LIPID CHARACTERIZATION OF STARVED AND GRASS SHRIMP-FED CUTTLEFISH (*Sepia officinalis*, L.), DURING THE FIRST 7 DAYS AFTER HATCHING

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Introduction

Sepia officinalis is a promising species for commercial aquaculture. Nonetheless, the hatchling stage is where most mortality occurs. Domingues *et al.* (2004) suggested that imbalances in the lipid profile of prey may result in lower growth and higher mortality. Grass shrimp has been used as prey for this first stage culture with good results and suggested as a model prey for nutritional studies (Sykes *et al.* 2006). Grass shrimp nutritional content displays a lipid profile almost similar to that of cuttlefish eggs during embryonic development (Sykes *et al.* 2009). Despite the work of Castro *et al.* (1992) to determine the use/mobilization of lipids in cuttlefish juveniles, no previous experiments studied cuttlefish lipid mobilization at the hatchling stage in starved and fed animals, and from the first day after hatching (DAH).

Objective:

- To obtain further insight on the lipid mobilization of cuttlefish at the hatchling stage under starvation and grass shrimp fed conditions.

Conclusions:

- Cuttlefish seems to display a metabolic adaptation capacity which is feeding related;
- Grass shrimp lipid profile seems to be suitable to cover structural and energetic demands at first feeding of cuttlefish hatchlings.

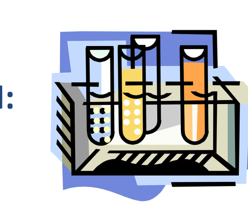
Material and Methods

- 700 fed cuttlefish hatchlings
- 700 starved cuttlefish hatchlings

- **Flow-through system**
- **Temperature** – 21.7±0.93°C
- **Salinity** – 39.2±0.21‰
- **Dissolved O_2** – 98.2±1.3%
- **Light Intensity** – 200 lux
- **14h Daylight cycle**

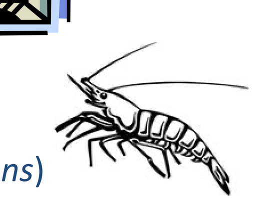
7 days

Sampling every 2 days from 1 DAH:



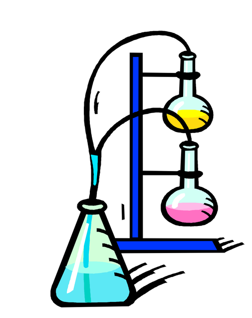
Samples:

- Fed and Starved Cuttlefish
- Prey - Live Grass Shrimp (*Palaemonetes varians*)

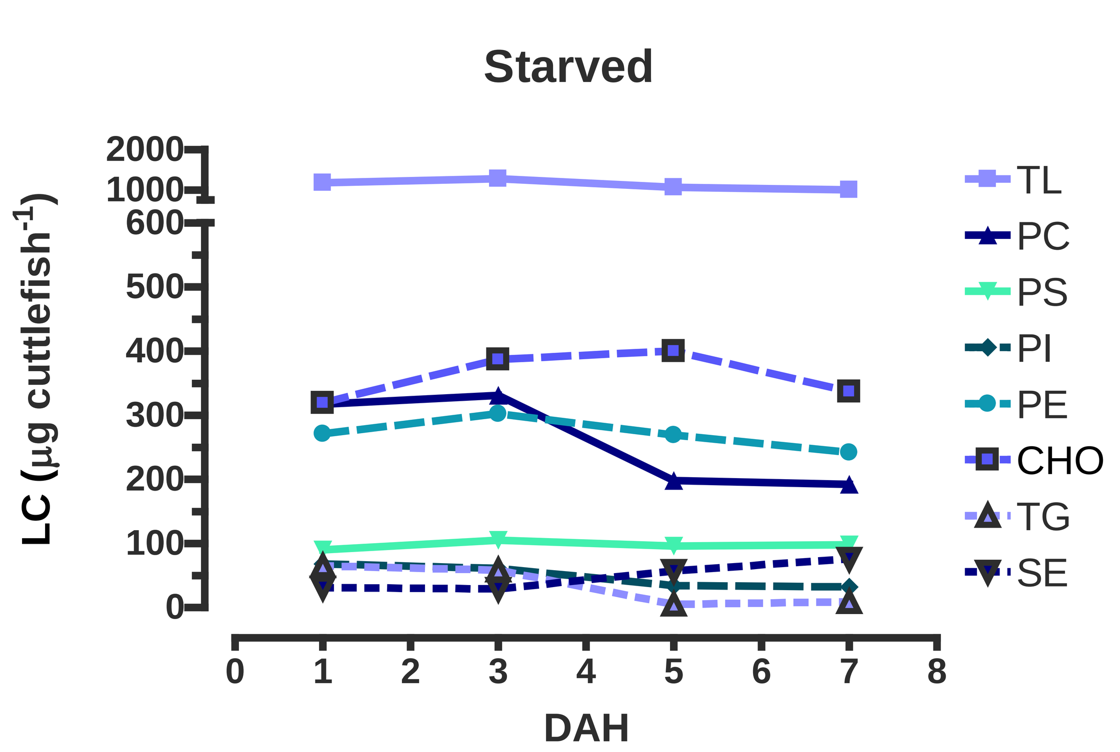


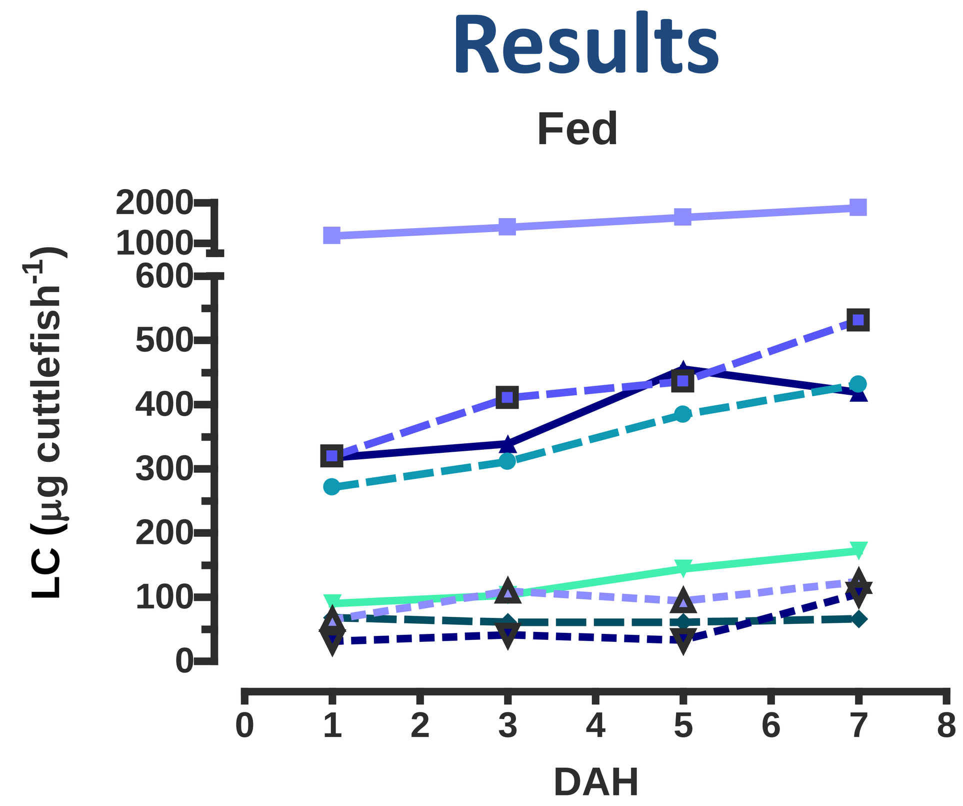
Biochemical Determinations:

- Total Lipid (TL) (Christie, 1982)
- Lipid Classes (Olsen & Henderson, 1989)
- Fatty Acids (Christie, 1982)



Results





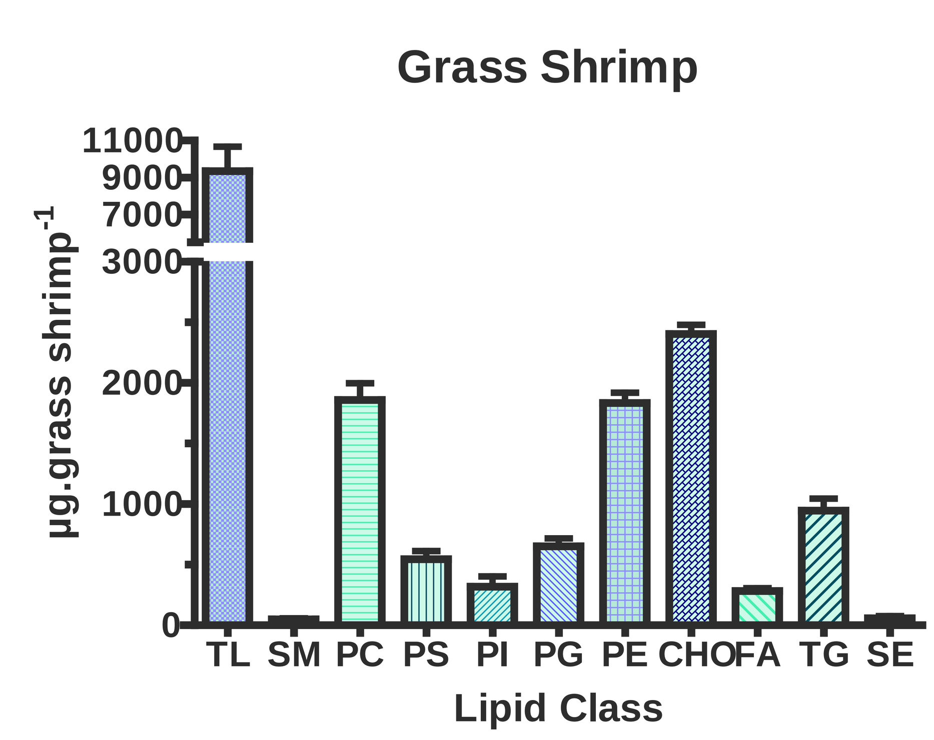


Table 1 - Fatty acid of TL (µg cuttlefish^{-1}) of starved cuttlefish hatchlings.

FA/DAH	STA1	STA3	STA5	STA7
16:0	112.3 ± 24.7[a]	115.2 ± 6.9[a]	92.5 ± 8.7[ab]	56.4 ± 3.6[b]
18:0	53.9 ± 10.7[ab]	58.1 ± 1.2[ab]	67.3 ± 5.6[b]	43.4 ± 2.9[a]
18:1 n-9	15.1 ± 1.9[a]	16.9 ± 0.3[a]	15.0 ± 1.4[a]	9.6 ± 0.5[b]
18:1 n-7	8.7 ± 1.0[a]	8.4 ± 0.0[a]	6.0 ± 0.4[b]	3.8 ± 0.1[c]
18:2 n-6	1.9 ± 0.1[a]	1.9 ± 0.2[ab]	2.5 ± 0.2[b]	1.9 ± 0.2[a]
20:4 n-6	5.5 ± 0.4[a]	9.2 ± 2.5[ab]	9.5 ± 0.7[b]	7.3 ± 1.0[ab]
20:5 n-3	77.1 ± 3.6[a]	76.7 ± 13.8[a]	69.9 ± 5.8[ab]	50.4 ± 7.4[b]
22:6 n-3	98.8 ± 3.8	105.5 ± 25.8	115.3 ± 7.9	83.2 ± 13.2
∑	459.6 ± 57.1[a]	487.8 ± 30.7[a]	469.6 ± 23.3[a]	316.0 ± 34.0[b]
Saturated	183.2 ± 39.3[a]	190.4 ± 9.3[a]	170.9 ± 15.4[a]	106.7 ± 6.9[b]
Monoenes	51.8 ± 6.7[a]	55.4 ± 1.2[a]	49.7 ± 4.5[a]	32.6 ± 1.4[b]
n-3	185.6 ± 7.4	193.0 ± 41.3	200.3 ± 14.7	144.8 ± 22.4
n-6	10.9 ± 0.9	15.3 ± 2.7	15.0 ± 1.0	11.4 ± 1.5
n-9	34.8 ± 3.6[a]	38.9 ± 1.2[a]	36.7 ± 3.7[a]	24.1 ± 1.3[b]
n-3 HUFA	181.7 ± 7.1	187.8 ± 40.5	190.6 ± 14.2	137.4 ± 21.3
n-3/n-6	38.6 ± 1.6[a]	29.0 ± 2.9[b]	28.1 ± 1.0[b]	25.0 ± 1.3[b]
EPA/DHA	0.78 ± 0.03[a]	0.74 ± 0.06[a]	0.61 ± 0.02[b]	0.61 ± 0.01[b]
AA/EPA	0.07 ± 0.00[a]	0.12 ± 0.01[b]	0.14 ± 0.01[bc]	0.14 ± 0,00[c]
AA/DHA	0.06 ± 0.00[a]	0.09 ± 0.00[b]	0.08 ± 0.01[b]	0.09 ± 0.00[b]

Results represent means ± S.D. (n≥3). Totals include minor components not shown. Superscript letter represent differences within the same row for p<0.05.

Table 2 - Fatty acid of TL (µg.cuttlefish^{-1}) of fed cuttlefish hatchlings.

FA/DAH	FED1	FED3		FED5		FED7	
16:0	112.3 ± 24.7[a]	147.1 ± 6.6[ab]	*	165.9 ± 8.5[b]	*	168.5 ± 4.6[b]	*
18:0	53.9 ± 10.7[a]	75.6 ± 5.6[b]	*	86.4 ± 4.2[bc]	*	103.8 ± 2.5[c]	*
18:1 n-9	15.1 ± 1.9[a]	24.6 ± 1.0[b]	*	25.9 ± 1.9[b]	*	43.9 ± 3.3[c]	*
18:1 n-7	8.7 ± 1.0[a]	13.5 ± 0.6[a]	*	13.4 ± 1.3[a]	*	26.6 ± 3.1[b]	*
18:2 n-6	1.9 ± 0.1[a]	3.5 ± 0.3[a]	*	3.3 ± 0.6[a]		13.3 ± 2.0[b]	*
20:4 n-6	5.5 ± 0.4[a]	14.0 ± 0.7[b]	*	16.1 ± 3.5[b]	*	18.3 ± 3.6[b]	*
20:5 n-3	77.1 ± 3.6	119.8 ± 17.2	*	119.8 ± 28.5	*	125.7 ± 21.0	*
22:6 n-3	98.8 ± 3.8	166.3 ± 22.3	*	174.5 ± 46.9		150.9 ± 30.5	*
∑	459.6 ± 57.1[a]	691.0 ± 39.3[b]	*	740.3 ± 106.1[b]	*	808.8 ± 51.6[b]	*
Saturated	183.2 ± 39.3[a]	243.7 ± 12.3[ab]	*	274.3 ± 13.3[b]	*	295.6 ± 7.1[b]	*
Monoenes	51.8 ± 6.7[a]	79.4 ± 1.9[b]	*	83.7 ± 6.6[b]	*	120.5 ± 6.7[c]	*
n-3	185.6 ± 7.4[a]	302.7 ± 41.0[b]	*	311.4 ± 79.5[b]	*	299.7 ± 55.6[b]	*
n-6	10.9 ± 0.9[a]	22.8 ± 1.6[b]	*	24.7 ± 5.1[b]	*	39.3 ± 6.2[c]	*
n-9	34.8 ± 3.6[a]	53.1 ± 1.2[b]	*	57.0 ± 4.1[b]	*	72.3 ± 2.5[c]	*
n-3 HUFA	181.7 ± 7.1	294.9 ± 40.9	*	303.2 ± 78.0	*	286.6 ± 53.8	*
n-3/n-6	38.6 ± 1.6[a]	27.5 ± 0.3[b]		27.3 ± 1.5[b]		18.4 ± 1.7[c]	*
EPA/DHA	0.78 ± 0.03[ac]	0.72 ± 0.01[ab]		0.69 ± 0.02[b]	*	0.84 ± 0.04[c]	*
AA/EPA	0.07 ± 0.00[a]	0.12 ± 0.01[b]		0.13 ± 0.00[bc]		0.14 ± 0.00[c]	
AA/DHA	0.06 ± 0.00[a]	0.09 ± 0.01[b]		0.09 ± 0.00[b]		0.12 ± 0.00[c]	*

Footnotes as Table 1. Asterisk represent differences between starved and fed hatchlings at the same period of time for p<0.05.

Table 3 - Fatty acid of TL(µg.grass shrimp^{-1}) of grass shrimp.

Shrimp Composition	
16:0	880.7 ± 106.3
18:0	437.5 ± 54.3
18:1 n-9	581.5 ± 67.0
18:1 n-7	416.1 ± 51.4
18:2 n-6	141.5 ± 28.8
18:3 n-3	102.3 ± 47.1
20:4 n-6	134.5 ± 24.2
20:5 n-3	1082.9 ± 197.9
22:6 n-3	621.4 ± 132.2
∑	5204.1 ± 803.9
Saturated	1496.6 ± 184.5
Monoenes	1224.6 ± 159.1
n-3	1769.1 ± 348.0
n-6	310.4 ± 59.6
n-9	628.3 ± 74.8
n-3 HUFA	1731.3 ± 340.0
n-3/n-6	14.6 ± 1.1
EPA/DHA	1.75 ± 0.07
AA/EPA	0.12 ± 0.00
AA/DHA	0.22 ± 0.01

Results represent means ± S.D. (n=12).

1) At hatching, cuttlefish displayed a lipid profile rich in phospholipids and CHO, as well as in 16:0, EPA and DHA;
2) During starvation, a general drop in PC, PI and TG, a preferential conservation of PE, PS and CHO, and an increment in SE were observed ;
3) A significant reduction in body contents of 16:0, 18:1n-9 and EPA, and the specific preservation of AA and DHA were also registered at the same period;
4) Differences in TL, LC and FAs among groups started to be noted when yolk reserves were exhausted (between the 3rd and 5th DAH);
5) The lipid profile of fed cuttlefish resembled that of dietary grass shrimp, which lead to increments of TL, structural lipids, 16:0 and the essential fatty acids EPA, DHA and AA.

- Grass shrimp displayed almost 10x more lipid content than day 1 hatchlings: was rich in PC, PE and CHO, and showed a moderate content in TAG.
- *P. varians* was highly rich in both 16:0 and 20:5 n-3 (EPA), and mildly rich in 18:0, 18:1 n-7, 18:1 n-9 and 22:6 n-3 (DHA);
- The overall FA profile of this prey was extremely rich in n-3 HUFA.

References: Castro et al. 1992. Mar. Biol. 114, 11-20; Christie, W.W., 1982. Lipid Analysis. Pergamon Press, Oxford; Domingues et al. 2004. Aquaculture. 229, 239-254; Olsen, R.E., Henderson, R.J., 1989. J Exp Mar Biol Ecol. 129, 189-197; Sykes et al. 2006. Vie Et Milieu-Life and Environment. 56, 129-137. Sykes et al. 2009. Aqua. Nut. 15, 38-53.

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