EFFECTS OF CULTURE DENSITY AND DIFFERENT LIVE PREY ON GROWTH AND SURVIVAL OF YOUNG CUTTLEFISH, *Sepia officinalis* (LINNAEUS, 1758).

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INTRODUCTION

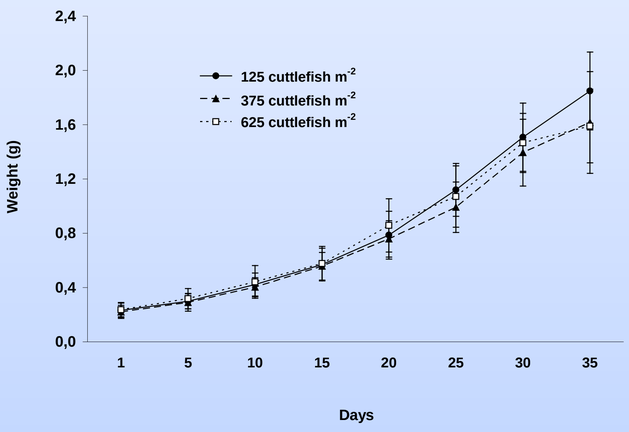
One of the key factors in large-scale aquaculture is to optimise growth while minimizing the culture space required (Forsythe *et al*., in press). Nevertheless, effects of tank size and animal density on growth, condition and reproduction in cephalopods are not known (Boal *et al*., 1999). For *S. officinalis*, which is a semi-solitaire species in nature, crowding may be a limiting factor in achieving successful large-scale culture of this species. Although the most common measure in determining optimum densities has been the carrying capacity, other aspects such as behavioural interactions could be of great importance (Boal *et al*., 1999).
Cephalopods have a predominant amino acid metabolism (Lee, 1994). Because of this, research on lipid and fatty acid requirements have been neglected, and there is little knowledge about them at the present stage (Navarro & Villanueva, 2000). Cephalopod paralarvae and juvenile must require a food rich in polyunsaturated fatty acids (PUFA), phospholipids and cholesterol, and a moderate content in neutral lipids (Navarro & Villanueva, 2000). The n-3 HUFA fatty acids are very important in the formation of the nervous and visual system, reproduction, growth and embryonic and larval development. (Sargent *et al*., 1995).
The objectives of this research were to determine the effects of several culture densities on growth and survival, and the effects of different live prey on growth and survival of *S. officinalis* up to the age of 40 days.

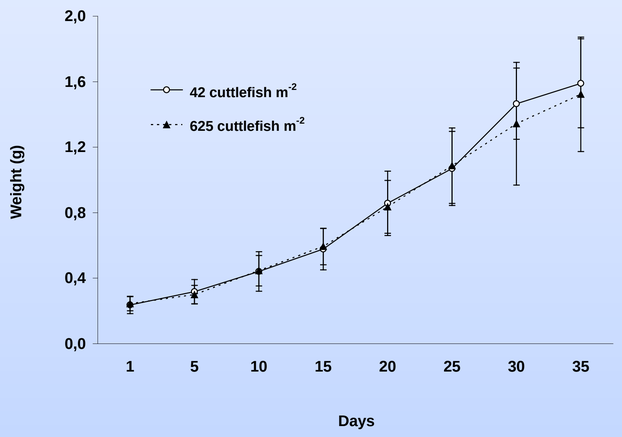
MATERIAL AND METHODS

Density experiments

120 newly born cuttlefish (0.233 ± 0.050 g) were used:
►24 were placed in isolation, in chambers with bottom area of 80 cm2, providing a density of 125 cuttlefish m^{-2} (density 1);
►36 hatchlings in groups of 3 were placed in identical containers providing 12 replicates, at densities of 375 cuttlefish m^{-2} (density 2);
►30 hatchlings in groups of 5 were placed in similar containers, providing 6 replicates and densities of 625 cuttlefish m^{-2} (density 3);
►30 hatchlings were grouped in 5, and placed in larger containers of 240 cm2 of bottom area providing densities of 42 cuttlefish m^{-2} (density 4).

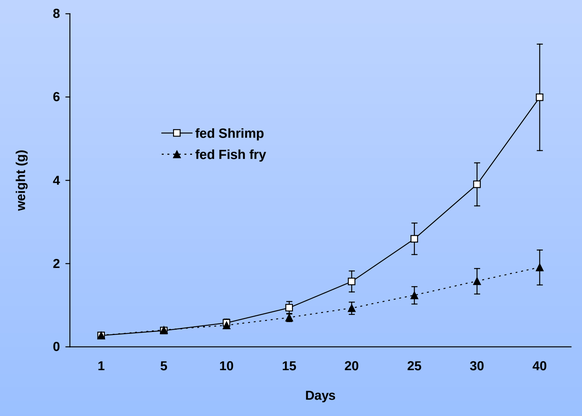
Hatchlings were fed live shrimp (*Crangon crangon*) at rates of approximately 25 %body weight d^{-1}. A recirculating system was used. About 50% of the water was exchanged every week. Water temperature was of 19ºC; salinity was 35 ppt. Hatchlings were weighed every 5 days, and the experiments lasted for 40 days.





Different prey (Shrimp vs fish fry)

Two groups of 25 newly born cuttlefish were cultured during the first 40 days of their life in a flow through system that was composed by two separate tanks with bottom area of 0,5 m^2, and water volume of 100 L. Water-flow was of 10 L h^{-1}. Water temperature was of 20±1ºC.
Both groups were fed small live shrimp (*C. crangon*) for the first 5 days. Afterwards, one group was fed live fish fry of several species (mainly *Blenius* sp. and *Gobius* sp.), while the other group continued to be fed live shrimp. Both groups were always fed *ad libitum* throughout the experiment. Hatchlings were weighed every 5 days. Total protein and lipid composition of the two diets as well as other two live prey used to feed cuttlefish hatchlings (Domingues *et al*., 2001). during the first weeks of their life cycle (the mysid shrimp, *P. nouvelli* and the grass shrimp, *P. varians*) were determined



Protein and Lipid determination

Moisture content was determined from 500 mg samples using the method of Horwitz (1980). Protein content was determined according to the Kjeldahl method (A.O.A.C., 1985). Total lipid from original tissue was extracted with chloroform:methanol (2:1 v/v) containing 0.01% of butylated hydroxytoluene (BHT) as antioxidant (Christie, 1982).

CONCLUSIONS

•Culture densities 2 and 3 were not adequate to culture cuttlefish weighing over 1.2 g

•Cuttlefish placed in isolation weighed ± 2.2 g could still be cultured in this density of 125 cuttlefish m^{-2}.

•Shrimp were easier to capture compared to fish fry. Nevertheless, fish fry were still easily captured and consumed by cuttlefish hatchlings. Therefore, the great differences in growth, condition and survival of hatchlings after 40 days must be associated to the composition of the shrimp and the fish fry, namely on fatty acid composition, phospholipids, cholesterol.

•The higher polar lipid content, especially due to the higher phosphatidylcholine (PC) and phosphatidylethanolamine (PE) levels observed in the common shrimp (*C. Crangon*), compared to fish fry (Table II) could possibly be one of the major factors to explain the significantly higher ($p<0.05$) growth rates for *S. officinalis* hatchlings fed shrimp, compared to the ones fed fish fry.
•The ratio DHA/EPA in the diet has been observed to be relevant on the early development and growth of several marine species. During our experiment, the 1/2 ratio that is found in the common shrimp and the other two crustacean species analysed, while fish fry, which promoted lower growth and survival, had a very different ratio (1.2/1) for these two important fatty acids. This could have some effect on the lower growth of cuttlefish fed fish fry.

RESULTS

Table I - Composition of proteins *(μg/g) (%DWB),* moisture (%), total lipids (% DWB), and lipid classes (% of total lipid) of live diets for cuttlefish hatchlings. Results represent means ± S.D. (n=3). [1] Contain phosphatydilglycerol, phosphatidic acid, and cardiolipin.

Live Diets	Mysid shrimp *P. nouvelli*	Grass shrimp *P. varians*	Fish fry	Common shrimp *C. crangon*
Total Protein	72,29 ± 2,97	70,75 ± 4,08	78,14 ± 3,32	74,95 ± 3,67
Moisture	82,31 ± 4,89	79,95 ± 0,78	80,07 ± 0,58	75,18 ± 0,47
Total lipid	8,49 ± 1,91	5,58 ± 0,19	11,19 ± 0,84	5,91 ± 0,81
Sphingomyelin	0,12 ± 0,11	0,41 ± 0,37	0,31 ± 0,15	0,22 ± 0,08
Phosphatidylcholine	11,61 ± 1,53	14,73 ± 0,81	3,40 ± 1,62	21,44 ± 1,05
Phosphatidylserine	3,01 ± 0,11	3,37 ± 1,45	1,50 ± 0,20	4,23 ± 0,48
Phosphatidylinositol	1,40 ± 0,04	2,25 ± 1,31	0,48 ± 0,13	2,21 ± 0,57
Phosphatidylglycerol [1]	2,28 ± 0,19	2,44 ± 0,29	0,20 ± 0,18	3,33 ± 0,41
Phosphatidylethanolamine	12,16 ± 0,34	13,61 ± 0,94	4,50 ± 1,17	16,00 ± 0,83
Diacylglycerol	2,17 ± 0,34	4,74 ± 0,30	2,24 ± 0,78	2,36 ± 0,67
Cholesterol	16,26 ± 0,45	26,59 ± 1,35	16,34 ± 0,52	25,02 ± 2,93
Free Fatty Acids	20,19 ± 1,63	25,26 ± 0,49	31,77 ± 2,39	13,31 ± 1,46
Triacylglycerol	28,08 ± 1,95	5,32 ± 3,12	30,33 ± 2,84	9,58 ± 5,97
Esterol Ester	2,71 ± 1,22	1,27 ± 0,23	8,94 ± 0,99	2,29 ± 0,78
Neutral Lipids	69,41 ± 1,34	63,19 ± 1,35	89,62 ± 3,15	52,56 ± 1,46
Polar Lipids	30,59 ± 1,34	36,81 ± 1,35	10,38 ± 3,15	47,44 ± 1,47

Table II - Fatty acid composition of total lipid (% weight) from different live prey used as food for cuttlefish hatchlings. Results represent means ± S.D. (n=3). [1] contains n-9 and n-7 isomers. [2] contains n-11 and n-9 isomers. Totals include some minor components not shown. [3] includes 17:0 and 20:0. [4] includes 14:1, 17:1n-7 and 22:1. [5] includes 20:3 n-3 and 21:5n-3. [6] includes 18:3n-6, 20:2n-6 and 22:4n-6. [7] 22:6n-3/20:5n-3. [8] 20:5n-3/20:4n-6; 0.0,values ≤ 0.3%.

Live Diets	Mysid shrimp *P. nouvelli*	Grass shrimp *P. varians*	Fish fry	Common shrimp
14 : 0	2,01 ± 0,09	1,80 ± 0,20	1,83 ± 0,15	1,50 ± 0,09
15 : 0	1,32 ± 0,08	0,46 ± 0,03	0,67 ± 0,03	0,89 ± 0,12
16 : 0	21,76 ± 0,65	18,89 ± 0,33	18,06 ± 0,50	16,50 ± 0,07
16 : 1 [1]	10,20 ± 1,10	4,30 ± 0,45	8,55 ± 0,78	7,68 ± 4,10
18 : 0	3,21 ± 0,10	6,24 ± 0,24	6,69 ± 0,36	6,02 ± 0,88
18 : 1 n-9	5,61 ± 0,08	9,38 ± 0,54	9,52 ± 0,45	6,85 ± 0,12
18 : 1 n-7	4,57 ± 0,30	9,42 ± 0,18	6,81 ± 0,21	6,85 ± 0,14
18 : 2 n-6	1,28 ± 0,08	2,95 ± 0,10	2,89 ± 0,09	1,93 ± 0,06
18 : 3 n-3	1,42 ± 0,05	2,28 ± 0,29	1,28 ± 0,24	1,16 ± 0,49
18 : 4 n-3	0,86 ± 0,02	0,47 ± 0,14	0,80 ± 0,26	0.0
20 : 1 [2]	0,57 ± 0,07	0,39 ± 0,11	0,44 ± 0,01	1,47 ± 0,58
20 : 4 n-6	2,87 ± 0,16	4,00 ± 0,22	3,30 ± 0,48	5,29 ± 0,51
20 : 4 n-3	0,38 ± 0,00	0.0	0.0	0.0
20 : 5 n-3	23,31 ± 0,89	20,68 ± 1,17	13,56 ± 0,53	22,60 ± 0,26
22 : 5 n-6	1,06 ± 0,04	0,47 ± 0,09	0,67 ± 0,02	0,86 ± 0,17
22 : 5 n-3	0,92 ± 0,02	0,62 ± 0,09	1,94 ± 0,07	2,58 ± 0,52
22 : 6 n-3	12,48 ± 1,12	11,90 ± 1,08	16,75 ± 0,84	10,61 ± 0,55
UK.	1,54 ± 0,28	2,00 ± 0,50	1,99 ± 0,24	2,74 ± 1,02
Totals				
Saturates [3]	29,37 ± 0,71	28,66 ± 0,39	28,29 ± 0,72	26,64 ± 1,55
Monoenes [4]	22,34 ± 1,36	26,40 ± 1,48	26,51 ± 1,45	26,89 ± 3,66
n-3 [5]	40,00 ± 2,09	36,53 ± 1,60	34,80 ± 0,77	37,37 ± 1,09
n-6 [6]	6,36 ± 0,24	7,96 ± 0,26	7,90 ± 0,52	8,93 ± 0,78
n-3 HUFA	37,51 ± 1,99	33,49 ± 2,08	32,71 ± 0,28	36,05 ± 0,83
n-3/n-6	6,31 ± 0,55	4,60 ± 0,34	4,42 ± 0,35	4,20 ± 0,24
DHA/EPA [7]	0,53 ± 0,03	0,57 ± 0,02	1,24 ± 0,11	0,47 ± 0,03
EPA/AA [8]	8,14 ± 0,70	5,17 ± 0,32	4,16 ± 0,60	4,29 ± 0,46

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REFERENCES

Boal, J.G., Hylton, R.A., Gonzales, S.A. & Hanlon, R.T. (1999). Effects of crowding on the social behaviour of cuttlefish (*Sepia officinalis*). *Contemporary Topics* 38 (1), 49-55.

Christie, W.W. (1982). *Lipids analysis*, 2nd edition. Oxford: Pergamon Press.

Domingues, P.M., Kingston, T., Sykes, A. & Andrade, J.P. (2001). Growth of young cuttlefish, *Sepia officinalis* (Linnaeus, 1758) at the upper end of the biological distribution temperature range. *Aquaculture Research* 32 (11), 923-930.

Forsythe, J., Lee, P., Walsh, L. & Clark, T. (in press). The effects of crowding on growth of the European cuttlefish *Sepia officinalis* Linnaeus, 1758 reared at two temperatures. *Journal of Experimental Marine Biology & Ecology*.

Horwitz, W. (1980). *Methods of analysis*, 13th edition. Washington D.C: Association of Official Analytical Chemists.

Kjeldahl (A.O.A.C., 1985). Official methods of analysis of the Association of Analytical Chemist. 14th Edition, Washington, U.S., 1018 pp

Lee, P.G. (1994). Nutrition of cephalopods: fuelling the system. *Marine and Freshwater Behaviour Physiology* 25, 35-51.

Navarro, J.C. & Villanueva, R. (2000). Lipid and fatty acid composition of early stages of cephalopods: an approach to their lipid requirements. *Aquaculture* 183, 161-177.