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Effect of Packing Density, Salinity and Temperature on the Survival and Duration of Oxygen Packed Seed of *Penaeus indicus* During Transporation

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Abstract

Transportation of large quantity of oxygen-packed shrimp seed involves several complex problems. This paper deals with the effect of four levels of packing density (200, 300, 400 and 500 PL/l), three levels of salinity (20, 25 and 30 ppt) and two levels of temperature ie., ambient (30 ±1°c) and lowered temperatures (23 ± 2° C) on the survival and duration of oxygen-packed seed (PL20) of the Indian white shrimp, Penaeus indicus during transportation. Specially designed hard plastic transparent jars fitted with facilities for oxygen packing under uniform pressure (0.2 Kg/cm²) were used. The survival and duration increased significantly at lowered temperatures. Salinity of 20-25 ppt was found to give longer duration (except at 100% survival) and higher survival rate. With increase in packing density, there was considerable reduction in the survival and duration. Significant difference was noted in the duration of 100% survival, referred to as the safe durationof transport of the oxygen-packed seed, amongst the different packing densities, salinities and temperatures. The water quality parameters *viz.*, pH, dissolved oxygen, free carbon dioxide and ammonia-N were also studied initially and finally at 70% survival and their changes taking place in the closed mileu, summarised.

Introduction

Transportation of shrimp post-larvae in see-through plastic bags partially filled with brackish water and oxygen (under pressure) is a common practice, well accepted by shrimp hatchery operators and farmers alike. Nature of the packing medium (water plus oxygen) and density of the seed packed is often based on a matter of experience and rarely on concrete scientific information. The congested mileu of the tightly closed, oxygen - packed bags may cause moulting of some individuals in transit. The moulted ones and any dead ones are likely to disappear after a while as a result of being preyed by the active shrimps in the bags. Packing more numbers on gratis by the hatchery operators in order to please their customers may only worsen the situation. The unaccountable loss of innumerable shrimp seed in transit cannot be treated lightly if one takes into account the financial returns obtainable from their grow out culture. So far, only little is known about the optimum requirements for the transportation of the valuable shrimp seed. Research work in this line include that of Shigueno (1975), De and Subrahmanyam (1975), Mammen *et al.*, (1978), Hamid and Mardjono (1979). Alikunhi *et al.*, (1980), Franklin *et al.*, (1982), Krishnakumar and Pillai (1984), Tenedero and Villaluz(1985) and Pillai *et al.*, (1992). More recently, transportation studies on the post-larvae of *Macrobrachium rosenbergii* were also carried out by Jayasree-Vadhyar *et al.* (1992). Nauplii of the tiger shrimp, *Penaeus monodon* had been transported experimentally under oxygen packing by Muthuraman *et al.* (1993).

There is paucity of information regarding the interrelationship of various factors such as packing density of the shrimp seed, salinity of the packing medium, temperature during transport and other water quality parameters, all of which may directly or indirectly govern the rate of survival of the shrimp seed and duration of their transport. Hence, the present study was taken up with the main aim of determining the effect of packing density, salinity and temperature together with the other water quality parameters on the survival and duration of transport of the post-larvae of *P. indicus* under oxygen packing.

Materials and Methods

Hatchery-reared post-larvae of *P. indicus* (PL20) of average weight 10 mg were used for the study. They were initially maintained in 1 ton capacity aerated tank under ambient temperature of 30 ± 1°c and were fed ad *libitum* using powdered dried clam and minced prawn meat. Twenty four hours prior to oxygen packing, the post-larvae were conditioned in the following manner. They were counted and divided into three lots of 800 each and were acclimatised to salinities of 20, 25 and 30 ppt in separate tanks. Feeding was not carried out in these tanks. Half the number of post-larvae from each tank was then separated into two lots. Pre-cooled isosaline water was slowly added to one of these lots, until the water temperature was lowered to 23± 2°c and the other lot was kept at ambient temperature of 30± 1°c. Transparent hard plastic jars of 600 ml capacity were used for oxygen packing. The screw type lid of each jar was fitted with a one-way valve for regulating the flow of oxygen as well as for facilitating the reading of oxygen pressure on a pressure gauge. A Bourdon type pressure gauge with a precision of 0.02Kg/cm² fitted with a pressure resistant hose, regulating knob and pressing nipple served to fill in oxygen from an oxygen cylinder. This facilitated confirmation of uniform initial oxygen pressure within the experimental jars (Jayasree-Vadhyar *et al.*, 1992).

The shrimp seed were packed at four different packing densities of 200, 300, 400 and 500 PL/1, each at three different salinities of 20, 25 and 30 ppt, at ambient temperature of 30 1oc and at lowered temperature of 23 ± 2°c. They were counted and transferred into the experimental jars with 100 ml water. Precooled water was filled in those jars which were observed at lowered temperature by placing them in troughs containing ice-cooled water. Immediately after the transfer, the jars were closed tightly and filled with oxygen from the oxygen cylinder under uniform pressure of 0.2 Kg/cm². While filling oxygen,care was taken to displace the air initially present inside the jar, with oxygen. To effect this, after filling oxygen initially, it was completely released by pressing the valve. This was repeated 3-5 times to ensure complete displacement of air with oxygen. The jars were periodically shaken to more or less simulate transport conditions. Jars which were filled with 100 ml water for each of the salinities and with oxygen at 0.2Kg/cm² at ambient and lowered temperatures, but without shrimp seed were opened immediately after filling oxygen, for collecting water samples initially.

Time of initial mortality of the oxygen-packed seed was recorded by making hourly observations. Thereafter, the number of survivors was counted at two hourly intervals until 70% survival occurred. The jars were then opened, samples of packing medium were collected for water quality analysis to determine the changes in their levels. The water quality parameters analysed were dissolved oxygen (Winklers method); free carbon dioxide (Alkalimetric titration method), ammonia-N (Phenol-hypochlorite spectrophotometric method) and pH (Potentiometric method) (Strickland and Parsons, 1972). A mercury bulb thermometer having a precision of 0.1oc was used for reading temperature and a salino-refractometer for reading salinity.

Results and Discussion

The effect of packing density on cumulative percentage survival was significant ($p<0.05$). The cumulative percentage survival at 12h was reduced from 90% to 70% when the packing density was increased from 200 to 500 PL/I. The decrease in percentage survival for a fixed duration of transport with increase in packing density is reported by De and Subrahmanyam (1975), Mammen *et al.*, (1978), Hamid and Mardjono (1979), Franklin *et al.*, (1982), Tenedero and Villaluz (1985) and Jayasree-Vadhyar et al. (1992). The major cause of mortality has been attributed to cannibalism. Post-larvae in transport container were reported to consume as much as 150% of their body weight by Alikunhi *et al.*, (1980). Addition of live food organisms was recommended by them to avoid cannibalism during transport. In the present study feeding was not done, to avoid pollution of the packed media. However, the mortality observed towards the later period of transportation at 200 and 300 PL/1 was not due to cannibalism.

The details of the duration of 100% survival referred to as safe durationof transport down to 70% survival of the oxygen-packed post-larvae are shown in Fig. 1. Packing density showed inverse relationship with the duration of transport. At packing densities of 200, 300, 400 and 500 PL/I, 100% survival could be observed for a duration ranging from 6.5 to 8.5h; 3.2 to 5h; 2 to 2.5h; 1.3 to 1.5h at ambient temperature, respectively. The corresponding safe duration at lowered temperature ranged from 22 to 42.5h, 8.25 to 9h; 6.25 to 6.75h and 4 to 4.75h. The effect of packing density on the safe duration of transport was significant ($P<0.05$) at ambient and lowered temperatures. Lowering of temperature, increased the safe duration of transport significantly ($P<0.05$). At lowered temperature, 300 PL/I could be transported with 100% survival for 8h, whereas at ambient temperature only 200 PL/I could be transported with the same survival for that duration. Temperature is an important factor which determines the survival of shrimp seed during transportation. Higher the temperature, higher is their cannibalistic tendency (Shigueno, 1975; Hamid and Mardjono, 1979; Alikunhi *et al.*, 1980), oxygen consumption (Bishop *et al.*, 1980) as well as ammonia and carbon dioxide excretion (Spaargaren *et al.*, 1982).

Duration of 70 -100% Survival of Oxygen - packed *P. indicus* Post - larvae at different Packing densities, Salinities and Temperatures.

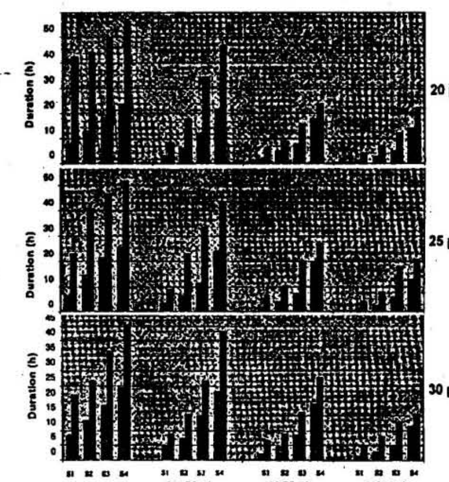

Fig. 1. Duration of 70-100% Survival of Oxygen - packed P.indicus Post - larvae at different Packing densities, Salinities and Temperatures.

The different salinity levels were found to have significant effect ($P<0.05$) on cumulative percentage survival and on safe duration. However, critical difference analysis of means showed that the salinities of 20 and 25 ppt formed the same homogenous group in the majority of percentage survival rates. So a salinity range of 20-25ppt apparently yields better survival rates in *P. indicus* seed transportation. The salinity-temperature interaction was also found to influence the survival of the shrimp seed during transportation. In general, the temperature seemed to affect the percentage of survival more than salinity. The salinity-temperature interaction effect was significant at lowered temperature. Towards the later period of transportation, cannibalism was not observed. The apparent cause of mortality in the final stages may be due to deteriorating water quality. The interaction of salinity and packing density influenced the survival of the shrimp seed significantly. The critical difference analysis revealed that packing density apparently affected the survival more than salinity during the earlier period of transportation. The interaction effect of these factors was significant towards the later period. The direct relationship between cannibalism and packing density has more influence on the survival soon after packing, than the interaction effect of salinity and packing density. During the later period the post-larvae were quite stressed, reducing their cannibalistic behaviour. The interaction of temperature and packing density was also significant. At the lowered temperature 500 PL/l could be transported for 12h against 300 PL/l.at the ambient temperature with 80% survival. By lowering of temperature, the number of post-larvae per unit volume of water could be increased as it enhances the survival by decreasing cannibalism, moulting and metabolic rate. At normal temperature when shrimps are crowded without food they tend to become more cannibalistic (Alikunhi *et al.*, 1980).

The details of the water quality parameters are summarised in Table 1. The initial dissolved oxygen in the oxygen-packed jars was 29.9 - 31 ppm at the ambient temperature and 33.1ppm at the lowered temperature. The final dissolved oxygen level in the jars was well above the lethal limits even at the highest packing density of 500 PL/l at 70% survival ie.,15ppm at the ambient temperature. The lowered temperature ensured a higher initial and final oxygen levels in the jars than the ambient temperature. Ammonia-N levels increased with increase in packing densities from 200 to 400 PL/l. At 500 PL/l, due to short duration of 70% survival, the ammonia-N levels were very low at the end. The highest ammonia-N levels of 60.38ppm was recorded at 400 PL/l at the end of 19 h. In the higher salinities, the levels of ammonia-N were generally higher. The reduction of temperature from $30\pm 1°c$ to $23 \pm 2°c$ lowered the accumulation of ammonia-N levels by 1.2 - 17.11 times. Increase in carbon-dioxide levels in the jars showed direct positive relationship with packing density at the ambient temperature. The initial carbon-dioxide values were nil, whereas the final values reached as high as 86 ppm at the highest packing density of 500 PL/l. In the lower packing densities of 200 and 300 PL/l, the increase in carbon-dioxide was only slightly higher than that at lowered temperatures whereas, there was remarkable difference in carbon-dioxide production with change in temperature in higher packing densities of 400 and 500 PL/l. Inspite of high carbon-dioxide values, the pH was not below 7. As long as the accumulation of carbon dioxide does not bring down the pH of the water to the

Table 1. Decrease in oxygen levels and increase in ammonia-N & free carbon dioxide levels in oxygen-packed jars with *P.indicus* post-larvae under different levels of salinities, packing densities and temperatuers at 70% survival.

Salinity (ppt)	Packing density (PL/l)	Temperature (°C)	Oxygen (ppm)	Ammonia-N (ppm)	Free carbon-diaoxide (ppm)
20	200	30±1	9.38	22.16	53.224
		23±2	8.27	4.57	49.28
	300	30±1	13.25	26.96	59.13
		23±2	11.6	9.41	55.19
	400	30±1	11.8	45.04	72.93
		23±2	8.6	12.13	59.13
	500	30±1	14.4	34.28	85.73
		23±2	11.03	27.16	78.84
25	200	30±1	6.9	16.08	49.28
		23±2	4.09	4.57	49.28
	300	30±1	11.18	50.16	59.13
		23±2	9.4	9.41	53.22
	400	30±1	12.6	60.38	74.90
		23±2	9.5	19.9	68.99
	500	30±1	13.2	50.67	83.77
		23±2	12.9	2.96	39.42
30	200	30±1	5.9	13.36	55.19
		23±2	3.1	2.96	49.28
	300	30±1	8.5	46.79	68.99
		23±2	9.4	9.416	59.13
	400	30±1	11.85	60.38	76.87
		23±2	10.3	14.25	59.13
	500	30±1	14.18	56.43	82.79
		23±2	8.55	6.19	39.42

acidic side, mortality due to its accumulation may not take place. Further, the high levels of dissolved oxygen help in reducing the harmful effects of carbon dioxide accumulation to some level.

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