REPRODUCTIVE PERFORMANCE OF WILD-CAUGHT SPINELESS CUTTLEFISH *Sepiella inermis* IN CAPTIVITY – PRELIMINARY RESULTS

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BACKGROUND

The aquaculture sector has recognized the potential of cuttlefish culture in the industry as it can reach market size quicker when compared to finfish. Several technological bottlenecks in cuttlefish culture were acknowledged, such as semelparity, low fertility and fecundity, and the inability to sustain consecutive generations. This work aims to assess the reproduction performance of *S. inermis* from a wild-caught breeding stock in a recirculation aquaculture system (RAS).

EXPERIMENTAL METHODS

Animal collection

Sri Lanka East Coast
(8°21'46.5"N 81°23'38.4"E)

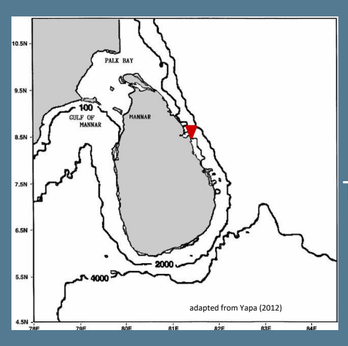


Fig. 1 – Bottom topography of Sri Lanka (depths are given in meters). Red arrow shows sampling location.

January 2024

Depth: 30 - 60 m
Temperature: 16 °C
Salinity: 30 ‰

Cuttlefish were collected with baited long scoop nets with a wooden handle by divers.

Experimental design

✓ *Recirculating aquaculture system*

Skid-mounted water treatment system connected to two tanks (A and B) with the volume of 2900 L (2.3 m diameter).

✓ *Cuttlefish*

31 wild-caught *S. inermis were* homogeneously distributed

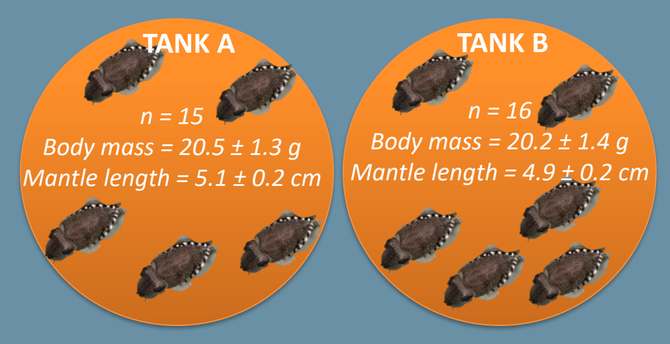


Fig. 2 – Diagram of the initial distribution of the cuttlefish. Results are shown as mean ± s.e.m.. No significant differences were found (unpaired t-test - Prism 10, GraphPad)

✓ *Experimental period:* 94 days (since animals arrive at the center, till the natural death of the last animal)

✓ *Feeding:* Krill (*Euphausia superba) ad libitum daily.*

✓ *Seawater parameters*

Table 1 – Seawater parameters during the experimental period. *Temperature, O_2, salinity and pH were measured daily (8 – 9 am), while ammonia, nitrites, nitrates and alkalinity were analyzed 2 times a week. Results are shown as mean ± s.e.m.*

Water parameter	mean ± s.e.m.
Temperature (°C)	27.2 ±0.07
Dissolved O_2 (mg·L^{-1})	6.59 ±0.01
Salinity (‰)	35.2 ±0.06
Ammonia (mg·L^{-1})	0.01 ± 0.00
Nitrites (mg·L^{-1})	0.016 ± 0.001
Nitrates (mg·L^{-1})	4.2 ± 0.16
pH	7.9 ± 0.01
Total Alkalinity (mg·L^{-1} as $CaCO_3$)	141 ± 1.7

RESULTS

Reproductive performance

✓ ***Reproduction Stage:*** 22 days
(n of days since the 1st batch of eggs layed till the last female dies)

✓ ***Fecundity*** *(total of eggs laid per tank)*
- Tank A = 0
- Tank B = 2008

▪ Individual Effective Fecundity (Fe)
(Fecundity/n of ♀ that reached the reproduction stage per tank)
- Tank A = 0
- Tank B = 401.6

▪ Individual Potential Fecundity (Fp)
(Fe + mean n oocytes)
- Tank A = 112.5
- Tank B = 479.8

✓ ***Biological data***

Table 2 – Biological data from S. inermis upon arrival and during the reproduction stage*, in which the Digestive Gland Index and Gonadosomatic Index were calculated by digestive gland mean mass·body mass^{-1} and gonad mass·body mass^{-1}, respectively. The Maturation Stage was according to Arkhipkin's (1992) scale. Results are shown as mean ± s.e.m.. Different superscript letters represent significant differences ($p < .05$, one-way ANOVA followed by the Tukey post-hoc test).*

		Tank A			Tank B		
		Total	Females ♀	Males ♂	Total	Females ♀	Males ♂
Initial	n cuttlefish	15	2	13	16	6	10
	Sex ratio (♀/♂)	0.15			0.6		
Reproduction stage	n cuttlefish	8	0	8	12	5	7
	Body mass (g)	21.1 ± 1.4	-	21.1 ± 1.4	20.0 ± 1.2	20.5 ± 2.1	19.6 ± 1.6
	Digestive Gland Index	0.02± 0.00	-	0.02 ± 0.00	0.03 ± 0.00^{ab}	0.04 ± 0.00^{a}	0.02 ± 0.00^{b}
	Gonadosomatic Index	0.01 ± 0.00^{a}	-	0.01 ± 0.00^{a}	0.03 ± 0.00^{cb}	0.04 ± 0.01^{c}	0.02 ± 0.00^{ab}
	Maturation Stage	VI (75%)	-	VI (75%)	V, VI (33%)	V, VI (40%)	IV, V, VI (29%)
	Sex ratio (♀/♂)	-			0.71		

✓ ***Reproductive Behaviour***

▪ Courtship

In tank B, it started at the end of the acclimation period. Observations of parallel swimming between pairs and males hovering over and alongside the females.

▪ Mating

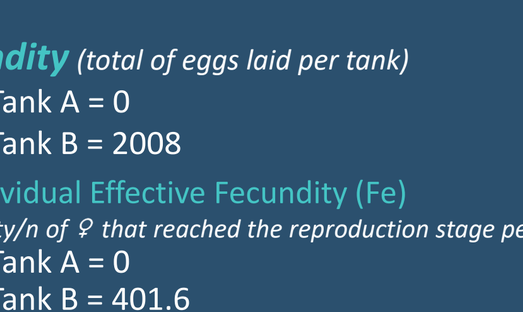


Fig. 2 – Cuttlefish mating before batch #2 was laid.

- Tank A = no mating was observed
- Tank B = started on 21st day of the experimental period.

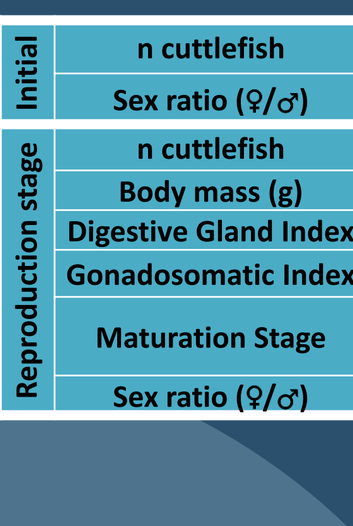


Fig. 3 - Female laying batch #2

▪ Egg laying

- Tank A = no eggs
- Tank B = started 7 days after mating observation. The female wrapped the stalk of each egg around the airlift (Fig. 3). Temporary mate guarding was observed.

✓ ***Eggs***
- n of batches
  - Tank A = 0
  - Tank B = 4
- Egg viability - Determined by *external morphology and color.*

✓ ***Embryonic phase***

The eggs were incubated at 27.5 ± 0.1°C in 35.2 ± 0.1 ‰.

▪ Duration of the embryonic phase (days)
- Batch #1 = 10-19
- Batch #2 = 9-21
- Batch #3 = 10-18
- Batch #4 = 9-19

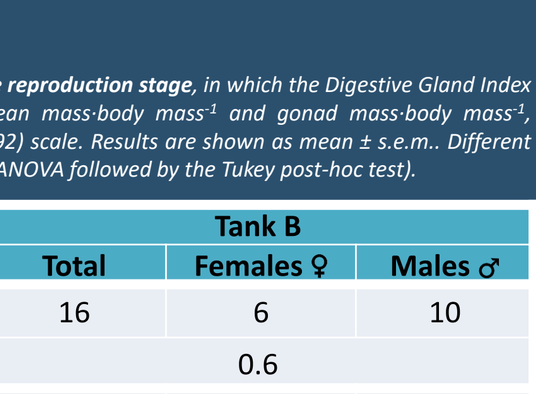
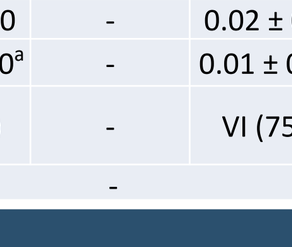


Fig. 4 – Different stages of the embryonic development in Sepiella inermis.



✓ ***Paralarvae***

Fig. 5 – S. inermis paralarvae (5-7 days after hatching) feeding with artemia.

Table 4 – Hatching data of S. inermis viable eggs and its paralarvae*. Results are shown as mean ± s.e.m.. Different superscript letters represent significant differences ($p < .01$, one-way ANOVA followed by the Tukey post-hoc test).*

Egg Batch	#1	#2	#3	#4
Hatching Rate %	57.7	58.5	88.1	87.7
n paralarvae	207	272	89	64
Paralarvae mass (mg)	14.2 ± 0.5^{a}	8.9 ± 0.6^{b}	9.4 ± 0.4^{b}	8.4 ± 0.4^{b}

Table 3 – Egg batch data from S. inermis breeding in RAS*, whereas Biomass = ∑ of each egg mass accounted per batch, Mean mass = average of individual egg mass. Significant differences were observed in all egg batches between viable and non-viable mean mass ($p < .01$, unpaired t-test). Different superscript letters represent significant differences ($p < .01$, one-way ANOVA followed by the Tukey post-hoc test).*

Egg Batch	#1		#2		#3		#4	
n	762		1030		123		93	
Biomass (g)	59.31		109.92		12.90		7.37	
Mean mass (g)	77.8 ± 1.5^{a}		106.7 ± 2.5^{b}		104.8 ± 3.4^{b}		79.3 ± 3.8^{a}	
% egg viability	47.1		45.1		82.1		78.5	
Viability	Viable	Non-Viable	Viable	Non-Viable	Viable	Non-Viable	Viable	Non-Viable
n	359	403	465	565	101	22	73	20
Biomass (g)	33.4	25.9	80.5	29.4	11.6	1.3	6.1	1.3
Mean mass (mg)	100.4 ± 1.9^{a}	60.4 ± 1.0^{A}	173.1 ± 3.7^{b}	52.1 ± 0.5^{B}	114.6 ± 3.4^{a}	59.9 ± 2.5^{C}	74.1 ± 1.4^{c}	64.9 ± 1.8^{AC}

CONCLUSIONS

- *S. inermis can be bred in recirculated aquaculture systems.*
- *Control over the sex ratio will define the fecundity obtained.*
- *Egg mass is an indicator of its viability*
- *% Egg viability is correlated with the hatching rate of each batch of eggs.*

Acknowledgments: We appreciate the assistance of our research team - Elise Marquis, Aadil Koothuparambil, Sajin Velakkandy and Fatima Zahra. Water quality analysis as invaluable help in sorting and weighting eggs was performed by the SeaWorld Animal Health team – Lab (Winelen, Hasitha, Poornima, Priya, Alberta, Mahnoor and Neil). Thank you SeaWorld Animal Life Support Systems Team for the technical input with the skids (Melbin, Resel, Naveen, Mahesh, Shijin, Krishnaraj, Ajay, Rayees, Ashiq, Kaniston, Resildon, Michael and Richard).