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

GROWTH OF *SEPIA OFFICINALIS* IN CAPTIVITY AND IN NATURE

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CUTTLEFISH
SEPIA OFFICINALIS
GROWTH

ABSTRACT. – This article presents a review on growth of the cuttlefish (*Sepia officinalis*) under laboratory conditions as well as in wild populations. Growth in captivity under different conditions relating to culture density, prey type and density, temperature and tank size is reported. Growth studies using artificial feeds are also reported. Growth in wild populations is focused upon considering recent results based on age-size frequency distributions and biochemical indices, where some new data are given. The effect of some environmental factors on growth is also reported.

INTRODUCTION

Individual growth, which is the increase in biomass or length, is an important measure in ecology. Growth rates indicate resource availability and asses the condition of individuals and the environment. Growth is influenced by biotic and abiotic factors; it is a complex process that occurs on many biological levels. Therefore, the form of lifetime growth curves and how biotic environmental factors alter growth can only be understood when the processes at lower levels of organization are known (Moltschaniwskyj 2004).

Cephalopods have fast growth rates, between 3 and 15% body weight per day (BW d^{-1}). Since protein is used mainly for energy, protein assimilation efficiency is high, with apparent protein digestibility greater than 85-90%. Therefore, there is a great amino acid requirement for protein synthesis (Lee 1994). Cephalopod bodies (flesh) are composed of 75 to 85% protein on a dry weight basis. In contrast to fishes, cephalopods contain 20% more protein, 80% less ash, 50-90% less lipid and 50-90% less carbohydrate (Lee 1994). Cephalopod water content is approximately 80% with protein (16.6%) being the next most abundant component (Iwasaki & Harada 1985). Carbohydrates are approximately 1% (Vlieg 1984) and lipids are usually less than 2%; neither are candidates for energy production (Hochachka *et al*. 1975). When considering overall composition of the whole animal, including visceral mass, protein percentage decreases and lipid increases. The lipid content of the digestive gland of *S. officinalis* can reach 13% (Semmens 1998). Cephalopods can feed at rates exceeding 20 to 50% of their BW per day (Boucher-Rodoni *et al*. 1987). Cephalopods are also characterized for having short life cycles and high feeding rates, which can vary between 20 and 50% BW day^{-1} (Boucher-Rodoni *et al*. 1987).

Sepia officinalis adapts easily to laboratory culture because it has (1) large eggs, (2) high hatchling survival, (3) voracious hatchlings, (4) sedentary behaviour, (5) tolerance to crowding and handling (6) acceptance of dead prey and (7) it easily reproduces in captivity (Forsythe *et al*. 1994). Therefore, its culture in the laboratory has been successful around the world, from the early 60's until the present day (Schröder 1966, Richard, 1966, 1971, 1975, Pascual 1978, Yim 1978, Boletzky, 1979, 1983, Boletzky & Hanlon 1983, DeRusha *et al*. 1989, Forsythe *et al*. 1994, Lee *et al*. 1998, Domingues 1999, Bettencourt 2000, Domingues *et al*. 2001b, 2002).

S. officinalis hatchlings are born as miniature replicas of adults, and have similar basic behaviour as adults, namely a marked benthic mode of life (Hanlon & Messenger 1996, Warnke 1994). During the first few weeks of their life, cuttlefish have to be fed live prey, usually mysid shrimp (Richard 1975, Forsythe *et al*. 1994, Domingues 1999, Domingues *et al*. 2001a). Afterwards, the animal accepts dead food, such as frozen shrimp, fish or crabs (De Rusha *et al*. 1989, Forsythe *et al*. 1991, Domingues *et al*. 2001b, Koueta & Boucaud-Camou 1999, 2001, Koueta *et al*. 2002). Some authors have cultured this species with this transition to dead food (Pascual 1978, Forsythe *et al*. 1994), while others fed live prey throughout the life cycle (Domingues *et al*. 2001a, 2001b, 2002).

Various mathematical equations have been used to describe cephalopod growth. Patterns of growth

included linear, asymptotic, cyclic, exponential, sigmoidal and exponential. Using a single equation to describe growth through the entire life span is desirable. This, however, has the disadvantage that growth during early or late life is not adequately described, and important details or even the form of growth can be obscured. Therefore, in some cases two equations, exponential followed by logarithmic, have been employed to describe growth throughout the life cycle of one species. This has also some disadvantages, two of the most important ones are that the intersection of the two equations is often not precise, and the second phase indicates continuous growth with no upper limit (Forsythe & Van Heukelem 1987 and Moltschaniwskyj 2004 for reviews)

Embryonic growth

The length of *Sepia officinalis* embryonic development varies with temperature. Under culture conditions, and considering that oxygen content is close to saturation around the eggs, the duration of embryonic growth ranges from 40-45 days at 20ºC to 80-90 days at 15ºC.

The stages of embryonic development can be identified using different staging systems proposed by Naef, Lemaire and Arnold (Boletzky 1983). It was observed that the late embryonic stages following organogenesis, last more than half of the total time of the embryonic development, and that the final growth from stages XVIII to XX (sensu Naef 1923, 1928) takes nearly 80% of the total time of the embryonic development of the species. During this late phase an accumulation of yolk transferred from the outer into the inner yolk sac occurs. Therefore, the hatchling always has a yolk reserve integrated in the circulatory system of the visceral mass that will continue to release nutritive material after the disappearance of the outer yolksac (Boletzky 1983). On the other hand, in the late embryonic stages the cuttlebone contains gas, but the embryo remains negatively buoyant, maintaining an upside down position until hatching.

Although premature hatching can occur under stress conditions, thus influencing the size of hatchlings, usually the hatchlings of this species have a mantle length ranging from 6 to 9 mm, depending primarily on the egg size. The general aspect and behaviour of the *S. officinalis* newly hatched are strikingly similar to the adults; there are, however, different proportions between the body, head and arms in hatchling and juveniles compared to adults, which is due to an allometric growth during the subsequent phases of the ontogenic development.

Growth in the laboratory

Growth of cuttlefish in the laboratory is generally lower, and the life cycle shorter when compared to wild populations. Pascual (1978) obtained an average weight of 210 g at the age of 210 days, while Domingues *et al*. (2002) reported an average 165 g at this age. Richard (1971) grew *S. officinalis* to 6.8±1.0 cm mantle lengths (ML) in 219 days and to 190 g in 285 days, at 15ºC. Nevertheless, long life spans were also obtained in the laboratory. Forsythe *et al*. (1994) reported average life cycles of 11, 14 and 17 months for cuttlefish cultured at temperatures varying between 20ºC and 24ºC. Richard (1971) reports life cycles of over one year, at 15ºC.

Domingues *et al*. (2001a, 2002, 2003a, 2004) never obtained cuttlefish larger than 400 g when culturing 6 consecutive generations in the laboratory, and the longest generation reported by Domingues *et al*. (2002) cultured in the laboratory was of 260 days, for cuttlefish cultured at average temperatures of 15ºC. Toll & Strain (1988) also obtained slow growth in captivity for this species. The exception has been the NRCC cephalopod research group in Galveston, Texas, that has regularly produced cuttlefish weighing over 2.5 Kg, and they cultured this species for 7 consecutive generations (Forsythe *et al.* 1994). This fact could be related to the tank size. While this last author uses large tanks, between 2.000 and 10.000 litres of volume, the former used small tanks, usually with less than 300 litres.

The effect of culture density on growth has been studied for hatchlings and juveniles by several authors (Forsythe *et al*. 1994, Nabhitabhata 1999, Nabhitabhata & Nilaphat 2000, Koueta & Boucaud-Camou 1999, Domingues *et al*. 2003b, Sykes *et al*. 2003), while for adults only Forsythe *et al.* (1994), Domingues *et al*. (2001a, 2002, 2003a), and Correia *et al.* (2005), provide information on culture stocking densities.

The effects of culture density on cuttlefish hatchlings, juveniles and adults at different temperatures are shown in Table I.

Sykes *et al*. (2003) determined the effects of enriched and non-enriched environments on growth of cuttlefish hatchlings, with similar growth rates of 10.1% and 9.7% BW d^{-1} for each environment, respectively.

The RNA/DNA ratio in cuttlefish muscle was shown to be directly correlated with growth rates (Clarke *et al.* 1989, Castro & Lee 1994, Koueta *et al*. 2000, Sykes *et al*. 2004). Nevertheless, according to Sykes *et al*. (2004), results obtained are weak, compared to the ones obtained from weight measurements, since the methodology is complex and errors can greatly influence final results.

Table I. – Growth (% BW d^{-1}) of *S. officinalis* hatchlings, juveniles and adults cultured at different temperatures and densities. Density is expressed as cuttlefish m^{-2}, size in g or mantle length (ML), period in days, temperature in ºC, tank volume in litres an d growth rate in % BW d^{-1}.

Density	Size	Period	T (ºC)	Volume	GR	Reference
250-300	< 0.5 g	28-42	20-24	90		Forsythe *et al*. 1994
20	10-15 cm	90	20-24	>90		Forsythe *et al*. 1994
2	15-22 cm		20-24	>2000		Forsythe *et al*. 1994
500	0.2 g	28	28		7	Nabhitabhata 1999
200	2 cm	180	28		3.4	Nabhitabhata 1999
5	<0.5 g	40	19	2	4-7	Koueta & Boucaud-Camou 1999
60	0.1 g	90-120	25	250	4.8-5.3	Domingues *et al*. 2001a
60	0.1	120	27	250	4	Domingues *et al*. 2002
60	0.1	210	15	250	6	Domingues *et al*. 2002
100	1.5 g	35	17	1.2	3.5	Forsythe *et al.* 2002
400	1.5 g	35	17	1.2	3.5	Forsythe *et al.* 2002
100	1.5 g	35	25	1.2	8.4-6.7	Forsythe *et al.* 2002
400	1.5 g	35	25	1.2	8.2-5.7	Forsythe *et al.* 2002
52	0.1 g	20	23	5.5	8.8	Sykes *et a*l. 2003
515	0.1 g	20	23	5.5	9.6	Sykes *et al*. 2003
1544	0.1 g	20	23	5.5	9.2	Sykes *et al*. 2003
10	1.5 g	30	23	5.5	5.9	Sykes *et al.* 2003
45	1.5 g	30	23	5.5	5.8	Sykes *et al.* 2003
120	1.5 g	30	23	5.5	5.6	Sykes *et al*. 2003
625	1.5 g	40	20	0.5	5.2	Domingues *et al*. 2003b
375	1.5 g	40	20	0.5	5.6	Domingues *et al*. 2003b
125	1.5 g	40	20	0.5	5.7	Domingues *et al*. 2003b
16	1.5 g	43	19	250	4.8	Correia *et al*. 2005
76	1.5 g	43	19	250	4.2	Correia *et al*. 2005
16	10.5 g	56	25	250	4.0	Correia *et al*. 2005
76	9.0 g	56	25	250	3.5	Correia *et al*. 2005

Recent rearing experiments have both verified the daily periodicity of statolith rings and invalidated the use of other hard structures such as the cuttlebone, eye lens and beaks (Bettencourt & Guerra 2001). These authors indicated, however, that in cuttlefish after more than 240 days the statolith rings were hardly visible. The fact that this difficulty is more important in older specimens than in younger ones could have several causes. One of the most important could be related with the lower percentage of organic matter found in these individuals, which determine statolith calcification and, thus, poor increment definition (Bettencourt & Guerra 2000). In this context, reading errors are likely to be higher and counting of increments should be carried out with extreme care and precision (Lipinski *et al*. 1998, González *et al.* 2000). Another important factor to be considered is that the rhythm of growth increment deposition was not influenced by temperature. The rhythmicity of statolith growth increments in individuals reared at two different temperature regimes was the same, although somatic growth was strongly influenced by temperature (Bettencourt 2000).

Biochemical indices based on qualitative and quantitative analysis of the chemical compounds such as proteins, lipids, carbohydrates, nucleic acids, organic matter and water content can be explored as an alternative to estimating growth rates from age-size-relationships. This is largely because statolith microstructure cannot be used to determine age in some species, but also to estimate instantaneous assessment of growth rather that lifetime estimates (Moltschaniwskyj 2004). Biochemical indices are also important to evaluate the nutritional stage and consequently the growth or survival potential of an individual. Changes in growth in response to food and temperature will be a reflection of alterations in the chemical compounds used as energetic substrates. Therefore, analysis of these biochemical compounds will be of great value to understand the influence of environmental factors on growth rates.

Effects of temperature on growth in laboratory culture

This species can be cultured at a wide range of temperatures; Richard (1971) obtained positive growth at temperatures as low as 9.5ºC. The same author suggested 30ºC as the possible upper temperature limit that *S. officinalis* could survive to, while Pascual (1978) reports lower growth at 30ºC compared to 22ºC. Nevertheless, Domingues *et al*. (2001) cultured *S. officinalis* in the laboratory at water temperatures that frequently reached 32ºC during the hottest part of the day. Temperature clearly plays a major role in determining the life span of *S. officinalis* (Richard 1971, Pascual 1978, Forsythe *et al*. 1991, 1994, Domingues *et al*. 2001a, 2001b, 2002, 2004). Table II shows different growth rates for cuttlefish grown during parts or the entire life cycle.

Table II. – Growth (% BW d^{-1}) of *S. officinalis* hatchlings, juveniles and adults cultured at different temperatures. Culture period is expressed in days, temperature in ºC, and growth rate in % BW d^{-1}, or mantle length (ML).

Initial size	Period	T (ºC)	GR	Reference
hatchling	240	20	14,8 cm ML	Richard 1966
hatchling	240	15	6,8 cm ML	Richard 1966
hatchling	240	10	4,7 cm ML	Richard 1966
30 days	30	21.5	5.4	Pascual 1978
hatchling	46	22.5	6.3	Pascual 1978
150 days		16	2,1	Pascual 1978
Hatchling		15-19	3.3 cm ML	Toll & Strain 1988
0.22 g	29	22	8.5	DeRusha *et al.* 1989
4.1 g	61	22	3.9	DeRusha *et al.* 1989
hatchling	90	24	6.5	Forsythe *et al.* 1994
hatchling	180	20	3.5	Forsythe *et al.* 1994
hatchling	40	20	8	Koueta & Boucaud-Camou 1999
hatchling	40	18	5.6	Koueta *et al.* 2000
hatchling	10	25-30	12.4	Domingues *et al.* 2001b
0.1-2 g	10	25-30	9.1	Domingues *et al.* 2001b
2-5 g	10	25-30	7.3	Domingues *et al.* 2001b
5-15 g	10	25-30	4.7	Domingues *et al.* 2001b
hatchling	First 10 d	27	11.2	Domingues *et al.* 2001a
hatchling	Life cycle	27	6.7	Domingues *et al.* 2001a
hatchling	First 20 d	27	9.5	Domingues *et al.* 2001a
hatchling	Life cycle	27	5.3	Domingues *et al.* 2001a
hatchling	First 14 d	22	> 10	Domingues *et al.* 2001a
1.5 g	35	17	3.5	Forsythe *et al.* 2002
1.5 g	35	25	6-8.5	Forsythe *et al.* 2002
hatchling	20	20	7.5-9.5	Koueta *et al.* 2002
hatchling	Life cycle	27	4	Domingues *et al.* 2002
hatchling	Life cycle	15	6	Domingues *et al.* 2002
hatchling	20	23	~ 9	Sykes *et a*l. 2003
1.5 g	30	23	~ 6	Sykes *et al.* 2003
1.5 g	43	19	~ 4.5	Correia *et al.* 2005
9-10.5 g	56	25	3.5-4.0	Correia *et al.* 2005

Although cephalopod growth is exponential during the first part of the life cycle and linear afterwards (Forsythe & Van Heukelem 1987), early results on growth for *S. officinalis* indicate linear growth at 20, 15 and 10ºC, at the end of 8 months (Richard 1966). Linear growth was also obtained for this species by Pascual (1978) at 16ºC. Similarly, Domingues *et al.* (2002) obtained linear growth during the first 120 days of the life cycle, when cuttlefish were cultured at 13ºC±1ºC. Growth rate is a function of body weight, and therefore it is expected to decrease with age (and size) of the cuttlefish. The decrease in growth rate with size for this species was reported by many authors (Richard 1971, Pascual 1978, Forsythe & Van Heukelem 1987, Forsythe *et al.* 1994, Koueta & Boucaud-Camou (1999, 2001), Domingues *et al.* 2001b, 2002, 2003a).

Differentiated growth by sexes is present in *S. officinalis* (Boletzky 1983). Female cuttlefish reduce their growth much faster than males, at the time of sexual maturation (Domingues *et al.* 2002, 2003a), as they invest more energy in reproduction. The total weight of the sexual organs in females can represent up to 16% of the BW, while in males it only reaches 5% of the total weight of the animal (Boletzky 1983).

Effects of prey type and density on growth in laboratory culture

Different prey has been used to culture early stages of cuttlefish, but mysids are the ones that produce better growth (Richard 1971, Pascual 1978, Boletzky & Hanlon 1983, DeRusha *et al.* 1989, Lee *et al.* 1991, Forsythe *et al.* 1994, Domingues *et al.* 2001a, 2002, 2003b, 2004, Sykes *et al.* 2003). Grass shrimp have been used as the only prey for the culture of *S. officinalis* during the whole life cycle (Sykes 2003). Domingues *et al.* (2002) reports that during the first 10 days (period of differentiated feeding), cuttlefish fed *Artemia* sp. had a considerably lower growth rate (4.4% BW d^{-1}) compared to the ones fed mysids (11.2% BW d^{-1}). The same author fed another group of hatchlings with *Artemia* and mysids, and obtained much higher growth rates for hatchlings fed mysids for 20 days, compared to the ones fed *Artemia* (11.4 and 4,7% BW d^{-1}, respectively). Artemia has been used by other authors as first food for cuttlefish hatchlings with poor results on growth and survival (Pascual 1978, Botelzky 1979, Boletzky & Hanlon 1983, DeRusha *et al.* 1989, Hanley *et al.* 1998).

Domingues *et al*. (2003a) fed cuttlefish hatchlings at 20ºC, until 40 days of age, using live shrimp or fish fry. Hatchlings fed live shrimp grew much faster and larger, compared to the ones fed live fish fry (7.9 and 4.8% BW d^{-1}, respectively). The natural diet of young cuttlefish (< 8,5 cm ML) captured from the wild was composed essentially of crustaceans (89%), while fish only represented 4.6% (Blanc *et al*. 1998). Nevertheless, Castro & Guerra (1990) reported that the importance of fish in the diet increased and importance of crustaceans decreased, as cuttlefish grow bigger. These findings strongly indicate that crustaceans are indeed a better food for hatchlings and juveniles of this species, and could explain the much better results obtained during this experiment when feeding them with shrimp, compared to fish fry.

Some authors report differences in growth when feeding live or frozen crustaceans to *S. officinalis* (Pascual 1978, DeRusha *et al.* 1989, Lee *et al.* 1991), while others report no differences (Domingues *et al*. 2003b). Koueta & Boucaud-Camou (1999) obtained higher growth rates (7.3% BW d^{-1}) for cuttlefish fed live prey, compared to the ones fed frozen mysids (4.6 BW d^{-1}). Similarly, DeRusha *et al*. (1989) reports that *S. officinalis* (30 to 160 g in wet weight) fed live prey grew 5 to 8% larger compared to others fed frozen diets.

The effect of frozen shrimp or frozen fish on growth of *S. officinalis* was also reported by Domingues *et al*. (2004) with higher growth rates (5.1% BW d^{-1}) for cuttlefish fed frozen grass shrimps, compared to frozen fish (3.3% BW d^{-1}). Domingues *et al.* (2004) studied the effects of different live prey on growth of cuttlefish aged 1, 30 and 60 days old, during 30 days periods. For newly born hatchlings, growth was of 6.2, 7.5 and 2.9% BWd^{-1} when feeding mysids, grass shrimps and fish, respectively, and different between the 3 diets. Growth was higher during the first two weeks with mysids, but afterwards grass shrimp promoted better growth. For 30 days old cuttlefish, growth rates were higher (5.7% BW d^{-1}) for cuttlefish fed grass shrimps, compared to fish (2.4% BW d^{-1}). Similarly, for 60 days old cuttlefish, growth rates were higher (5.8% BW d^{-1}) for cuttlefish fed grass shrimps, compared to fish (3.5% BWd^{-1}).

Prey density can affect growth of *S. officinalis*. A few studies reporting different prey density for cuttlefish hatchlings were conducted (Koueta & Boucaud-Camou 1999, 2001), they obtained higher growth rates at higher prey density (21, 30 and 35%BW d^{-1}), where differences appeared only after 10-days of age (the lower density promoted lower growth) and after 20 days (the higher density promoted higher growth). Koueta & Boucaud-Camou (2001) obtained growth rates of 12.8% BW d^{-1}, with optimal prey rations (40% BW d^{-1}). At that age ingestion increased to 46% BW d^{-1}, with growth rates of 13.2% BW d^{-1}. Similarly, Koueta *et al*. (2000) conducted experiments with different live mysid densities fed to juvenile *S. officinalis* and obtained higher growth at higher densities (2.6, 4.4 and 5.6% BW d^{-1}, respectively).

Enrichment of live prey with polyunsaturated fatty acids (PUFA) before feeding them to young cuttlefish (less than 4 days old) promoted better growth rates at 20ºC (Koueta *et al.* 2002). This author reported higher growth rates in animals fed enriched mysids (2.8% BW d^{-1}) compared to non enriched mysids (2.0% BW d^{-1}), while enriched shrimp promoted higher growth rates (9.5% BW d^{-1}) compared to non enriched shrimp (7.5% BW d^{-1}). Koueta & Boucaud-Camou (2003) also found that the combination of feeding frequencies enhanced growth when cuttlefish receive short periods of light per day.

Effects of artificial diets on growth in laboratory culture

In the last few years, feeding experiments using *S. officinalis* have been conducted with either moist or dry pellets (Castro 1990, Lee *et al*. 1991, Castro *et al*. 1993) or surimi (fish myofibrillar protein concentrate; Castro *et al*. 1993, Castro & Lee 1994, Domingues 1999, Domingues *et al*. 2005), demonstrating that cuttlefish readily accept prepared diets. Feeding rates on prepared diets have been considerably lower than with a normal laboratory maintenance diet of crustaceans (Pascual 1978, Richard 1971, 1975, Boletzky 1979, Castro *et al*. 1993, Castro & Lee 1994, Lee *et al*. 1991, Forsythe *et al*. 1994, Domingues *et al*., 2001b, 2002, 2003a, 2003b, 2004, Koueta & Boucaud-Camou 1999, 2001, Koueta *et al*. 2000), and also considerably lower than rates during transition periods when cuttlefish were fed thawed catfish fillets, during these transition periods, feeding rate varied between 3.5% and 10% BW d^{-1} (Domingues 1999).

Despite the acceptance of the prepared diets, negative growth with artificial diets was common, and the highest growth rates reported in the literature, close to 0.5% BW d^{-1} (Castro 1990, Castro *et al*. 1993, Castro & Lee 1994, Lee *et al*. 1991, Domingues 1999, Domingues *et al*. 2005) are almost 10 times lower than growth rates recorded during normal laboratory maintenance of this species (5% BW d^{-1}) (Pascual 1978, Lee *et al*. 1991, Forsythe *et al*. 1994, Domingues *et al.* 21001b, 2002, Sykes *et al*. 2003). Also, mortality rates when feeding artificial diets are usually higher compared to natural diets (DeRusha *et al*. 1989, Lee *et al*. 1991, Castro *et al*. 1993).

Growth in the wild

Growth analysis based on statolith-ageing techniques

Important advances in cephalopod growth analysis have been carried out in the last two decades with individual age determination through quantification of statolith increments (Jackson 1994, González *et al*. 1998, González *et al*. 2000, Bettencourt & Guerra 2000, Durholtz *et a*l. 2002). Statolith increments in *S. officinalis* are difficult to visualise and initially other hard structures such as the cuttlebone were examined for ageing (Le Goff *et al*. 1998, Ré & Narciso 1994). The results found by Bettencourt & Guerra (2001) in statolith increments of cuttlefish cultured in the laboratory can be extrapolated to wild specimens, but bearing in mind that this should be done with caution because (1) the hypothesis 1 day = 1 increment has not yet been validated for the entire life cycle of the species and (2) the influence of environmental factors must be considered. For example, temperature has a profound impact on statolith growth and microstructure, and, consequently, on the interpretation of putative daily increments (Durholtz & Lipinski 2000).

The first attempt to apply statolith-ageing techniques to *S. officinalis* collected in the wild was carried out by Challier *et al* (2002). In this study, age and growth were estimates using statolith increments only for juveniles of 53-90 mm mantle length, collected between October and December 2000 in the French part of the English Channel and from August to December in the Bay of Seine. The general pattern of juvenile growth of the species with statolith analysis was consistent with previous studies based on length-frequencies distributions (Medhiboud 1986, Duun 1999). Thus, (1) the cohort 2000 was recruited at an age between 2 and 5.5 months, the bulk of recruits ranging from 60 to 120 days, (2) an increasing trend in average age was observed in the fall, (3) the maximum of hatching occurred between June and August, and (4) the life-span of the species is about two years. Several authors had reported a different growth rate by hatching month in *S. officinalis* (Bouchaud & Daguzan 1989, Le Goff & Daguzan 1991). Challier *et al* (2002) provided the first evidence of a large inter-individual growth variation consisting in a reduction of the growth rate in juveniles born in late summer compared with those born early in the hatching period (1.18 mm mantle length.d^{-1} in June, 0.69 mm ML.d^{-1} in July and 0.46 mm ML.d^{-1} in August). The juvenile cuttlefish hatched the same spring-summer period showed in autumn a size range with two modes (53-90 mm ML and 103-128 mm ML). The animals born early in the hatching period were larger than the animals of equivalent age born later. Temperature fluctuations seemed to be the most important environmental factor, which caused seasonal growth variation. However, reared *S. officinalis* also showed relatively strong growth variations although they were maintained at a constant temperature (Richard 1969, Bettencourt 2000, Domingues *et al.* 2001, 2002). These observations may limit interpretations of the influence of temperature on cuttlefish growth variation. This first study by Challier *et al*. (2002) was carried out considering only one cohort, but it suggested that other cohorts could follow a similar pattern accounting for inter-annual growth variations. Inter-annual growth variations in juveniles could cause stock fluctuations, and hence be an important factor in the development of predictive models for fishery management advice.

On the other hand, the growth curve estimated by Challier *et al*. (2002) was linear during the studied period (40-170 days). Since growth was not measured before and after this period, the overall nature of the growth curve of this species estimated from wild samples is still unknown.

The most recent study on *Sepia officinalis* growth in the wild has been carried out by Challier (2005). In this study age was determined from statolith analysis. It was observed that, although the main recruitment of this species to the trawl fisheries in the English Channel takes place during the autumn, seasonal differences existed on the order of 11-17 days in the age at recruitment, and relative back-calculated hatching dates indicated that waves of individuals enter the fishery throughout the year. Recruitment occurred at an age ranging from 3 to 4 months, which agrees with that previously observed by Challier *et al* (2002). This indicated most cuttlefish hatched during the summer, but some hatching took place throughout the year. Fitted growth models (both exponential and linear) indicated that growth rate of pre-recruit specimens was significantly higher in 2002 (2.3 to 2.9 cm ML. $month^{-1}$) than in 2000 (1.4 to 1.9 cm. ML. $month^{-1}$). The last growth rate is consistent with the one estimated by Medhioub (1986). In spite of this significant inter-annual growth difference, a similar growth trend was found between the different micro-cohorts identified within both years. That trend consisted in highest growth rates for cuttlefish hatched in July, and lower growth rates for those hatched in June and August. Growth differences between micro-cohorts seem to be influenced by both feeding opportunities and environmental conditions such as temperature (Forsythe 2004), and, consequently, through environmental heterogeneity, differences in growth rates may be related to different hatching grounds and hatching periods.

Challier (2005) also identified significantly spatial instantaneous growth rate differences in both years sampled. Thus, growth rates in the Bay of Seine were 0.018 mm ML. d^{-1} for 2000 and

0.0328 mm ML. d^{-1} for 2002, whilst they were 0.0295 mm ML. d^{-1} and 0.0294 mm ML. d^{-1} for 2000 and 2002, respectively, in the northern coast of the English Channel. These authors suggest that these differences could be due to the influence of low salinity and high turbidity in the Bay of Seine and/or density-dependent effects, which varied between areas.

Growth seasonal variation based on biochemical indices

The relationship between biochemical composition and biological parameters including sex, maturation and growth was not yet studied in wild *S. officinalis*. We here present some unpublished results obtained by Bettencourt (2000) on the biochemical composition (DNA, RNA, protein and water content) in wild *S. officinalis*. This text also offers some discussion about the problems involved in the use of biochemical indices for estimating growth, and the relationships between somatic growth, gonad development and environmental conditions.

Two samples of 40 specimens of *S. officinalis* were collected in the Ría de Arousa (Galicia, NW Spain) in October-November 1999 and in April 2000, respectively. Cuttlefish were measured (ML – dorsal mantle length in mm) and weighed (TW – total body weight in grams). Sex was recorded and the sexual maturity stages were identified according to Mangold-Wirz (1963) and Richard (1971). Pieces (± 2 cm^2) of mantle muscle were cut and maintained at –80ºC until further biochemical analyses. Water content was determined by drying samples of mantle muscle in a dry oven for 24h at 105ºC. The extraction and quantification of nucleic acids was carried out using the procedure of Schmidt-Thannhauser (Munro & Fleck 1966). Total protein was determined according to the Bradford and Smith procedure (Smith *et al.* 1985) using Bovine serum albumin (BSA) as standard.

Water content (%) in the muscle was constant for all sampled individuals. However, comparing dry weights (%) between specimens sampled in spring and in autumn significant differences ($t=-29.634$; $p<0.01$) were detected, showing that the spring cuttlefishes have more water in the muscle than the autumn individuals.

There were no significant differences between male and female (Table III) for all biochemical indices studied, except for the DNA content of spring specimens where females showed higher DNA concentration than males.

In autumn, the highest RNA value was 1.1µg/mg obtained from the muscle tissue of one cuttlefish of 80g TW. The tendency of RNA content was to decrease with the increase of individual size. The largest cuttlefish (1564g TW) had 0.36µg of RNA/mg of muscle tissue

DNA concentration showed the same pattern, with values decreasing with increasing individual size. However, the decrease observed in DNA was not so evident as in RNA. DNA values showed higher inter-individual variability. This variability was higher in juvenile cuttlefishes, with values between 0.26 and 0.52µg/mg of muscle tissue, than in adult individuals, that presented DNA values lower than 0.1µg/mg of muscle tissue in specimens of more than 1000 g TW.

Protein content ranged between 35 and 147µg/mg of muscle tissue. However, in immature juveniles a high variability was found with more than 100µg of variation. Individuals with more than 200g TW showed a constant protein content around 100µg of protein/mg of muscle tissue. Individuals with more than 1000g TW showed a slight increase of protein content reaching a maximum of 147µg of protein/mg of tissue.

The same biochemical pattern was found during spring. However, all spring values were slightly smaller. The highest RNA value (0.94µg/mg) was obtained in a small individual. A strong decrease was detected in individuals with TW smaller than 300g. Larger individuals seem to present a more constant RNA content around 0.25µg/mg. The same pattern was observed for the DNA indices. These values decreased abruptly from 0.44 to 0.20µg/mg in individuals smaller than 250g TBW, achieving a constant value around 0.20µg/mg, and then showing a slight decrease until a minimum value of 0.10µg/mg of DNA in the largest individual. Total protein contents tend to be constant, with an average value of 83.52µg/mg ± 16.62 of muscle tissue, and with a high variability in small individuals.

Table III. – t-Student (t) and significance level (p) from different biochemical indices between females (0) and males (1) of *Sepia officinalis* (n.s. no significant at $p<0.05$).

	Autumn				Spring			
	n: 0	1	t	P	n: 0	1	t	P
RNA	22	16	-1.783	0.083n.s	19	29	1.594	0.084n.s
DNA	21	15	0.287	0.388n.s	19	28	2.215	0.016 *
Proteins	22	13	1.665	0.053n.s	18	31	1.403	0.084n.s

Males and females from spring samples reached sexual maturity at smaller sizes than individuals sampled in autumn. Spring individuals grow slowlier than autumn ones, being in accordance with RNA values for these seasons. The autumn individuals have RNA values higher than spring ones with the same weight. Moreover, DNA values in spring specimens decreased more abruptly until they reached 250g TW, which corresponds to totally mature individuals. However, autumn cuttlefishes only reach sexual maturity with 550g. This result could explain the non-existence of an abrupt decrease of DNA and RNA indices in autumn.

The mean water temperature in Ria de Arousa during the studied period was 14ºC ± 1.62. The highest value of 19.5ºC was recorded during the summer months (August & September) while the lowest temperature was 11.7ºC, recorded in the winter months (December & January). *S. officinalis* collected in spring passed through a cold season and, consequently, they show a slower growth rate in comparison with the growth rate of those specimens collected in autumn that passed through a warm season.

Several authors started that growth rates in cephalopods are related with temperature (Forsythe & Van Heukelem 1987, Moltschaniwskyj & Martínez 1998, Martínez *et al.* 2000, Domingues *et al.* 2002, Forsythe 2004). In fact, growth rates of *S. officinalis* reared at low temperatures were lower than those observed at high temperatures (Richard 1971, Forsythe *et al.* 1994, Bettencourt & Guerra 2001, Domingues et al. 2002).

Water content in the muscle of *S. officinalis* is influenced by the season of the year. Individuals sampled in spring had more water in the muscle than the autumn ones, revealing a minor protein growth rate. This situation corroborates the results obtained with reared individuals in starvation condition that presented an increase in water content (Castro *et al.* 1993).

From the results obtained in the present paper it can be concluded that sex does not influence the DNA, RNA or protein concentrations. This suggests that growth patterns between males and females are identical whatever the season of the year.

Moreover, for any biochemical parameter analysed, the values obtained were lower in specimens sampled in spring than in individuals sampled in autumn. These individuals just passed through a cold season, where temperatures ranged between 11.5ºC and 14.5ºC. Growth is slow within this range of temperature. Clarke *et al.* (1989) concluded that juveniles of *S. officinalis* reared in starvation conditions at 12.5ºC did not reach negative growth rates due to a decrease of metabolism and activity. At temperatures below 10ºC the individuals do not feed, stay inactive and will die in a couple of days. On the other hand, studies carried out with American plaice *(Pseudopleuronectes americanus)* larvae showed a linear positive relationship between RNA/DNA and both growth rate and ambient temperature (Bucley 1982). The author concluded that temperature is the main growth factor for the larvae in normal feeding conditions.

RNA values obtained in both seasons of the year were between 1.2 µg/mg for the smallest individuals and 0.32 µg/mg for the largest ones. These RNA values are in agreement with other studies published on *S. officinallis* (Castro & Lee 1994), *Eledone cirrhosa* (Siegert *et al*. 1994, Houlihan *et al.* 1998) and *Loligo forbesi* (Siegert *et al.* 1994, Pierce *et al*. 1999). However, the estimated RNA values obtained by Clarke *et al*. (1989) in *S. officinalis* are higher than the normally accepted values for cephalopods. This could be explained by the different methodologies applied. Also, RNA concentration in mantle tissue of immature squid *Todarodes sagittatus* estimated by Pierce *et al*. (1999) is higher than the concentration found in the present study. The explanation for this discrepancy could be that all *T. sagittatus* analysed were immature and consequently they were in the faster growth phase of their life cycle.

RNA concentration decreases throughout the life of cuttlefish independently of the season of the year. This result evidences the decrease of ribosome number and concentration in the tissue. Primarily, this result reveals that the capacity for an individually realized protein synthesis decreases with growth. This behaviour is corroborated by previous results obtained from fishes. Mathers *et al*. (1992) found that RNA concentration decreases with the increase of body size of saithe, *Pollachius virens*, which means that RNA concentration decrease is not followed by the change of protein content. The protein content was practically constant for all *S. officinalis* sizes. This could be related to the methodology used to measure the RNA content in the tissue, independently of the RNA efficiency. This suggests that cuttlefish could change the ribosomal efficiency for protein synthesis or change the efficiency in retention of the new proteins synthesized or a combination of both, as was mentioned by Mathers *et al.* (1994) for herring larvae, *Clupea harengus*. Houlihan *et al.* (1990) studied the influence of changes in RNA concentration or activity in the protein synthesis stimulation in *Octopus vulgaris* tissue. The authors concluded that fast growth rates in this species are due to high protein synthesis rate and high efficiency of protein retention and consequently small protein degradation. Taking into account this conclusion, it seems to be probable that the high protein content comparing with the low RNA values in *S. officinalis* mantle muscle can be explained by the high retention capacity of synthesized proteins. This positive relationship between RNA concentration in cuttlefish tissues and enzymes involved in

nucleic acid synthesis, for example, aspartate transcarbamylase, has been also observed in early growth (Koueta *et al*. 2000).

The slight decrease of DNA throughout the life cycle of *S. officinalis* (0.5 – 0.1µg/mg of tissue) could be explained by the fact that growth is due not only to cellular multiplication, but also to the increase of its volume (Martínez & Moltschaniwskyj 1998), which will decrease the DNA concentration by unit of cell. Bulow (1970), working with golden fish (*Notemigonus crysoleucas*), suggested that the small decrease of DNA values observed in fish muscular tissue during growth was probably due to changes in the cytoplasmatic volume. Therefore, the DNA concentration decrease, that reveals an increase of individual cellular mass, is responsible for the increase of the protein/DNA relationship.

Another interesting aspect to consider is the comparison between the biochemical parameters, the individual body weight in both seasons, and the maturation process. In spring, individuals pass through different stages of sexual maturation with relatively small changes in weight when compared with individuals collected in autumn. Moreover, the nucleic acids concentration decrease occurs inversely to the progress of sexual maturation. From these results it can be concluded that sexual maturation is responsible for the decrease in the nucleic acid concentration. The changes of nucleic acid concentration in both seasons of the year could be explained by different growth rates. Individuals collected in autumn, which passed through a warm season, present a faster growth than the specimens from spring due to the temperature effect or higher food availability (Van Heukelem 1979). This fast growth would promote a smooth decrease in nucleic acid concentration. Inversely, in spring the slow growth combined with the fast sexual maturation would promote a sharp decrease in nucleic acids concentration.

A possible explanation for the fast progress of sexual maturation in individuals collected in spring is the photoperiod, since the dark period is longer in winter in this latitude. Richard (1967) showed that photoperiod is a main factor inducing the maturation process in cuttlefishes from the English Channel, and concluded that in dark periods equal or superior to 12 hours the optic glands could start activity increasing the sexual maturation process. Kelly (1993) reported that the RNA concentration in *E. cirrhosa* decreases with the maturation process. Siegert *et al.* (1994) found immature *L. forbesi* with a RNA content significantly higher than mature individuals, which agrees with the fact that somatic growth occurs in immature individuals. Moreover, in both studies the protein content does not present any significant difference between maturation stages. These results are in agreement with those obtained in the present work, in which it seems that the concentration of protein in muscle tissue of *S. officinalis* is not related, neither with the maturation process nor with the individual body weights.

Final considerations

Although culture conditions always differ from those in the wild, the fact that *Sepia officinalis* has been brought into captivity and successfully bred and grown in experimental tanks allowed obtaining new insights into aspects of their biology and ecology that cannot be gained in field work. Some of these insights are: a) The evidence for the very high individual growth rates, which have been subsequently, evidenced by means of fine analysis of growth increments on the statoliths of animals belonging to different cohorts; b) The decisive influence of temperature in growth rates and life span of the species. Growth rates of *S. officinalis* in the wild are generally lower than in the laboratory, possibly due to the lower temperatures, and lower availability of food, compared to the ones most often used in the laboratory. Nevertheless, duration of life cycle in the wild is usually much higher (up to 2 years), and cuttlefish reach larger sizes, although with lower growth rates. Overall growth rates for the whole life cycles of >6%BW d^{-1} are obtained in the laboratory at average temperatures over 22º C, with life cycles as short as 6 months, and mature females as small as 50 g (3 to 4 months), which does not occur in the wild. Nevertheless, life cycles of over 1 year and animals over 500-600 g body weight at lower temperatures (15ºC) and in relatively small tanks can be obtained in captivity. An exception are the results of the Galveston research group, where cuttlefish cultured at >20º C in large tanks (2.000-10.000 l) average a life span of one year, but the animals attained body weights over 1 Kg, and in some cases almost reaching 3 Kg. c) The negative influence of density on growth rates and life span, an aspect which is very difficult to study in the wild. During 7 years of constant culture under 22ºC, in small culture tanks and under different density rates, cuttlefish larger than 500 g was never obtained. This indicates that space and density, besides temperature, also play an important role in the life cycles of the species in laboratory culture. Cuttlefish are usually solitary, and in confined spaces throughout the life cycle, the presence of other specimens contributes to early maturation, while competition, lack of space and stress within the tanks are likely some of the reasons contributing to the much shorter life cycles in captivity than in the wild; d) The possibility to validate the 1 ring = 1 day hypothesis in the statoliths, and, therefore, ageing animals, at least until 240 days old; and to study the mechanisms of ring formation in the statoliths; e) To reject the validity of the cuttlebone growth increments for aging cuttlefish due to their high

temperature dependence; f) The possibility of timing the change from exponential to logarithmic growth and to study the influence of abiotic (mainly temperature and photoperiod) and biotic factors (density, type of diet, food intake, efficiency and conversion rate of food into somatic growth, size, sex and maturity stage) on the growth pattern in different phases of the life cycle of the species; g) The influence of different environmental conditions on the biochemical composition of digestive gland and mantle muscle, which provide some degree of calibration explaining the seasonal variations found in the wild in RNA:DNA ratios or proteins: nucleic acid indices; and h) The effect of starvation on growth and biochemical composition of different organs and tissues.

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