STANDARDIZATION OF OXYGEN PACKING PROCEDURE OF *CHANOS CHANOS* SEED

By

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THESIS

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TO

MY PARENTS

DECLARATION

I hereby declare that this thesis entitled "STANDARDIZATION OF OXYGEN PACKING PROCEDURE OF CHANOS CHANOS SEED" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title, of any other University or Society.

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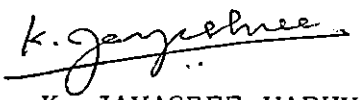

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I. INTRODUCTION

1. INTRODUCTION

Milk fish - Chanos chanos (Forskal) - constitutes one of the most important fin fishes for brackish water culture. Extensive brackish water fish farms are exclusively devoted to commercial milk fish culture in several south-east Asian countries. From 1931 onwards, culture of C.chanos was attempted in India on experimental basis, but no appreciable progress has been achieved commercially. As plentiful milk fish seed resources have been located throughout the coasts of peninsular India by Smith (1981), there is a great potential for commercial cultivation of this species in our country with about 2 million hectares of brackish water area. For better utilisation of the milk fish fry resources, standardized methods for packing and transporting are highly warranted.

With the advancement of aquaculture technology, transportation of aquatic animals alive is gaining importance. During the past decade, several devices have been evolved for the transportation of aquatic animals, primarily based on empirical knowledge and later on scientific lines. Subsequently, concerted efforts have been directed by several researchers in solving the problems on the transportation of live fishes and their seed. Although several simple conventional methods of transporting milk fish seed in open containers such as 'palayok' in the Philippines, cement or tar-coated bamboo baskets in Indonesia, galvanised cans in Taiwan,

the 'hundies' in India (mainly used for transporting carp seed) were in vogue for centuries, all these were means of short-distance transportation and involve a lot of manual labour and constant care.

The packing of fish seed in sealed plastic bags under oxygen pressure for transportation is a more recent innovation in aquaculture which originated ever since the production and transport of hatchery-bred seed in the Western countries during the mid 18th century. By the early 1950's, oxygen-filled plastic bags have been widely used for transporting live fish all over the world. (Fry and Norris, 1960). Although this type of packing for transportation of seed has proved to be handy and economical, it is not free from the risk of mortality. The major problems of this method are changes in temperature, depletion of dissolved oxygen, increase in acidity and carbon dioxide, and accumulation of nitrogenous wastes (Froese, 1986).

The use of insulated shipping boxes or of extra plastic bags filled with ice or hot water alleviated the temperature problem. The replacement of air above the transport water with pure oxygen was a straight forward method for maintaining a sufficient concentration of dissolved oxygen. Different methods have been suggested for the control of carbon dioxide and ammonia, such as the use of buffers, ion-exchangers, adsorbents, anaesthetics and bacteriological nitrification. However, none of the methods has been standardized.

Information on the various aspects of packing of C.chanos fry in oxygen-filled bags is scarce. The oxygen requirement of individual fry (0.01 g and 14 mm) was estimated as 0.0002 c.c./hour (Mammen, 1966). The packing rate of the fry of 0.01 g and 13.5 mm was estimated as 150-175 fry per 100 ml water (Durve and George, 1963) and that of the fry of 13-15 mm as 115 fry per 100 ml water (Mammen, 1966). Mammen (1966) also reported the necessity of conditioning the fry for 6 hours before packing. However, there is lacuna in the knowledge on the different aspects of oxygen packing of C.chanos seed for transportation.

For the transportation purposes, packing of fish seed in plastic bags under oxygen pressure basically requires, first of all, careful conditioning of the seed prior to packing; secondly, right quantity as well as quality of the water medium; and thirdly, the optimum quantity of oxygen. Success of the operation apparently depends on the careful manoeuvring of all these factors, and in addition, on the control of hyperactivity of the seed which otherwise results in accumulation of too much of the metabolites in the bag, which in turn upsets the health and hygiene of the seed. The present study is aimed at obtaining fresh - hand information on the above mentioned aspects in order to standardize the oxygen-packing procedure of C.chanos seed. Mammen (1966) highlighted the inefficiency of the methods used for transporting the seed as one of the main difficulties in utilising the seed resources. Clearly, the risk element involved in the transportation

of milk fish seed can be reduced to a minimum level by resorting to standardized procedures for oxygen packing.

1.1 Objectives of the study

i. To find out the optimum conditioning method of C.chanos seed before packing in oxygen-filled containers.

ii. To determine the optimum packing density of the seed.

iii. To determine the effect of salinity and pH on oxygen packing of the seed.

iv. To determine the effect of oxygen pressure on packing the seed.

v. To find out the feasibility of using a cheap and locally available sedative, tertiary butyl alcohol in oxygen packing of the seed.

vi. To find out the feasibility of using chitosan, which is a by-product of prawn shell, as an absorbent in removing toxic metabolites of the seed accumulated in the oxygen-packed plastic containers.

2. REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Oxygen packing and transportation of live fishes

The history of transportation of live fishes could be traced back from literature recorded ever since the 1870's. Norris (1874) mentioned about the historical records of fish culture and transport of fish spawn by referring to Chinese and Roman history. Norris et al., (1960) reviewed the then existing practices of transporting live fishes. The first major shipment of live fishes consisting of about 12,000 American shad, Alosa sapidissima fry were transported in barrels, from the Hudson river in New York to Tehama in California, way back in 1871. Later in 1879, Dr.Livingston Stone transported 133 striped bass, Roccus saxatilis from New Jersey to California, in fish cans cooled with ice and aerated with hand; of these only 25 fishes reached the destination alive. An ingenious device for oxygenation of water, described by Raveret-wattel (1880) consisted of a blacksmith's bellow which was geared to the axle of a cart, so that the movement of the cart resulted in pumping a stream of air into the transporting cans. Yet another shipment was made by . . Woodbury in 1882 in U.S.A.. in a modified device, by using a cylinder with finely perforated bottom which was manually pushed into the water in the transporting can, allowed to fill and then lifted, resulting in a fine shower of water in the can. (see review by Norris et al., 1960). Anon. (1883) explained a transportation tank in which sea water was circulated through a filter from an

overflow and then pumped into an overhead storage tank, where it was aerated and returned to the main tank by gravity. Using this tank, trips of 72 hours duration from Triesta to Berlin were highly successful. Thomas Moore in 1887 reported the first endeavour in the transport of marine fishes using the flat fish, Solea sp., from England to the east coast of North America. (see review by Norris et al., 1960).

Lorenz (1903) devised an apparatus in which a small oxygen cylinder was fitted to the bottom of a tranportation tank and the oxygen was forced through an air stone. Holder (1908) described a fish transportation container lined with a sponge inside and capped with a lid having a perforated false bottom. Any movement of the container resulted the water to flow into the lid and cascade back into the container, thereby aerating the water. The sponge-lining was provided to make the walls of the containers free from causing injury to the fish. Mead (1908) outlined the essential factors for the transport of live fishes. He mentioned, "The principle is, briefly, to provide at the start native 'unmodified' water; to maintain proper temperature and density, and in some cases current; to secure continuous 'respiration' of the water, including egress of waste gases of the metabolism of contained fishes and often bacteria as well as the access of oxygen, and to avoid contact with injurious metallic substances." This statement provided a precognition to the present day thinking on live fish transportation problems,

as it delineated a summary of several important factors involved in transportation.

Seale (1910) devised one of the first motor-driven pumps for aeration for a month-long transport of black bass, Micropterus sp. from California to Bagio in the Philippine Islands. Osburn (1910) reported the use of oxygen in fish packing and transportation. Fisher (1915) described his techniques and equipment for carrying trout fry in California and emphasized the fact that temperature and aeration of water were the most important factors affecting trout fry during transport. The use of oxygen in fish transportation was first employed in Europe towards the end of 19th century. (Shebley, 1927). Birtwistle (1931) used ordinary petrol cans with a hermetically sealed screw-cap, and fitted with two small pipes for introducing oxygen, for the carp fry transportation. Buschkiel (1937) vividly described various methods of transportation. Tanks having different systems of aeration were described by several workers (Olson, 1940; Copeland , 1947; Wall, 1948; Stubblefield et al., 1949; Sykes, 1951; Carlson, 1955; Skinner, 1955; Greene, 1956 and Macklin, 1959). Tanks with refrigeration unit for temperature control were described by Olson (1940) and Stubblefield et al., (1949). Mitra (1942) described the advantage of transporting fish fingerlings in a suitably designed container filled with oxygen, which could be despatched through rail as an ordinary parcel. Khan (1946)

explained a type of oxygenated container for transporting carp seed. This was made of galvanised iron of the size 45.72 cm x 35.36 cm, with two air tight openings at the top; one to let in oxygen and the other to let out the displaced water. Jagannadham (1947) used tin carriers of 12 gallon capacity containing 10 gallon water and 50-60 fingerlings or 130 fry of Catla catla. Sundara Raj and Cornelius (1948 & 1949) transported a consignment of 60 fingerlings of mirror carp, from Ootacamund in Nilgris to Kumaun hills in the Himalayas by aeroplane in sealed petrol tanks of 10 gallon capacity and with a special oxygen carrier. Burrows (1949) described the typical transport methods and equipments for salmon eggs. Ranganathan and Ganapati (1949) dealt with the collection, acclimatization and transportation of the fry and fingerlings of C.chanos. Based on the experiments done in experimental flasks with oxygen on the transportation of the larvae of Indian carps, Basu (1950) indicated that inspite of the presence of oxygen, larvae died due to high carbon dioxide, bacterial population and decomposition of dead larvae inside the flasks. Ganapati et al., (1950) reported about the acclimatization and transportation of C.chanos seed in inland waters of Madras. Basu (1951 a) studied the physiological requirements of the eggs, larvae and fry of Indian carps during transportation. Ganapati and Chacko (1951) made a comparative study of the transport of carp seed in ordinary tin carriers with and without oxygen. An account of the air transport of live fishes like tilapia and carps in sealed drinking-water jerry cans was given by Vaas (1951). Basu

(1952) studied the effect of different surface area on the survival and transport of the larvae of Indian major carps and found that 400 larvae per litre can survive for 48 hours when the surface area per unit depth is between 16 to 470 under experimental condition. Punwani (1952) reported on the surface transport of the fingerlings of mirror carp, leather carp and scale carp from Nilgris to West Bengal. Schuster (1952) mentioned that when C.chanos fry from freshwater were directly transferred to salt water of less than 40 ppt salinity, no mortality occurred; but beyond a salinity of 40ppt, total mortality set in. Davis (1953) described the transport methods and equipments for trout eggs. Mints (1956) determined the optimal packing density of mullet fry during transportation in rail-road cars.

The use of plastic bags for transporting live fishes apparently started in the U.S.A. after the second world war. (see Martin, 1980). Miller (1956) gave an account on the general use of plastic bags for carrying and shipping live fish. Ranade and Kewalramani (1956) conducted experiments on the air shipment of carp fry in plastic bags. Saha et al., (1956 a) studied the correlation between the number of fry, their size and weight to the exposed surface, pressure of oxygen and water volume of the transport medium. Sarojini (1958) gave an account of the collection and transport of mullet seed in West Bengal.

A preliminary report on the transportation of C.chanos fingerlings with aerating system was given by Djajadiredja (1958).

He developed a simple, economical and safer method of C.chanos seed transportation in ordinary fish tins equipped with aerator pumps and a cooling system. Kawamoto et al., (1958) studied the physiological and biochemical factors affecting the transportation of live rainbow trout. They found that mortality during tranportation could be reduced by removing the fish excretions, lowering the water temperature and adding oxygen. Saha and Sen (1958) made a study on the comparative economy of different types of containers such as glass carboy, metal containers, plastic and latex-rubber bags for long distance transportation of carp spawn and fry. Clark (1959) reported the results of experiments in transporting several species of fish in oxygen-filled polyethylene bags supported by cardboard boxes. In one case, 10,000 wall eyes, Stizostedion vitreum fry were carried in one gallon of water in polyethylene bags for 9 hours with 100% survival. Furnish (1959) successfully transported fish fry at the rate of one and a half pounds in one gallon of water in polyethylene bags with oxygen for short duration of 4 hours. Macklin (1959) used direct drive gasoline-engine-powered pumps for temperature control in the transportation tanks. Garcia and Demetrio (1960) determined the maximum number of C.chanos fingerlings that can be safely and economically transported in plastic bags, and it was 200 to 250 fingerlings per bag. Schuster (1960) reported that 500 to 1000 C.chanos fry per litre of water can be safely packed and transported without artificial aeration at a temperature of 25°C. Sison et al., (1960) reported the use of polyethylene bags as an aid in holding and transporting several species of fish fry like milk

fish and mullets. Rao (1961) reported the use of small polyethylene bags filled with oxygen by the Fisheries Extension Department of Government of India for fish fry transport. Wohlfarth et al. (1961) mentioned the possibility of transporting 1 kg of small carp with an average weight of 30 g in 2-2.5 litres of water taken in polyethylene bags and filled with oxygen, for a period of 4 to 8 hours. Hora and Pillay (1962) gave an account on the theoretical considerations important in fish fry transport and the methods employed in their transport.

Durve and George (1963) gave an account on the physiological changes in C.chanos fry during their transportation in plastic containers. He also worked out the packing density for transportation; C.chanos fry of transparent stage could be transported at a density of 100 fry in 100 ml freshwater without oxygen pressure, and 150-175 fry per 100 ml freshwater could be transported under oxygen pressure. Mammen (1966) estimated the packing density of C.chanos seed for a period of over 24 hours. He reported that 200 fry of 13-15 mm length could be safely transported in 175 ml of water for over 24 hours. He also studied the oxygen requirement of individual C.chanos fry of 13-15 mm length as 0.002 ml/hour. Mammen (1967) designed a 'splashless tank' for broodfish transport. Another type of live fish carrier was designed by Patro (1968), which was a double-barrel type carrier. Ramachandran (1969) discussed tne factors affecting survival during transportation of carp spawn, fry,

fingerlings and breeders. Bardach et al., (1972) described the transport of the larvae and fry of carps. Huet (1972) explained different types of receptacles for transport and also discussed the general rules for transportation. Huq et al., (1972) discussed the use of oxygen-filled polyethylene bags for transporting carp fry in Bangladesh. Ramanathan and Jayamaha (1972) reported successful transportation of C.chanos fry packed in polyethylene bags filled with oxygen and sealed with rubber bands. Bensam (1974) discussed the transportation of the fry of C.chanos, mullets and pearl spot in unglazed earthen ware jars, tin vessels and galvanised cans.

An open transport van improvised with a small semi-rotary pump has been devised by the State Fisheries Department, Orissa. By using this pump, fish fry can be transported in semi-insulated road vans upto a distance of about 500 km with mortality as low as 5%. (Jhingran, 1975). For transporting major carp breeders in Punjab and Madhya Pradesh, open canvas containers of the size, 1m x 1m x 1.25m, as well as galvanised iron drums of 180 litre capacity have been used. (Jhingran, 1975).

Marathe et al., (1975) used hydrogen peroxide as a source of oxygen supply in the transportation of Cyprinus carpio fry. Twelve drops of hydrogen peroxide per litre of water containing 50 fry has been reported as the safe limit; its indiscriminate use has been found harmful to the fry. Rao and Gopalakrishnan (1975) reviewed

the then existing information on the methods employed in the collection, acclimatization and transportation of cultivable brackish water fishes. Chen (1976) gave a brief account of the transportation methods and carrying density of C.chanos fry in Taiwan. Kim et al., (1977) carried out a series of indoor experiments to find out the causes of mortality for conger eel, Astroconger myriaster during transportation and determined the minimum dissolved oxygen level necessary to sustain the fish for 48 hours during transportation. Singh (1977) studied the oxygen consumption of fingerlings of Indian major carps and estimated the amount of oxygen required by them during a safe transport for given periods. Also, he suggested that a mixture of 80% oxygen and 20% nitrogen may prove as a better packing medium than pure oxygen, as nitrogen is an inert respiratory gas reducing the effect of metabolic carbon dioxide in water and air.

An improved method for transporting young American shad was devised by Meinz (1978). In this method, an oxygen bottle with regulator was used to trickle down oxygen into the covered chests during transportation. Snow et al., (1978) reported the transport of fish in plastic bags containing water and oxygen as a well established technique used for several years. Anon. (1979) mentioned that viscous water (1% methyl cellulose) safeguard trout eggs during transportation. Johnson (1979 a) described the requirements and limiting factors in the packing and transportation of live fishes in plastic bags. Kulkarni and Ogale (1979) successfully air-lifted the

eggs of Mahseer, Tor khudree from Pune to Bombay at a distance of 100 km. Laird and Wilson (1979) observed high survival of rainbow trout eggs immersed in a highly viscous solution (1% methyl cellulose or polysucrose) during simulated transport. Bogdan and Waluga (1980) reviewed the transport methods employed for eel stocking material. Martin (1980 and 1981) gave a detailed account on the plastic bag hauling of small live fishes. Hamman (1981) successfully transported the fry and fingerlings of endangered fish species-colorado squaw fish (Ptycocheilus lucius) and hump back chub (Gila cypha)-in plastic bags. Preliminary observations on the transport of carp fry under air pressure was given by Selvaraj et al., (1981), who explained an inexpensive and practical method of transporting fish fry (Cirrhinus mrigala) in plastic bags using an ordinary cycle pump for pumping in atmospheric air. At a stocking density of 300 fry per 6 litres of water, no mortality was observed for 96 hours. Smith (1981) described the transportation methods of the fry and fingerlings of C.chanos in Philippines, where transportation was done in buckets or 'palayok' for shorter distances and in plastic bags for longer distances.

The transportation technique for blue black herring was described by Guest and Prentice (1982) who carried out both air and truck transport of the herring. Sarnita et al., (1982) studied the transportation of milk fish yearlings in Indonesia using three kinds of containers and two methods of aeration. Hettler (1983)

reported successful transportation of Menhaden larvae for seven days in plastic bags filled with oxygen and with daily rotifer feeding. A report on the long distance transportation of intensively reared large mouth bass, Micropterus salmoides in trucks was given by Carmichael (1984). Dupree and Huner (1984) explained in detail the basic transportation systems employed for live fish transportation, types of transportation equipment, water quality considerations and the hauling aids important in transportation. Villaluz (1984) described the collection, storage and transportation techniques of milk fish fry and fingerlings existing in different countries. Villaluz et al., (1984) described the existing methods and practices of milk fish fry and fingerling transport in Philippines. Siraj et al., (1985) studied the effects of packing densities in plastic bags on the survival of larvae and fry of Helostoma temmincki. Thakurta and Pakrasi (1985) carried out experiments on the transportation of the mullet, Liza parsia fry. Thayaparan and Chakrabarty (1985) transported C.chanos fry in double polyethylene bags filled with oxygen in the ratio of 1 part water to 2 parts oxygen; 200-2000 fry were packed in each bag containing 4 to 6 litres of sea water.

The relationship between body weight and loading densities in fish transportation using plastic bag method was explained by Froese (1985 and 1986), who derived a formula for calculating the fish density for packing freshwater fishes, number of fishes per litre and the required water volume for individually packed adult fish. De et al., (1986) carried out preliminary trials on the

transport of Hilsa ilisha fry in both open and closed systems; open system transport was found better than closed system transport for Hilsa ilisha fry as very high mortality was encountered in the closed system. Sampson and Macintosh (1986) conducted studies on the problems associated with packing and transporting silver carp fry, Hypophthalmichthys molitrix in oxygenated and sealed polyethylene bags; discussed the difficulties of interpreting the effects of water quality on fish survival during transportation. Villaluz (1986) described the procedure involved in the storage, transport, acclimation and stocking of milk fish fry. Hamza and Zaki (1987) successfully transported mullet fry in polyethylene bags of the size 70 cm x 35 cm filled with 5 litre water, packing 200 fry and 10 litres oxygen; the bags were firmly sealed with elastic bands. They emphasized the importance of collection, packing and transportation during the early hours of the day to avoid the effect of high temperature. Garcia and Toledo (1988) studied the effects of loading density, length of transit time, temperature and salinity on milk fish eggs during simulated transport. Taylor and Ross (1988) discussed the use of hydrogen peroxide as the source of oxygen for the transportation of live fish fry, Oreochromis niloticus. A simple and cost-effective system for transporting the fry using oxygen derived from hydrogen peroxide was described, in which oxygen was generated separately from the fish container.

2.2 Conditioning of fishes for oxygen packing

Because of the limited space in the transportation container, the packed fishes undergo environmental as well as physiological stress, which may contribute to the large scale mortalities in fish seed transportation. Another factor determining the survival of fishes during transportation is their initial condition. So, before packing for transporting to long distances, the fish seed are conditioned in order to get rid of the excreta and to acclimatize them to a restricted area. (Haskell, 1941; Miller, 1952; Saltzman, 1953; Wales, 1954; Brock and Takata, 1955; Stroud and Bitzer, 1955; Horton, 1956; Mc Farland and Norris, 1958; Norris et al., 1960; Fry and Norris, 1960; Bardach et al., 1972; Rao and Gopalakrishnan, 1975; Specker and Schreck, 1980; Robertson et al., 1988).

Jagannadham (1947) reported that clean, natural water at a temperature of approximately 20.5°C was suitable for the conditioning of catla fry for a duration of 48 to 72 hours, in specially designed wire-meshed boxes. He also mentioned that during conditioning and packing for transportation, the fry and fingerlings should not be handled with bare hands as it would remove the slime and scales covering the body rendering them vulnerable to bacterial and fungal infections. According to Schuster (1952), the fry and fingerlings of milkfish should be conditioned for 24 hours before packing for transportation. Miller (1952),

Saltzman (1953), Wales (1954), Stroud and Bitzer (1955) and Horton (1956) reported significant post-transport mortality in different game fishes, and this stimulated a series of studies on pre-treatment of fishes. Saha and Chowdhury (1956) mentioned that the conditioning enclosure should be installed at a depth of 30-35 cm in ponds or still parts of the river, and recommended a conditioning period of 48 to 72 hours for Indian major carps' fry in general. Alikunhi (1957), while discussing the need for conditioning the carps' fry, was of the opinion that the fry which were fed on animalcules like cladocerans during conditioning and transport, survived better and stood the strain of transportation better than the unfed fry during long distance transportation.

Djajadiredja (1958) emphasized the necessity of conditioning milk fish fry before packing and transportation. He conditioned the fry by keeping them in catching pond of 30 m^2 for 1 or 2 days before packing so as to empty the gut and prevent spoilage of water by excreta during transportation. Wohlfarth et al., (1961) used a disinfecting dip before packing fishes in polyethylene bags which resulted in good survival during a nine hour transit. Hora and Pillay (1962) reported that the fry collected from nurseries should be conditioned by keeping them in nets fixed in ponds, and water should be splashed from all directions in order to frighten the fry to pass excreta and vomit. Srivastava and Karamchandani (1964) observed that when carp fry of 8 - 23 mm were conditioned for 24 hours before packing in a limited volume of water (1.8-2.0

ml/fry), a minimum of 0.88 ppm oxygen was sufficient for their survival. Mammen (1966) reported that C.chanos fry collected from sea water should be gradually acclimatized to lower salinities of 20 ppt; further dilution to freshwater should be done after reaching the destination.

Jhingran (1975) mentioned about the various types of conditioning containers, namely, boxes made of wire meshes, bamboo or cane wicker work, barrels or boats with perforated bottom, temporary enclosures made of netting or bamboo matting, cloth hapa etc. He emphasized the adoption of prophylactic and quarantine measures involving the use of antiseptics, antibiotics and germicidal chemicals as a pre-treatment measure against infectious diseases, parasites, predatory insects and aquatic weeds. He also reported that prior to transportation, only short duration baths in chemicals such as copper sulphate, methylene blue, acriflavine, potassium permanganate, chloromycetin, common salt or formalin were desirable Hattingh et al., (1975) described a method for the packing and transport of freshwater fishes, resulting in little or no mortality. In this method, commercial salt was added to the water to give a final concentration of 0.5% to prevent the incidence of any infection; compressed air was bubbled through water.

Prinsloo and Schoonbee (1985) mentioned the steps taken prior to packing and transportation of fishes, which included the disinfection of fishes against parasites and use of sedatives to

minimise handling stress. Thakurta and Pakrasi (1985) emphasized that mullet fry should not be transported without conditioning and recommended a conditioning period of 4 hours in plastic pool. De et al., (1986) was of the opinion that the fry of Hilsa ilisha should be acclimatized for 3 to 4 hours in plastic pools before packing for transportation. Froese (1986) also emphasized the need for pre-treatment of fishes before packing and transportation. Depending on the size of the fish, the starvation period was varied and the fishes were kept in clean water in separate tanks, rejecting the diseased and weak fishes. Sampson and Macintosh (1986) reported that the conditioning of fish and the type of water used for packing were the factors determining the survival of fish during transportation. He also stated that conditioning was more effective in removing the weaker fish than in raising the tolerance of all fishes to transportation.

2.3 Use of sedatives in packing of live fishes for transportation

Perhaps, the first work recognising the potential use of sedatives for transporting fishes appears to be that of Aitken (1936), who used 4% solution of chloral hydrate. Osborn (1951) used thiouracil as an aid in holding and transporting fish, and succeeded in lowering oxygen consumption of the fish by 20% and thus helped to increase the packing density in transportation tanks by 15-20%. Burrows (1952) used chloretone to anaesthetize salmon fingerlings. Little work was done until Calhoun (1953) and Reese (1953) reported

sodium amytal in enhancing the packing density of trout during transportation. Reese (1953) suggested that sodium amytal was beneficial in trout transport as it reduced the activity of fishes, slowed down their body functions and oxygen requirements, and resulted in doubling their packing density.

Phillips and Brockway (1954) demonstrated the reduction in the rate of excretion and oxygen consumption of brook trout - Salvelinus fontinalis using sodium amytal. Saha et al., (1955) reported that sodium amytal at a dose of 21-28 mg/litre water reduced the metabolic rate of fry and thereby increased the packing density for transportation by 30% and in turn reduced the cost of transportation. Wood (1956) considered urethane to be undesirable for fish transportation due to its low potency and carcinogenic nature. The clear experimental demonstration of the value of sedatives in transporting fishes, however, came from Nemoto (1957) who indicated that sodium amytal caused a one-third reduction in the rate of oxygen consumption of the cichlid - Oreochromis mossambicus sealed in containers. However, Onkst et al., (1957) doubted the value of sodium amytal for transport of marine fishes as it was antagonised by calcium ions.

The need for more fundamental data on the effects of sedatives on fishes for transportation purpose was indicated by the results of Webb (1958) who used tricaine methane sulfonate (MS - 222) as an aid to increase the loads of blue gills, Lepomis

macrochirus carried. Mc Farland (1958) studied the effect of several sedatives on two species of marine teleosts. Several drugs were found beneficial in fish transportation by decreasing the fishes' reaction to external stimuli, resulting in a decreased metabolic activity and lower the rates of oxygen consumption, of carbon dioxide production and of excretion of nitrogenous wastes, all of which act as limiting factors in fish transport. (Mc Farland, 1958; Mc Farland and Norris, 1958; Fry and Norris, 1960). Martin and Scott (1959) used tricaine methane sulfonate (MS-222) in the transport of live fish without water. Messerby (1959) used sodium amytal in the transport of albino brook trout fingerlings. Thompson (1959) narcotized cut throat trout using MS-222 for their transportation.

Mc Farland (1960) recommended three sedatives, viz., methyl parafynol, tertiary amyl alcohol and chloral hydrate for use in fish transportation. He found that by using these drugs, the packing density in transport containers could be doubled or tripled. Natarajan and Ranganathan (1960) studied the possibilities of utilising quinaldine in transporting tilapia fry and fingerlings at a dose of 5-10 ppm for fry and 25 ppm for fingerlings. Sreenivasan (1962) studied the effect of different drugs on the reduction of oxygen consumption of fish and found quinaldine as a good narcotizer in the transportation of fingerlings of Catla catla and Labeo fimbriatus at a dose of 5-10 ppm. Bell (1964) found that quinaldine was inexpensive, highly effective at very low concentrations, had

a low toxicity and the fish recovery time from the sedation was rapid. Collins and Hulse (1964) showed that 0.5% salt and 1 g MS-222 per 12 gallon water reduced thread fin shad hauling mortality.

Durve and Dharma Raja (1966) studied the effects of different sedatives on the behaviour of mullet fingerlings and their scope in different aquaculture activities. Of the six chemicals studied, viz., chlorobutanol, chloral hydrate, tertiary butyl alcohol, sodium amytal, sodium barbital and urethane, chloral hydrate was the most suitable for transportation purpose. Kewalramani and Gogate (1968) carried out experiments on the sedation of brooder tilapia and major carps using novocaine, barbital sodium and amobarbital sodium, and their transportation. Durve (1970) carried out experiments to study the effect of drugs like tertiary amyl alcohol, quinaldine, ether, paraldehyde, phenobarbital sodium, pentobarbital sodium and MS-222 sandoz on the behaviour of mullet fingerlings.

Vijayagupta and Sharma (1974) tested the efficacy of MS-222 in the transportation of adult breeders of Chinese carps-Hypophthalmichthys molitrix and Ctenopharyngodon idella ; they successfully transported 3-3.6 kg Chinese carp breeders in open, galvanised iron tanks mounted on a truck to a distance of 310 km involving 16 hour journey under sedation with MS-222 at 1.6 ppm. Durve (1975) studied the use of several drugs in the transportation of live mullet seed and found that MS-222, chloral

hydrate, tertiary amyl alcohol, tertiary butyl alcohol, chlorobutanol, quinaldine and paraldehyde to be effective for transportation of mullet seed; their doses were also determined. Dick (1975) tried sedation of adult grey mullet using MS-222 sandoz for fish transportation and found it as very effective.

Murai et al., (1979) studied the effect of valium, MS-222 and sodium chloride on the mortality induced by handling and transporting fingerlings of American shad - Alosa sapidissima. Taylor and Solomon (1979) discussed the efficacy of several fish tranquillizers and recommended tertiary amyl alcohol and benzocaine as the best and cheapest tranquillizer in the transportation of fishes.

Alvarez-Lajonchere and Moreno (1982) studied the effects of MS-222, tertiary amyl alcohol and tertiary butyl alcohol on Mugil trichodon., Poey, post larvae for their transportation. With these three anaesthetics, an increase in their concentration decreased the induction time. Guest and Prentice (1982) found that MS-222, quinaldine, etomidate and imidazole carboxylate were successful in the transport of blue black herring for as long as 10 hours. Mishra et al., (1983) reported that Labeo rohita fry could be safely transported with carbonic acid anaesthesia at 500 ppm for 215 hours. Takeda and Itazawa (1983) discussed the possibility of causing sedation by carbon dioxide in the transportation of carps. Three methods were used for this purpose of causing sedation, ie., (1) by adding sulphuric acid to water, (2) by adding sodium

carbonate to water and (3) by bubbling carbon dioxide and oxygen into the water for a short period until fish were sedated.

Dupree and Huner (1984) tested different chemicals viz., quinaldine, MS-222, tertiary amyl alcohol, methyl pentynol, carbonic acid and sodium bicarbonate to reduce the hyperactivity of fishes for better survival during handling and transportation. The potential use of benzocaine-hydrochloride as an aid in the transport of live fish, Oreochromis mossambicus was investigated by Ferreira et al., (1984). At a concentration of 25 mg/L, the drug caused reduction in the excretion of ammonia and carbon dioxide by the fish; also the pH and alkalinity values of the transport water remained fairly constant as a result of the reduced activities of the fish. Siwicki (1984) tested the practical application of Propandid-POLFA during handling of fishes at commercial farms. The influence of quinaldine on some tilapias was studied by Sado (1985). He determined a safe dose of 5-10 ppm and 25 ppm of quinaldine for the transportation of tilapia fry and fingerlings respectively. Thomas et al., (1990) efficiently used quinaldine (0.0002%), tertiary amyl alcohol (0.004%) and tertiary butyl alcohol (0.006%) in the transportation of grass carp, Ctenopharyngodon idella seed of 20-30 mm length for 40 hours without mortality ; 1.5-2.0 fold increase in density per unit volume of water as against the conventional practices was possible with these sedatives. Also, paraldehyde (0.005 - 0.009%) was found effective in the transportation of the grass carp seed of 10-20 mm length for 25-30 hours, with 2-3 fold increased

densities per unit volume of water.

2.4 Management of water quality during packing and transportation of live fishes

During long distance transportation of live fishes, several factors like excretory products, slime, deciduated scales, decomposition products from dead fish and regurgitated food reduce water quality to critical levels. Some measure to control deterioration in water quality brought in by these factors may be achieved by chemical means. The methods that have received some attention in the control of water quality during transportation are buffering, chemical absorption of nitrogenous wastes, adsorbents and the use of bactericidal and fungicidal agents.

Basu (1951 b) studied the effect of addition of soil in the transport medium of Indian carps' larvae and concluded that the soil reduced the extent of pollution by organic matter, as the soil attract the dead larvae infested with bacteria to the bottom of the container and get them buried under the soil. Vaas (1951) demonstrated the bacterial build up in closed system transport and proved the addition of sodium phosphate buffer to be effective in counteracting the carbon dioxide accumulation in the transport medium, ie., fresh water. Different fishes like Oreochromis mossambicus, Cyprinus carpio and Puntius javanicus were used for the study. Phillips and Brockway (1954) found that the best method

to control ammonia was to reduce its rate of excretion by decreasing the metabolism of fish. They reported a three-fold decrease in the accumulation of ammonia in tanks containing brook trout when the water temperature was reduced from 24°C to 13°C.

Srinivasan et al., (1955) experimented on the utility of sodium phosphate in the transport of fingerlings of Indian carps in both ordinary open tin carriers and in closed oxygen-filled tin carriers. The addition of 1.5 g/litre sodium phosphate in the closed oxygen containers was found to be beneficial for the survival of fish and will help in the transport of fingerlings to greater distances and for longer durations than without the addition of buffer. Saha and Chowdhury (1956) studied the effect of red soil (specific soil) in the water medium to check mortality during transportation of carp spawn and found that the red soil was not superior to the ordinary soil in finely pulverised condition. Saha et al., (1956 b) reported the addition of the buffer, secondary sodium phosphate ($Na_2H\ PO_4$) had a negative effect, as its addition reduced the transportation time from 64 hours to 40 hours in the case of carp spawn. Also, they observed that absorbents like pulverised earth, activated charcoal, and amberlite absorbed gases like carbon dioxide and ammonia from the medium, thereby increasing the survival period; but the absorbent-permutit was not beneficial in prolonging survival period. Nemoto (1957) reported the doubtful utility of sodium phosphate in the transport of fishes. Tanizaki et al., (1957) used ion-exchange resins to remove ammonia in the

transport water.

Mc Farland and Norris (1958) reported that inorganic buffering compounds were ineffective in sea water, since they cause either precipitation of calcium salts or buffer in undesirable ranges of acidity. At the same time, they reported that an organic buffer, tris-hydroxymethyl-aminomethane was effective for p^H control in both salt and fresh water closed or open system transportation. Also, they found that the buffer - 'tris-buffer' inhibited bacterial growth in closed system transport, thereby maintaining the clarity of water and fresh odour in comparison to non-buffered controls. Haskell and Davies (1959) reported carbon dioxide to be the limiting factor in trout transportation and recommended methods of purging the excess carbon dioxide including the addition of lime water.

Use of several bactericides and fungicides has been reviewed by Norris et al., (1960). The most commonly used bactericide is acriflavine. S.T. Snieszko found that calphomycin (active ingredient oxytetracyclin) controlled bacteria in transport water. Terramycin at the level of 50mg/gallon prevented bacterial disease during transportation. Dr. Clark - Hubbs reported the use of methylene blue to remove Ichthyopthirus parasites during transportation, and the application of acriflavine for the control of fungus. (See review by Norris et al., 1960).

Jorgensen et al., (1976) stated that clinoptilolite could be used for ammonia removal, because of its action by a combination of ion-exchange and adsorption. He also found that when clinoptilolite was combined with activated carbon, 90% removal of organics and ammonium-ion was effected. Woiwode and Fairgrieve (1980) reported that terramycin acting as an antibacterial agent could be directly added to the transport medium to prevent the mortality of Tilapia fingerlings during transportation; 10.0 mg/L was found to be the optimum treatment level. Amend et al., (1982) described methods to control ammonia, carbon dioxide, pH and bacterial growth during the transportation of aquarium fishes in closed systems. Clinoptilolite at dose of 14 g/litre controlled ammonia accumulation, tris-buffer (0.017 M) at pH 8.0 controlled the accumulation of free carbon dioxide, and neomycin sulphate at a dose of 20 mg/litre prevented bacterial blooms. The data indicated that by using these, the fishes could be transported at higher loading densities for longer duration in better condition than that achieved in earlier practices. The transportation of marine fishes in buffered sea water containing nitrifying bacteria attached to a solid substrate like crushed oyster shell, granulated activated carbon or polyeurythane foam appeared as a practical and effective method for preventing the accumulation of ammonia and decrease in pH (Turner and Bower, 1982). However, its effectiveness in reducing shipping mortalities was not studied. Dupree and Huner

(1984) reported that acriflavine at 1-2 ppm, nitrofurazone at 5-10 ppm or oxytetracycline at 20 ppm reduced bacterial growth and thus the amount of carbon dioxide and ammonia produced during transportation. Also, it was observed that 0.5% common salt alone or in combination with MS-222 at 0.1 - 1.0 g/gallon or quinaldine at 15-30 ppm often aids in the transit of fish.

Froese (1986) highlighted the addition of ion-exchangers like Amberlite IR 120 and Dowex 50 x 8 to remove poisonous ammonia from transport water and thus the fish density in transportation bags could be raised by 20%. Teo et al., (1989) investigated the application of clinoptilolite, tris-buffer and 2-phenoxy ethanol in controlling the aquatic environment of a closed system involving guppies in polyethylene bags containing water overlaid with oxygen. A combination of 2-phenoxyethanol (0.1 g/l) with clinoptilolite (20g/l) or tris-buffer (0.02 M) was effective in lowering the mortality rates of guppies during transportation.

3. MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Experimental fish

C.chanos seed used for the present studies were obtained from the coastal region of Puduveypu in the Vypeen Island, Kerala. The fish seed that enter the ditches and tidal flats in this region were collected using surf net and stocked temporarily (approximately one week) in mosquito netting hapas kept in a nursery pond at the Instructional Farm, Fisheries Station, Kerala Agricultural University, Puduveypu. The salinity of the water in the nursery pond ranged from 15 to 25 ppt, and the pH ranged from 7.5 to 8.5. The seed were lifted from Puduveypu to the College of Fisheries, Panangad in oxygen-filled PVC bags and were acclimatized in a fibre glass tank containing brackish water having a salinity of 20 ppt, a pH range of 7.5 to 8.5, and a temperature range of 28 to 32°C. The seed were fed daily in the morning with powdered mixture of rice bran, ground nut oil cake and dried prawns (1:1:1), after removing the detritus settled at the bottom of the tank, by siphoning using a PVC tube. Sufficient aeration was also provided in the tank. The seed thus acclimatized uniformly for three days were used for the different experiments.

3.2 Experimental containers and other accessories

Two types of containers were used during the course of the

study because of their ease of handling and operation - (1) Specially designed jar-type hard plastic air tight containers and (2) Soft PVC air tight transportation bags patented and supplied by Plastic Crafts Corporation, Bombay.

The jar-type containers were of 300 ml capacity and with a tight screw-type lid (Fig.1). These were made air tight by means of a thin plastic flap tightly fitting inside the lid, which was kept pushed down at the mouth of the container. For regulating the flow of oxygen and reading the oxygen pressure inside the container, a short PVC tube of 3 mm diameter fitted with a one-way valve (the valve used was the one used for pneumatic tyre tube) at the distal end, was firmly fixed to the plastic flap by boring it in the centre. The lid was bored to pass the PVC tube with the valve. M - seal was used as the adhesive for fixing the PVC tube with the plastic flap for complete sealing.

The transportation bags used (Fig. 2) were of 2 litre capacity and made of H/ gauge, soft non-toxic PVC with a firm base and a leak proof internal valve on the wall of the bag. A PVC tube of 3 mm diameter fitted with a one - way valve at the distal end as described earlier, was fixed at the opening of the internal valve on the wall of the bag using Araldite as the adhesive.

Other accessories included a medical oxygen cylinder and

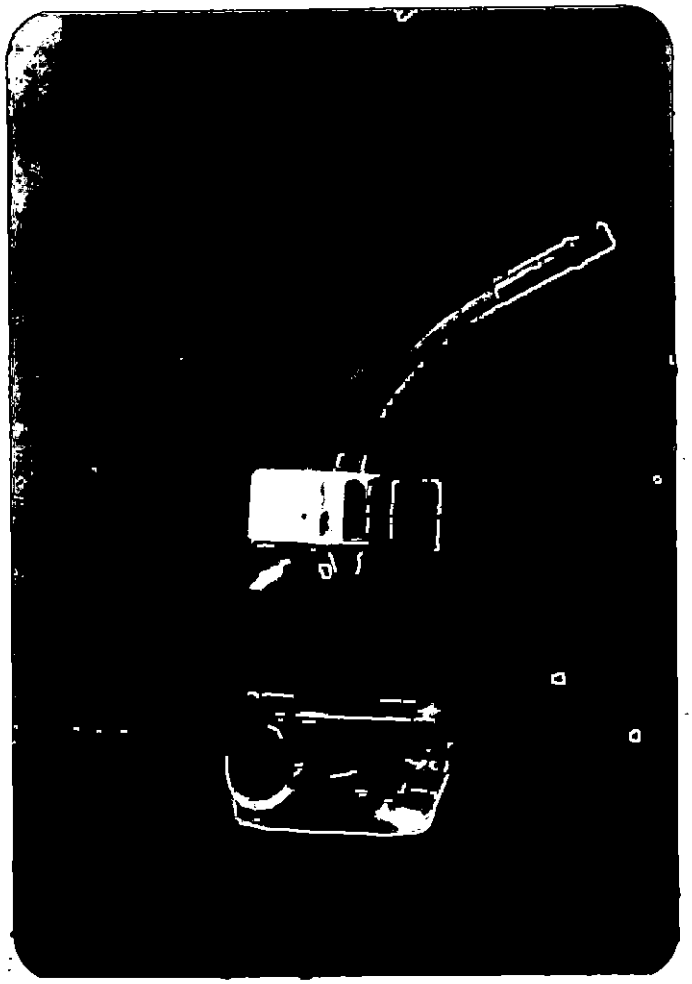

Fig.1. Specially designed hard plastic container used for oxygen packing.

Fig.2. Soft PVC transportation bag used for oxygen packing.

a Bourdon - type pressure gauge, which could indicate a maximum of 1 kg/cm² and a minimum of 0.02 kg/cm². The pressure gauge was fitted with two pieces of pressure - resistant hose, one leading to the cylinder, and the other fitted with a nozzle for pumping in and reading the oxygen pressure inside the containers (Fig.3).

3.3. Experimental procedures for oxygen packing of C.chanos seed

3.3.1 Optimum conditioning prior to oxygen packing.

An experiment was conducted to study the optimum conditioning of the seed prior to oxygen packing. Four treatments, viz., control (A), congestion (B), gut voiding (C), and gut voiding and congestion (D), were used for this experiment. The experiment was statistically designed using Completely Randomised Design (C.R.D.) and each treatment was replicated five times.

The seed acclimatized in the fibre glass tank were collected using a hand net, counted and transferred to plastic basins. The seed used for this experiment were of the size range of 14-20 mm and 0.01 - 0.06 g. In the case of control, the seed without any conditioning, ie, those collected from the acclimatization tank were directly used for oxygen packing. For congestion, 100 seed were congested in 250 ml filtered water of 20 ppt salinity in a plastic basin using nylon net for 15 minutes, and were then used for oxygen packing. For gut voiding, 100 seed were kept in two plastic basins,

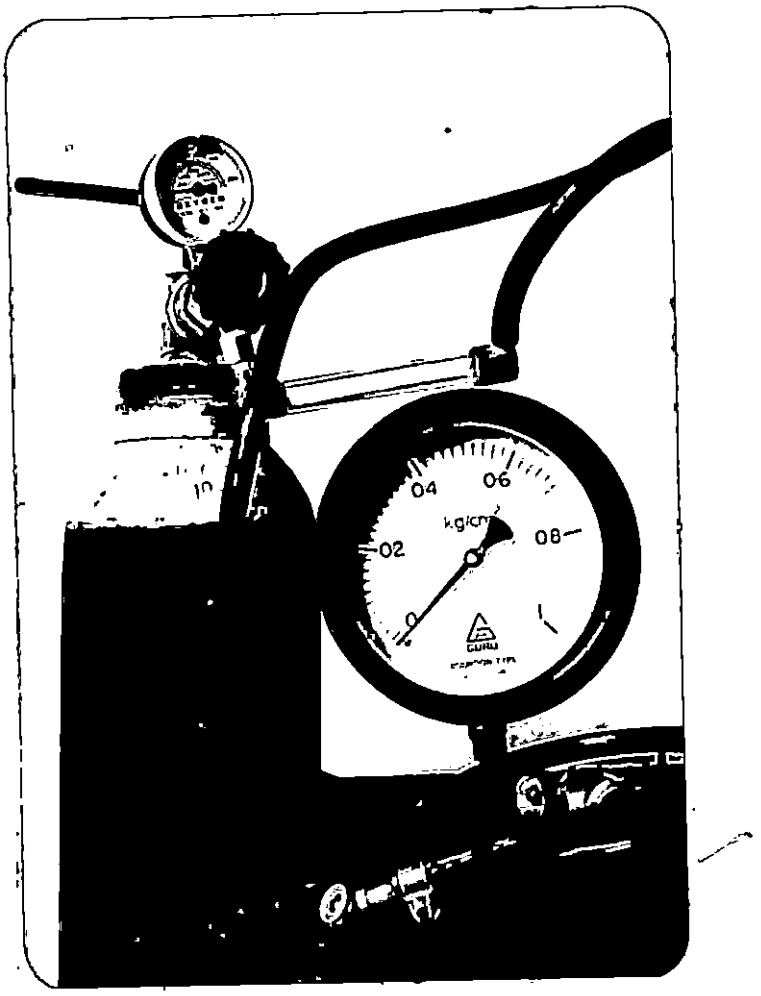

Fig.3. Assembly used for pumping in oxygen and reading the pressure inside the containers.

each containing 50 numbers of seed and 2 litres of filtered water of 20 ppt salinity. The faeces settled at the bottom of the basin was removed at half-hourly interval by siphoning it with a PVC tube. The water taken out during siphoning was measured and equivalent quantity was replaced in the basins each time. The gut was found completely empty within 24 hours of observation. Thereafter, the seed were packed under oxygen pressure. For the fourth treatment viz., gut voiding and congestion, 100 seed were kept in two plastic basins, each containing 50 seed and 2 litres of filtered water of 20 ppt salinity for 24 hours for complete voiding of the gut as explained earlier. The seed were then congested in 250 ml filtered water of 20 ppt salinity in a plastic basin using nylon net for 15 minutes, and were then used for oxygen packing.

The jar-type hard plastic containers were used for oxygen packing. In each container, 100 ml filtered water of 20 ppt salinity was taken and 20 seed from each treatment were carefully transferred to each container. The containers were then closed tightly and oxygen was pumped in through the one-way valve fitted to the inner flaps of the containers, till the oxygen pressure inside each container reached 0.2 kg/cm^2 (Fig.4). It was ensured that air in the containers was displaced completely with oxygen by repeatedly pumping in oxygen and pressing the valve to release the air inside. The packed seed were then transported to a distance of 20 km by road and carefully observed at four hourly

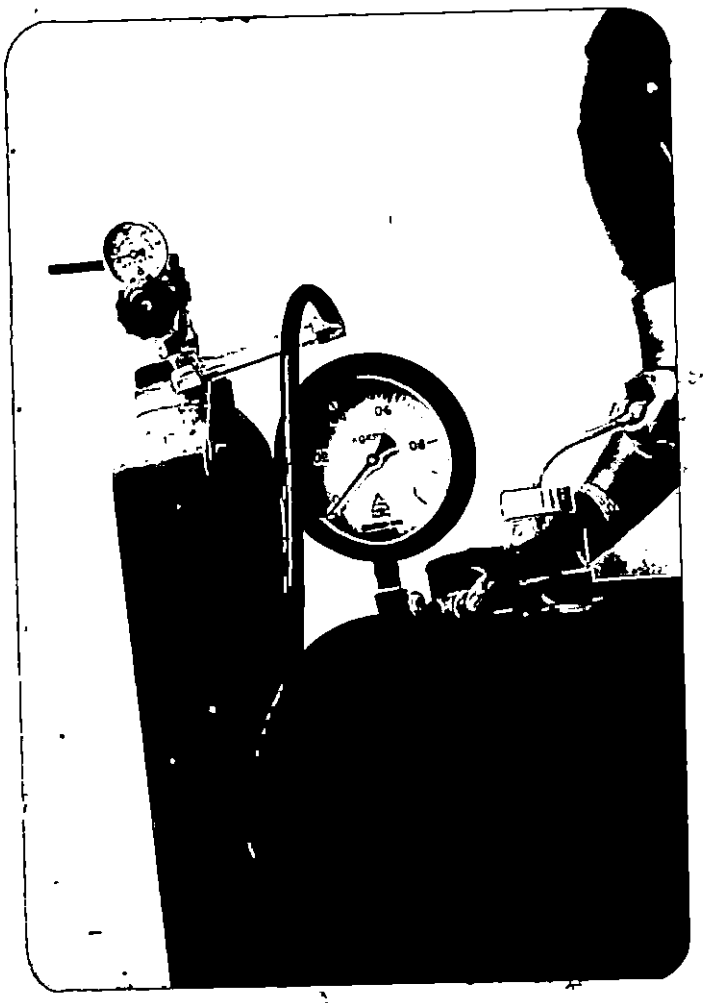

Fig.4. Oxygen packing of seed in hard plastic container.

intervals for any mortality. The containers were periodically shaken to simulate transport conditions. The experiment was terminated when 70 to 77% mortality was observed. The initial and final water quality parameters were also determined to find out their effect on the mortality of the seed in the containers. The water temperature range during the experiment was 28 to 31°C.

3.3.2 Optimising the seed packing density.

Durve and George (1963) reported that 150-175 fry of 0.01 g size could be transported in 100 ml water under oxygen pressure. Taking this as the basis, acclimatized seed of average size of 20 mm and 0.0362 g were packed at densities of 20, 30, 40 and 50 numbers in 50 ml water, ie., 0.724, 1.086, 1.448 and 1.810 g in 50 ml water respectively. The experiment was statistically designed using C.R.D and replicated five times. The jar-type, hard plastic containers were used for this experiment. The corresponding numbers of seed were packed in each container in filtered water of 20 ppt salinity, at an oxygen pressure of 0.2 kg/cm^2, replacing air inside as explained earlier. The packed seed were then transported to a distance of 20 km by road and observed for mortality at four hourly intervals. The containers were periodically shaken to simulate transport conditions. The experiment was wound up uniformly after 52 hours when 100% mortality was observed in the containers packed at 50/50 ml, by which time, 65%, 40% and 30% mortality was observed in the containers packed at 40/50 ml, 30/50

ml and 20/50 ml respectively. The initial and final water quality parameters were also determined. The water temperature range during the experiment was 29 to 32°C.

3.3.3 Effect of salinity on oxygen packing.

The experiment was carried out to determine the effect of different salinity levels on the survival of oxygen-packed C.chanos seed. In this experiment, salinity was kept at 0 ppt, 10 ppt and 20 ppt. The experiment was statistically designed using C.R.D and replicated four times. The seed used for this experiment were obtained from the acclimatization tank and were of the size range, 17-22 mm and 0.01-0.04 g.

The jar-type hard plastic containers were used for this experiment. In each set of containers, 100 ml filtered water of the corresponding salinity was taken and 20 seed were carefully transferred to each container. The containers were then filled with oxygen till the pressure inside each container reached 0.2 kg/cm^2, replacing the air as mentioned in the previous experiment. The containers were then transported to a distance of 20 km by road and carefully observed at two hourly intervals. Any mortality observed at these intervals was noted. The containers were periodically shaken to simulate transport conditions. The experiment was concluded when 70 to 75% mortality was reached in each container. The initial and final water quality parameters were also

determined. The water temperature during the experiment ranged between 28°C and 31.5°C.

3.3.4 Effect of initial pH on oxygen packing.

The experiment was done to study the effect of pH on the duration and survival of the oxygen-packed C.chanos seed. Three ranges of pH , viz., 6.5-7.5 (A), 7.5-8.5 (B) and 8.5-9.5 (C) were tried. Water of the pH 7.5-8.5 and 8.5-9.5 was obtained from the nearby brackishwater ponds of the College of Fisheries, Panangad. Water of the pH 6.5-7.5 was obtained by adjusting the pH with 1 N HCl. The experiment was statistically designed using C.R.D and each treatment was replicated six times. The acclimatized seed of the size range 14-18 mm and 0.01-0.03 g were used for this experiment.

The soft PVC transportation bags were used for oxygen packing in this experiment. In each set of bags, 1 litre water of the corresponding pH range having a salinity of 20 ppt was taken and 10 seed were carefully transferred to each bag. The bags were then filled with oxygen till the pressure reached 0.4 kg/cm^2, replacing the air inside as explained in the earlier experiment (Fig.5). The packed seed were then transported to a distance of 20 km by road and carefully observed for any mortality at four hourly intervals. The bags were periodically shaken to simulate transport conditions. The experiment was terminated when 70 to 75% mortality was observed

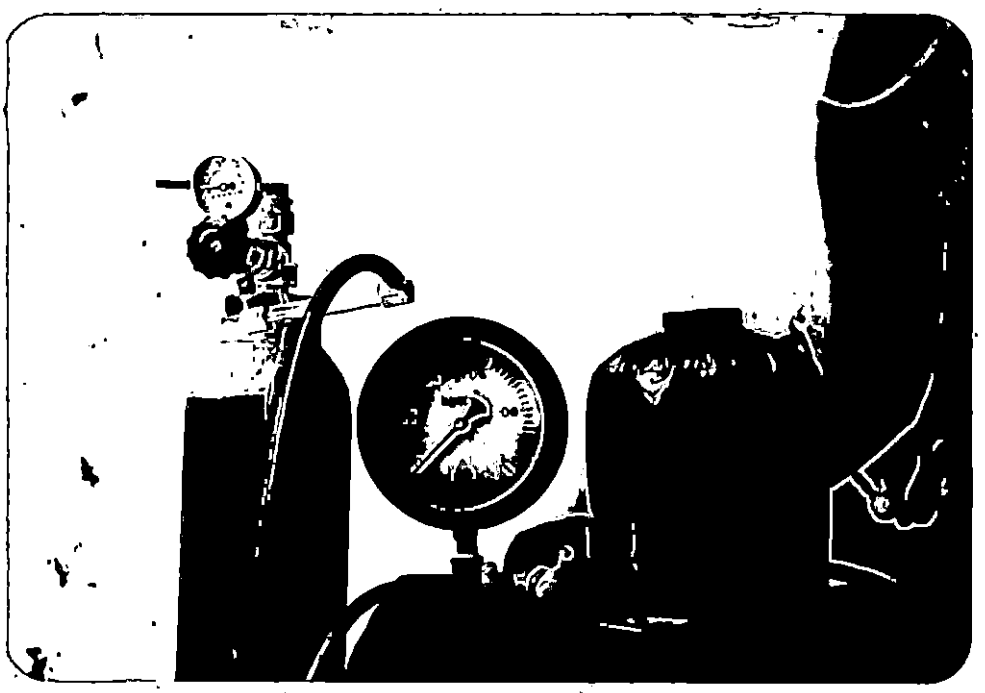

Fig.5. Oxygen packing of seed in soft PVC transportation bag.

in each bag. The final pH in all the bags was also noted. The water temperature range during the experiment was 29-31°C.

3.3.5 Effect of oxygen pressure on packing

The experiment was carried out to find out the effect of two varying pressures of oxygen on the duration and survival of packed C.chanos seed for transportation. One set of containers were filled with oxygen at atmospheric pressure (A) and the other set at 0.2 kg/cm² oxygen pressure (B). The experiment was statistically designed using C.R.D. and each treatment was replicated six times. Acclimatized seed of the size range, 14-18 mm and 0.01-0.03 g were used in this experiment.

The jar-type hard plastic containers were used in this experiment. In each container, 100 ml filtered water of 20 ppt salinity was taken and 20 seed were carefully transferred to each container. One set of six containers were then filled with oxygen by replacing the air inside the containers. The oxygen in these containers were maintained at atmospheric pressure. This was confirmed by checking the normal tension of the container walls. In the second set of six containers, oxygen was filled at a pressure of 0.2 kg/cm². The packed seed were then transported to a distance of 20 km by road and carefully observed at two hourly intervals for any mortality. The containers were periodically shaken to simulate transport conditions. The experiment was wound up when

60-65% mortality was reached in each container. The initial and final water quality parameters were also determined. The water temperature range during the experiment was 29-32°C.

3.3.6 Feasibility of using a sedative in oxygen packing.

An experiment was carried out to study the feasibility of using a commmonly available and cheap sedative, tertiary butyl alcohol in the oxygen packing of C.chanos seed for transportation. A pilot study was carried out to determine the approximate dose of sedative for the seed of the size range, 14-18 mm and 0.01 -0.04 g, based on the information available for mullet fry (Durve, 1975), from which two doses of (1) 0.3 ml/100 ml water and (2) 0.35 ml/100 ml water were selected in packing the seed. In the former case, the seed were lightly sedated, ie, the seed remained stationary in the water column; in the latter case, the seed were deeply sedated, ie, the seed settled at the bottom of the container.

The soft PVC transportation bags were used for this experiment. Three treatments, viz., control (A), 0.3 ml/100 ml water (3.0 ppm, B) and 0.35 ml/100 ml water (3.5 ppm, C) were tried. The treatments were duplicated. In each bag, 1 litre filtered water of 20 ppt salinity was taken and 20 seed were carefully transferred to each bag. In the first set of bags used as the control, sedative was not added. In the other two sets of bags, the two selected doses of the sedative were added. The bags were

then tightly closed and oxygen was filled in through the one-way valve fitted on the internal valve of the bag, till a pressure of 0.4 kg/cm², replacing the air inside as done in the previous experiment. The seed were then transported to a distance of 20 km by road and observed for any stress and mortality at two hourly intervals. The containers were periodically shaken to simulate transport conditions. The experiment was concluded when 70-75% mortality was observed. The initial and final water quality parameters were determined. The water temperature during the experiment ranged between 29°C and 31°C.

3.3.7 Feasibility of using chitosan as an absorbent in oxygen packing.

An experimental trial was conducted to study the feasibility of using chitosan as an absorbent in removing the toxic metabolites accumulated by *C. chanos* seed in the oxygen-filled plastic containers. Two treatments were used for this experiment, one with chitosan (A) and the other without it (B); the treatments were duplicated. The trial was carried out using acclimatized seed of the size range, 24-32 mm and 0.02-0.29 g. The chitosan in fluffy form was thoroughly washed with distilled water, dried and then packed in coarse cotton bags, each weighing 0.5 g.

The jar-type hard plastic containers were used for this experiment. In each container, 50 ml filtered water of 20 ppt salinity

was taken and two seed were carefully transferred to each container. In one set of containers, the small bag of 0.5 g chitosan was added. In the other set of containers, chitosan was not added. The containers were then tightly closed and oxygen was filled till the pressure inside each container reached 0.2 kg/cm², replacing the air inside as explained earlier. The packed seed were then transported to a distance of 20 km by road and observed at hourly intervals for any stress and mortality. The containers were periodically shaken to simulate transport conditions. The experiment was terminated after 12 hours of observation as the seed died in the absorbent-packed containers by about that time. The initial and final water quality parameters in the containers were determined to study their effect on mortality. The water temperature during the experiment was 28-31.5ºC.

3.4 Determination of water quality parameters

The following water quality parameters were analysed using the method mentioned against each.

Dissloved oxygen	:	Standard Winkler's method. (Strickland and Parsons, 1972).
pH	:	Colourimetric method using universal pH indicator solution , which in turn periodically checked with pH meter.

Carbon dioxide : Alkalimetric titration method. (APHA et al., 1981).

Ammonia-nitrogen : Photometric measurement using indophenol method . (Grasshoff et al., 1983).

3.5 Statistical analyses

The percentage survival observed upto a particular period of packing under different treatments of conditioning were compared using the Analysis of variance (ANOVA) technique. The percentage values were first transformed using the arc sine transformation given by :-

$\theta = \sin^{-1}\sqrt{x}$, where x is the percentage value divided by 100 (Snedecor and Cochran, 1967). The various water quality parameters were analysed separately using the ANOVA technique to find their effect on the different treatments of conditioning. For the treatments with significant difference at 5% level, pair-wise comparisons were made using critical diffference values, given by

$C.D. = \sqrt{\frac{2\ EMSS}{r}} \times t_{5\%}$, where r is the number of replications and $t_{5\%}$ is the value of students' t at 5% level with degrees of freedom of error mean sum of squares (EMSS).

The relationship between packing density and time of initial

mortality was studied using regression method. (Snedecor and Cochran, 1967). Also, the water quality parameters were analysed separately using ANOVA technique to find their effect on the different packing densities. For the treatments with significant difference at 5% level, pair-wise comparisons were made using critical difference values.

In the experiment to study the effect of salinity on packing, the water quality parameters, viz., carbon dioxide and dissolved oxygen were analysed separately using ANOVA technique to find their effect on the C.chanos seed packed under the three different levels of salinity.

4. RESULTS

4. RESULTS

4.1 Optimum conditioning method prior to oxygen packing

The details of the cumulative percentage mortality of C.chanos seed packed after conditioning them under four treatments, viz., control (without any conditioning, A), congestion (B), gut voiding (C) and gut voiding and congestion (D) are given in Table 1 and Fig.6. At 24 hours of packing, the seed packed after the treatments B, C and D did not show any mortality, while those packed after treatment A resulted in 8% mortality. At 48 hours of packing, the seed packed after treatment D did not show any mortality, while those packed after the treatments A, B and C showed 36%, 26% and 14% mortality respectively. At 72 hours of packing, the seed packed after the treatments A,B,C and D showed 74%, 55%, 40% and 22% mortality respectively. The rate of mortality of the seed packed under each of the four treatments at four hourly intervals was gradual, ranging from 3-10%.

The duration of 100% survival for each of the four treatments is represented in Fig.7. The seed packed after treatment D showed 100% survival for the longest duration of 52 hours after packing, followed by treatment C for 36 hours, treatment B for 24 hours and treatment A for 16 hours of packing. It is obvious that treatment D is the best conditioning method prior to oxygen packing of C.chanos seed as it showed 3.25 fold increase in the duration

Table 1. Cumulative percentage mortality* of C.chanos seed packed after conditioning them under four different treatments, at four hourly intervals.

Time interval (hours)	Percentage Mortality			
	Control	Congested	Gut voided	Gut voided and congested
Upto 16	0.0	0.0	0.0	0.0
20	5.0	0.0	0.0	0.0
24	8.0	0.0	0.0	0.0
28	13.0	5.0	0.0	0.0
32	17.0	10.0	0.0	0.0
36	22.0	14.0	0.0	0.0
40	26.0	19.0	5.0	0.0
44	31.0	22.0	8.0	0.0
48	36.0	26.0	14.0	0.0
52	40.0	31.0	18.0	0.0
56	45.0	33.0	23.0	5.0
60	51.0	38.0	28.0	8.0
64	56.0	44.0	32.0	13.0
68	64.0	49.0	37.0	17.0
72	74.0	55.0	40.0	22.0
76	-	60.0	45.0	26.0
80	-	70.0	50.0	31.0
84	-	79.0	55.0	35.0
88	-	-	60.0	40.0
92	-	-	65.0	46.0
96	-	-	75.0	54.0
100	-	-	-	61.0
104	-	-	-	71.0
108	-	-	-	77.0

* Each value is a mean of five replicates.

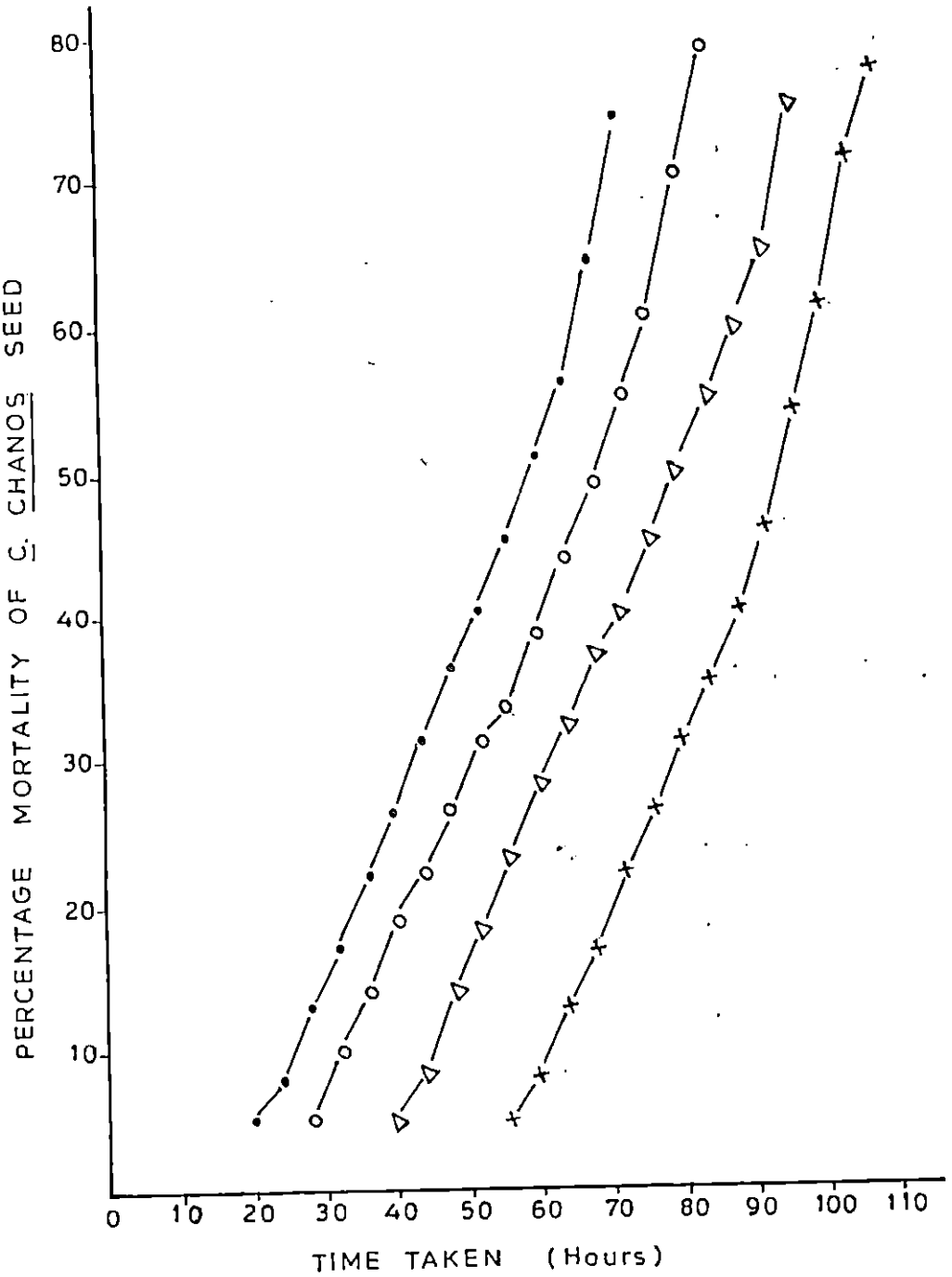
PERCENTAGE MORTALITY OF C. CHANOS SEED
80
70
60
50
40
30
20
10
0
10
20
30
40
50
60
70
80
90
100
110
TIME TAKEN (Hours)

Fig. 6

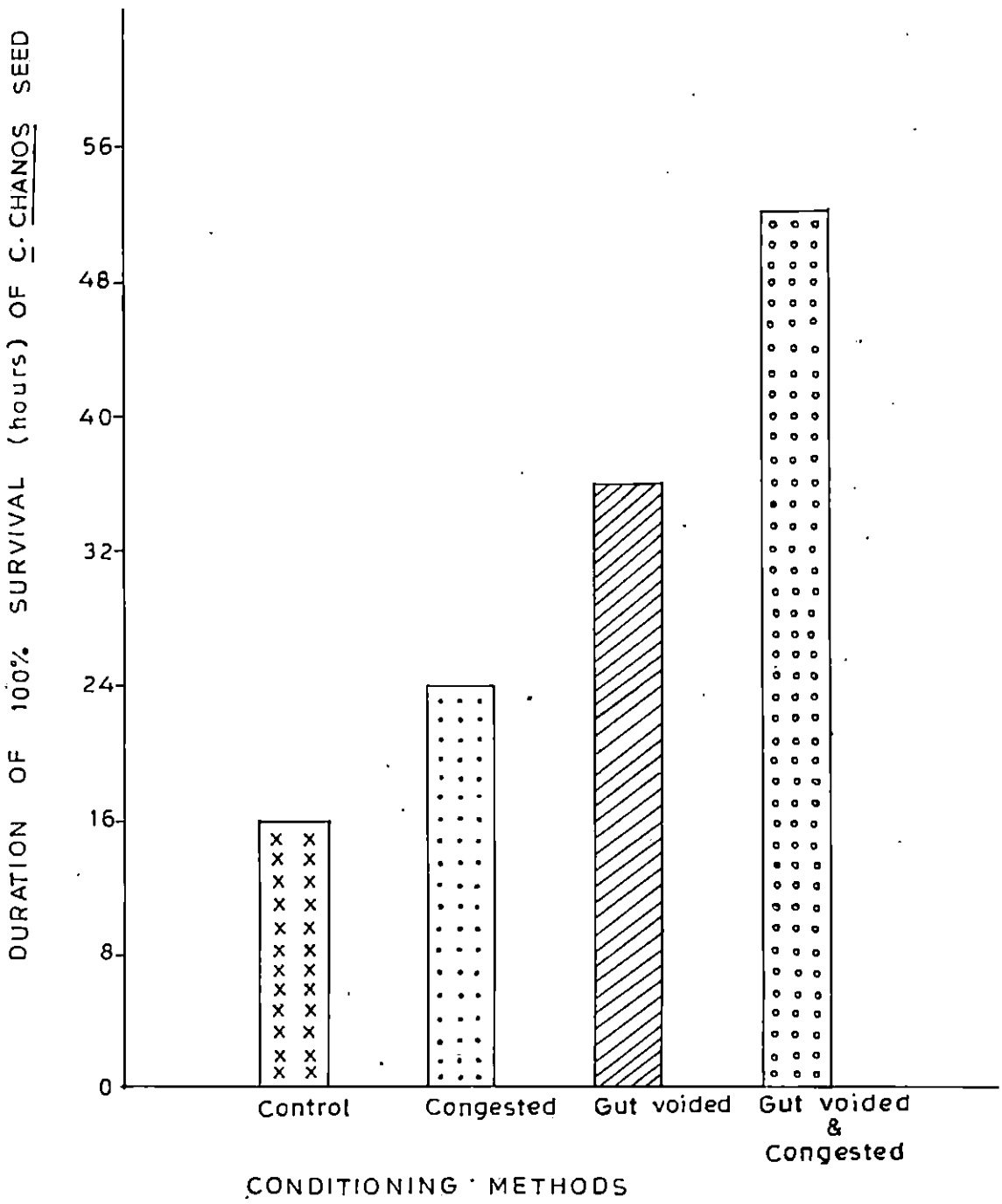
DURATION OF 100% SURVIVAL (hours) OF C. CHANOS SEED
0
8
16
24
32
40
48
56
Control
Congested
Gut voided
Gut voided & Congested
CONDITIONING METHODS

Fig. 7

of 100% survival compared to the seed packed without any conditioning.

The effect of the four treatments of conditioning on the survival of C. chanos seed at different periods of packing from 16 hours upto a maximum of 72 hours was studied using ANOVA technique (Table 2). It is clear that significant difference exists at 5% level. Pair-wise comparisons show that treatments A & D and B & D differ significantly unlike the treatment pairs of A & B, A & C, B & C and C & D.

The results of the chemical analyses of water quality parameters determined initially and at the end of the experiment are given in Table 3. It was observed that in treatment D, the final levels of ammonia-nitrogen (36.20 mg/L) and carbon dioxide (12.57 mg/L) were the lowest of all the treatments; the final dissolved oxygen content (14.50 mg/L) was the highest. The initial and final pH remained almost uniform in all the treatments. In the case of treatment A, the final levels of ammonia-nitrogen (50.10 mg/L) and carbon dioxide (71.69 mg/L) were the highest and the dissolved oxygen content (4.99 mg/L) was the lowest. Figures 8 - 11 show a comparison of the final levels of the water quality parameters under the four different treatments.

ANOVA for each of the water quality parameters, except pH which remained almost uniform in all the four treatments, to reveal

Table 2. ANOVA for testing the significance of the four different treatments of conditioning on the survival of the oxygen - packed C.chanos seed at 8 - hourly intervals from 16 to 72 hours.

Source	S.S.	D.f	M.S.S	F value Computed	F value Tabular (5%level)
Conditioning method	2621.86	3	873.95	3.29	2.93*
Error	7432.15	28	265.43		
Total	10054.01	31			

* Significant at 5% level.

The percentage values were transformed using the arc sine transformation, given by $\theta = \sin^{-1} \sqrt{x}$, where x is the percentage value divided by 100.

Conditioning method survival mean.

$\bar{x}_A$: 57.72

$\bar{x}_B$: 65.07

$\bar{x}_C$: 72.16

$\bar{x}_D$: 82.25

The calculated C.D value was 16.68.

Table 3. Initial (I) and final (II) levels* of water quality parameters in the containers with C.chanos seed packed after conditioning them under four treatments.

Parameters	Treatments							
	A		B		C		D	
	I	II	I	II	I	II	I	II
NH_3-N (mg/L)	Nil	50.10	Nil	45.70	Nil	43.50	Nil	36.20
CO_2 (mg/L)	Nil	71.69	Nil	53.54	Nil	19.09	Nil	12.57
DO (mg/L)	32.00	4.99	32.00	7.87	32.00	9.42	32.00	14.50
pH	7.5	7.2	7.5	7.2	7.5	7.5	7.5	7.5

* Each value is a mean of five replicates.

A - Control (no conditioning); B - congested;
C - Gut voided; D - Gut voided and congested.

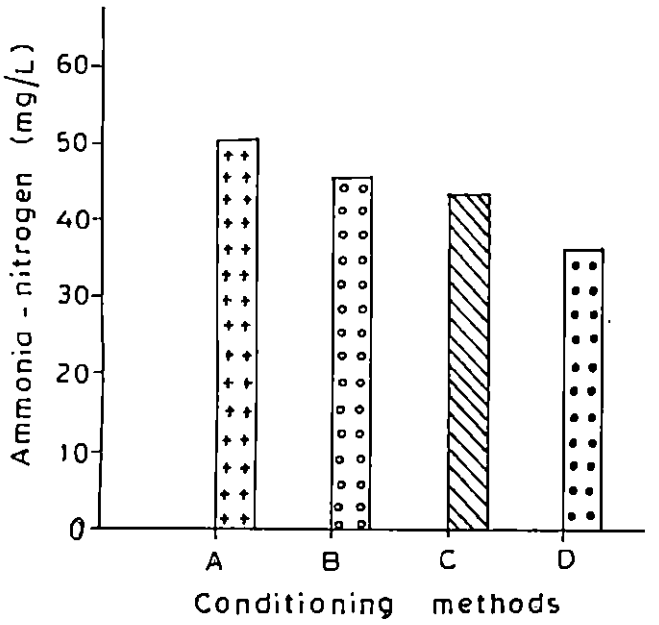

Fig. 8

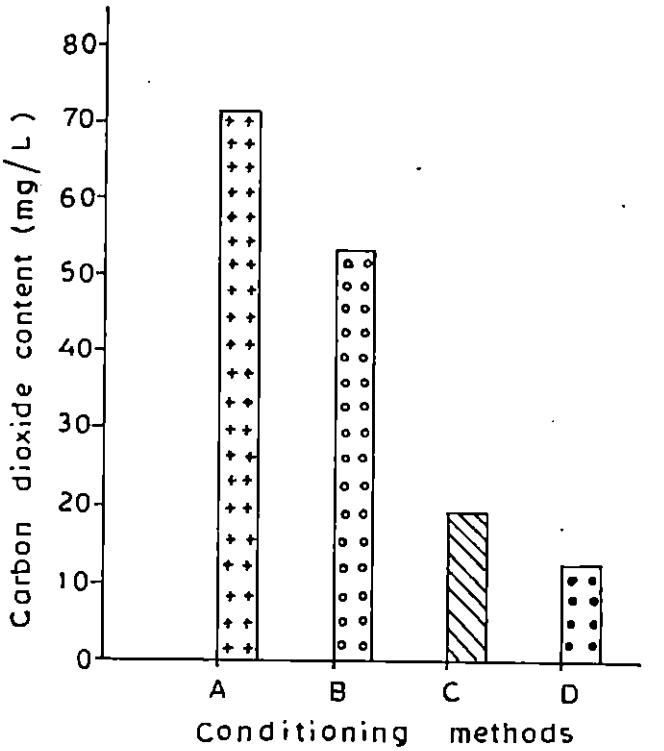

Fig. 9

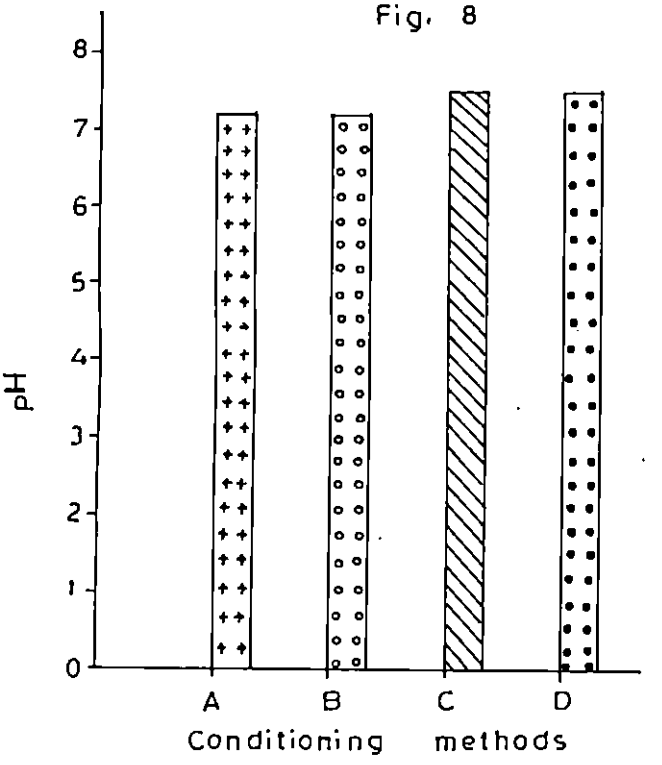

Fig. 10

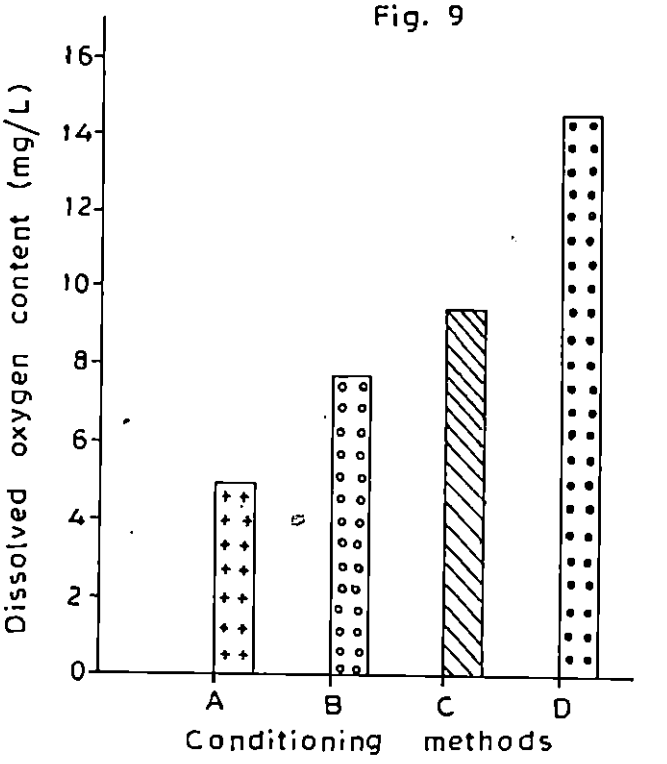

Fig. 11

their effect on the different conditioning methods shows significant difference at 5% level. In the case of ammonia-nitrogen, pair-wise comparisons show that treatments A & C, A & D, B & D and C& D differ significantly unlike the pairs of treatments A & B and B & C (Table 4). In the case of carbon dioxide and dissolved oxygen, pair-wise comparisons reveal that all treatment pairs differ significantly (Tables 5 and 6).

4.2 Optimum density of seed for oxygen packing for a given duration

The cumulative percentage mortality of C.chanos seed of average size, 20 mm and 0.0362 g, packed at four different densities, viz., 20,30,40 and 50 numbers per 50 ml, upto 52 hours of packing is given in Table 7 and Fig.12. It is obvious from the data obtained that the duration of 100% survival decreases with increase in the packing density of C.chanos seed. At the packing density of 20,30,40 and 50 numbers per 50 ml, 100% survival was observed upto 34,28,20 and 12 hours respectively. The duration of 100% survival of the seed at the four different packing densities is depicted in Fig.13.

A relationship between packing density (g/50 ml) of C.chanos seed and time (hour) of starting the initial mortality was established (Fig.14).The relationship was found to be linear. The regression equation establised was Y = 51.398-20.44X; where Y stands for time

Table 4. Analysis of variance of the effect of Ammonia-nitrogen on the four different treatments of conditioning.

Source	S.S	D.f	M.S.S	F value Computed	Tabular (5%level)	
Treatment	505.638	3	168.546	6.47	3.49	*
Replication	96.625	4	24.156	0.93	3.26	NS
Error	312.675	12	26.056			
Total	914.938	19				

* Significant at 5% level.

NS Not significant .

Treatment mean.

$\bar{x}_A$: 50.10

$\bar{x}_B$: 45.70

$\bar{x}_C$: 43.50

$\bar{x}_D$: 36.20

The calculated C.D. value was 5.75.

Table 5. Analysis of variance of the effect of Carbon dioxide on the four different treatments of conditioning.

Source	S.S.	D.f	M.S.S	F value Computed	F value Tabular (5%level)	
Treatment	11874.292	3	3958.10	1168.93	3.49	*
Replication	17.879	4	4.47	1.32	3.26	NS
Error	40.633	12	3.39			
Total	11932.803	19				

* Significant at 5% level.

NS Not significant.

Treatment mean.

$\bar{x}_A$: 71.69

$\bar{x}_B$: 53.54

$\bar{x}_C$: 19.09

$\bar{x}_D$: 12.57

The calculated C.D value was 1.3401.

Table 6. Analysis of variance of the effect of Dissolved oxygen on the four different treatments of conditioning.

Source	S.S	D.f	M.S.S	F value Computed	F value Tabular (5%level)	
Treatment	237.843	3	79.2810	2763.05	3.49	*
Replication	0.014	4	0.0035	0.123	3.26	NS
Error	0.344	12	0.0287			
Total	238.201	19				

* Significant at 5% level.

NS Not significant.

Treatment mean.

$\bar{x}_A$: 4.99

$\bar{x}_B$: 7.87

$\bar{x}_C$: 9.42

$\bar{x}_D$: 14.50

The calculated C.D. value was 0.1909.

Table 7. Cumulative percentage mortality* of C.chanos seed packed at various densities, at two hourly intervals.

Time interval (hours)	Percentage Mortality			
	20/50 ml (0.724 g)	30/50 ml (1.086 g)	40/50 ml (1.448 g)	50/50 ml (1.810 g)
Upto 12	0.0	0.0	0.0	0.0
14	0.0	0.0	0.0	4.8
16	0.0	0.0	0.0	6.4
18	0.0	0.0	0.0	14.4
20	0.0	0.0	0.0	20.8
22	0.0	0.0	2.0	24.4
24	0.0	0.0	4.0	28.8
26	0.0	0.0	6.5	32.8
28	0.0	0.0	10.5	37.6
30	0.0	4.0	17.0	42.4
32	0.0	4.7	21.0	47.2
34	0.0	8.7	26.0	52.0
36	2.0	12.0	30.5	56.8
38	3.0	16.0	36.0	61.2
40	8.0	17.3	41.5	68.0
42	11.0	20.0	45.5	73.6
44	15.0	22.0	48.5	81.6
46	19.0	24.7	52.0	88.0
48	24.0	30.0	57.0	94.0
50	28.0	35.3	63.0	97.6
52	30.0	40.0	65.0	100.0

* Each value is a mean of five replicates.

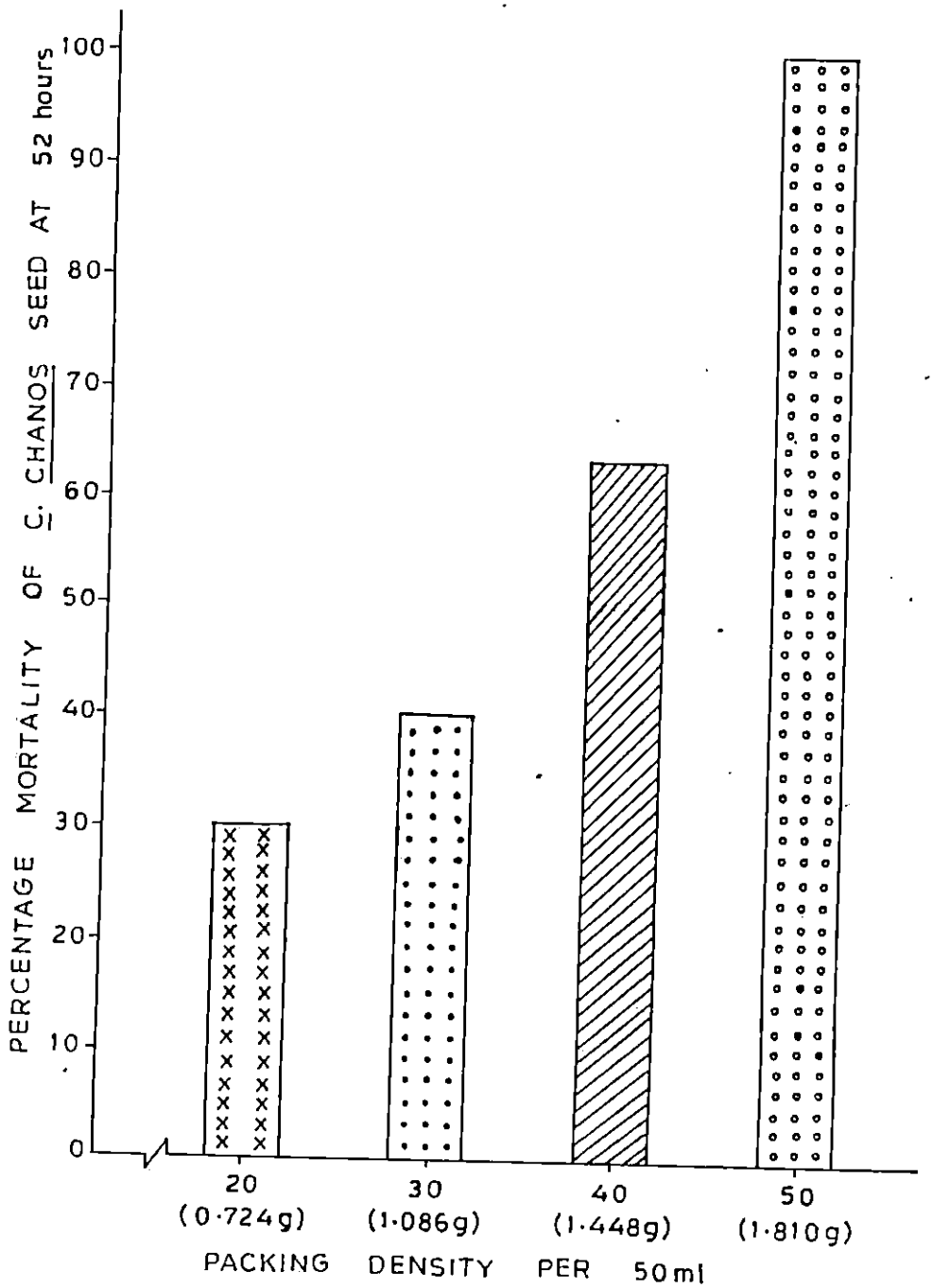
PERCENTAGE MORTALITY OF C. CHANOS SEED AT 52 hours
100
90
80
70
60
50
40
30
20
10
0
20
(0·724g)
30
(1·086g)
40
(1·448g)
50
(1·810g)
PACKING DENSITY PER 50ml

Fig. 12

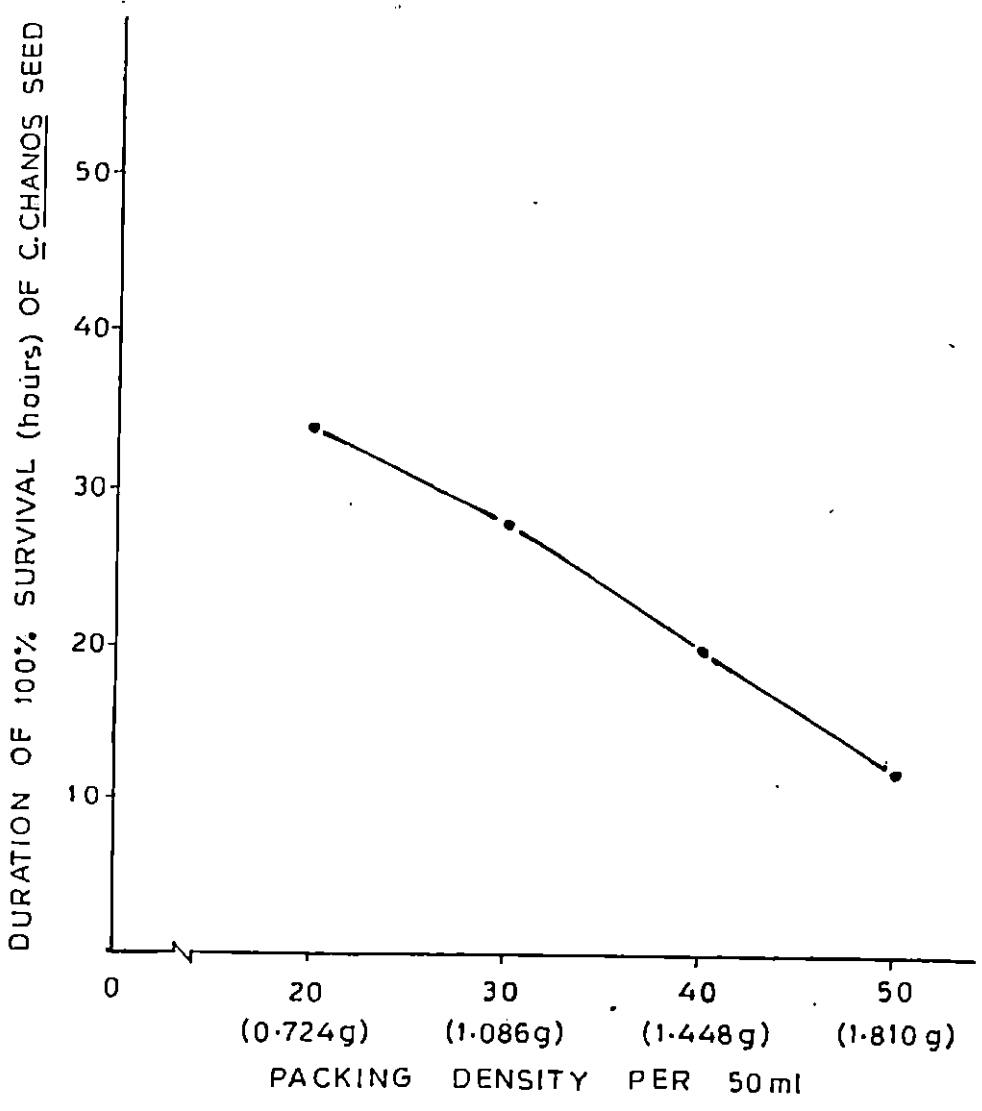
DURATION OF 100% SURVIVAL (hours) OF C. CHANOS SEED
50
40
30
20
10
0
20
(0·724g)
30
(1·086g)
40
(1·448g)
50
(1·810g)
PACKING DENSITY PER 50ml

Fig. 13

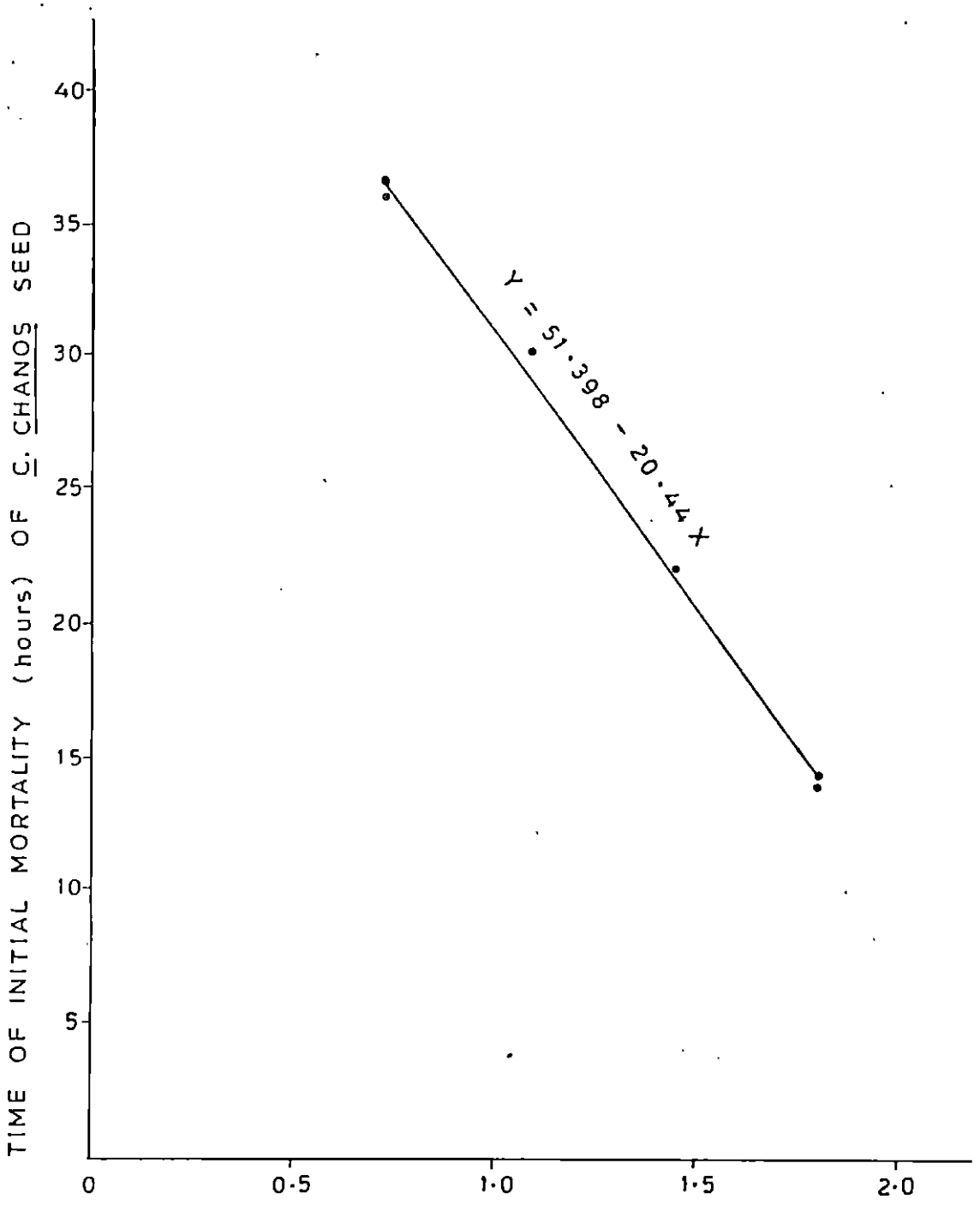
TIME OF INITIAL MORTALITY (hours) OF C. CHANOS SEED
40
35
30
25
20
15
10
5
0
0·5
1·0
1·5
2·0
Y = 51·398 - 20·44 X
PACKING DENSITY (g/50 ml)

Fig. 14

(hours) at which initial mortality starts and X stands for packing density in g/50 ml.

In the 50 per 50 ml group, the walls of the containers were seen slightly withdrawn at the end of the experiment. The results of the chemical analyses of the initial and final water quality parameters are given in Table 8. The final level of carbon dioxide in the 50 per 50 ml group was the highest (214.14 mg/L). The ammonia-nitrogen content was also the highest (58.10 mg/L); the final pH dropped to 6.0 and the dissolved oxygen content was zero. For the 20 per 50 ml group, the final carbon dioxide and ammonia-nitrogen were the lowest (40.96 mg/L and 4.88 mg/L respectively); the pH remained almost uniform and the dissolved oxygen content (24.96 mg/L) was the highest of all groups. Figures 15-18 show the comparison of the final levels of the water quality parameters for the four different packing densities.

ANOVA for each of the water quality parameters to study their effect on the different packing densities show that significant difference exists among the four treatments for ammonia-nitrogen, carbon dioxide and dissolved oxygen (Tables 9-11). Pair-wise comparisons also show significant difference among all the treatment pairs for ammonia-nitrogen, carbon dioxide and dissolved oxygen. In the case of pH , the total variation is completely explained by the treatment variation (Table 12).

Table 8. Initial (I) and final (II) levels* of water quality parameters in the containers packed with C.chanos seed at four different packing densities.

Parameters	Packing density							
	20/50 ml		30/50 ml		40/50 ml		50/50 ml	
	I	II	I	II	I	II	I	II
NH_3 - N (mg/L)	Nil	4.88	Nil	18.30	Nil	29.60	Nil	58.10
CO_2 (mg/L)	Nil	40.96	Nil	76.34	Nil	141.52	Nil	214.14
DO (mg/L)	32.00	24.96	32.00	16.64	32.00	6.08	32.00	0.00
pH	8.0	7.5	8.0	7.0	8.0	6.5	8.0	6.0

* Each value is a mean of five replicates.

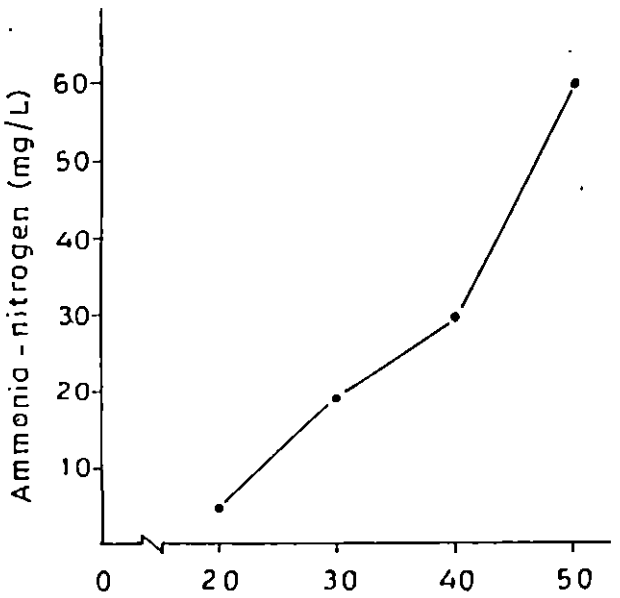

Fig. 15

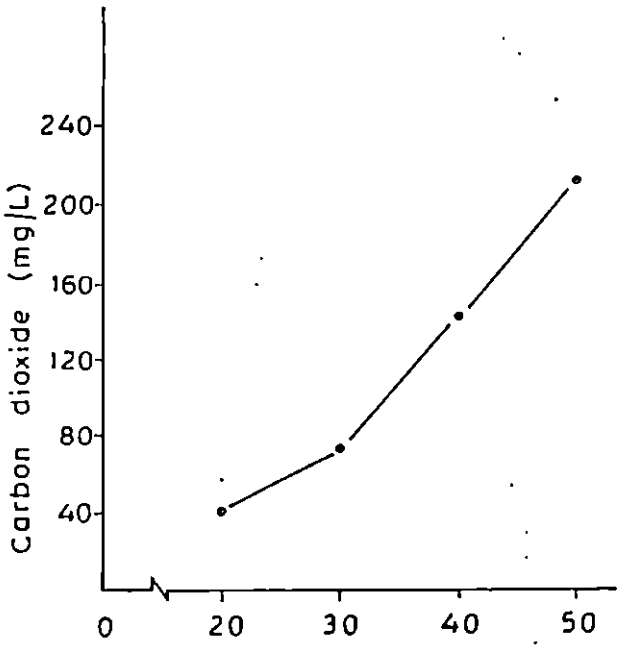

Fig. 16

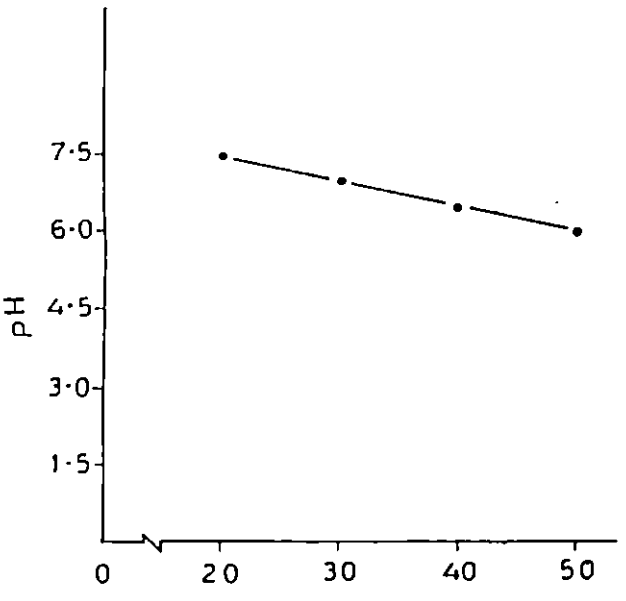

Fig. 17

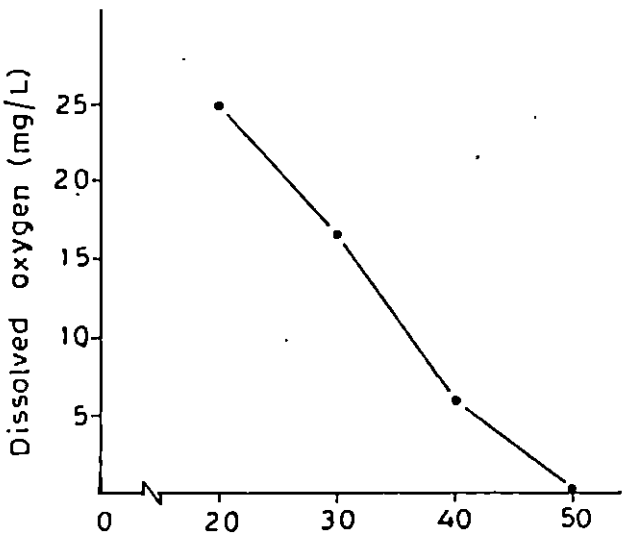

Fig. 18

Table 9. Analysis of variance of the effect of Ammonia-nitrogen on the different packing densities.

Source	S.S.	D.f	M.S.S	F value Computed	F value Tabular (5%level)	
Treatment	7684.404	3	2561.48	871.25	3.49	*
Replication	31.943	4	7.99	2.72	3.26	NS
Error	35.280	12	2.94			
Total	7751.627	19				

* Significant at 5% level.

NS Not significant.

Treatment mean.

$\bar{x}_A$: 4.88

$\bar{x}_B$: 18.30

$\bar{x}_C$: 29.60

$\bar{x}_D$: 58.10

The calculated C.D. value was 1.932.

Table 10. Analysis of variance of the effect of Carbon dioxide on the different packing densities.

Source	S.S.	D.f	M.S.S	F value Computed	Tabular (5%level)	
Treatment	87329.42	3	29109.81	719.65	3.49	*
Replication	450.72	4	112.68	2.79	3.26	NS
Error	485.38	12	40.45			
Total	88265.52	19				

* Significant at 5% level.

NS Not significant.

Treatment Mean.

$\bar{x}_A$: 40.96

$\bar{x}_B$: 76.34

$\bar{x}_C$: 141.52

$\bar{x}_D$: 214.14

The calculated C.D value was 7.168.

Table 11. Analysis of variance of the effect of Dissolved oxygen on the different packing densities.

Source	S.S.	D.f	M.S.S	F value Computed	F value Tabular (5%level)	
Treatment	1842.56	3	614.19	1919.3	3.49	*
Replication	1.79	4	0.45	1.4	3.26	NS
Error	3.84	12	0.32			
Total	1848.19	19				

* Significant at 5% level.

NS Not significant

Treatment mean.

$\bar{x}_A$; 24.96

$\bar{x}_B$: 16.64

$\bar{x}_C$: 6.08

$\bar{x}_D$: 0.00

The calculated C.D value was 0.638.

Table 12. Analysis of variance of the effect of pH on the different packing densities.

Source	S.S.	D.f	M.S.S	F value
Treatment	3.438	3	1.146	The total variation is completely explained by the treatment variation.
Replication	0.0	4	0.0	
Error	0.0	12	0.0	
Total	3.438	19		

4.3 Effect of salinity on oxygen packing

The details of the cumulative percentage mortality of C.chanos seed packed in three different salinities, viz., 0 ppt, 10 ppt and 20 ppt are given in Table 13. Until 32 hours of packing, 100% survival of the seed was observed in all the three treatments. The pattern of mortality in all the three treatments was almost uniform.

The results of the initial and final levels of the water quality parameters analysed are given in Table 14. In all the three treatments, the final ammonia-nitrogen content was uniform, ie., 43.88 mg/L. The final content of carbon dioxide and dissolved oxygen varied slightly, but were found to be statistically insignificant. (see Tables 15 and 16). The pH dropped from an initial level of 8.0 to a final level of 6.25 at 0 ppt and 20 ppt, and to 6.5 at 10 ppt. The final levels of the water quality parameters in the three treatments are compared in Figs.19-22.

4.4 Effect of initial water pH on oxygen packing

Table 17 reveals the cumulative percentage mortality of C.chanos seed packed under three different ranges of pH , viz., 6.5-7.5 (A), 7.5-8.5 (B) and 8.5-9.5 (C). For all the three treatments, the duration of 100% survival was 76 hours of packing. The rate of mortality in all the three cases was gradual, ranging from 1.7 to 10% at four hourly intervals. At 96 hours of packing,

Table 13. Cumulative percentage mortality* of C.chanos seed packed in three different salinities, at two hourly intervals.

Time interval (hours)	Percentage mortality		
	0 ppt	10 ppt	20 ppt
Upto 32	0.00	0.00	0.00
34	13.75	13.75	13.75
36	25.00	26.25	25.00
38	36.25	36.25	35.00
40	51.25	52.50	50.00
42	60.00	62.50	60.00
44	72.50	73.75	72.50

* Each value is a mean of four replicates.

Table 14. Initial (I) and final (II) levels* of water quality parameters in the containers packed with C. chanos seed under three different levels of salinity.

Parameters	Level of salinity					
	0 ppt		10 ppt		20 ppt	
	I	II	I	II	I	II
NH_3 - N (mg/L)	Nil	43.88	Nil	43.88	Nil	43.88
CO_2 (mg/L)	Nil	125.69	Nil	114.05	Nil	118.71
DO (mg/L)	30.00	9.13	32.00	10.29	32.00	9.96
pH	8.0	6.25	8.0	6.5	8.0	6.25

* Each value is a mean of four replicates.

Table 15. Analysis of variance of the effect of Carbon dioxide on C. chanos seed packed under three different levels of salinity.

Source	S.S	D.f	M.S.S	F value Computed	F value Tabular (5%level)
Treatment	2	274.498	137.249	2.192	4.26 NS
Error	9	563.443	62.605		
Total	11	837.941			

NS Not significant

Table 16. Analysis of variance of the effect of Dissloved oxygen on C.chanos seed packed under three different levels of salinity.

Source	D.f	S.S	M.S.S	F value Computed	F value Tabular (5%level)	
Treatment	2	2.866	1.433	3.77	4.26	NS
Error	9	3.417	0.38			
Total	11	6.283				

NS Not significant

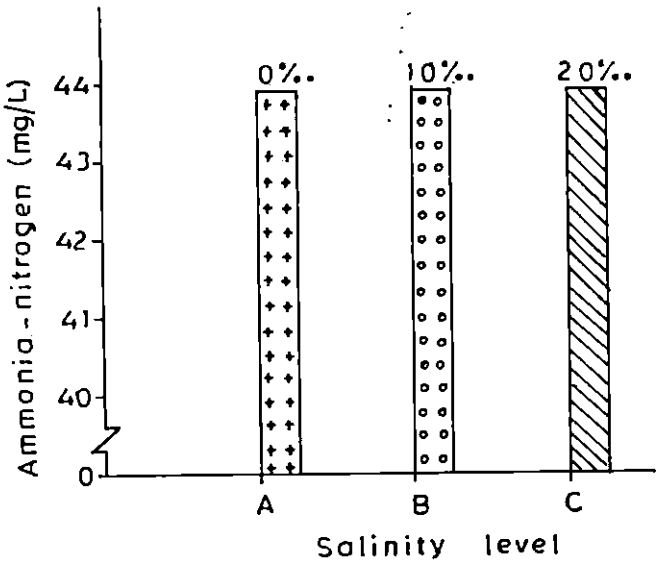

Fig. 19

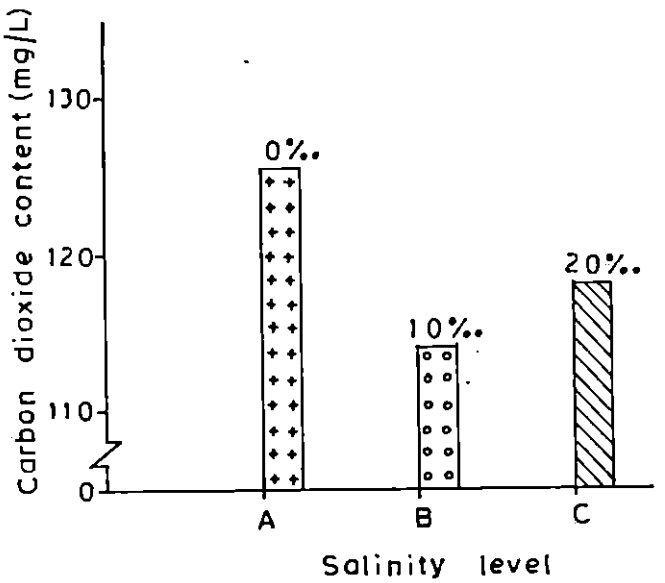

Fig. 20

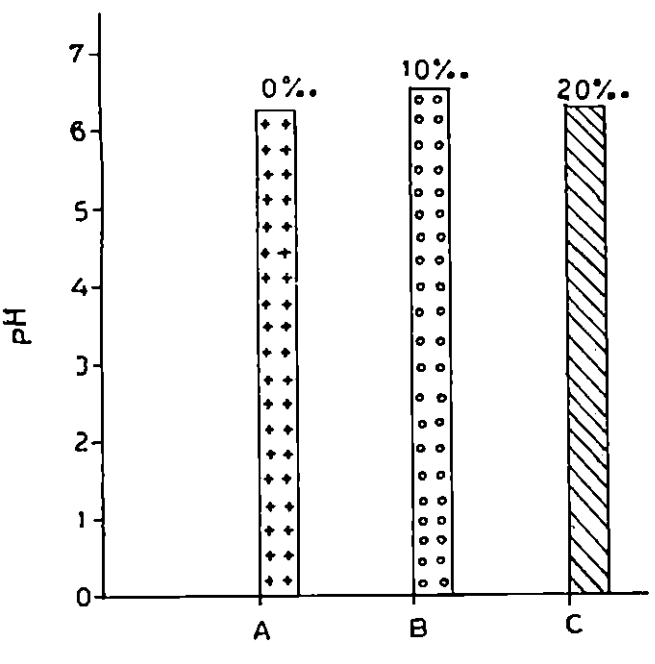

Fig. 21

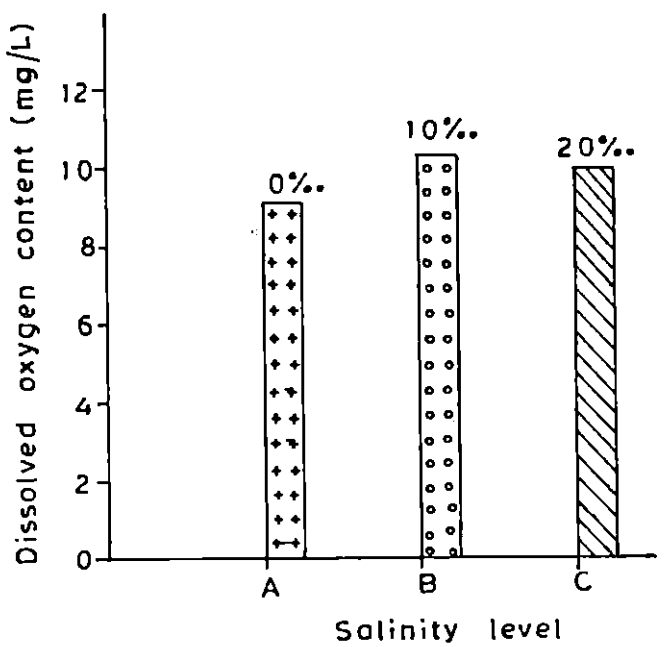

Fig. 22

Table 17. Cumulative percentage mortality* of C.chanos seed packed under three different ranges of pH , at four hourly intervals.

Time interval (hours)	Percentage mortality under different pH ranges		
	6.5-7.5	7.5-8.5	8.5-9.5
Upto 76	0.0	0.0	0.0
80	10.0	6.7	8.3
84	15.0	11.7	15.0
88	16.7	15.0	16.7
92	25.0	18.3	21.7
96	26.7	25.0	23.3
100	30.0	28.3	30.0
104	35.0	30.0	31.7
108	38.3	35.0	35.0
112	43.3	38.3	41.7
116	45.0	48.0	45.0
120	50.0	51.7	51.7
124	56.7	56.7	55.0
128	66.7	61.7	63.0
132	70.0	68.3	71.7
136	71.7	71.7	73.3

* Each value is a mean of six replicates.

the mortality of the seed in all the three treatments was almost uniform, ie., 26.7%, 25.0% and 23.3% in treatments A, B and C respectively. At 120 hours of packing, the corresponding mortality rates were also almost uniform ie., 50%, 51.7% and 51.7%. At the time of termination of the experiment ie., at 136 hours, the corresponding mortality rates were 71.7%, 71.7% and 73.3%.

The final pH levels in all the three treatments ranged between 6.5 and 7.0.

4.5 Effect of oxygen pressure on packing

The cumulative percentage mortality of C.chanos seed packed under two levels of oxygen pressure, viz., oxygen at atmospheric pressure (A) and at 0.2 kg/cm^2 (B) is given in Table 18. In both cases, the duration of 100% survival was 34 hours of packing. Subsequently, the rate of mortality at two hourly intervals in both the treatments was almost uniform, ranging from 1 to 8.35%. At 36, 48 and 64 hours, the mortality rates were also uniform in both the treatments, ie., 5.0%, 24.15% and 60.85% respectively.

The initial and final levels of the water quality parameters under the two levels of oxygen pressure are given in Table 19. It was observed that in both the cases, the final levels of the water quality parameters were almost uniform (see Figs.23-26).

Table 18. Cumulative percentage mortality* of C.chanos seed packed under two levels of oxygen pressure, at two hourly intervals.

Time Interval (hours)		Percentage Mortality	
		O_2 at atmospheric pressure	O_2 at 0.2 kg/cm² pressure
Upto	34	0.00	0.00
	36	5.00	5.00
	38	7.50	8.35
	40	10.00	12.50
	42	13.50	13.50
	44	17.50	18.35
	46	20.85	20.85
	48	24.15	24.15
	50	27.50	25.85
	52	31.65	30.85
	54	35.85	36.65
	56	39.15	40.00
	58	43.35	44.15
	60	48.35	49.15
	62	51.65	52.50
	64	60.85	60.85

* Each value is a mean of six replicates.

Table 19. Initial (I) and final (II) levels* of water quality parameters in the containers packed with C. chanos seed under two different levels of oxygen pressure.

Parameters	Level of oxygen pressure			
	Atmospheric Pressure		0.2 kg/cm²	
	I	II	I	II
NH_3 - N (mg/L)	Nil	35.50	Nil	34.92
CO_2 (mg/L)	Nil	72.47	Nil	72.16
DO (mg/L)	32.0	4.96	32.0	4.99
pH	8.0	7.5	8.0	7.5

* Each value is mean of six replicates.

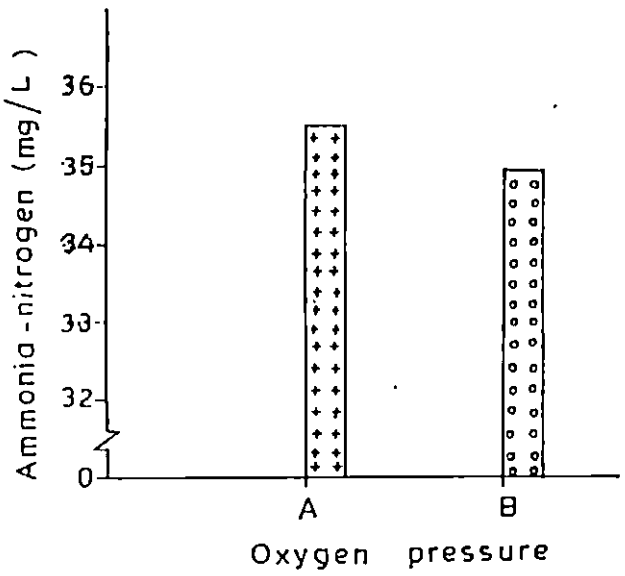

Fig. 23

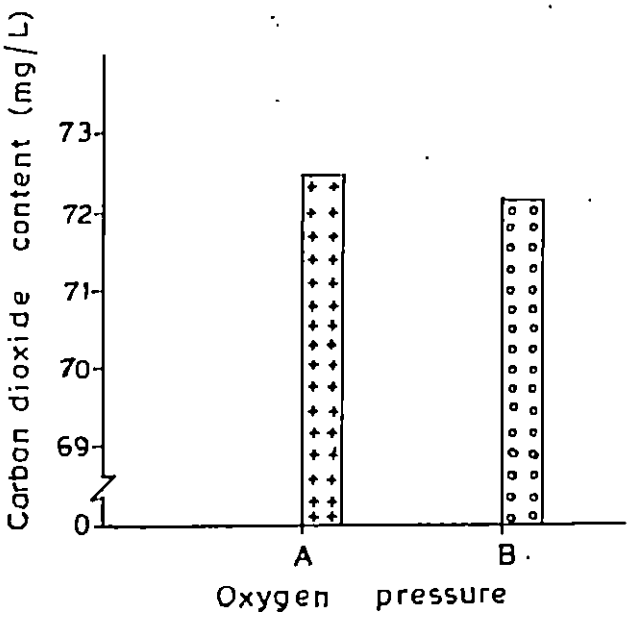

Fig. 24

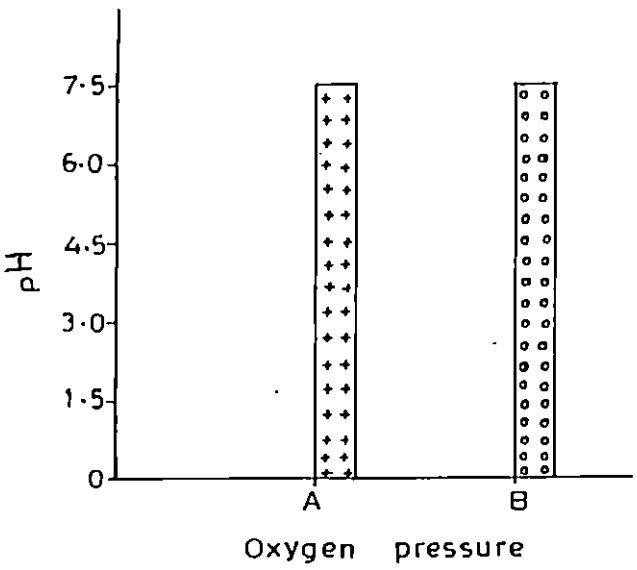

Fig. 25

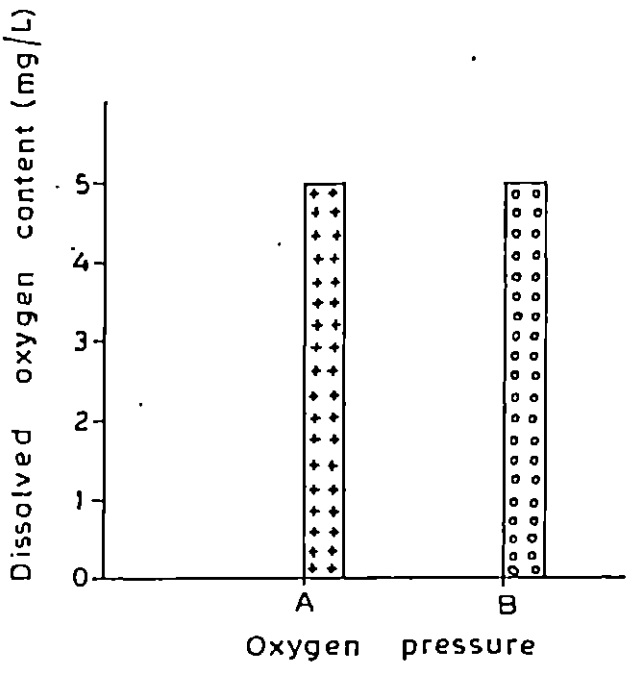

Fig. 26

4.6 Feasibility of using the sedative - tertiary butyl alcohol - in oxygen packing

Table 20 and Fig.27 provide the details of the cumulative percentage mortality of C.chanos seed packed with two doses of tertiary butyl alcohol, ie., one at 3.0 ppm and the other at 3.5 ppm, and without it (control). Upto 48 hours of packing, 100% survival was observed in all the three treatments. At 60 hours of packing, the seed packed with the two doses of sedative did not show any mortality, but the seed packed without sedative showed 37.5% mortality. The rate of mortality of the seed packed with and without tertiary butyl alcohol at two hourly intervals was gradual, ranging from 5-10%.

Fig.28 shows the duration of 100% survival of the seed packed with two doses of tertiary butyl alcohol and without it. The seed sedated at 3.0 ppm showed 100% survival for the longest duration of 80 hours of packing, followed by those sedated at 3.5 ppm for 60 hours of packing and finally by those without sedation for only 48 hours of packing. A dose of 3.0 ppm of this sedative has been found feasible for increasing the duration of survival of C.chanos seed in oxygen packing by 1.67 fold.

The initial and final levels of the water quality parameters in the packed containers with the two doses of sedative and without it are given in Table 21. The final levels of the water quality

Table 20. Cumulative percentage mortality* of C.chanos seed packed with two doses of sedative-tertiary butyl alcohol and without it (control), at two hourly intervals.

Time interval (hours)	Percentage Mortality		
	3.5 ppm	3.0 ppm	Control
Upto 48	0.0	0.0	0.0
50	0.0	0.0	7.5
52	0.0	0.0	12.5
54	0.0	0.0	15.0
56	0.0	0.0	22.5
58	0.0	0.0	30.0
60	0.0	0.0	37.5
62	7.5	0.0	47.5
64	12.5	0.0	57.5
66	20.0	0.0	62.5
68	22.5	0.0	67.5
70	25.0	0.0	72.5
72	32.5	0.0	-
74	35.0	0.0	-
76	37.5	0.0	-
78	40.0	0.0	-
80	42.5	0.0	-
82	47.5	5.0	-
84	50.0	7.5	-
86	60.0	12.5	-
88	67.5	22.5	-
90	72.5	30.0	-
92	-	35.0	-
94	-	45.0	-
96	-	52.5	-
98	-	57.5	-
100	-	62.5	-
102	-	67.5	-
104	-	75.0	-

* Each value is a mean of duplicates.

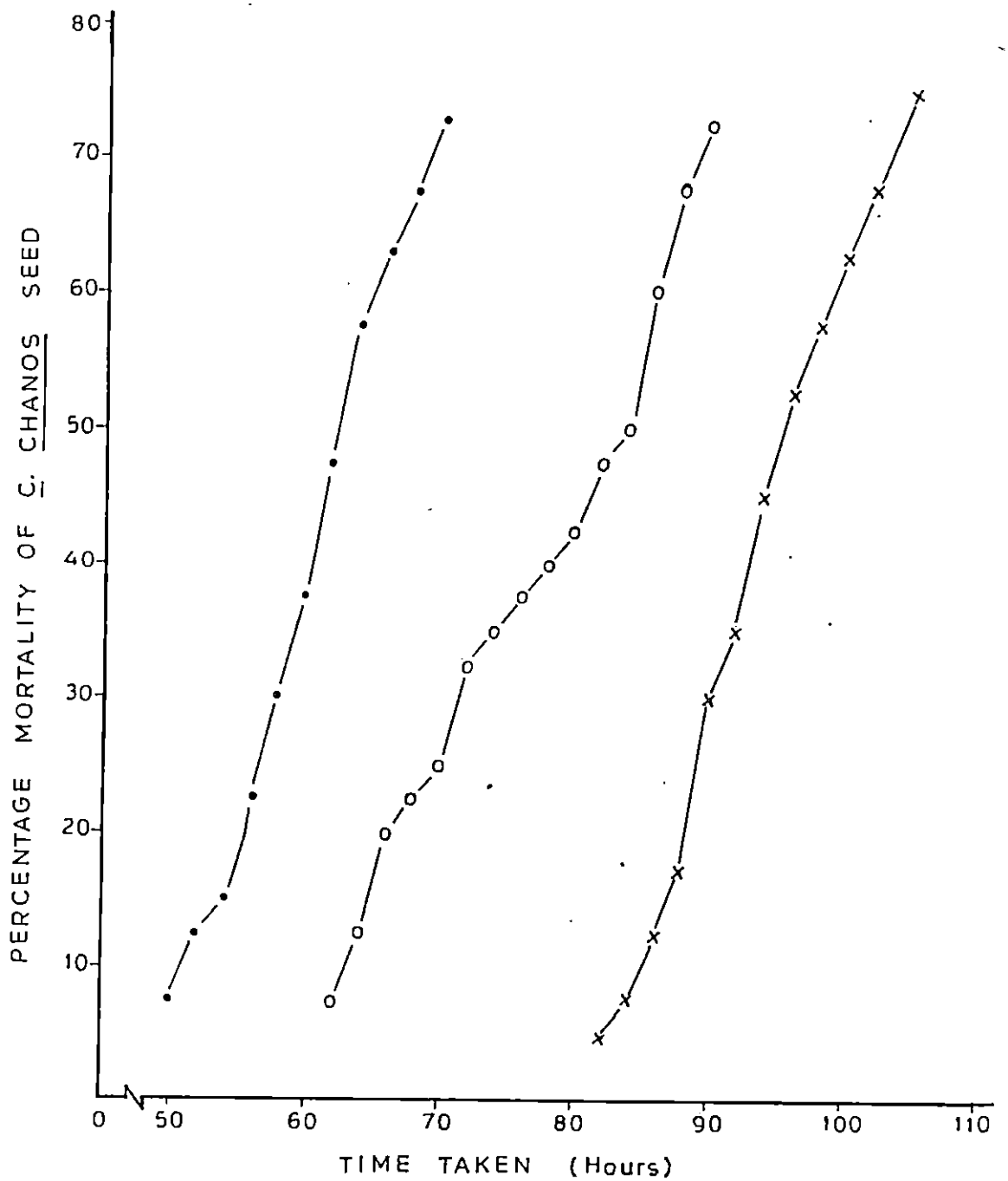
PERCENTAGE MORTALITY OF C. CHANOS SEED
80
70
60
50
40
30
20
10
0
50
60
70
80
90
100
110
TIME TAKEN (Hours)

Fig. 27

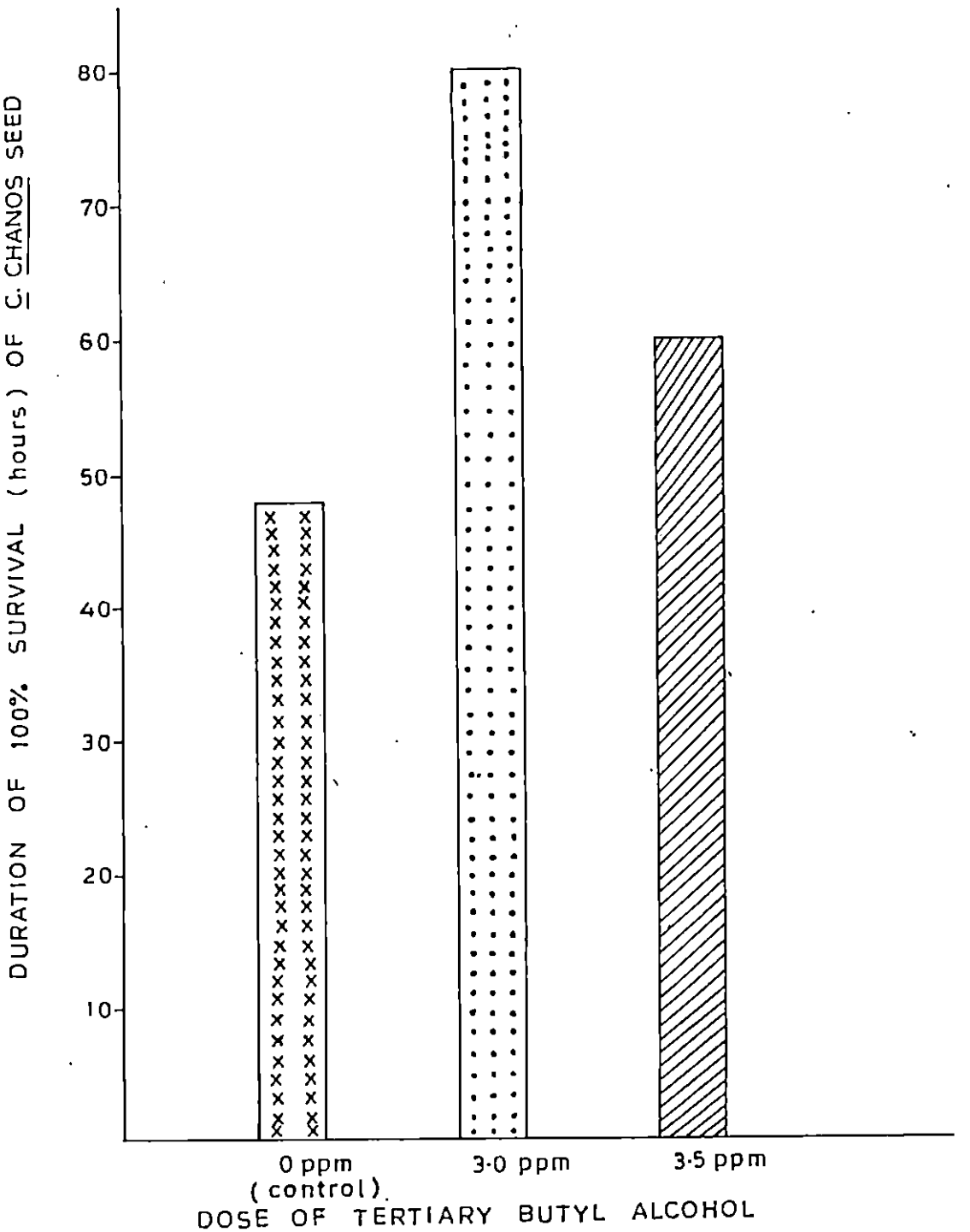
DURATION OF 100% SURVIVAL (hours) OF C. CHANOS SEED
80
70
60
50
40
30
20
10
0 ppm
(control)
3·0 ppm
3·5 ppm
DOSE OF TERTIARY BUTYL ALCOHOL

Fig. 28

Table 21. Initial (I) and final (II) levels* of water quality parameters in the containers packed with *C.chanos* seed with two doses of sedative - tertiary butyl alcohol and without it (control).

Parameters	Dose of sedative					
	3.5 ppm		3.0 ppm		0 ppm (control)	
	I	II	I	II	I	II
NH_3 - N (mg/L)	Nil	28.00	Nil	16.00	Nil	46.50
CO_2 (mg/L)	Nil	14.98	Nil	8.99	Nil	16.98
DO (mg/L)	32.00	6.64	32.00	8.63	32.00	5.31
pH	8.00	6.5	8.0	7.0	8.0	6.5

* Each value is a mean of duplicates.

parameters are compared in Figs.29-32. It was found that in the containers with seed sedated at 3.0 ppm, the ammonia-nitrogen (16.00 mg/L) and carbon dioxide (8.99 mg/L) contents were the lowest among all the three treatments. Also, the dissolved oxygen content (8.63 mg/L) was the highest among all the three treatments.

4.7 Feasibility of using chitosan as an absorbent in oxygen packing

In the containers with chitosan, the fishes showed signs of stress, which was indicated by weak movements and lying on their sides, after 6 hours of packing; 100% mortality occurred at 12 hours of packing, while those in the containers without chitosan did not show any stress or mortality at that time.

The water in the containers with chitosan became turbid and pale yellow in colour. The results of the chemical analyses of the water quality parameters initially and at the end of the experiment are given in Table 22. It was observed that the final ammonia-nitrogen and carbon dioxide content in the containers with chitosan (28.00 mg/L and 71.16 mg/L respectively) were much higher than in the control (4.8 mg/L and 34.91 mg/L respectively). The final pH in the containers with chitosan dropped from initial 7.5 to 6.5, while in the control it dropped from initial 7.5 to only 7.0. The final dissolved oxygen content in the chitosan - packed containers (2.4 mg/L) was much lower than that in the control (12.0 mg/L).

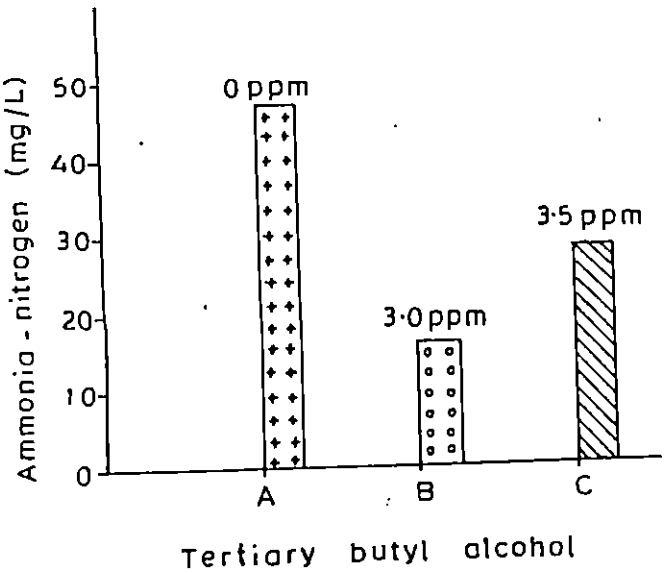

Fig. 29

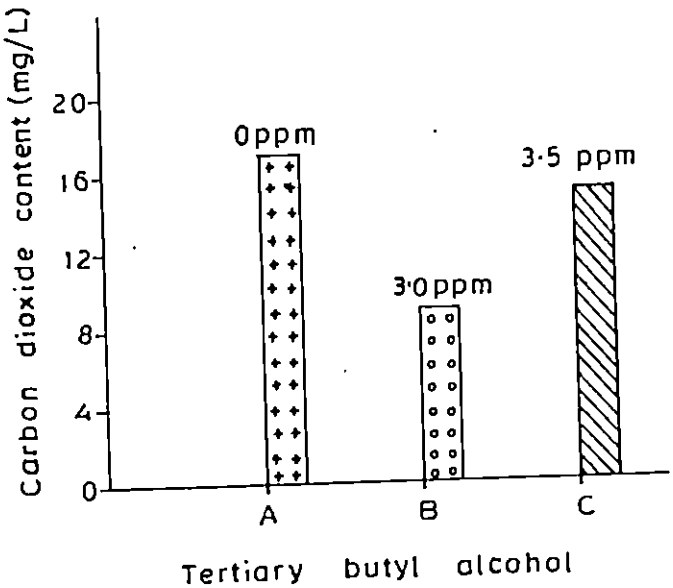

Fig. 30

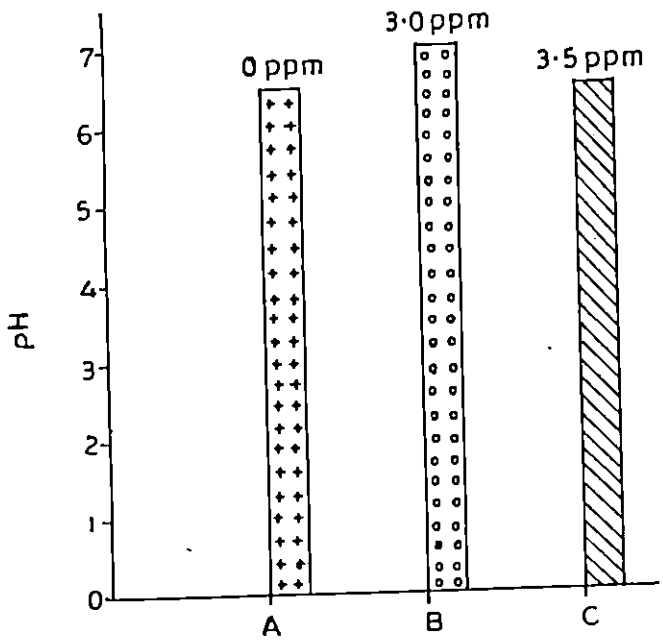

Fig. 31

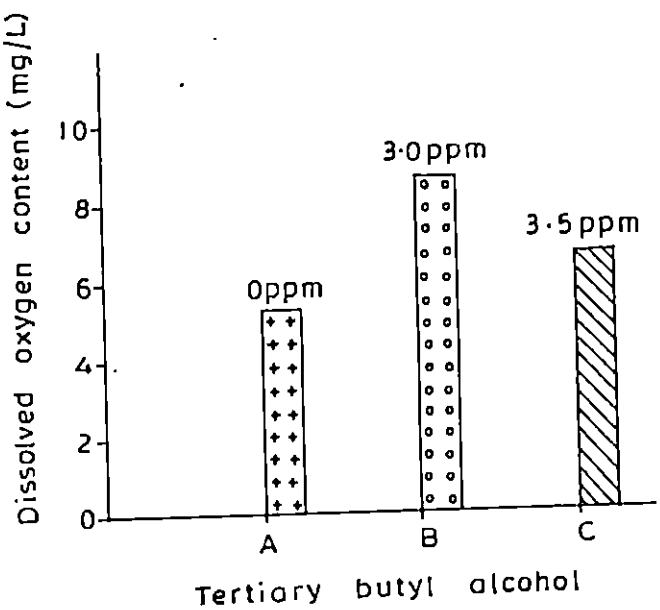

Fig. 32

Table 22. Initial (I) and final (II) levels* of water quality parameters in the containers packed with C.chanos seed with chitosan (A) and without it. (control,B).

Parameters	Treatment			
	A		B	
	I	II	I	II
NH_3- N (mg/L)	Nil	28.00	Nil	4.8
CO_2 (mg/L)	Nil	71.16	Nil	34.91
DO (mg/L)	32.00	2.40	32.00	12.00
pH	7.5	6.5	7.5	7.0

* Each value is a mean of duplicates.

Figures 33-36 show a comparison of the final levels of the water quality parameters in the two treatments.

Based on the above results, the feasibility of using chitosan as an absorbent in the oxygen packing of C.chanos seed can be ruled out.

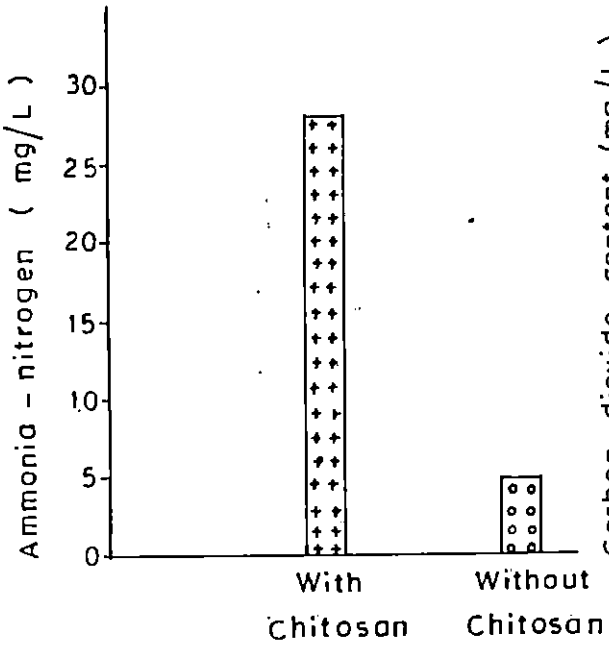

Fig. 33

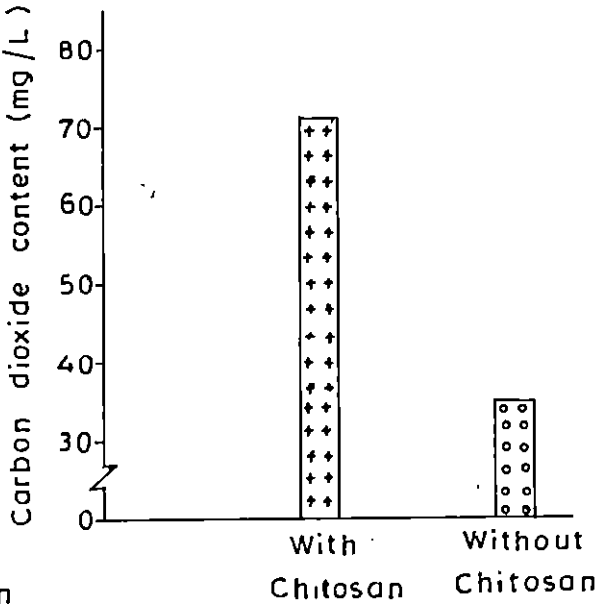

Fig. 34

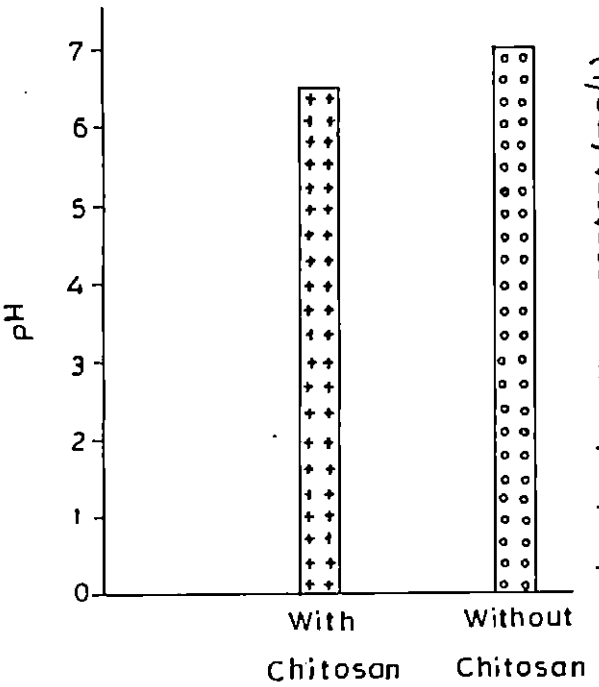

Fig. 35

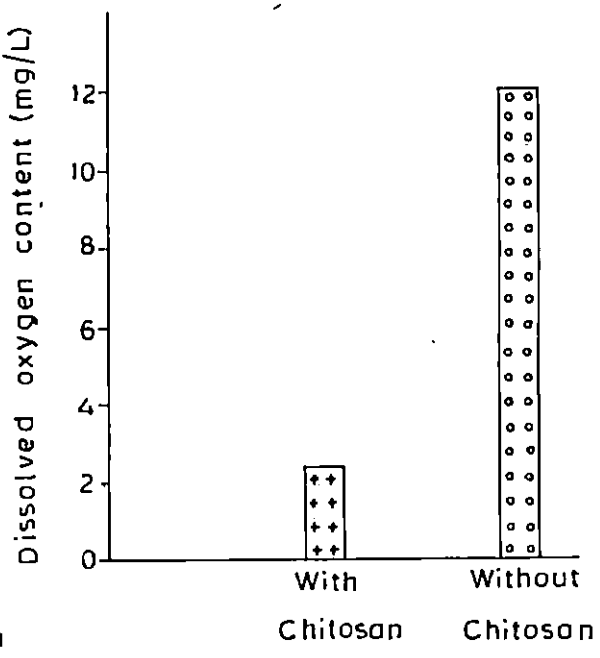

Fig. 36

5. DISCUSSION

5. DISCUSSION

5.1 Conditioning of fish seed before oxygen packing

The importance of conditioning fish fry in a limited area without feeding prior to packing has been highlighted by several workers (Jagannadham, 1947; Schuster, 1952; Saha and Chowdhury, 1956; Djajadiredja, 1958; Hora and Pillay, 1962; Srivastava and Karamchandani, 1964; Mammen, 1966; Hamid and Mordjono, 1978; Jobling, 1981; Thakurta and Pakrasi, 1985). This apparently effects in emptying the gut, thereby lowering the rate of excretion and oxygen consumption of the fry. However, Alikunhi (1957) suggested that feeding the fish fry with small animalcules during conditioning and transport would enable the fry to withstand the strain of long distance transport in a better way. Of the four different treatments of conditioning of the C. chanos seed tried in the present study, a combination of gut voiding (24 hours) and congestion (15 minutes) was found to be the best method prior to oxygen packing. Statistical analysis showed that there was a significant difference ($P < 0.05$) in the survival of the seed packed after the four different treatments of conditioning.

The chemical analyses showed that the water quality parameters in the containers packed with seed after gut voiding and congestion were also the best among the four treatments . Mc

Farland and Norris (1958) found that oxygen deficiency and carbon-dioxide excess were the major factors affecting the survival of fish seed during transportation. Durve and George (1963) reported that carbon dioxide level of more than 20 ppm was harmful to C.chanos fry in the transportation containers and heavy mortality occurred when the carbon dioxide level increased beyond 30 ppm, irrespective of the final oxygen level. In the containers packed with seed without conditioning, the carbon dioxide content was the highest and the dissolved oxygen content was the lowest (ie., 71.69 mg/L and 4.99 mg/L respectively). In the containers packed with seed after gut voiding and congestion , the carbon dioxide content was the lowest and the dissolved oxygen content was the highest (ie., 12.57 mg/L and 14.50 mg/L respectively). There was a significant difference in the dissolved oxygen and carbon dioxide content ($P < 0.05$) in the containers packed with seed after the four different treatments of conditioning.

Saha et al., (1956 b) found that carp spawn could withstand a concentration of 2.5 ppm of dissolved free ammonia and 15 ppm of dissolved ammonia in the form of inorganic salts in the transport medium. In the containers packed with C.chanos seed without conditioning, the ammonia-nitrogen content was the highest (50.10 mg/L); but in those packed after gut voiding and congestion, the ammonia-nitrogen content was the lowest (36.20 mg/L).Nevertheless, both values are beyond the tolerance limit. Brockway (1950),

Phillips and Brockway (1954) and Doudoroff (1957) reported that nitrogenous wastes, particularly un-ionized ammonia to be highly toxic in the transport media causing mortality of fish seed. Significant difference ($P < 0.05$) in the ammonia-nitrogen levels was noted in the containers packed with C.chanos seed after the four different treatments of conditioning.

There was a slight decrease in the pH value in the containers packed with C.chanos seed without conditioning and with congestion; but no decrease was observed in the other two treatments. This decrease may be due to the dissociation of carbonic acid to release bicarbonate and hydrogen ions, causing reduction of pH . (Alias and Siraj, 1985).

The longest duration of 100% survival in the containers packed with C.chanos seed after gut voiding and congestion coincide with the relatively lowest levels of carbon dioxide and ammonia and the highest level of dissolved oxygen in the containers. Conceivably, the voiding of gut (24 hours) might have resulted in the lower rate of excretion and oxygen consumption, which in turn might be attributed to the better water quality parameters in the containers. The congestion done after gut voiding might have made the seed get accustomed to a restricted volume of water available in the packing containers. With this treatment, the duration of 100% survival could be increased by 3.25 fold to that of the seed packed without conditioning.

5.2 Optimising the seed packing density for a given duration

The experiment conducted with four different densities of C.chanos seed (20 mm and 0.0362 g average size) viz., 20,30,40, and 50 numbers per 50 ml showed that at highest density of 50 numbers per 50 ml, the safe period of transport will be 12 hours. The regression between the packing density (g/50 ml) and the time of initial mortality (hours) was tested to compare the previous results. At a density of 0.724 g/50 ml, the time of initial mortality observed was 36.6 hours. This is in conformity with the results obtained by Durve and George (1963) and Mammen (1966). Durve and George (1963) reported that a density of 150-175 C.chanos fry per 100 ml water (ie., 1.5-1.75 g/100 ml) can be used for 24 hour transportation. Using the regression equation obtained in the present study, the time of initial mortality for this packing density, ie., 0.75-0.875 g/50 ml water varied from 36 hours to 33.5 hours. Mammen (1966) concluded that a packing density of 200 fry per 175 ml water (2 g/175 ml) as the safe limit of transportation for 24 hours. Applying the present regression equation, it may be seen that the time of initial mortality for this packing density (ie., 0.571 g/50 ml) is 39.7 hours.

Chemical analyses of water from the containers packed at the density of 50 numbers per 50 ml (1.81 g/50 ml) after 52 hours

of packing, when 100% mortality occurred in these containers showed that the oxygen content was zero; the carbon dioxide and ammonia-nitrogen content was the highest (214.14 mg/L and 58.10 mg/L respectively); the pH dropped from initial 8.0 to final 6.0. Vaas (1951), Saha et al., (1956 a), Mc Farland and Norris (1958) and Norris et al., (1960) had also reported that when the pH of the medium in transporting containers decreases in combination with higher levels of carbon dioxide, large-scale mortality of fish seed occurs. Decrease of pH and dissloved oxygen and increase of ammonia and carbon dioxide concentration in closed containers have been also reported by Srinivasan et al., (1955); Fry and Norris (1960); Ramachandran (1969); Hattingh et al., (1975); Johnson (1979b); Turner and Bower (1982); Siraj et al., (1985).

Statistical analyses showed significant differences ($P < 0.05$) in the dissolved oxygen, carbon dioxide and ammonia-nitrogen levels in the containers packed with the seed at the four different packing densities. In the case of pH , the total variation can be completely explained by the treatment variation.

5.3 Effect of salinity on oxygen packing

C.chanos being a euryhaline species could be transferred from salt water to fresh water without any mortality (Ranganathan and Ganapati, 1949; Ganapati et al., 1950; Alikunni, 1957;

Djajadiredja, 1958; Thayaparan and Chakrabarty, 1985). Schuster (1952) reported that no mortality of C.chanos seed occurred when they were transferred from freshwater to salt water of less than 40 ppt, beyond which mortality occurred. In the present study, the seed originally acclimatized at 20 ppt and then packed at 0 ppt, 10 ppt and 20 ppt salinity did not show any significant difference in the duration and percentage of survival.

Mammen (1966) reported that in transporting containers, the fry packed in low saline water, mortality set in earlier (after 24 hours) than that in high saline water (after 40-70 hours). The author highlighted that this difference in duration of setting in mortality may be due to the after effect of acclimatization or the effect of metabolic waste products; in the higher salinity of 20 ppt, osmotic pressure was more or less similar to that of the body fluid of the fry. Contrary to this finding, in the present study, there was no difference in the duration of 100% survival among the seed packed under the three different salinity levels. Hence it may be deducted that salinity has no effect on increasing the duration of 100% survival of C.chanos seed under oxygen packing.

During transportation in closed system, carbon dioxide as well as ammonia concentration increase and pH as well as dissolved oxygen concentration decrease causing mortality of fish seed in the containers (Fry and Norris, 1960; Ramachandran, 1969; Hattingh et al., 1975; Johnson, 1979b; Turner and Bower, 1982; Siraj

et al., 1985). Such a trend was also observed in the present study at all the three salinities tried.

5.4 Effect of initial pH on oxygen packing

The present study revealed that initial pH within the range of 6.5 to 9.5 had no effect on the survival of the oxygen-packed C. chanos seed. The mortality rate, duration of 100% survival and the final pH were almost uniform at the three initial pH ranges tried. From the information available it may be gathered that in the case of C.chanos seed packing for transportation, the initial water pH commonly used varied between 7.0 and 8.5 (Sreenivasan, 1962; Durve and George, 1963; Mammen, 1966; Ramanathan and Jayamaha, 1972; Bensam, 1974; Smith, 1981; Villaluz, 1984).

5.5 Effect of oxygen pressure on packing

Durve and George (1963) found that 100 C.chanos fry of transparent stage could be packed in a container with 100 ml water without oxygen; while with oxygen pressure, the density could be increased to 150-175 fry in 100 ml water, Mammen (1966) reported that the duration of 100% survival was longer when C.chanos fry were packed with oxygen than with air under mild pressure, 100 fry in 175 ml water could be transported for 96 hours when packed with oxygen under mild pressure, but for only 36 hours when packed with air under mild pressure. Selvaraj et al., (1981) found that oxygen supplied through atmospheric air was sufficient to maintain the standard metabolic activities of fish, till a period when there was no mortality; 500

fry could be safely transported in 6 litre water for 24 hours with negligible mortality. In the present study, C.chanos seed packed under oxygen at atmospheric pressure and at 0.2 kg/cm² oxygen pressure behaved uniformly. These results could not be compared for paucity of previous comparable information. Water quality parameters in both the treatments were almost uniform.

5.6 Use of tertiary butyl alcohol in oxygen packing

Durve and Dharma Raja (1966), Durve (1975) and Alvarez-Lajonchere and Moreno (1982) recommended a dose of 3.0 ppm and 3.5 ppm of tertiary butyl alcohol for light and deep sedation respectively, in the oxygen packing of carp and mullet fry. The present study revealed that a dose of 3.0 ppm was found to cause light sedation and 3.5 ppm to cause deep sedation in the oxygen packing of C.chanos seed also.

The seed packed with 3.0 ppm tertiary butyl alcohol showed the longest duration of 100% survival of 80 hours, compared to 60 hours for those sedated at 3.5 ppm and 48 hours for those which were not sedated. This increase in the duration of 100% survival for the seed sedated may be due to the decreased activity of fishes with the resultant reduction in oxygen uptake, lowering of the rate of production of carbon dioxide and excretion of nitrogenous wastes, all of which are limiting factors in fish transportation. (Osborn, 1951; Reese, 1953; Phillips and Brockway, 1954; Nemoto, 1957; Mc Farland and Norris, 1958; Mc Farland,

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1960; Durve and Dharma Raja, 1966; Durve, 1975; Ferreira et al., 1984). This was noticed in the water quality parameters in the containers packed with seed sedated at 3.0 ppm. In these containers, the ammonia-nitrogen and carbon dioxide content were the lowest (16.00 mg/L and 8.99 mg/L respectively) and the dissolved oxygen content was the highest (8.63 mg/L) of all the three treatments. Also, the pH drop was less compared to that of the others, ie., it dropped from initial 8.0 to final 7.0; while in the other two treatments, it dropped from initial 8.0 to final 6.5. In the case of containers packed with seed sedated at 3.5 ppm, deep sedation occurred and this may be the reason for the shorter duration of 100% survival than to those sedated at 3.0 ppm. In the containers packed with seed without any sedative, the lowest duration of 100% survival was noted and this may be due to the hyperactivity of fishes resulting in higher accumulation of metabolic wastes and depletion of dissolved oxygen, causing rapid mortality of the seed . In this case, the ammonia-nitrogen and carbon dioxide were the highest (46.50 mg/L and 16.98 mg/L respectively) and dissolved oxygen content was the lowest (5.31 mg/L).

The present study revealed that the dose of 3.0 ppm of tertiary butyl alcohol was feasible to enhance the duration of 100% survival of C. chanos seed in the oxygen packing by 1.67 fold. Durve and Dharma Raja (1966), Durve (1975) and Alvarez-Lajonchere and Moreno (1982) also reported that 3.0 ppm

tertiary butyl alcohol was feasibile in carp and mullet fry packing and transportation. Thomas et al., (1990) found tertiary butyl alcohol (0.006%) to be effective in the transportation of grass carp seed. Mc Farland (1958) and Durve and Dharma Raja (1966) found a very low narcotic potency for tertiary butyl alcohol and so, recommended it to be safe in live fish transportation. But, Mc Farland (1960) reported tertiary butyl alcohol to be least desirable for transporting fishes due to its low potency .

5.7. Feasibility of using chitosan as an absorbent in oxygen packing

Hirano and Tokura (1982) reported the use of chitosan in removing gases like hydrogen sulphide from water. In the present study to find the feasibility of chitosan to remove the metabolic wastes accumulated by C.chanos seed in the oxygen-packed containers, it was found to have a negative effect on oxygen packing of C.chanos seed. The seed in the chitosan-packed containers died after 12 hours of packing; while in the containers without chitosan, the seed were very active even after 12 hours of packing. The death of the individuals in the chitosan-packed containers may be due to the stress caused by high turbidity and pale yellow colour of the water in the containers. The stress might have resulted in the increased oxygen consumption and the increased excretion of metabolic wastes. This was indicated by

the chemical analyses of water in the chitosan-packed containers, which showed a very low dissolved oxygen (2.4 mg/L) and comparatively higher ammonia-nitrogen and carbon dioxide content (28.00 mg/L and 71.5 mg/L respectively). Also, the pH dropped from initial 7.5 to final 6.5.

6. SUMMARY

6. SUMMARY

1. The objectives of the study were to find out (i) the optimum conditioning method of C.chanos seed before packing in oxygen-filled containers, (ii) their optimum packing density, (iii) the effect of salinity, pH and oxygen pressure on their packing and (iv) the feasibility of using a sedative (tertiary butyl alcohol) and of using chitosan as an absorbent in removing toxic metabolites of the seed in the oxygen-packed containers.

2. Two types of packing containers were used during the course of study, because of their ease of handling and operation - (a) Specially designed jar-type hard plastic air tight containers of 300 ml capacity and (b) Soft PVC air tight transportation bags of 2 litre capacity patented and supplied by Plastic Crafts Corporation, Bombay. For regulating the flow of oxygen and reading the oxygen pressure inside the container, a short PVC tube of 3 mm diameter fitted with a one-way valve (the valve used was the one used for pneumatic tyre-tube) at the distal end was firmly fixed to the inner plastic flap and lid by boring them in the centre.

3. An assembly consisting of a pressure gauge (Bourdon type) having a capacity of 1 kg/cm^2 and 0.02 kg/cm^2 sensitivity, an oxygen cylinder and pressure-resistant hose with a nozzle was used for pumping in oxygen and reading the pressure in the experimental containers.

4. The experiment conducted to find out the optimum conditioning method prior to oxygen packing of C.chanos seed, showed that a

combination of gut voiding for 24 hours and thereafter congestion (100 seed in 250 ml of water for 15 minutes) was the best among the four treatments tried, since it showed the longest duration of 100% survival. In this case the duration of 100% survival was increased by 3.25 fold compared to the seed packed without conditioning.

5. The experiment carried out to study the optimum density of C.chanos seed for a given duration showed that a linear relationship could be established between the packing density of C.chanos seed (g/50 ml) and the time of initial mortality (hour). The regression equation established was Y=51.398-20.44X, where Y = Time (hour) at which initial mortality starts and X = Packing density in g/50 ml. At the highest packing density of 50 numbers (1.810 g) per 50 ml water, the safe period of transport was 12 hours.

6. The study to determine the effect of three different levels of salinity, viz., 0 ppt, 10 ppt and 20 ppt, on the duration and survival of the oxygen-packed C.chanos seed, showed that the packed seed behaved uniformly at these three levels of salinity. A sudden drop in salinity from 20 ppt to 0 ppt did not show any effect on the oxygen-packed seed.

7. The experiment carried out to study the effect of initial pH at three ranges, viz., 6.5-7.5, 7.5-8.5 and 8.5-9.5, on the survival and duration of the oxygen-packed C.chanos seed, revealed that there was no significant effect of these three ranges of pH on the survival and duration of the packed seed.

8. The study conducted to find out the effect of oxygen pressure on the packing of C.chanos seed indicated that the seed packed with oxygen at atmospheric pressure and at 0.2 kg/cm^2 oxygen pressure behaved in a similar manner and the duration of 100% survival was uniform in both cases.

9. The experiment aimed at studying the feasibility of a sedative, tertiary butyl alcohol, in oxygen packing of C.chanos seed, showed that this sedative at a dose of 3.0 ppm is effective. It caused light sedation of the seed at this level, thereby increasing the duration of 100% survival by 1.67 fold to that of the control.

10. The experiment carried out to study the feasibility of chitosan as an absorbent in removing the toxic metabolic wastes accumulated by C.chanos seed in the oxygen-filled plastic containers revealed that it had a negative effect since total mortality of the packed seed occurred at 12 hours of packing. At that time the seed packed without it did not show any stress and were active.

11. The analyses of water quality parameters in the containers packed with C.chanos seed at the termination of all the experiments revealed a general trend of decrease in dissolved oxygen content and increase in ammonia-nitrogen as well as carbon dioxide content.

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STANDARDIZATION OF OXYGEN PACKING PROCEDURE OF *CHANOS CHANOS* SEED

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ABSTRACT OF A THESIS

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ABSTRACT

The present study was aimed at finding out the optimum conditioning method of C.chanos seed before packing, their optimum packing density, the effect of salinity, pH and oxygen pressure on their packing and the feasibility of using a sedative, tertiary butyl alcohol and chitosan as an absorbent in removing toxic metabolites accumulated in the oxygen-packed containers. Specially designed air tight plastic containers were used for packing the seed.

Of the seed packed after four treatments, viz., control, congestion, gut voiding and gut voiding plus congestion, the fourth treatment was the best with 3.25 fold increase in the duration of 100% survival compared to the control.

From packing densities of 20,30,40 and 50 numbers per 50 ml water, a linear relationship between packing density in g/50 ml (X) and time of initial mortality in hours (Y) was established as $Y = 51.398-20.44\ X$.

No significant effect on the duration and survival of the seed was observed at different levels of salinity (0 ppt, 10 ppt and 20 ppt), initial pH (6.5-7.5, 7.5-8.5 and 8.5-9.5) and oxygen pressure (oxygen at atmospheric pressure and at 0.2 kg/cm^2 pressure).

The use of tertiary butyl alcohol at 3.0 ppm and 3.5 ppm resulted in light and deep sedation of the seed respectively. It also increased the duration of 100% survival by 1.67 and 1.25 fold at the respective doses, compared to the control. The feasibility of using chitosan as an absorbent in oxygen packing of the seed was ruled out.