable. The technology of producing grass shrimp as an easy and low cost feeding method is under study. Results obtained will provide data that will be used to compose a dry pellet, different from those tried previously (which gave some disappointing results). Moreover, the study of the number of generations achievable until eggs become infertile is of extreme importance to determine brood stock renewal. Further research regarding the viability of different eggs in terms of colour and shape combinations is also needed.

In order to successfully design artificial diets for cephalopods, their particular metabolism has to be taken into consideration. Cephalopods are composed mainly of protein (75-85% dry weight) (Iwasaki & Harada 1985, Boucaud-Camou 1990), suggesting that this is an extremely important nutrient in their development. According to Lee (1994), cephalopod metabolism is mainly protein and amino acid driven. Contrary to fish and crustaceans, cephalopods exclusively use protein for both growth and energy supply. So, total protein content and AA composition are considered key factors in the design of artificial diets for this species. Besides, higher grow rates (between 3 and 15% BW d^{-1}) imply an elevated AA requirement for protein synthesis during the life cycle (Lee 1994). Protein digestibility of the diet is also a relevant factor to be considered in diet formulation. Related to AA composition (D'Aniello *et al.* 1995), proportions of essential AA (which cannot be synthesised from their precursors) should cover requirements in all stages of cuttlefish development. Also, levels of proline and alanine (non-essential AA) in the diet must be reinforced, given that both AA represent one of the most important energy resources in cephalopods with a production not sufficient to cover catabolic demands (Lee 1994).

Lipids represent less than 2% DW (Boucaud-Camou 1990) and are considered to be of lower importance in energy metabolism (Lee 1994). Because of this, research on lipid and fatty acid requirements has been neglected, and there is little knowledge at present (Navarro & Villanueva 2000). Nevertheless, the importance of lipids for cephalopod nutrition, especially during early development, has been shown by several authors (Bouchaud & Galois 1990, Boucher-Rodoni *et al.* 1987, Castro *et al.* 1992, Domingues *et al.* 2003b, 2004, Koueta *et al.* 2002, Navarro & Villanueva 2000, Perrin *et al.* 2004, Sinanoglou & Miniadis-Meimaroglou 2000). Cuttlefish hatchlings and juveniles require prey rich in PUFA, phospholipids and cholesterol, and a moderate content in neutral lipids. Within PUFA, the fatty acids eicosapentaenoic acid (20:5n-3, EPA), docosahexaenoic acid (22:6n-3, DHA) and arachidonic acid (20:4n-6, AA) are very important for development, growth, reproduction, and other physiological functions in marine species (Sargent *et al.* 1995). These fatty acids are essential since they cannot be synthesised from their precursors (linolenic acid - 18:3n-3 for EPA and DHA or linoleic acid - 18:2n-6 for AA) and must be included in the diet of these species (Sargent *et al.* 1995, Arts *et al.* 2001).

Another important nutrient is carotenoids that must be incorporated in the diet, since they are not produced by cephalopods. Carotenoids are widely distributed in nature and have shown their influence on growth in marine species. The antioxidant effect of these pigments is one of the main reasons for their importance, especially in PUFA- rich tissues, to avoid lipid peroxidation (Liebler 1993). In cephalopod nutrition, crustacean species are the main source of carotenoids.

Finally, to formulate an optimal diet, other nutrients and micronutrients such as carbohydrates, vitamins, and minerals must be taken into consideration to satisfy the main nutritional requirements for the cuttlefish. Experimental design of diets should not only take nutrient profiles and proportion into consideration, but also nutrient sources and the protein/energy ratio. Other aspects are also important, such as different palatability, texture, colour, form, and moisture content, all of which can influence the acceptability and performance of the designed diet.

The development of artificial diets for the European cuttlefish will open the doors to cephalopod commercial aquaculture, and will be eventually required with increasing demand of fishery products and shortage of fish stocks.

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