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STUDIES ON SURVIVAL OF POSTLARVAE OF *PENAEUS INDICUS* H. MILNE EDWARDS UNDER OXYGEN PACKING

K. KRISHNA KUMAR AND N. N. PILLAI

Central Marine Fisheries Research Institute, Cochin 682 031

ABSTRACT

A series of studies and experiments on the longevity of postlarvae of *Penaeus indicus* in flexible polyethelene containers with oxygen have indicated that a survival rate of 70% could be obtained at a packing density of 250 postlarvae (20 day old) per litre after 24 hours under oxygen packing. When the duration was increased to 36 and 48 hours 70% survival was obtained at 100 postlarvae per litre. However for the 8 day old postlarvae the same survival rate was obtained at packing densities of 300 and 150 postlarvae per litre for 24 and 36 hours respectively. Mortality of the postlarvae under oxygen packing has been attributed to cannibalism, accumulation of carbondioxide (indicated by the reduced pH) and increase in the ammonia.

INTRODUCTION

CONSEQUENT to the increasing demand for prawn, a major foreign exchange earner for the country, there has been an upsurge of interest in large scale culture of prawns. However the success of propagation and intensive prawn culture would largely depend on a viable seed collection from the wild or from the hatchery and its transportation techniques. Mohanthy and Patro (1974) have presented an account on the collection, transport and rearing of postlarvae of penaeid prawns from Chilka Lake in Orissa. Seed of *Penaeus monodon* and *P. indicus* have been transported in plastic bags under oxygen packing (De, 1977; De and Subrahmanyan, 1975). Subsequently Chakroborti (1978), Varghese (1978), Ali (1978), Dwivedi (1978), Mammen *et al.* (1978) Alikunhi (1980 a, b) and Selvaraj *et al.* (1980) have presented accounts on transportation of penaeid prawn seeds. However, only little is known about the requirements for transportation of seeds of cultivable prawns in India as also the factors that contribute to mortality in transit in a closed system. The present study on the survival of *Penaeus indicus* postlarvae of different sizes under oxygen packing has been undertaken with a view to elucidate some of the important environmental factors that contribute to the mortality and to find out the optimum density of postlarvae that could be packed in a unit volume of water for different durations.

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MATERIAL AND METHODS

For the present study, postlarvae of *P. indicus* of different sizes, reared at the Narakkal Prawn Culture Laboratory of CMFRI, Cochin were used. The transportation bags used were of 4 litre capacity (194 mm dia., 165 mm ht) made of H/guage, soft non-toxic PVC with a firm base and a double safety internal valve and caps provided with airtight washers.

Three experiments (Expt. I, II and III) were conducted using 20 day old (14-18 mm), 8 day old (8-12 mm) and 16 day old (13-15 mm) postlarvae respectively. The first two experiments (Expt. I and II) were carried out using clear sediment free filtered brackishwater of salinity 22-25 ppt and the third experiment with seawater of 33 ppt collected from inshore sea off Narakkal. Experiments I and II were carried out for three different durations - 24 hours, 36 hours and 48 hours. Expt. III was conducted for 48 hours. Packing densities tested were 25, 50, 100, 150, 200 and 300 per litre for Expt. I and III and 50, 100, 150, 200, 250, 350 and 400 per litre for the second experiment (Expt. II). All experiments were carried out in triplicate.

The postlarvae were collected in early morning hours from the nursery pools using velon screen and kept in basins with well aerated brackishwater/seawater. Feed was provided in these basins and the postlarvae were kept for a minimum of 2-3 hours, by which time they got well fed and acclimatized. While transferring them into the bags, care was taken to exclude the excess food material and excreta and the postlarvae alone were transferred, by gently washing through a wide-mouth funnel. The volume of water in all the bags were constant at two litres. Before closing the bags, pure oxygen was bubbled through the water for one minute and then the bags were tightly closed and filled with oxygen through the valve. The quantity of oxygen filled in the bags were kept constant at two litres. The packed postlarvae were kept in a jeep and transported to the headquarters of CMFRI, Cochin. The duration of this transport was about one hour covering a distance of 16 kilometres. In the laboratory the bags were kept in a stationary condition for the remaining period of the experiments. After the duration of each experiment the bags were opened and the surviving postlarvae were counted. The water was analysed for dissolved oxygen, pH and ammonia ($NH_3 + NH_4$). Water temperature was also noted as soon as the bags were opened.

The analysis of dissolved oxygen was carried out by Winkler's method (Strickland and Parson, 1968); the pH using a pH meter (EIL portable analog pH meter model No. 3030 with a combination pH electrode 0-14 pH and a temperature compensation electrode) and temperature using an ordinary thermometer. Ammonia was analysed by the method described by Solorzano (1968). The un-ionised NH_3 was calculated from this by the relation given by Whitefield (1975) as: % un-ionised ammonia $= 100\ [1 + \text{antilog}\ (pks - pH)]^{-1}$

Results

Experiment I

At a packing density of 25 postlarvae per litre (25/1) 96.7% of the postlarvae were found to be surviving after 24 hours under oxygen packing. For the same duration when the packing density was increased, the survival was found to decrease gradually upto a packing density of 250/1 (Fig. 1). The survival rate was above 70% upto the packing density of 250/1. When the packing density exceeded 250/1, the survival declined considerably with almost total mortality of the postlarvae. In the case of 36 hours the highest survival rate of 92.7% was found in the lowest packing density of 25/1, but it decreased to 76.3% at a packing density of 50/1. The mortality of the postlarvae between a packing density of 50 and 150 per litre was found to be gradual, unlike the heavy mortality noticed when the density was changed from 25 to 50 per litre. In the case of 150/1, the survival rate decreased to 64.3%. At a packing density of 200/1 and above the survival was very poor and was below 50%. Complete mortality was recorded at 300/1. The survival rate in the different packing densities for the 48 hours duration trial showed a similar pattern as in the case of the 36 hour duration experiment. The survival rate of above 60.0% was obtained upto 150/1.

The initial oxygen content of the water after filling was 15.0 - 16.0 ml O_2/l. From the oxygen values recorded at the termination of the experiment (Fig. 1) it was noted that the depletion of oxygen was observed only in the ent that in the longer duration experiments (36 and 48 hours) for the same packing rate, almost complete mortality was recorded at 24 hours most probably due to the high level of CO_2 (indicated by the low pH); afterward

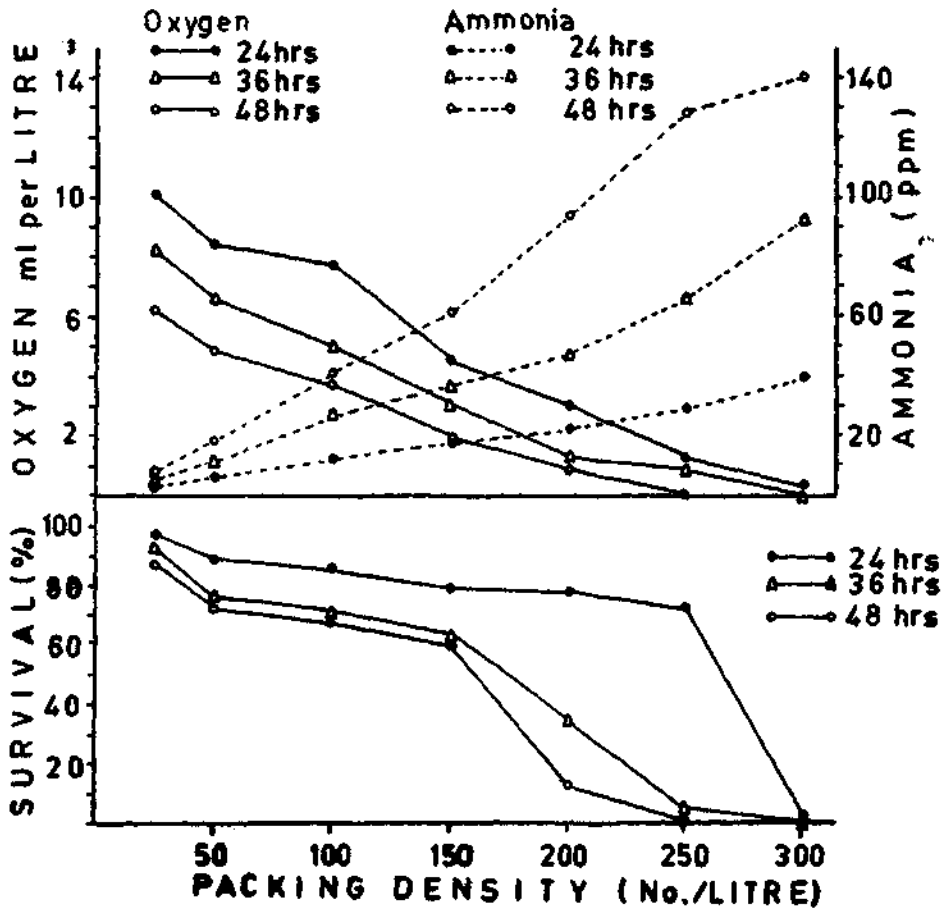

Fig. 1. Survival rate of *Penaeus indicus* seeds (20 day old) at different packing densities and durations and the final oxygen and total ammonia concentrations after the close of the experiment.

higher packing densities of over 200/l. This is particularly evident in the experiments of longer durations (36 and 48 hours). Thus in the 24 hour experiment, when the packing density was 300/l, the final oxygen level was 0.37 ml O_2/l and the survival was 1.6%. It is evid- the dead and decomposing postlarvae alone would have contributed to the oxygen depletion and the high values of ammonia recorded at the termination of the experiment.

Table 1 gives the pH recorded at the termination of the experiments. In all the higher

packing densities the pH has come down to the acidic level. Below a pH of 6.6 considerable mortality was recorded. The decrease in pH noticed in the higher packing densities may be due to the accumulation of carbondioxide.

The initial level of ammonia (NH_3+NH_4) in the brackishwater used for the experiment was 0.25 ppm. At the end of the experiment, the ammonia content was observed to be gradually increasing with the increase in the number of postlarvae in the 24 hour duration experiment (Fig. 1). However, in the 36 and 48 hours duration experiments, ammonia increased suddenly to a high level in the packing densities of over 200/1; this increase being greatly contributed by the dead and decomposing postlarvae observed in these bags.

Experiment II

The survival of the postlarvae in the 24 hour experiment was above 80% in the packing densities between 50 and 150/1 (Fig. 2). It was reduced slightly and was 73% when the packing density was increased to 250/1 for the same duration. At still higher densities of 300, 350 and 400 per litre the survival did not decline considerably and was 69, 62 and 57 percent respectively. When the duration was increased to 36 hours, 80% survival was recorded at a packing density of 50/1. It was varying between 70-77% when the packing density was raised to 150/1, about 63-64% at a density of 200-250/1 and above this packing density the survival declined to less than 60%. In the 48 hours duration experiment about 70% survival was observed at a packing density of 50/1. Above this packing densities, higher mortality was recorded compared to 24 and 36 hour, the survival rate being 50 and 60% at 100-200/1 and between 40-50% at 250-400/1.

There was no complete oxygen depletion in all the experiments and the minimum level of oxygen recorded was 1.3 ml O_2/1 in the highest packing density involving a duration of 48 hours (Fig. 2).

The trend of decline in pH was found to be similar to that in the previous experiment (Expt. I); the pH value falling below 7.0 in higher stocking densities (Table 1).

TABLE 1. *The pH values* recorded at the end of experiment*

Packing density numbers/L	24 hrs	36 hrs	48 hrs
EXPERIMENT I			
25	7.1	7.1	7.0
50	7.0	7.0	6.9
100	6.9	6.9	6.8
150	6.8	6.75	6.75
200	6.7	6.7	6.55
250	6.6	6.6	6.5
300	6.5	6.45	6.4
EXPERIMENT II			
50	7.4	7.35	7.2
100	7.3	7.18	7.08
150	7.2	7.09	7.0
200	7.1	7.0	6.9
250	7.0	6.88	6.78
300	6.9	6.8	6.72
340	6.8	6.7	6.62
400	6.7	6.6	6.48

* Each value represents average of three

In the 24 hour packing experiment the ammonia showed a gradual increase from 1.7 ppm at a packing density of 50/1 to 8.7 ppm at a packing density of 250/1. It was observed that the ammonia content increased at a rapid rate, beyond a packing density of 150/1 in the 36 and 48 hour experiments (Fig. 2).

Experiment III

The results of this experiment are summarised in Table 2.

At a packing density of 25 postlarvae per litre the survival was 93.3%. Ten percent decrease in survival was noticed when the packing density was doubled (50/1). A survival rate of 72% was recorded at 150/1 and about 60% at 200/1. Above this packing density, mortality was high.

At the beginning of the experiment the temperature of the medium was less than 27°C, pH 7.03 and oxygen 15.13 ml O_2/l.

There was no oxygen depletion even in higher packing densities and was found to be above 2.5 ml O_2/l at the termination of the experiment. The final pH of the medium in all the packing densities remained above 7.0 in contrast to the drastic reduction in pH to acidic levels observed in higher densities

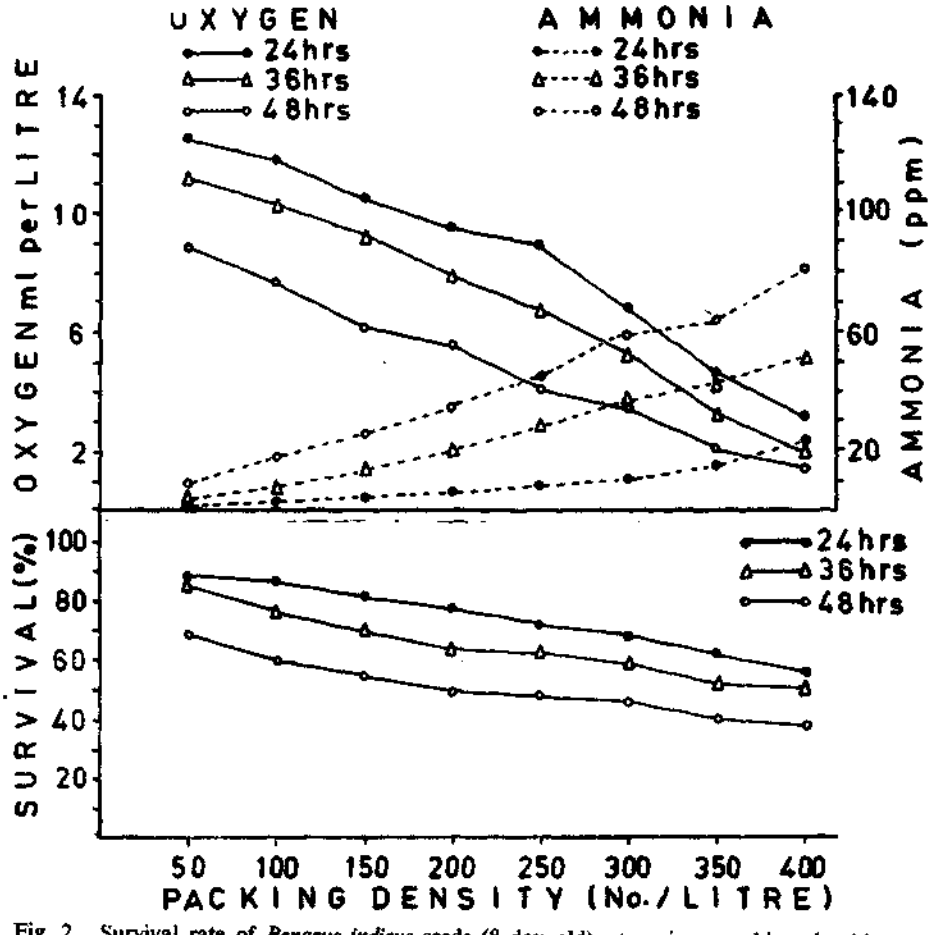

Fig. 2. Survival rate of *Penaeus indicus* seeds (8 day old) at various packing densities and durations and the final oxygen and total ammonia concentrations after the close the of experiment.

TABLE 2. *Survival rates of* P. indicus *seeds* (16 *day old*) *at different packing densities and final pH and oxygen values recorded in the medium* (values in parenthesis represents average)

Packing density Nos/l	Survival (%)		pH		Oxygen (ml O_2/l)	
25	90		7.55		11.28	
25	94	(93.3)	7.60	(7.58)	10.51	(11.31)
25	96		7.60		12.16	
50	77		7.55		8.51	
50	82	(81.0)	7.55	(7.53)	7.47	(8.04)
50	84		7.50		8.13	
100	74.5		7.45		7.68	
100	84.0	(75.8)	7.46	(7.45)	7.03	(7.17)
100	69.0		7.45		6.79	
150	70.3		7.45		5.45	
150	79.3	(72.1)	7.45	(7.43)	6.10	(5.82)
150	66.6		7.40		5.91	
200	72.0		7.35		5.19	
200	49.3	(60.5)	7.35	(7.36)	5.17	(5.08)
200	60.3		7.40		4.87	
250	26.4		7.20		3.08	
250	46.2	(39.3)	7.15	(7.16)	4.44	(3.80)
250	35.2		7.15		3.89	
300	31.93		7.08		2.57	
300	30.00	(31.9)	6.95	(7.06)	2.32	(2.69)
300	34.00		7.10		3.16	

in the experiments using brackishwater (Expt. I & II).

DISCUSSION

Packing density of postlarvae per litre of water is one of the factors to be looked into carefully while transporting the seeds to ponds to make prawn culture more economical and viable. When the packing density is more, survival will be less and vice versa. Therefore, to make the transportation economical and indirectly the prawn farming a profitable avocation, it is essential that maximum number of postlarvae must be packed in minimum quantity of water, and at the same time get good survival. The data of the present experiments were analysed keeping this in view. Table 3, shows the number of postlarvae (20 day old) required for initial packing (at different packing densities) and the quantity of water required for packing them to get approximately one lakh of postlarvae after 24, 36 and 48 hours under oxygen packing. Considering the number of postlarvae required and the volume of water needed for packing, the packing density at which 70% survival is obtained seems to be the best for transportation of *P. indicus* postlarvae.

The results of the present investigation suggesting a packing rate of 250/l for 20 day old *P. indicus* postlarvae for 24 hr duration and 100/l for 36 and 48 hours duration at a survival rate of 70% are comparable with that of De (1977). The high survival reported for same size seeds at a packing density of 100/l for 36 and 48 hours by Selvaraj *et al.* (1980), is obviously due to the continuous oxygenation provided in these experiments. The higher packing densities (450-900/l) suggested by Chakroborti (1978) would be useful only for short duration transporation. In general the survival of the 8 day old postlarvae was found to be relatively poor. This could be due to the more frequent moulting and the cannibalistic behaviour of the early postlarval stage as documented by Mammen *et al.* (1980) and Alikunhi (1980 b).

Though duration of transportation was only 14 hours, Alikunhi (1980 b) could take 1786 seeds/l by feeding with *Moina* sp. As the present investigations were carried out for longer durations of 24, 36 and 48 hours, feed was not provided in the bags. The fact that increase of ammonia and decrease in pH were noticed as the duration increased from 24 to 48 hour in the present study even without feeding, suggests doubtful utility of feed in closed system, as it may further add to the pollution of the medium. However, as suggested by Alikunhi (1980 a, b) for short duration transportation of small seeds (less than 10 day old) feeding and low temperature may reduce cannibalism and thus increase the survival to a considerable extent. That reduced temperatures between 17-18°C increased the survival of prawn seeds during transport has also been recorded by Shigueno (1980), Mammen *et al.* (1978), and Dwivedi (1980). Perhaps this explains the better survival recorded in Expt. III, where a survival of 70% was obtained at a packing density of 150/l compared to 100/l (Expt. I) and 50/l (Expt. II) for 48 hours duration experiment, since the temperature during the course of Expt. III was 26-27 °C and 30-31°C during Expt. I and II. The effect of temperature is also reflected in better pH and oxygen levels recorded at the end of the third

TABLE 3. *Initial requirement of number of postlarvae (20 day old) to get approximately one lakh of postlarvae after 24, 36 and 48 hours at different packing densities computed from the survival rate recorded in Expt I and the quality of water in litres required to pack the given number of postlarvae*

Packing density Nos/l	24			36			48		
	No. of post-larvae required	Quantity of water (litres)	% survival recorded in the present study	No. of post-larvae required	Quantity of water (litres)	% survival recorded in the present study	No. of post-larvae required	Quantity of warer (litres)	% survival recorded in the present study
25	1,03,413	4137	96.7	1,078,75	4315	92.7	1,14,548	4582	87.3
50	1,11,483	2230	89.7	1,31,062	2621	76.3	1,36,986	2740	73.0
100	1,17,925	1179	84.8	1,39,860	1399	71.5	1,42,248	1422	70.3
150	1,26,103	841	79.3	1,55,521	1037	64.3	1,62,602	1084	61.5
200	1,27,551	638	78.4	2,84,091	1420	35.2	–	–	–
250	1,37,174	549	72.9	–	–	–	–	–	–

experiment compared to the other two experiments. The better pH level could also be partly attributed to the buffering capacity of seawater.

It is well known that when prawn seeds are transported under oxygen packing, dissolved oxygen is not found to be the main cause of mortality (De and Subrahmanyam, 1975; De, 1977; Alikunhi, 1980). The minimum level of oxygen at which prawn seeds can survive ranges between 0.6-0.8 ml O_2/l (De and Subrahmanyam, 1975; Chakroborti, 1978). It is also known that the seeds of *P. indicus* can survive in healthy condition even when the oxygen level is at 2.5 ml O_2/l and the lethal level is at 0.2 ml O_2/l or below (Selveraj *et al.*, 1980). In the present investigation depletion of oxygen was found only in higher packing densities and when duration involved was longer; the oxygen depletion being obviously due to the dead and decomposing postlarvae remaining in the bags. Evidently the mortality of postlarvae is not due to the depletion of oxygen.

It is fairly well known that the pH of the medium during transport decreases concommittant with the accumulation of carbondioxide and cause large scale mortality of fish seeds (Mc Farland and Norris, 1958; Saha *et al.*, 1956). These workers have also reported that even when the dissolved oxygen of the medium is fairly high the accumulation of carbondioxide brings in marked decline in pH causing mortality of the seeds. In the present investigation high mortality was observed when the pH fell below 6.6 even when the oxygen level was within survival limits.

It has been reported that total ammonia measuring above 80 ppm at a pH of 6.83 (which is a NH_3 concentration of only 0.27 ppm, well below the toxic level reported) is found to be lethal to the larvae of *Macrobrachium rosenbergii* (Armstrong *et al.*, 1978). The present observation agree with this, as most of the postlarvae died when the ammonia (NH_3 + NH_4) reached above 80 ppm. Nevertheless the toxicity would not have been due to un-ionised ammonia (NH_3) as pH was on the acidic side in these packing densities. From Table 4

TABLE 4. *Concentration of un-ionised ammonia (NH_3) in the total ammonia observed in the present investigation (in ppm*)*

Stocking density number/L	24 hrs	36 hrs	48 hrs
EXPERIMENT I			
25	0.0309	0.0499	0.0470
50	0.0415	0.0775	0.0985
100	0.0662	0.1445	0.0738
150	0.0793	0.1439	0.2104
200	0.0782	0.1658	0.2571
250	0.0806	0.1758	0.2796
300	0.0868	0.1929	0.2429
EXPERIMENT II			
50	0.0290	0.0549	0.0942
100	0.0395	0.0901	0.1502
150	0.0531	0.1163	0.1799
200	0.0535	0.1415	0.1929
250	0.0598	0.1470	0.1855
300	0.0623	0.1560	0.2148
350	0.0661	0.1541	0.1739
400	0.0757	0.1398	0.1674

*Each value represents average of three

it can be seen that in the higher packing densities where the total ammonia is high, the un-ionised ammonia is at a very low concentration as the pH is low. Therefore it is reasonable to assume that the higher content of the ionised (NH_4) form itself would have affected the postlarvae adversely.

The results of the present investigation have provided certain basic information on the survival of *P. indicus* postlarvae at different packing densities over varying periods under

oxygen packing. The results indicated a survival of 70% could be obtained at 250/1 after 24 hours for the 20 day old postlarvae. When the duration was increased to 36 and 48 hours the same survival rate was obtainable at 100/1. However for the 8 day old postlarvae, same survival rate was obtainable at 300/1 and 150/1 packing densities for 24 hours and 36 hour experiments. The survival was poor in the 48 hours experiment even in lower packing densities. The relatively poor survival of the smaller postlarvae, particularly when the duration involved was longer has been attributed to cannibalism. It has also been able to collect information on certain environmental factors which cause mortality in a closed system. With the ratio of oxygen to water maintained at 1:1 as in the present investigation, depletion of oxygen was not found to be the cause of mortality. Mortality of the postlarvae has been related to accumulation of carbondioxide (indicated by the low pH) and increase in ammonia, besides cannibalism.

References

ALIKUNHI, K. H. 1980 a. Summary. *Proceedings of First National Symposium on shrimp farming*. Session 1. Publ. MPEDA. p. 17.

———— 1980 b. Observation on mass rearing of penaeid and *Macrobrachium* larvae at the regional shrimp hatchery, Azhikode, during 1979-1980. *Bull. Dep. Fish., Kerala*, **11** (1): 20-24.

ALI, HAMEED 1980. Summary. *Proceedings of First National Symposium on Shrimp Farming*. Session 1. Publ. MPEDA, p. 18.

ARMSTRONG, A.D., D. CHIPPENDALL, A. W. KNIGHT AND J. E. COLT 1978. Interaction of ionised and unionised ammonia on short term survival and growth of prawn larvae, *Macrobrachium rosenbergii*. Biol.Bull. (*Woods Hole*), **154**: 15-21.

CHAKROBORTI, R. K. 1978. On the oxygen requirement of brackishwater prawn seeds and their transport without oxygen packing. *Science and Culture*, **44** (9) 422-423.

DE, D. K. AND M. SUBRAHMANYAM 1975. Transport of Badga *P. monodon* seeds under oxygen packing. *Ibid.*, **41** (12): 588-589.

————1977. On the procurement and transportation of Chapra, *P. indicus* seed under oxygen packing. *Journal of the Inland Fisheries Society of India*, pp. 189-190.

DWIVEDI, 1980. Summary. *Proceedings to the First National Symposium on Shrimp Farming*. Session 1. p. 17.

MAMMEN, T. A., G. N. PANICKER, A. S. SHENOY, ELIZABATH LEELA GEORGE. A. V. VARGHESE AND K. K. CHANDRAN 1978. Procurement of prawn fry by MPEDA. *Proceedings to First National Symposium on Shrimp Farming*. p. 83-86.

MC FARLAND. W. N. AND K. S. NORRIS 1958. Control of pH and carbondioxide buffers in fish transport. *Calif. Fish and Game.*, **44** (4): 291-310.

MOHANTHY, S. K. AND J. N. PATRO 1974. A note on collection, transport and rearing of postlarval prawns (Penaeids) of Chilka Lake in Orissa. *Proc. Symp. Maricult. Mech. Fish.*, p. 40.

SAHA, K.C., D.P. SEN AND MAZUMDAR 1956. Studies on the mortality in spawn and fry of Indian major carps during transport. Part III. Effect of inimical substances in the medium, on spawn life and their control. *Indian J. Fish.*, 3 (1): 135-140.

SELVARAJ, G.S.D., K.J. MATHEW AND K. N. GOPALAKRISHNAN 1980. Techniques for the collection and transportation of prawn seeds. *Marine Fisheries Information Service Technical and Extension Service*, **19**: 11-12.

SHIGUENO, K. 1975. *Shrimp culture in Japan*. Publ. Association for International Technical Promotion, Tokyo, Japan p. 153.

SOLORZANO, L. 1969. Determination of ammonia in natural waters by the Phenol-hypochlorite method. *Limnology and Oceanography*, **14** (5): 799-801.

STRICKLAND, J. D. H. AND T. R. PARSONS 1968. *A manual of sea water analysis*. Fisheries Research Board of Canada, Ottawa.

VARGHESE, P. O. 1978. Trade in cultivable prawn seeds. *Indian Sea Foods.*, **14** (1): 6-18.

WHITEFIELD, M. 1974. The hydrolysis of ammonium in sea water - a theoretical study. *J. Mar. Biol. Ass. U. K.*, **54**: 565-580.