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Effect of salinity and fly-ash on the embryonic development of the bigfin squid, *Sepioteuthis lessoniana*

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Abstract

Cephalopods breed in the near shore waters where the salinity fluctuations are more likely. Also, pollution from the adjoining industries is common in some of these areas. During the study, an attempt was made to find out the effect of salinity on the hatching of eggs of the bigfin squid, *Sepioteuthis lessoniana*. Embryonic development was found to be good at salinities 28ppt, 32ppt and 36ppt, while it was poor at 40ppt. No development was observed in 24ppt. Like wise, the effect of fly ash on the eggs of this species was tested at various concentrations in gram per liter of seawater. Mortality and premature hatching were observed at concentrations 25g, 10g, 5g, 1g, 0.5g and 0.1g. At 0.01g concentration, the hatching was normal but the hatchlings died within two days.

Key words: Effect of salinity and fly-ash, bigfin squid *Sepioteuthis lessoniana*

Introduction

Pollution of the coastal seas is becoming increasingly common with the rapid development of shore based industries. It is a usual practice that many of these industries situated on the banks of water bodies empty their highly lethal wastes into the coastal environment. At present the marine flora and fauna in some of such areas are compelled to survive in a polluted environment filled with several life threatening chemicals and trace metals, which are derived from the industrial and urban runoff. In the present study, the effects of various saline conditions which are resultant of the innumerable saltpans in Tuticorin, as well as the effect of fly - ash dumped by the Tuticorin Thermal Power Plant (TTPP), on the egg capsules of the bigfin squid *Sepioteuthis lessoniana* were studied. Cephalopods, in general, are short-lived and carnivorous. They show rapid growth rates and play an important part in oceanic and coastal food webs (Boyle, 1990). They are described as the "Climax of the invertebrate evolution" on account of their efficient neuro-sensory system (Nabhitabhata, 1996). Usually they try to avoid highly polluted areas by swimming away. But the magnitude of pollution is so high that these places and other coastal virgin grounds could get transformed into highly polluted areas in the near future and would be a testing time for marine life. The hyper saline water from the saltpans of Tuticorin is making life difficult for the near-shore living cephalopods. There is all possibilities that the various chemicals and heavy metals can get into human beings by consumption of cephalopods as they are considered to be good accumulators. Laboratory studies offer the only viable option for studying the growth of cephalopods during their delicate early life stages (Forsythe and Hanlon, 1989). The present study, therefore, was undertaken to find out the impact of various salinities and different concentrations of fly-ash on the egg capsules of *S. lessoniana*.

Material and methods

Sea water was taken in plastic PET bottles of 1.5l capacity and the salinities were adjusted to 24ppt, 28ppt, 32ppt, 36ppt and 40ppt. Freshwater was added to make up lower salinities while, for higher salinities aged seawater evaporated under the sunlight was used. The prepared salinities were checked with the help of a refractometer. Ten egg capsules containing 51, 52, 51, 52 and 49 embryos were introduced into salinities of 24ppt, 28ppt, 32ppt, 36ppt and 40ppt respectively (in triplicates). Fly-ash of quantities varying from 25g, 10g, 5g, 1g, 0.5g, 0.1g and 0.01g was mixed with 1l of seawater taken in 1.5l capacity PET bottles (in triplicates). Ten egg capsules with 4 embryos each were introduced into each concentration. The changes in the length of the egg capsules exposed to different salinities and different concentrations of fly-ash were measured with the help of a Vernier caliper to the accuracy of 0.1 mm. The incubation period and hatching rates at different salinities and the survival at various concentrations of the fly-ash were noted down. The mouths of the PET bottles were covered using polythene sheets and vigorous aeration was provided throughout. Water of the same salinity was exchanged whereas water for fly-ash test was not exchanged. Empty egg capsules and eggs that did not enlarge during the experiment were removed to prevent the growth of bacteria and fungi (Paulij, 1962). The significance of differences between the survival of the embryos at different salinities and concentrations of fly-ash were tested using two-way analysis of variance (ANOVA).

Results

Effect of salinity on developing embryos

At 24ppt salinity, the growth was very slow. On the 5th day, the number of live embryos got reduced drastically from 51 to 10. None of the embryos hatched out and cent percent mortality occurred on the 9th day. All the embryos changed into a white coloured mass. The length and diameter of the egg capsules kept on decreasing and came to a stagnant point at 100% death of the embryos.

Fifty-two embryos were maintained at 28ppt salinity. The time taken for hatching was found to be more (17 days) than all other salinities. The development was slow but clear and distinct. The length and diameter of the capsules increased gradually. At the time of hatching, the embryos were in well-developed compartments with well-defined boundaries. In this salinity, 4 embryos turned into white mass, while the remaining 48 embryos hatched within a period of 3 days. Until hatching the egg capsules remained healthy and intact.

At 32ppt salinity, out of the 51 embryos, 2 died and turned as white masses. The time taken for the first hatchling to come out was 13 days. Here also the length and diameter of the egg capsules increased gradually. The hatching period was 4 days and till hatching all the egg capsules were in good condition.

At 36ppt salinity, out of 52 embryos only 1 died. The incubation period was found to be 15 days and the hatching time 5 days. Here also the length and diameter kept on increasing till hatching. Distinct compartmentalization was observed in comparison to other salinities. The conditions of the embryos remained excellent till hatching.

Forty-nine embryos were maintained at 40ppt salinity. The development was very slow and compartmentalization was weak. The length and diameter of the egg capsules remained more or less same and tend to reduce slightly by the time of hatching. The egg capsule was found to degenerate on both ends with brown coloration and only 30 embryos hatched. The incubation period was found to be 11 days and hatching time just 2 days. The other embryos were under-developed and white in color. A small number of embryos were found to hatch out prematurely with yolk sacs hanging down (Fig 1). They sank to the bottom and died in a few hours. The length increment and the survival rate recorded at different salinities are given in Tables 1 and 2 respectively. Statistical analysis showed that there was significant difference between the survival of the embryos at different salinities at P<0.01 level.

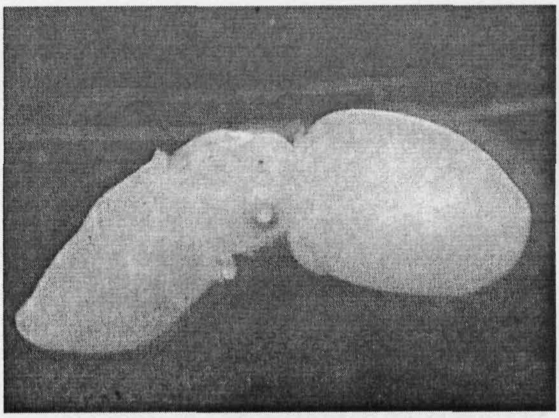

Fig 1. *Premature hatchling developed from the eggs exposed to 40 ppt salinity*

Effect of fly-ash on embryos

The egg capsules in the tanks containing higher concentrations of the fly-ash (25g, 10g, 5g and 1g) were found to be twisted and degenerated after 5 days of experiment (Fig 2). Setups with lesser concentrations of fly-ash had comparatively healthier eggs. Eggs exposed to higher concentrations did not show uniform growth and the development was observed to be very slow. Also the degenerated capsule wall led to premature

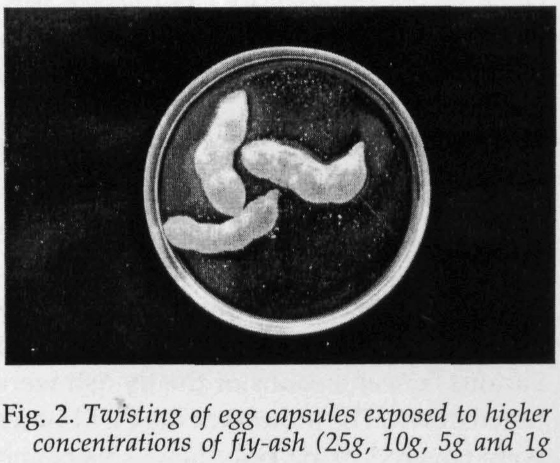

Fig. 2. *Twisting of egg capsules exposed to higher concentrations of fly-ash (25g, 10g, 5g and 1g*

Table 1. *Length increment (mm) in the egg capsules of* S. lessoniana *at different salinity*

Days	Control	24ppt	28ppt	32ppt	36ppt	40ppt
0	54.9	55.2	54.7	56.4	55.2	53.1
5	59.8	49.5	55.7	59.1	60.2	56.3
10	63.9	-	62.4	62.5	63.9	51.3
13	66.5	-	63.8	63.9	66.4	-
15	68.1	-	66.4	-	68.0	-
17	-	-	68.2	-	-	-

('-'denotes no reading)

Table 2. *Survival rate (%) of the embryos in the egg capsules of* S. lessoniana *at different salinity*

Days	Control	24ppt	28ppt	32ppt	36ppt	40ppt
0	100	100	100	100	100	100
5	100	18	100	100	100	100
10	100	0	94	98	98	73
15	100	0	92	96	98	61
17	-	-	92	-	-	-

('-' denotes no reading)

was observed but all the hatchlings died in two days.

At 25g concentration of fly-ash, 25 embryos died within the egg capsule. The remaining hatched out prematurely. The hatchling swam erratically and failed to survive. At 10g, 5g and 1g concentrations premature hatching was 17, 22 and 25 numbers respectively on the 10th day. At hatching on the 10th day of the experiment. Whereas at lesser concentrations (viz. 0.5g and 0.1g), premature hatching was found to occur on the 12th day of incubation. However, the egg capsules at 0.01g fly-ash concentration remained intact and did not show any premature hatching. The embryos at this concentration developed normally and hatched out on the 17th day. Cent percent hatching lower concentrations (0.5g, 10 and 0.1g) 6 embryos failed to develop while on the 12th day 30 and 34 embryos hatched out prematurely.

The length increment and survival rate at each fly-ash concentration is shown in Tables 3 and 4. As in the case of salinity, statistical analysis showed that there was significant difference between the survival

Table 3. *Length increment (mm) in the egg capsules of* S. lessoniana *at different concentrations of fly-ash*

Days	Control	25g/l	10g/l	5g/l	1g/l	0.5g/l	0.1g/l	0.01g/l
0	42.8	41.8	42.5	42.6	43.4	42.3	41.9	42.8
5	49.8	47.1	46.7	48.5	47.8	47.7	48.4	49.8
10	59.2	50.8	53.5	59.2	59.6	51.8	56.7	59.1
15	63.1	-	-	-	-	-	-	62.3

('-'denotes no reading)

Table 4. *Survival rate (%) of the embryos in the egg capsules of* S. lessoniana exposed to different concentrations of fly-ash

Days	Control	25g/l	10g/l	5g/l	1g/l	0.5g/l	0.1g/l	0.01g/l
0	100	100	100	100	100	100	100	100
5	100	37.5	42.5	55	62.5	90	95	100
10	100	0	0	0	0	76	85	100
15	100	0	0	0	0	0	0	100

rate at different concentrations of fly-ash at $P<0.01$ level.

Discussion

The eggs of cephalopods are highly sensitive to fluctuation in salinities. In the present study, cent percent mortality of the embryos occurred on the 9th day after exposure to 24ppt. Whereas only one death was observed among the embryo exposed to 36ppt. However, an earlier study indicated that highest survival and growth of the embryos of *Sepiella inermis* were obtained in the range of 28-32ppt salinity (Danakusumah, 2001). It was observed that at 36ppt, the length and diameter of the embryos kept on increasing till hatching. At 40ppt, there was 61.2% hatching which is far higher than reported by Omar *et al.* (2001) who observed 100% mortality for the capsules exposed to the same salinity. Nabhitabhata *et al.* (2001) found hatching rate over 85% in 24-32ppt and highest percentage of hatching was in 32ppt when compared to 36ppt observed in the present study. Also cuttlefish eggs incubated in 29, 32, 35 and 38ppt hatched at the rates of 52%, 84%, 93% and 62% respectively which was observed to be lower than what obtained in the current study. The hatching percentage was 0, 92.3, 96.1, 98.1 and 61.2 at salinity 24ppt, 28ppt, 32ppt, 36ppt and 40ppt respectively. The authors opined that the optimal salinity for incubation of cuttlefish eggs ranged between 28 and 36 ppt which appeared true even in the present work. Nabhitabhata *et al.* (2001) have found out that abnormal development and mortality of embryos occurred within 7 days before organogenesis that caused unsuccessful hatching of cuttlefish eggs and premature hatching was also reported at salinities outside the optimum range. In the present study also premature hatching was observed outside the optimum range of salinity (40ppt), where as at 24ppt the embryos failed to develop. Also statistical analysis revealed that there was a significant difference in the mortality exposed to different salinities. The hypersaline conditions which prevail in Tuticorin coastal waters due to the industrial effluents and those from saltpans could negatively affect the dynamics of cephalopod population. An impact similar to this has been observed in the growth of mangroves and molluscs (Kumar and Edward, 2001).

Fly-ash, by far is the major waste constituting to 88% of the total burned out products from power plants (Sandelin and Backman, 2001). Elements present in the coal fly-ash are not fully bound to the particles (Querol *et al.,* 2001). Thermal effluent not only can produce adverse effects on the coastal water but also affects the aquatic organisms such as planktonic community and bottom fauna (Naylor, 1965; Evans *et al.,* 1986; Markowski, 1960). Due to their sedentary or sessile nature, usually the benthic fauna bears the casualties of any environmental change (Philips and Segar, 1986; Bilyard, 1987). Major amounts of cephalopod eggs are laid on the structures which emerge from the sea floor. The normal incubation period for the eggs of *S lessoniana* is about 15-17 days, but in the experiment where fly-ash was introduced, premature hatching was observed. In the present study, at higher concentrations of fly-ash (25, 10, 5 and 1g), the growth of the embryos was found to be slow and not uniform. This could be due to the reason that metals, including Iron, Vanadium and Nickel are present in high concentrations as water-soluble salts in fly-ash (Schroeder *et al.,* 1987). The ovary of cuttlefishes, *Sepia officinalis* was reported to show a concentration of 12.7ppm of Cu and 28ppm of Zn. There is a quantitative transfer of Cu from the yolk to the embryonic body during development (Decleir *et al.,* 1970). The presence of fly-ash could be a possible reason for the premature hatching, degradation of the egg capsule and the ultimate death of the hatchlings. In the present experiment, at a concentration of 25g of fly-ash, the embryos died within the egg capsule. This observation is supported by an earlier report, which documented a high deposition of ash from the Tuticorin Thermal Power Plant (TTPP) at the rate of 4000ml/ day. There is no benthic fauna at the Tuticorin Bay which lie in close proximity to the Thermal Effluent Discharge Point of TTPP since the bottom was continuously disturbed by the deposition of ash (Kailasam and Sivakami, 2004). The present finding is in agreement with the views of Byrne and Kosta (1978) who have reported a highest concentration of fly-ash in lower marine animals (shellfish). The study revealed a positive correlation between the concentration of fly-ash and the mortality rates of the embryos. It was found out that there was a significante difference in the mortality of the embryos exposed to different concentrations of fly-ash as evidenced by the statistical analysis. The accumulation of fly-ash is very high and would be increasing day by day. Converting this waste into bricks has to be encouraged to meet the challenge of this type of pollution to some extend. The irrational expulsion of the hyper saline water from the salt pans too could be equally responsible for the decline in cephalopod population.

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