EFFECTS OF INCREASED TANK BOTTOM AREAS ON CUTTLEFISH (*Sepia officinalis*, L.) REPRODUCTION PERFORMANCE

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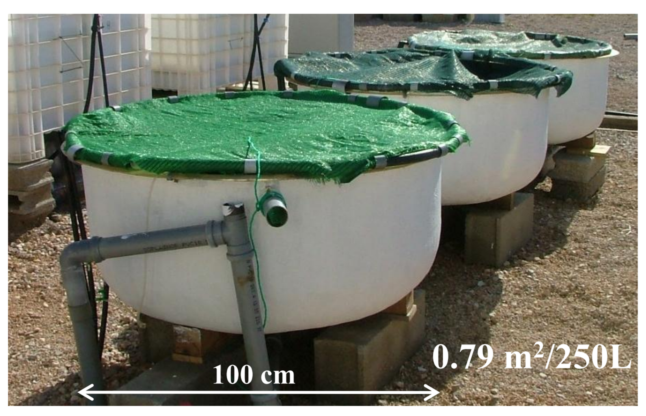
Research Questions:

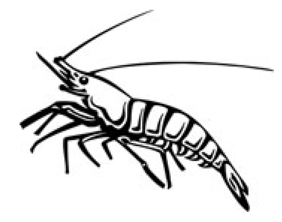
- How do different tank bottom areas / volumes influence cuttlefish growth and reproduction stages?
- Which tank setup is the best to enhance reproduction?

Conclusions:

- Lower mortality in bigger tanks;
- 9000L tanks seem to promote higher reproduction performance in terms of increased fecundity and egg viability.

Material & Methods





Replicates of 23 cuttlefish juveniles fed *ad libitum* on frozen *Palaemonetes varians*

Table 1 – Experimental conditions.

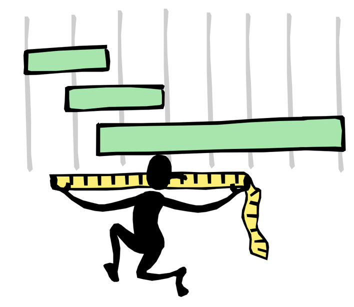
Tank Volume	250 L	750L	9000 L
Growth			
Temperature (°C)	15.4±1.52	15.0±1.53	15.0±1.50
Salinity (all)		36.0±0.89gL^{-1}	
Dissolved Oxygen (all)		98.2±1.3%	
Reproduction			
Temperature (°C)	21.2±3.38^{a}	19.0±2.17^{b}	20.5±2.87^{c}
Salinity (all)		36.8±0.83gL^{-1}	
Dissolved Oxygen (all)		95.0±5.2%	

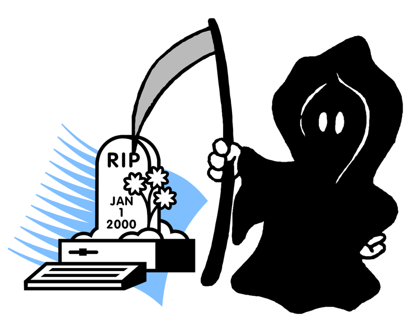
Superscript letter represent differences within the same row for p<0.05

Growth Stage

Sampling every 15 days:

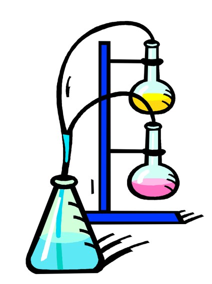
- Mean Wet Weight
- Instantaneous Growth Rate
- Biomass
- Mean Biomass Increase
- Total Absolute Mortality
- Mean Cumulative Mortality





Reproduction Stage

- Duration
- Fecundity
- Egg Biomass
- Number of batches
- ♀, ♂ , egg and hatchling weights
- Fertility
- Egg proximate composition



Results

Table 2 – Mean Wet Weight (MWW), Mean Absolute IGR (MAIGR), Total Absolute Mortality (TAM), Mean Cumulative Mortality (MCM), Biomass (B) and Mean Biomass Increase (B%) values for cuttlefish at the end of growth stage.

Tank volume	250L	750L	9000L	Anova/Kruskal-Wallis
MWW (g)	117.6 ± 59.99	100.6 ± 48.42	114.8 ± 48.14	p>0.05
MAIGR (%BW/d)	0.9 ± 0.08	0.7 ± 0.04	0.9 ± 0.11	p>0.05
TAM (cuttlefish)	23	12	2	-
MCM (%)	33.3 ± 9.05	17.4 ± 15.68	4.3 ± 6.15	p>0.05
B (g)	1802.8 ± 367.78	1911.7 ± 237.14	2526.4 ± 123.04	p>0.05
B% (%BW/d)	0.66 ± 0.323	0.75 ± 0.257	1.37 ± 0.122	p>0.05

Superscript letter represent differences within the same row for p<0.05

- **No differences found in growth;**
- **Lower mortality in replicates of 9000L.**

Table 3 – Duration of Reproduction Stage (DRS), Fecundity (F), Egg Biomass (EB), Individual Fecundity (IF), Eggs Sampled, Mean Egg Weight (MEW), Maximum and Minimum Individual Egg Weight (MaxIEW and MinIEW, respectively), Mean Female and Male Weight (MW ♀ and MW ♂, respectively), Sex Ratio, Number of Batches (Ba), Eggs per Batch (EBa), Viable and Non-viable Eggs, Egg viability and Mean Hatchling Weight (MHW) values for cuttlefish at the end of reproduction stage.

Tank Volume	250L			750L			9000L		ANOVA /
	T1	T2	T3	T4	T5	T6	T7	T8	Kruskal-Wallis
DRS (days)	78	112	66	19	34	47	38	89	p>0.05
F (eggs)	3014	1154	2465	28	973	3030	1397	16593	p>0.05
EB (g)	1443.7	441.4	843.2	10.9	383.7	914.7	613.9	7319.4	p>0.05
IF (egg.female $^{-1}$)	431	192	352	4	108	337	175	1383	p>0.05
Eggs sampled (n)	1779	908	1947	28	519	1652	837	5654	p>0.05
MEW (g)	0.548 ± 0.1107^{f}	0.397 ± 0.0612^{abc}	0.487 ± 0.0910^{de}	0.388 ± 0.0221^{ab}	0.379 ± 0.892^{bcd}	0.387 ± 0.0743^{a}	0.508 ± 0.0629^{e}	0.443 ± 0.1161^{cde}	**p<0.05**
MaxIEW (g)	1.197	0.733	0.898	0.436	0.775	0.730	0.974	1.034	p>0.05
MinIEW (g)	0.216	0.149	0.101	0.346	0.075	0.124	0.247	0.071	p>0.05
MW ♀ (g);(n)	185.9 ± 69.06; 7	120.3 ± 35.35; 6	137.4 ± 32.84; 7	125.1 ± 45.78; 7	113.1 ± 41.39; 9	133.8 ± 36.30; 9	148.7 ± 46.88; 8	173.8 ± 48.18; 12	**p<0.05**
MW ♂ (g);(n)	177,0 ± 109,25^{ab};6	154,0 ± 117,13^{ab};7	152,5 ± 61,35^{ab};8	236,6 ± 112,50^{ab};7	133,9 ± 56,11^{a};10	168,0 ± 75,28^{ab};8	353,5 ± 166,19^{b};9	228,4 ± 64,56^{ab};8	**p<0.05**
Sex ratio (♀/♂)	1.17	0.86	0.88	1.00	0.90	1.13	0.89	1.50	p>0.05
Ba (n)	19	14	23	1	5	17	9	23	p>0.05
EBa (eggs.batch^{-1})	159	82	107	28	195	178	155	721	p>0.05
Viable eggs (n)	2398	1114	2382	28	882	2936	1256	16537	p>0.05
Non-viable eggs (n/%)	616/25.7	40/3.6	83/3.5	-	91/10.3	94/3.2	141/11.2	56/0.3	p>0.05
Egg viability (%)	31	30	84	-	52	80	85	72	p>0.05
MHW (g)	0.101 ± 0.0318^{b}	0.072 ± 0.0127^{a}	0.107 ± 0.0104^{b}	-	0.110 ± 0.0156^{b}	0.088 ± 0.0111^{ab}	0.109 ± 0.0762^{b}	0.106 ± 0.0115^{b}	**p<0.05**

- **One of the 9000L tanks displayed the highest overall and individual fecundity and egg viability ever obtained in our facilities;**
- **Differences obtained within 9000L tanks might be related to sex ratios and differences in weight between females and males;**
- **Further studies regarding reproduction and by using a multidisciplinary approach are needed.**

Table 4 – Egg proximate composition for each tank (%).

Tank Volume	250L			750L			9000L		ANOVA
	T1	T2	T3	T4	T5	T6	T7	T8	
Moisture	80.12 ± 3.96	81.53 ± 3.31	77.94 ± 7.39	-	78.39 ± 5.41	77.87 ± 5.26	80.94 ± 5.46	84.68 ± 1.84	**P<0.05**
Total Protein	39.94±1.37^{bc}	46.20±0.79^{d}	35.27±0.23^{a}	-	39.65±1.86^{bc}	43.04±1.22^{c}	41.46±0.36^{bc}	39.23±0.99^{b}	**P<0.05**
Total Lipid	12.3 ± 3.10	9.8 ± 0.37	19.8 ± 8.34	-	17.1 ± 3.70	13.6 ± 1.75	16.1 ± 3.13	7.8 ± 1.52	**P<0.05**
Total Carbohydrate	21.4±4.50^{b}	21.7±1.06^{a}	12.8±8.07^{d}	-	15.7±2.35^{bc}	20.6±2.24^{a}	13.4±3.24^{c}	25.4±0.59^{bc}	**P<0.05**
Ash	26.4 ± 0.77	22.4 ± 0.10	32.1 ± 0.61	-	27.5 ± 1.36	22.7 ± 0.15	29.0 ± 0.28	27.6 ± 0.26	**P<0.05**

Superscript letter represent differences within the same row for p<0.05. Similar letter represents tank grouping using the Scheffé post-hoc test.

- **Differences in egg composition might have derived from spawners condition which depend on sex ratios and rearing conditions.**

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SEVENTH FRAMEWORK PROGRAMME

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