Impact of ocean acidification effects on marine clownfish sperm behavior, fertilization and embryonic developmental stages of *Amphipiron sebae*

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Abstracts

Atmospheric elevated carbon dioxide (CO_2) levels can cause ocean acidification. In the present study, ocean acidification effects on sperm behavior, fertilization and embryogenesis of clownfish *Amphipiron sebae* was assessed in captive condition. It was found that significant effects of decreased pH doesn't influence much on sperm behavior, whereas slight substantial changes in egg mortality and hatch rate were observed. The present study highlights that increasing level of ocean acidification may affect the population dynamics of *A. sebae*.

Introduction

The concentration of CO_2 in the atmosphere is increasing due to the anthropogenic activities and can decrease pH of ocean (Feely et al., 2004). The fishes and cephalopods have the efficient acid-base regulatory system whereas their early stages are more vulnerable to reducing hydrogen ion concentration of the ocean (Melzner et al., 2009). However, the studies on effect of reducing pH on early developmental stages of marine fishes are meager. Hence the present study was designed to investigate the sperm behavior and embryonic developmental stages of *A. sebae* in captive condition.

Materials and Methods

- **Sperm behavior was studied, followed by Frommel et al. (2010).**
- **Experimental and Embryonic developmental studies were done, followed by Munday et al. (2009) and Ramamathu et al. (2012)**

Results

Table1. Summary of carbon parameters system: At total alkalinity, Ct– total dissolved inorganic carbon pCO_2 - partial pressure of carbon dioxide T– Temperature S– Salinity

Carbon parameters										
Treatments	A_T (µmol kg^{-1})	C_T (µmol kg^{-1})	$_pCO_2$ (µatm)	Co_2 (µmol kg^{-1})	HCO^{3-} (µmol kg^{-1})	Co_3^{2-} (µmol kg^{-1})	pH	T (°C)	S	Ω_{arag}
Control	2195.052	1871.527	294.95	8.094	1628.185	235.248	8.15	27.7	28.5	3.931
Treatment 1	2045.785	2380.9	793.171	21.767	2199.491	159.641	7.83	27.7	28.5	2.667
Treatment 2	1956.564	2906.117	1564.753	42.942	2737.796	125.379	7.65	27.7	28.5	2.095

Table 2. Sperm motility and viability in control (C) and acidified conditions.

A. sebae Sperm behavior in control (C) and acidified (A) Conditions								
S. No	Total length (cm)	Total Weight (g)	% mot C	% mot A		Egg Viability C	Egg Viability A	
			Control pH 8.15	Treatment 1 pH 7.84	Treatment 2 pH 7.63	Control pH 8.15	Treatment 1 pH 7.84	Treatment 2 pH 7.63
1	9.8	20.71	79.23	77. 65	75.98	80.51	57.46	48.78
2	10	19.95	80.16	78.96	76.93	81.23	53.95	49.32
3	9.5	15.6	75.87	74.94	73.87	72.45	55.01	50.39
4	8.8	14.36	78.98	76.98	75.98	77.83	58.39	51.78
5	9	13.42	79.07	79.98	78.94	81.65	55.54	49.09
6	8.5	13.57	80.78	81.76	79.01	79.47	59.24	47.38
7	9.3	16.2	81.08	79.03	78.95	74.87	54.55	47.49
8	9.5	15.23	82.94	79.54	75.09	78.95	55.56	49.29
9	8.2	12.95	80.84	78.93	76.04	78.32	56.39	50.04
10	7.9	12.14	77.93	77.56	75.01	73.18	54.45	46.04
11	9.6	15.97	77.08	76.32	74.98	75.32	53.84	48.49
12	9.8	19.89	78.93	77.06	75.76	80.98	53.99	49.95
13	8.9	14.15	79.04	77.89	76.95	76.45	54.88	48.58
14	8.6	14	78.08	80.07	75.98	79.48	55.34	47.32
15	9.2	14.54	79.47	78.56	77.38	80.18	53.56	49.56
Mean		9.107	15.512	79.299	78.399	76.457	78.058	55.477	48.9
SD		0.622	2.571	1.736	1.753	1.567	2.857	1.708	1.439

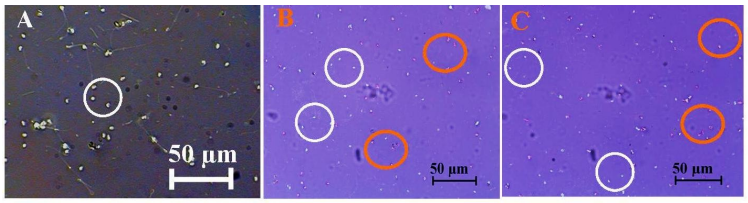

Fig.1. Sperm viability in control (pH 8.15) and treatment pH (7.64 and 7.84); image A with white round shows the viable sperm (un stained sperm cells) and B and C image orange round shows the non viable sperm (unstained sperm cells).

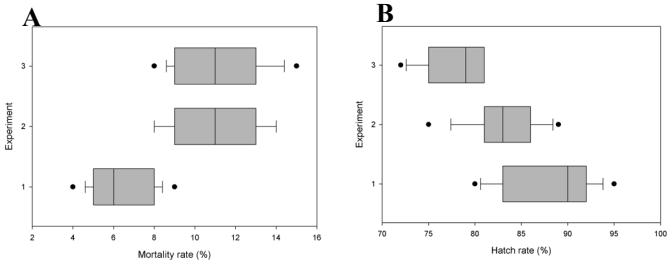

Fig. 2. Mortality rate (A) Egg hatch rate (B) in control (8.15) and treatment pH (7.84 and 7.63) conditions.

Fig. 3. *Amphipiron sebae*

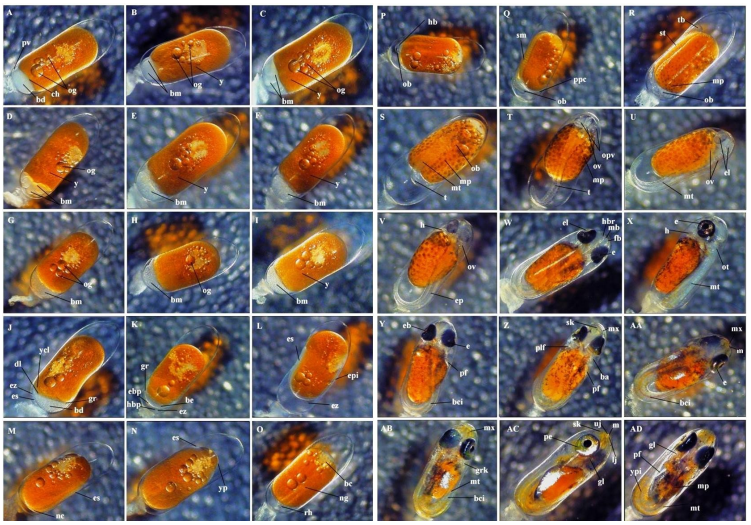

Fig. 4. Blastodisc formation stage (00:75 h); (B) 2- blastomere stage (00.95 h); (C) 4-blastomere stage (01.10 h); (D) 8- blastomeres stage (2.00 h); (E) 16- blastomeres stage (3.00 h); (F) 32- blastomeres stage (3.30 h); (G) 64- blastomeres (4.00 h); (H) Early blastula (6.00 h); (I) Late blastula/Morula (6.30 h); (J) Early gastrula (10.30 h); (K) late gastrula (17.30 h); (L) Epiboly (21.00 h); (M) Nerve cord formation (21.45 h); (N) Yolk pulg stage (23.00 h); (O) Blastopore closing (24.45 h); (P) Initiation of organogenesis (27.00 h); (Q) Notocord formation (30.00 h); (R) Somatogenesis - formation of head bud and optic bud (34.00 h); (S) Inversion of head (42.30 h); (T) Eye lens formation (51.30 h); (U) Myotomes formation (57.00 h); (V) Heart and heart beat formation (66.30 h); (W) Differentiation of fore brain mid brain and hind brain (72.30 h); (X) Appearance of otolith and muscle (93.00 h); (Y) Appearance of pectoral fin and blood circulation (96.00 h); (Z) Prominent pectoral fin, slight formation of pelvic fin and appearance of branchial arches (103.00 h); (AA) Advanced maxilla and pelvic fin (119.00 h); (AB) Formation of mouth and advanced gill rackers (157.00 h); (AC) difference of mouth upper jaw and lower jaw and appearance of pigmented eye (166.00 h) ; (AD) Embryo just before hatching (172.00 h).

Conclusion

Present study concludes that slight decrease in the pH of water in captive condition, doesn't affect the sperm behavior significantly. However, viability rate was subtly decreased.

Notably, there was no substantial difference in the developmental stages of fish egg due to the reduced pH of the water. On the other hand egg mortality rate was increased and egg hatch rate was decreased.

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Reference

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