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Optimization of packing and transportation of the fingerlings of cobia (*Rachycentron Canadum* (Linnaeus, 1766)) and silver pompano (*Trachinotus blochii* (Lacepède, 1801))

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In mariculture, often the locations of fish hatcheries and farming are distantly situated, and hence, there is a need to transport the seeds/seedlings to a longer distance with maximum survival rate. In the present study, length and weight of fingerlings, duration of starvation, packing density, water volume, water temperature and oxygen level in the packing bags were standardized to ensure maximum survival during transportation. The cobia fingerlings with mean length and weight of 48±12 mm and 3.0±1.0 g, respectively were transported for a duration 48 h at 25 °C with 100 % survival at a packing density of 2.5 fingerlings per litre (L) (7.5±2.5 g/L). Similarly, silver pompano fingerlings with mean length and weight of 24±0.48 mm and 0.6±0.08 g, respectively were transported for a duration of 48 h at 25 °C with 100 % survival at a packing density of 18.75 fingerlings/L (11.25±1.9 g/L). Experiments on bulk transportation of pompano fingerlings in 1000 litres capacity HDPE tanks containing 600 litres of seawater with continuous aeration using oxygen cylinders fitted in a transportation vessel revealed that the silver pompano fingerlings can be transported for a duration of 48 h at 25 °C and 5 – 6 ppm dissolved oxygen concentration with 100 % survival at a packing density of 8.3 fingerlings/L (4.98±0.8 g/L).

[**Keywords**: Cobia, Packing density, Seed transportation, Silver Pompano, Survival rate]

Introduction

In mariculture, the actual farming locations are generally situated at far places from the hatcheries, especially in countries like India; thereby generating a necessity to transport the fingerlings either by road, rail or air with minimal transportation cost and maximum survival of fish fingerlings/seedlings. Handling stress leading to increased metabolic rate of the fish, low oxygen levels and rapid accumulation of toxic metabolites such as ammonia and carbon dioxide in a closed system are the important factors contributing to fish mortalities during transportation[1-4]. A cost-effective packing method that would require less water volume and higher packing densities is very much needed for efficient packing and transportation of the seedlings. However, the accumulation of metabolites in a small and restricted environment deteriorates the water quality quickly, resulting in a low survival rate during transport.

Starving the fish to be transported for a certain period of time plays a major role in maintaining the water quality during transport as it avoids stress, oxygen demand for digestion, faecal excretion and regurgitation of food materials while transiting. Similarly, water temperature to a tolerable level plays an important role in the transport of live fishes as it reduces the excitement in fishes and metabolic activities; thus, reducing the metabolic wastes and aids sustaining the water quality[1]. Various transport techniques have already been documented for multiple fish species[1-6] from different regions around the globe. However, there is no documented report on this aspect for the hatchery-reared cobia and silver pompano fingerlings except Resley *et al.*[7], Benetti *et al.*[8], Colburn *et al.*[9] and Stieglitz *et al.*[10] which are on live transport of the cobia fingerlings from the hatchery to farm with varying salinity and packing biomass. Reports suggest that the use of anaesthetics did not produce a significant reduction in the stress during fish transport[11,12] and the fishes lose their equilibrium and settle down during higher levels of anaesthesia, which leads to asphyxia and mechanical damage[9]. This necessitates a suitable transport protocol for fish seeds without the use of chemicals, particularly anaesthetics.

Since there is no standard method for shipping these species in India, and due to an ever-increasing demand for these fishes from the farmers, experiments were carried out to standardize the packing density for transport of hatchery-reared cobia and silver pompano with respect to the following parameters *viz.*, length and weight/biomass of fingerlings, duration of starvation, duration of transport, volume & temperature of water and oxygen levels in the packing bag which influence the carrying capacity of transport containers and thereby optimizing the procedure for their transport without the use of any anaesthetics and chemical buffers.

Materials and Methods

The study is conducted on the hatchery-produced seeds of cobia and silver pompano using two different modes of transportation *viz.*, oxygen-filled polythene bags and bulk containers fitted with aerators.

Hatchery rearing of the cobia and the silver pompano larvae

Newly hatched larvae of cobia and silver pompano were reared separately in 2000 l capacity Fibreglass Reinforced Plastic (FRP) tanks with a water volume of 1800 l. After 45 days of hatchery rearing as per the protocols of Gopakumar *et al.*[13], the cobia fingerlings attained a mean length and weight of 48±12 mm and 3.0±1.0 g, respectively. Whereas, the silver pompano fingerlings raised as per the protocols of Nazar *et al.*[14] attained a mean length and weight of 24±0.48 mm and 0.6±0.08 g, respectively. The fingerlings thus raised were used for the present study. The water quality parameters were recorded using multi-parameter kit (Eutech instruments – PCD 650 model) at the time of packing and at every 24 h of transportation.

Experiment 1: Transport of hatchery-reared cobia fingerlings in polythene bags

The fingerlings of cobia with a mean length and weight of 48±12 mm and 3.0±1.0 g, respectively were starved for 24 h before packing in polythene bags. The polythene bags were 17 inches wide and 70 inches in length, and each bag was made double layered by making a knot in the centre and by reversing one half inside out. Each bag was filled with 8 litres of filtered seawater (salinity: 34 ppt, pH: 7.5 – 8.5, oxygen: 5 – 6 ppm) and pre-cooled to 25±2.0 °C. Fishes were packed in the bags at varying densities of 10, 15, 20, 25, 30, 35 and 40 numbers per bag. These bags were then filled with medical-grade oxygen gas in a ratio of three-fourth of oxygen and one-fourth of water with the fishes. The survival of fingerlings was recorded at every 6 h interval. Triplicates were maintained in each density. A set of small ice packs were kept uniformly in between the bags in order to prevent a drastic rise in temperature.

Experiment 2: Transport of hatchery-reared silver pompano fingerlings in polythene bags

The fingerlings of silver pompano, having a mean length and weight of 24±0.48 mm and 0.6±0.08 g, respectively were starved for 24 h prior to packing and packed in the same manner as described above (except the salinity, which was maintained at 25 ppt) with varying packing densities of 50, 100, 150, 200, 250, 300 and 350 fingerlings per bag. Triplicates were maintained at each density and the survival of the fingerlings was recorded at every 6 h interval.

Experiment 3: Transportation of the cobia and silver pompano fingerlings in bulk containers

Bulk transportation of live fishes, whether fingerlings or adults, is a laborious and challenging task since any misstep could lead to devastation. Preliminary trials for the bulk transportation of the cobia and the silver pompano fingerlings were carried out in large tanks of 1000 l capacity to identify the prerequisites and assess the conditions of transportation with minimum mortality and consequently optimize the protocols.

The cobia fingerlings of the same length and weight as mentioned above were starved for 24 h prior to transportation and were kept at varying densities of 500, 700 and 900 numbers in a 1000 l capacity HDPE tank filled with 900 l of filtered seawater. The pompano fingerlings of the same length and weight as mentioned above were also starved for 24 h prior to packing and were kept at varying densities of 2000, 3000, 4000, 5000 and 6000 numbers in a 1000 l capacity HDPE tank filled with 600 l of filtered seawater. The tanks were supplied with oxygen through aeration tubes connected to the oxygen cylinders fitted in the vehicle. The level of aeration is maintained in such a way that the dissolved oxygen is maintained at an optimum level (5 – 6 ppm), and the water temperature close to 25 °C during transport. Duplicates were maintained in each density, and the survival of fingerlings was recorded at every 6 h interval for the entire 48 h of transportation in the vehicle.

Statistical analysis

A completely randomized design was used in all the experiments. Treatment means were compared using Analysis of Variance (ANOVA) followed by

Duncan's Multiple Range Test (DMRT) when the difference was significant at 5 % level ($P < 0.05$). All the percentage data were normalized using arcsin or square root transformation prior to statistical analysis[15].

Results

Experiment 1: Transport of hatchery-reared cobia fingerlings in polythene bags

The survival of cobia fingerlings was recorded up to a maximum duration of 48 h in all the packing densities. Statistical analysis revealed that there was no significant difference ($P > 0.05$) among the packing densities of 10, 15 and 20, but showed a significant difference ($P < 0.05$) from the higher packing densities of 25, 30, 35 and 40. One hundred per cent survival for the entire period of 48 h was observed in the packing densities of 10, 15 and 20 fingerlings per bag and the lowest survival of 12.5 % in the density of 40 fingerlings per bag (Fig. 1). Survival rates decreased gradually with the increased packing densities from 25, 30, 35 and 40. However, the decrease was gradual at the packing densities of 30 and 35, with mortality starting after 18 h and a rapid decrease in survival was noted in the packing density of 40 fingerlings per bag, in which the mortality commenced after 12 h of packing. If the duration of transportation is less, the packing density of the fingerlings can be increased, which would have an added economic advantage. The water quality parameters analyzed and recorded at the time of packing as well as at every 24 h interval are given in Table 1. Overall, the increased values of temperature and ammonia and the decreased values of pH, dissolved oxygen and salinity were observed, as anticipated in the transportation process. The variations in these parameters are due to relatively higher metabolic rate of cobia.

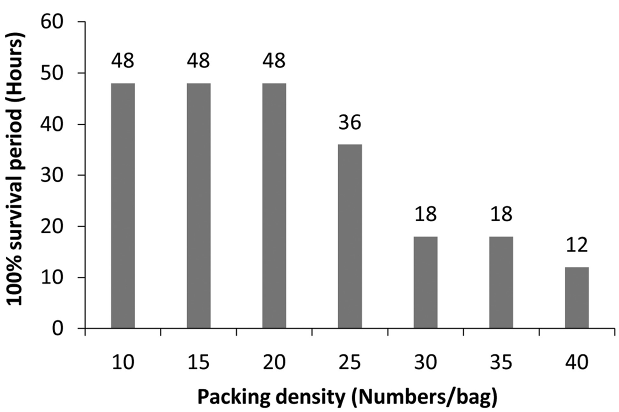

Fig. 1 — Survival of hatchery reared cobia fingerlings in polythene bags during transportation with reference to duration and packing density

Experiment 2: Transport of hatchery-reared silver pompano fingerlings in polythene bags

One hundred per cent survival was recorded in the packing densities of 50, 100 and 150 fingerlings per bag for the entire period of 48 h (Fig. 2). Statistical analysis revealed that there was no significant difference ($P > 0.05$) among the packing densities of 50, 100 and 150; however, showed significant difference ($P < 0.05$) from the higher densities of packing (200, 250, and 300). Incremental increase in the number of fishes per bag beyond 150 initiated the onset of mortality from 18 h, 12 h and 6 h, corresponding to the packing densities of 200, 250, and 300 fingerlings per bag. The lowest survival was recorded in the packing density of 350 fingerlings per bag, in which the mortality commenced after 6 h of packing and a significant decline in the survival was noted between 12 to 36 h, *i.e.*, from 80 to 21 %. However, the decrease in the survival rate was gradual, and the number of fingerlings that could be packed with 100 per cent survival for the longest duration (48 h) experimented was found to be 150

Table 1 — Water quality parameters (Mean±SE) recorded at the time of packing and various time intervals of transportation

Parameters	Values at the time of packing	Values during transportation in polythene bags filled with oxygen				Values during bulk transportation in tanks with continuous oxygen supply			
		Cobia		Pompano		Cobia		Pompano	
		24 h	48 h	24 h	48 h	6 h	12 h	24 h	48 h
Temperature (°C)	24.5±0.5	25.0±0.7	26.3±0.6	26.0±0.5	27.3±0.6	25.1±0.6	26.9±0.7	26.5±0.5	27.4±0.7
pH	7.9±0.3	7.6±0.2	7.3±0.3	7.4±0.4	7.2±0.3	7.8±0.5	7.2±0.6	7.7±0.2	7.5±0.2
Dissolved Oxygen ($mg\ l^{-1}$)	5.9±0.2	5.1±0.3	4.7±0.3	5.2±0.4	4.8±0.3	5.7±0.7	5.4±0.8	5.8±0.1	5.6±0.2
Ammonia ($mg\ 100ml^{-1}$)	0.01±0.00	0.19±0.04	0.28±0.10	0.13±0.04	0.23±0.09	0.08±0.07	0.15±0.09	0.13±0.07	0.19±0.09
Salinity-Cobia (ppt)	34.0±0.5	34.0±0.8	33.8±0.7	—	—	33.7±0.5	33.1±0.7	—	—
Salinity-Pompano (ppt)	25.0±0.5	—	—	24.5±0.6	24.1±0.5			24.6±0.4	24.4±0.4

fingerlings per bag for 48 h. Similar to cobia, if the duration of transportation is less, the packing density of the silver pompano fingerlings can be increased to have an economical advantage. The notion that the higher the packing density for a longer period of transit, the higher the mortality holds. The water quality parameters analyzed and recorded at the time of packing as well as at every 24 h interval are given in Table 1. Overall, the increased values of temperature and ammonia and the decreased values of pH, dissolved oxygen and salinity were observed, as anticipated in the transportation process. The variations in these parameters are due to the fast-moving behaviour of silver pompano though the packing density also has a significant role.

Experiment 3: Transportation of the cobia and silver pompano fingerlings in bulk containers

The fingerlings of cobia did not yield favourable results in the bulk transportation experiments, whereas the silver pompano yielded positive results with maximum survival. The bulk transportation of cobia fingerlings showed 100 % survival only up to 12 h in the lowest density (500/tank) and up to 6 h and 4 h in the densities of 700 and 900 per tank, respectively. The survival was significantly affected beyond 6 h in the higher densities and beyond 12 h in the lowest density. As the transportation duration is limited due to packing density, the bulk transportation of cobia was not found economical.

The silver pompano fingerlings showed hundred per cent survival up to 48 h, at all the packing densities except for the highest density studied (6000/tank). The highest packing density of 6000 per tank showed mortality to a minor degree up to 42 h (98.9 % survival at 42 h) followed by a further 2 % reduction at 48 h (Fig. 3). Considering these results, which demonstrate a meagre mortality, it is prudent to transport the fingerlings of silver pompano at a maximum packing density of 6000 per tank provided the transport period is less than 36 h and above which the mortality occurs.

The water quality parameters analyzed and recorded at the time of packing as well as at every 24 h interval are given in Table 1. The values are given for 6 h and 12 h in the bulk transportation of cobia as the fishes did not survive beyond 12 h in all the packing densities which might be due to the higher metabolic rate and the morphological orientation of the body of cobia. The variations in the water quality parameters recorded in the silver pompano transportation were not distinct up to the packing density of 6000 numbers per tank beyond which the changes in these parameters might have caused the mortality.

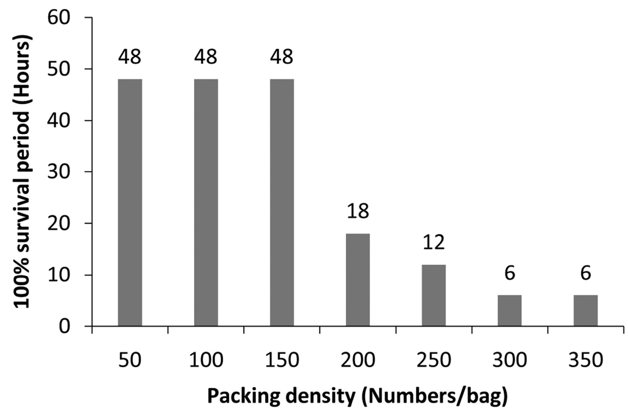

Fig. 2 — Survival of hatchery reared silver pompano fingerlings in polythene bags during transportation with reference to duration and packing density

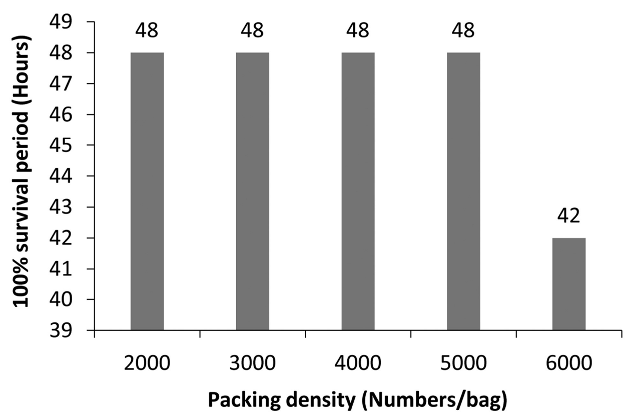

Fig. 3 — Survival of the silver pompano fingerlings in bulk containers during transportation with reference to duration and packing density

Discussion

Based on the results of the present study, it is recommended that the best packing density for transporting the cobia and the silver pompano fingerlings in polythene bags with hundred per cent survival for 36 to 48 h duration is 2.5 fingerlings of cobia per litre (with a mean weight of 3.0±1.0 g) and 18.75 fingerlings of silver pompano per litre (with a mean weight of 0.6±0.1 g); provided the bags are filled with one-fourth filtered seawater (34 ppt salinity) and three-fourth oxygen under the optimum transport temperature of 25 °C. The bulk transportation of silver pompano fingerlings with the same weight can be transported for 36 h at a packing density of 8.3 per litre with 100 % survival rate. In terms of biomass, 7.5±2.5 g/L of cobia fingerlings and 11.25±1.9 g/L of pompano fingerlings can be transported using polythene bags, and 4.98±0.83 g/L of silver pompano can be transported using bulk transportation tanks. The packing biomass can be further increased or decreased based on the transport duration in an inverse manner, and it is recommended to use the packing densities a little below the optimum level to ensure the maximum survival of the seeds in case of any delay in transportation. Berka[1] reported that the packing density could be reduced in case of transport of any endangered fishes or fish species of survival interest where 100 % survival is a primary concern but not the economy. The above-recommended packing densities and water quality parameters have been followed for the past five years (since 2015) with a 100 % success rate.

Concerning transportation of live seedlings, Benetti *et al.*[8] reported that the packing biomass for cobia can be greater than 6.67 g/l and less than 50 g/l, with temperature not less than 16 °C for a duration of 12 to 20 h. This conclusion has been found to be in agreement with the results of the present study, where a hundred per cent survival was achieved with 7.5±2.5 g/L packing biomass at 25 °C for 48 h of transportation. Results of the present study show better survival rates for cobia fingerlings than the report of Stieglitz *et al.*[10], in which a 24 h shipping trial with a packing biomass of 5 g/l showed hundred per cent survival in 32 ppt and 12 ppt salinity, but packing biomass of 10 g/l showed less than 90 % survival at 32 ppt salinity and greater than 90 % at 12 ppt.

The cobia and the silver pompano fingerlings can survive at a considerable level of salinity reduction, where the former fish species requires a minimum of 8 – 11 ppt of salinity[16] for survival, and the latter species requires 4 ppt of salinity[17]. Following these two studies, the packing biomass can be further increased by reducing the salinity of packing water as this helps to reduce the metabolic demand and osmoregulatory stress because when the medium of transport is isotonic or near isotonic, the energy demand for the osmoregulation can be reduced[18]. This statement has been demonstrated successfully by Stieglitz *et al.*[10], where the cobia fingerlings showed 100 % survival after 24 h following abrupt transfer, without acclimatization, from full-strength seawater (35 ppt) to salinities ranging from 11 ppt to 45 ppt. In another trial using two different salinities (12 ppt and 32 ppt), survival rate was significantly enhanced at 12 ppt relative to rates in the higher salinity (32 ppt) even with increased biomass of cobia fingerlings under the same time period of 24 h[ref. 19].

The packing density of the cobia fingerlings recommended in this study is found to be in agreement with the results of Colburn *et al.*[9], where they determined that the packing biomass of 20 g/L is found to have the highest survival for 24 h of transport under the transportation temperature range of 19 – 25 °C. The cobia larvae metamorphose from cutaneous mode to the gill respiration during the 11 – 15 dph[8], and hence the transportation should be avoided during this period in order to eliminate the loss posed due to metamorphosis related stress.

Preparing the fish for transport, which involves nutritional prophylaxis during hatchery rearing, health prophylaxis, proper starvation and increasing stress resistance, ensures the health and survival of the fish during transport and also during the post transport acclimation period[20]. Nutritional prophylaxis before the shipment for a considerable period improves the stress tolerance and disease resistance of the fish fingerlings. Supplementing vitamin C with the feed increases stress tolerance and disease resistance in various fish species[21]. Increased production of corticosteroids (cortisol) due to stress compromise the fish immune system, thereby leading to pathogenic infections resulting in mortality[22], and this chance is high when the parasite or bacterial infected fishes are under transport stress. Nowadays, as the transport methods evolve, the bactericides and parasiticides are added directly into the transport water[20]. Starving the rainbow trout fingerlings for 72 h showed lower metabolite excretion and increased fish survival than 24 h starvation group[23]. The stress tolerance of a marine fish can be estimated by subjecting it to an osmotic shock with freshwater or using formalin as a stress inducer in order to evaluate the stress resistance. Eliminating the fishes with lower stress tolerance can help reduce earlier shipment mortality, which eventually results in higher mortality if not eliminated[20]. In other cases, the anaesthetics and buffers are also used to prevent physical injury and reduce metabolism during the transportation of fishes; however, a heavier dose or miscalculation for appropriate dosage may lead to lethal effects, causing mortality[24]. Transporting at high density in low water volume is also practiced as a cost-effective method in fish transport, but due to the accumulation of toxic metabolites in combination with the metabolic stress during high-density fish transport induces mortality[9]. Augmenting these factors (nutritional prophylaxis, health prophylaxis, proper starvation and stress resistance) will help reduce mortalities during high-density fish transport. Piper *et al.*[25] reported tank transportation of various fish species but to a shorter period of transport wherein they reported a maximum of 24 h transport of striped bass (*Morone saxatilis*) at 60 g/L, which is quite higher than the packing biomass of silver pompano recommended in this study (4.98±0.83 g/L).

Attempts have also been made to standardize the transport of yolk sac larvae of silver pompano as the yolk sac with oil globule has to be absorbed within 48 h of post-hatching. Multiple trials have been carried out with different packing densities, and better results were obtained at the packing density of 200 larvae/l for 36 h of transport in open tanks. Transport trials by Benetti *et al.*[8] for the cobia yolk sac larvae gave the best results at the packing density of 700/l at 21 °C. In the case of bag transport, transport water should be maintained without much agitation to avoid the physical damage of the yolk sac larvae[1].

Conclusion

The results of the present study indicated that the cobia and the silver pompano fingerlings can be transported in polythene bags with 100 % survival up to 48 h with a stocking density of 2.5 and 18.75 fingerlings per litre, respectively. If the duration of transportation is less than 48 h, the packing density of the fingerlings can be increased to have an economic advantage. The survival of the fishes can further be ensured by employing measures like nutritional prophylaxis, increased starvation period, avoiding handling stress, health prophylaxis and eliminating fishes with low-stress tolerance and diseased individuals.

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Conflict of Interest

The authors declare that there is no conflict of interest in the manuscript.

Author Contributions

RJ, GT & AKAN – Conceptualization of the work; RJ & MS – Design of experiment, data analysis and preparation of the manuscript; PR, KKA, MSr & BJ – Supervision of the experiments; GHR, TT, NK, NM & AAM – Execution of the experiments; MS, RJ & GT – Review and editing.

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