EFFECT OF CARBON DIOXIDE ANAESTHESIA ON *LABEO ROHITA* (HAMILTON) FRY DURING TRANSPORTATION.

By

LEESHA O.S., *B.F.Sc.*

THESIS

Submitted in partial fulfilment of the requirement for the degree

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COLLEGE OF FISHERIES,
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2000

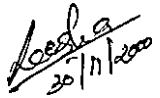
Dedicated to

My

PARENTS & GRANDMA

DECLARATION

I hereby declare that this thesis entitled " **EFFECT OF CARBON DIOXIDE ANAESTHESIA ON *LABEO ROHITA* (HAMILTON) FRY DURING TRANSPORTATION** " 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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Certified that this thesis entitled **"EFFECT OF CARBON DIOXIDE ANAESTHESIA ON *LABEO ROHITA* (HAMILTON) FRY DURING TRANSPORTATION"** is a bonafide record of research work done independently by Kum. LEESHA. O.S., under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

K. Jayasree 30/11/2000

Dr. K. JAYASREE VADHYAR,
(Chairperson, Advisory Committee),
Associate Professor,
Department of Aquaculture,
College of Fisheries, Panangad,
Cochin.

Panangad,
30th November 2000.

Dr. K. Jayasree Vadhyar
Associate Professor
Department of Aquaculture
College of Fisheries, Panangad.

MEMBERS

Dr. P.M. Mathew
Professor & Head
Department of Aquaculture
College of Fisheries, Panangad.

Shri. T.M. Sankaran
Associate Professor & Head
Department of Management Studies
College of Fisheries, Panangad.

Shri. P.S. Mrithyunjayan
Associate Professor
Department of Fishery Hydrography
College of Fisheries, Panangad.

EXTERNAL EXAMINER
Dr A. LAKSHMANAN
Principal Scientist &
Chief Training Organiser CTTC
CMFRI
Cochin-18

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INTRODUCTION

1. INTRODUCTION

Availability of healthy seed is one of the prime requirement in any aquaculture operation. The procurement of quality seed is highly essential for successful and profitable results. Whether the fry are from natural sources or from hatcheries, they are to be transported with maximum survival and least stress with minimum mortality to the farm sites.

The transportation and handling of live fish form an integral part of aquaculture. Transportation of live fish is reported to have started in the year 1874 (Norris *et al.*, 1960). Several methods are developed for the transportation of live fish. The traditional mode of transport was in open earthern pots and metal containers (Jhingran, 1975). Now - a - days plastic bags are widely used for the transportation of live fish seeds . Aquaculturists carry as many fish in as little water as possible with minimum mortality.

The mortality of fish seed during transportation is mainly due to the depletion of dissolved oxygen and accumulation of gases like ammonia and carbon dioxide in the packing medium. Other reasons may be handling stress and hyper activity of the fish . Low oxygen level of the packing medium has been solved by the addition of pure oxygen. However, carbon dioxide and metabolic waste levels must be low enough to allow sufficient oxygen uptake (Mc Farland and Norris , 1958).

Fishes could be anaesthetized for transportation for ensuring better survival rate . The pioneer worker to recognize the potential use of anaesthetics for transporting fishes was Aitken (1936). The very purpose of anaesthetizing fish seed, is to minimize the consumption of oxygen and the concentration of toxic gases like ammonia and carbon dioxide in the medium by lowering their metabolic rate. This, in turn, may lead to longer period of their survival. Anaesthetics at the correct dosages are very useful for the transportation of fish seed. The weight of fish per unit volume of water could at least be doubled during transport by lowering metabolic rate of the fish using anaesthetics. Reese (1953) and Mc Farland (1960) reported that doubling and occasionally tripling of the normal total weight of fishes could be done in transport containers by using anaesthetic.

Labeo rohita (Hamilton), is one of the most important freshwater food fish. The major reasons for the mortality of the seed of rohu during and after transportation are claustrophobia, and the resultant exhaustion / injury, increase in excretion of metabolic products, and deterioration of water quality in transporting packs. Both hyper activity and metabolic activity could be depressed by the action of anaesthetics which would in turn facilitate the safe and efficient transport of these fishes.

Transportation of large quantity of fish seed covering long transit entails several complex problems, such as maintenance of water quality, biological and physiological requirements of the fish transported, optimum packing density during transportation, conditioning them before transportation and acclimatization soon after reaching the destination. It is in this context, that concerted effort is made

through this study to investigate these vital problems, so as to ensure high survival rate and to minimize the economic loss.

Although a lot of chemicals are used as anaesthetics in fishes, carbon dioxide anaesthesia is reported to be more safe, because it is effective, inexpensive, non toxic and it leaves no residues in the tissues and requires no withdrawal time. In fisheries, the current trend is to discourage or even ban the use of certain chemical anaesthetics and to encourage the use of electroanaesthesia and carbon dioxide anaesthesia.

The present work was planned to evaluate the effect of carbon dioxide anaesthesia on rohu fry during oxygen - packed transportation.

Main objectives of the study :

1. To evaluate the effect of carbon dioxide anaesthesia on the *L . rohita* fry
2. To determine the effect of anaesthetization and different packing densities on the survival rate of rohu fry and duration of transport .
3. To find out the important changes in the water quality parameters of the oxygen - packed containers caused by anaesthetization and different packing densities.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 . Anaesthetics and anaesthesia

Anaesthetics are chemical or physical agents which with increasing exposure or concentration, initially calm (sedate) an animal, then cause it successively to lose mobility, equilibrium, consciousness and finally reflex action. In fisheries and aquaculture, the major use of anaesthetics is to reduce the activity of fish during transportation and to immobilize them so that they can be handled more easily. Anaesthesia is a state caused by an external agent (anaesthetic) which causes a loss of sensation in a part or all of the body, due to the depression of nervous function (Williams & Wilkins Company, 1982).

Fish anaesthesia and the nature of various anaesthetics have been reviewed many times. McFarland (1959), Lumb (1963), Klontz (1964), Westhunes and Fritsch (1965), Smith and Bell (1967), Klontz and Smith (1968), Mc Farland and Klontz (1969), Randall and Hoar (1971), Jolly *et al.* (1972), Houston and Corlett (1976), Johansson (1978), Stuart (1981) and Ross and Ross (1984) updated information on kinds of anaesthetics, dosages, toxicity and residues.

Anaesthetics can be classified as general, local and regional. General anaesthesia affects the entire body; its manifestation varies from mild sedation to loss of equilibrium, consciousness, and reflex action. General anaesthesia is the form usually applied to fish, which must be immobilized before a local or regionally active agent can be applied.

Local anaesthesia occurs when the loss of sensation is limited within a restricted segment of the body by action on sensory nerve endings; while the animal is still conscious. A local anaesthetic may be administered to produce a temporary block of the olfactory nerve or to immobilize a fin for the study of swimming performance (Horrobin , 1968). Regional anaesthesia is accomplished by blocking the sensory innervation to an area with an anaesthetic (Medical Economics Company, 1987).

2.2. Use of anaesthesia in fisheries

Anaesthesia has many experimental and other uses in fisheries, primarily to immobilize animals so they can be handled faster and less stressfully. The principal uses of anaesthesia are to facilitate operations to weigh and measure fish, to mark and tag them, to study their physiology and behaviour, to perform surgery, to photograph them, to prepare them for live shipment, to transport them, to manually spawn them, to inject them with vaccines and antibiotics and to collect blood and other tissues from them.

2.3. Stages of anaesthesia (Summerfelt and Smith, 1990)

Stage	Description	Behavior, ventilation rate and reflex action
0	Normal	Reactive to external stimuli; opercular rate and muscle tonenormal.
1	Light sedation	Slight loss of reactivity to external visual and tactile stimuli;opercular rate slightly decreased; equilibrium normal.
2	Deep sedation	Total loss of reactivity to external stimuli except strong pressure; slight decrease in opercular rate; equilibrium normal.
3	Partial loss of equilibrium	Partial loss of muscle tone; swimming erratic; increased opercular rate, reactive only to strong tactile and vibrational stimuli.
4	Total loss of equilibrium	Total loss of muscle tone and equilibrium; slow but regular opercular rate; loss of spinal reflexes.
5	Loss of reflex activity	Total loss of reactivity; opercular movements slow and irregular; heart rate very slow; loss of all reflexes.
6	Medullary collapse	Opercular movements cease; cardiac arrest usually follows quickly.

2.4. Induction time and recovery time

Induction time is the number of minutes required to reach a given stage of anaesthesia. Recovery time is the time required for the animal to return to full mobility after it is removed from the anaesthetic solution (Schoettger and Julin, 1969).

2.5. Characteristics of an ideal anaesthetic

Selection of a general anaesthetic for fish involves consideration of toxicity, efficacy, cost, restrictions on use and intended use. Marking and Meyer (1985) listed several characteristics of an ideal anaesthetic. They are as follows:

1. It has an induction time of less than 15 minutes and preferably less than 3 minutes.
2. Recovery time after its use is short, 5minutes or less.
3. It is non toxic to fish.
4. It is easy to handle and not harmful to humans during normal use.
5. It has no persistent effect on physiology and behaviour.
6. It is rapidly excreted or metabolized, leaving no residues and requiring no withdrawal time.
7. It is inexpensive and easily available.

2.6. Anaesthetics commonly used in fisheries

Many chemicals and other compounds have been used as anaesthetics for fish. The concentration needed to obtain sedation or full anaesthesia by a given anaesthetic

can be affected by many factors, principally by the species of fish to be anaesthetized. Some anaesthetics may be more suitable for certain species than for others (Dupree and Huner, 1984). A concentration of anaesthetic that produces a light sedation is desirable for transporting fish. When oxygen consumption and carbon dioxide and ammonia production are decreased two to three times the normal weight of fish per volume of water can be accommodated (Piper *et al.*, 1982). An ideal fish sedative should reduce oxygen consumption and stress during transportation (Marking and Meyer, 1985). Some of the fish anaesthetics are:

2.6.1. Tricaine (MS-222)

It is also called finquel. It is a white crystalline powder easily soluble in water (Merck and Company, 1983). It is the only anaesthetic which is registered for use with food fish by the Food and Drug Administration in the United States (Marking and Meyer, 1985). It is an expensive drug and it requires a 21 day withdrawal period.

Bove (1962) gave the most effective concentration of tricaine as 333-500 mg/l. Schoettger and Julin (1969), Murai and Catacutan (1981), Gilderhus and Marking (1987), Takeda *et al.* (1987), Mattson and Riple (1989), Kidd and Banks (1990), Molinero and Gonzalez (1995) and Massee *et al.* (1995) recommended different concentrations for tricaine that varied with species, induction time, purpose and temperature.

Dupree and Huner (1984) reported tricaine concentrations that ranged from 26.4 to 264.2 mg/l for transporting fish. *Cyprinus carpio* seeds require a dose of 250-300 ppm of tricaine to induce sedation for the transportation purpose (Jain, 1987).

2.6.2. Quinaldine

It is a colourless (Merck and Company, 1968) or light yellow (Marking , 1969 a) oily liquid, that darkens to reddish brown after exposure to air (Merck and Company, 1983) and should be protected from light. It is slightly soluble in water but soluble in acetone and ethanol (Bell, 1967). It is a potential carcinogen and it is not approved by FDA for use on food fish. Quinaldine anaesthesia is given as injection.

Effective quinaldine concentration varies with species among warm water fishes. Muench (1958) indicated that gold fish, golden shiners, yellow bullheads were anaesthetized in 0.5 - 4 minutes by 2.5 - 20 mg/l exposures. Dupree and Huner (1984) gave 15 - 30 mg/l for use in warm water fishes.

Bell (1967) and Locke (1969) reported that a quinaldine concentration of 5 - 12 mg/l was sufficient to anaesthetize salmonids.

2.6.3. Quinaldine sulfate

It is also called quinate. It is a light yellow crystalline powder, soluble in water (Merck and Company, 1983). The effective dosage varies widely with species, size and temperature. Larger fish are more heavily sedated at a given dose and the recovery is longer at higher temperatures (Blasiola, 1977). Gilderhus and Marking (1987) determined that a dose of 25-40 mg/l quinaldine sulfate was effective for fingerling and adult rainbow trout, which recovered in 5-6 minutes.

2.6.4. Benzocaine

Water soluble form of benzocaine is benzocaine hydrochloride. It is harmless and it is used as a topical and local anaesthetic for veterinary purposes (Merck and Company, 1989). The efficacy of benzocaine has been affected by the size of the fish, where the smallest fish require the lowest dose, as well as the temperature of the water (Gilderhus, 1989).

Ferreira *et al.* (1984) observed that a benzocaine hydrochloride concentration of 25 mg/l caused a reduction in the excretion of ammonia and carbon dioxide by the fish during transportation.

2.6.5. Metomidate

It is a water soluble powder which has the properties of a hypnotic or sleep inducing drug (Merck and Company, 1989). Mattson and Riple (1989) reported the effective concentration of metomidate as 5 mg/l in *Gadus morhua* . Metomidate does not cause hyperactivity in the fish. In Canada, metomidate is the only anaesthetic registered with the Bureau of Veterinary Drugs, for use with non food fish.

2.6.6. Lidocaine

It is also called xylocaine and is soluble in acetone or alcohol. Lidocaine has been used in combination with sodium bicarbonate to anaesthetize *Cyprinus carpio, Oreochromis mossambica* and *Ictalurus punctatus* (Carrasco *et al.*, 1984). Addition of sodium bicarbonate at 1g/l, enhanced the anaesthetic effects of lidocaine. Rodriguez and Esquivel (1995) used xylocaine anaesthesia for the handling of breeding carp and the optimum dose was 0.18 g/l.

2.6.7. Ketamine hydrochloride

It is a white crystalline powder. It has been widely used as an anaesthetic both in human and veterinary medicine, and is safe for the handler (Merck and Company, 1989). It is an injectable drug, which is dissolved in saline and administered intravascularly. The effect is variable with respect to the depth and length of anaesthesia (Graham and Iwama, 1990). It is appropriate for long- term anaesthesia.

2.6.8. 2 - phenoxy ethanol

It is a colourless, oily, aromatic liquid soluble in water. It is often used as a topical anaesthetic (Merck and Company, 1989). The efficacy of 2-phenoxy ethanol varies with the size of the fish and with the temperature of water (Schoettger and Julin, 1969).

Guo *et al.* (1995) studied the effect of 2-phenoxy ethanol in platy fish *Xiphophorus maculatus* and it was found that a concentration of 220 - 440 ppm was effective to anaesthetize the fish. Weyl *et al.* (1996) studied the efficacy and mode of action of 2-phenoxy ethanol as anaesthetic in gold fish and the optimum dose was 0.5 ml/l at 30°C.

2.6.9. Urethane

It is a crystalline powder and is soluble in water (Merck and Company, 1989). It is a popular fish anaesthetic and it has no ill effects to the fish with repeated exposures (Mc Farland and Klontz, 1969). The effective concentration of urethane is 100mg/l (Jhingran and Pullin, 1988).

2.6.10. Chloral hydrate

It is an aromatic, acrid smelling powder with a bitter taste. It is soluble in water. It is more useful when deep anaesthesia is needed such as in transport or various research applications (Mc Farland and Klontz, 1969). Recommended concentration of chloral hydrate for deep anaesthesia is 0.75 g/l (Jhingran and Pullin, 1988).

Vimala (1998) studied the effect of chloral hydrate as an anaesthetic in the transportation of *Liza parsia* fry and she found that a dose of 0.3 g/l was effective to induce anaesthesia.

2.6.11. Propandid

It is a pale yellow liquid which is insoluble water, but soluble in alcohol (Merck and Company, 1989). Siwicki (1984) tried this chemical as anaesthetic in common carp and he found that addition of this anaesthetic to the water did not change water pH and CO_2 content and was ideal to induce anaesthesia. Jeney *et al.* (1986) also used propandid in the artificial propagation of common carp.

2.6.12. Barbiturates

They include amobarbital, barbital sodium, butabarbital, hexobarbital, thiopental sodium etc. (Merck and Company, 1983; Griffith, 1987). Barbiturate drugs are used for general anaesthesia (Warren, 1983). Their strong lipid solubility makes barbiturates useful as pre-anaesthetics or as ultra short - acting depressants of the central nervous system , but their subsequent slow release from fatty tissue, causes prolonged effects (Medical Economics Company, 1987).

Sodium amytal is one of the most tried drug among barbiturates. Saha *et al.* (1955) reported that sodium amytal at 21 to 28 mg/l reduced the metabolic rate of fry and that 30% more fry could be transported in a given volume of water, there by reducing the cost of transport. Sreenivasan (1962) found 50 ppm of sodium amytal to be adequate for transporting live fish.

2.6.13. Clove oil

It is a dark brown liquid resulting from the distillation of flowers, flower stalks, and leaves of clove trees (*Eugenia aromatica*). It has been used by humans as a topical anaesthetic. It consists of primarily the compounds like the phenols , eugenol , eugenol acetate and kariofilen (Burhanuddin *et al.*, 1989).

Soto and Burhanuddin (1995) successfully used the clove oil as fish anaesthetic for the handling of *Siganus lineatus.* Keene *et al.* (1998) studied anaesthetic effect of clove oil derived eugenol in juvenile *Oncorhynchus mykiss.* It was found that a dose of 40-60 ppm eugenol was effective to induce rapid anaesthesia with a relatively short time for recovery.

Vimala (1998) determined the effect of clove oil as anaesthetic in *Liza parsia* fry and the optimum dose was 8.5 mg/l to induce anaesthesia. Clove oil appears to be highly effective as a fish anaesthetic with potentially no side effects.

2.6.14. Hypothermia

Hypothermia is also employed as anaesthesia in fishes. It is induced by lowering the ambient temperature of the fish, with ice or cold water. Reported temperatures of cold anaesthesia of freshwater and marine temperate fishes ranged from 0 to 10°C according to acclimation temperature and species (Mittal and Whitear, 1978). Hypo-

thermic anaesthesia is more effective for fish acclimated to water above 10°C, as sedative effects are not induced if acclimation temperatures are lower than this. In such cases, an additional chemical anaesthetic may be necessary to induce deep anaesthesia (Mittal and Whitear, 1978). Hypothermia results in a slow, light anaesthesia which is characterized by an absence of motion , reduced power of exertion and diminished nerve sensitivity (Bell ,1987). This is useful for transport, but it is not deep enough for any type of lengthy surgery.

Yoshikawa *et al.* (1989) evaluated the efficacy of cold anaesthesia in the transportation of carp acclimated at 23°C and found that 14°C was the optimal temperature to keep the live fishes in sedate state for transportation.

Salin (1997) studied the effect of cold anaesthesia in the live transportation of *Penaeus monodon* out – of - water in chilled sawdust and reported the optimum temperature as 14±1°C.

2.6.15. Electroanaesthesia

Electric current is also used as an anaesthetic in fishes. Most forms of electrical current used for anaesthetic purposes can be classified into three categories such as alternating current, direct current and rectified pulsating current. The induction is immediate, but the recovery time can vary, depending on the conditions of the current, water chemistry and body size of the fish. Larger fish tend to be more affected within a given voltage gradient field compared to smaller fish, since their larger body mass presents a greater potential difference across their bodies (Lamarque *et al.*, 1971; Barham *et al.*, 1987;

Mazur *et al.*, 1992). Recovery from direct current is immediate, while recovery from alternating current or rectified pulsating current is slow (Ross and Ross, 1984). Physiologically, electroanaesthesia produces effects similar to those resulting from chemical anaesthesia.

2.7. Carbon dioxide anaesthesia in fishes

Other names for carbon dioxide are CO_2, carbonic acid and carbonic anhydride. Carbon dioxide is a colourless , odourless, non flammable gas which is soluble in water (Bell, 1987). It can be derived from a chemical reaction of sodium bicarbonate with an acid in water or delivered into the water as a gas. Carbon dioxide is reportedly useful as an anaesthetic because it is safe , effective, soluble in water, inexpensive and non toxic. It is a flexible anaesthetic, because depth of anaesthesia and time to recover can be altered with minor changes in the concentration of carbon dioxide in the water or in exposure time (Post, 1979). Carbon dioxide leaves no residues and requires no withdrawal period.

Carbon dioxide anaesthesia is an old method and it was first described by Fish (1943). He produced carbon dioxide concentrations of 150-650 mg/l with sodium bicarbonate and sulphuric acid (soda - acid technique); and reported that 200 mg/l was optimum for anaesthetizing fingerling and adult salmon.

Booke *et al.* (1978) determined that a 642 mg/l solution of sodium bicarbonate at a pH of 6.5 was the most effective medium for causing rainbow trout, brook trout and

common carp to cease swimming and to slow down respiration within 5 minutes. They hypothesized that the mechanism was a pH - controlled release of carbon dioxide.

Post (1979) recommended mixing 6.75% (weight/volume) sodium bicarbonate solution and 3.95% (weight/volume) sulphuric acid solution to obtain the desired concentration of carbonic acid for anaesthetizing the fish fingerlings and adults of salmon.

Kumar *et al.* (1981) and Mishra *et al.* (1983) tried carbonic acid anaesthesia in fish fry transport. Mishra *et al.* (1983) reported that *Labeo rohita* fry at 50 No. / l could be safely transported with carbonic acid anaesthesia at 500 ppm for 251 hours with 5% mortality.

Takeda and Itazava (1983) discussed the possibility of causing sedation by carbon dioxide in the transportation of carps. The methods tried by them were (1) by adding sulphuric acid and sodium bicarbonate to water and (2) by bubbling carbon dioxide and oxygen into the water for a short period until the fish were sedated.

To reduce the hyper activity of fishes and for better survival during handling and transportation, Dupree and Huner (1984) tried carbonic acid and sodium bicarbonate separately to anaesthetize the fishes.

Yoshikawa *et al.* (1988) studied carbon dioxide anaesthesia in the transportation of carp fry. For long-term anaesthesia in carp, Yokoyama *et al.* (1989) applied carbon dioxide anaesthesia combined with low temperature.

Zhao and Chen (1994) determined the efficacy of carbonic acid as anaesthetic for grass carp (*Ctenopharyngodon idella)* fry transportation and defined the optimum density of the anaesthetic as well as the survival time under the varied densities. They found that the optimum density of carbonic acid was 500 ppm in the medium of transportation of grass carp fry and the safety survival time was 201 hours with 10% mortality.

In preparing adult salmon for surgical procedures, the required stage - four anaestheisa (immobility) was achieved by Prince *et al.* (1995) using sodium bicarbonate activated by glacial acetic acid. They determined that 40g of sodium bicarbonate and 15 ml of glacial acetic acid in 30 l of river water yielded carbon dioxide concentrations that ranged from 195 to 328 mg/l. This range of carbon dioxide concentrations was sufficient to bring the fish to stage – four anaesthesia within 7 minutes.

Jenyferck *et al.* (1997) tried carbonic acid as an anaesthetic in the transport of ornamental fish fry. They reported that 200 ppm of carbonic acid was most effective for the transportation with 80 - 100% survival.

Bernier and Randall (1998) studied the physiological and anaesthetic effect of carbon dioxide anaesthesia in *Oncorhynchus mykiss* during handling and Gelwicks *et al.* (1998) determined effective concentration ranges for carbonic acid as an anaesthetic for this species across a wide range of water temperatures and alkalinities. Induction time decreased and recovery time increased with increasing concentration. Effective concentration range decreased as temperature increased.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Experimental animals

L. rohita fry produced at the hatchery of the College of Fisheries , Kerala Agricultural University , Kochi, were used for the experiments. The required number of fry were maintained in a flat bottomed oval fibre glass tank of 1.2 tonne capacity. The average length and weight of the fry were 25mm and 750mg respectively. Aeration was provided in the tank. The fry were fed *ad libitum* using rice bran and groundnut oil cake in the ratio 1:1. Fifty percent of the tank water was exchanged every day. The feed remnants and faecal matter were removed from the tank bottom by siphoning once daily.

3.2. Experimental containers

Air tight, transparent, hard plastic jars each of 600 ml capacity with screw type lid fitted with one way valve similar to those described by Jayasree - Vadhyar *et al.* (1992), were used for the oxygen packing of the fry under uniform oxygen pressure and water quality conditions.

3.3. Experimental procedure

3.3.1. *In situ* production of carbon dioxide anaesthesia

Sodium bicarbonate and glacial acetic acid were used for *in situ* production of carbon dioxide in freshwater as the packing medium. Three different concentrations of

sodium bicarbonate viz., 0.2, 0.4, 0.6 g/l of water, mixed with acetic acid (ml/l) in the ratio 2:1 were tested in the study, on the basis of the available literature on salmon (Prince *et al.*, 1995). These concentrations were considered as the first (T_1) second (T_2) and third (T_3) treatments respectively.

3.3.2. Packing

The fry were packed under uniform oxygen pressure and water quality at five different packing densities of 100, 200, 300, 400 and 500 fry/l each with three replications. The experiment was conducted at ambient temperature of 28 ±2°C.

Twenty four hours prior to starting the experiment, the feeding was stopped to reduce the quantity of metabolic waste products in the oxygen filled packing jars.

The fry maintained in the fibre glass tank, were taken at random , counted i.e., 10, 20,30,40 , 50 fry and acclimatized to the restricted space in a beaker of 250ml capacity with 100ml water for 10 minutes. These were introduced into the packing jars containing 100ml water treated with three different concentrations of acetic acid and sodium bicarbonate, at five different packing densities. Immediately after transferring them the jars were closed tightly and filled with oxygen from an oxygen cylinder under uniform pressure of 0.2 kg/cm2. The oxygen pressure in the jars was measured through a pressure gauge with a precision of 0.2kg/cm^2 (Bourdon type). The jars were shaken periodically to simulate transporting conditions.

Simultaneously, controls were maintained in a similar way at five different packing densities, but without the addition of chemicals in the packing medium.

3.3.3. Induction time and safe duration of survival .

The induction time i.e., the time taken by the fishes for light sedation indicated by lying on their sides with only slight movement and no response to external stimuli, which is favourable for transportation ,was noted for each set of concentration at each packing density.

Time of initial mortality of the oxygen - packed fry was recorded by making hourly observations. This was considered as the safe duration of 100% survival. The duration of subsequent survival down to 70% were noted by taking three hourly observations. The behavioural pattern of the fry under treatments and control was observed closely .

3.3.4. Recovery time and post – treatment survival.

The survivors from the jars were released into circular fibre glass tanks of 83 litre capacity containing aerated freshwater without the anaesthetic. The fry were then observed closely to note the recovery time, i.e., the time taken by the fishes to return to normalcy with active movement in upright position as well as response to external stimuli. The post -treatment survival including feeding activity was observed for a period of 96 hours after release.

3.4. Determination of water quality

Jars containing 100ml packing medium with and without chemicals (control) with no fry , were filled separately with oxygen at 0.2 kg/cm2 as described above .

These jars were opened immediately after filling oxygen and the water samples of each treatment as well as control were collected for analyses of initial water quality parameters .For the determination of initial free carbon dioxide concentration of the packing medium, samples of each treatment were collected from the jars before and after filling oxygen. Samples of the packing medium for the final water quality analyses were collected from the jars soon after 70% survival of the fry was recorded .

Initial and final quality of the packing medium were analyzed using standard procedures. The parameters analyzed were dissolved oxygen, ammonia nitrogen, free carbon dioxide, pH and total alkalinity. Of the three replicates of each treatment and control , one was used for analyzing dissolved oxygen, one for free carbon dioxide and one for ammonia - N, because the determination of these parameters from the same jar might yield erroneous values for the subsequently measured parameter. pH was noted from all the replicate jars.

The following methods were used for analyzing the water quality parameters.

Dissolved Oxygen	-	Winkler method (Strickland and Parsons, 1972)
Free carbon dioxide	-	Alkalimetric titration method (Strickland and Parsons , 1972)
Ammonia - N	-	Phenol-hypochlorite spectrophotometric method (Strickland and Parsons, 1972)
Total alkalinity	-	Alkalimetric titration method (Strickland and Parsons ,1972)
pH	-	Using universal pH indicator solution.
Temperature	-	Using mercury bulb thermometer having a precision of 0.1°C

3.5. Statistical analyses

Randomized block design (RBD) with, dose of anaesthetic and control as treatments and packing densities as blocks , was used for planning the experiment and analyses of results. Each treatment were replicated three times.

Data obtained from all the observations were analyzed statistically by analysis of variance. Data on dissolved oxygen, free carbon dioxide, ammonia - N and total alkalinity were analyzed. Pair - wise comparisons using critical difference values were made for those treatments which were found statistically significant.

RESULTS

4. RESULTS

4.1. Effect of carbon dioxide anaesthesia and packing density on the oxygen – packed fry of *L. rohita*

4.1.1. Behavioural pattern

Behavioural pattern of the fry subjected to three treatments of carbon dioxide anaesthesia using sodium bicarbonate at 0.2 g/l (T_1) ,0.4 g/l (T_2),0.6 g/l (T_3) ,mixed with acetic acid (ml/l) in the ratio 2:1 are summarized in Table 1. In the first treatment, it was found that the movement of the fry slowed down after 10 minutes of adding the chemicals and the fry responded to external stimuli. But, in the case of the second treatment, the fry lay on their sides after 2 minutes of adding the chemicals and then returned to normal upright condition; they showed slow movement and were not reactive to external stimuli. In the third treatment the fry lay on their sides immediately after adding the chemicals and returned to normal upright condition within 4 – 5 minutes. They did not respond to external stimuli and showed slow movement. The behavioural pattern of the fry was more or less similar at all the five packing densities tried viz; 100, 200, 300, 400 and 500 fry / l. The recovered fry survived for the observed period. They were found to be active with normal movement and feeding activity.

Table 1. Behavioural pattern of *L. rohita* fry subjected to three treatments of carbon dioxide anaesthia using sodium bicarbonate at 0.2 g/l (T_1) ,0.4 g/l(T_2), 0.6 g/l (T_3) mixed with acetic acid (ml/l) in the ratio 2:1 at five packing densities.

Treatment	Packing density (No. /l)	Behaviour of the fry
T_1	100 200 300 400 500	Responded to external stimuli. Remained in the water column with slow movement after 10 minutes of adding the chemicals.
T_2	100 200 300 400 500	Lay on their sides at the surface after 2 minutes of adding the chemicals and then returned to normal up - right condition within another 2 minutes , but did not respond to external stimuli. Remained in the water column with slow movement.
T_3	100 200 300 400 500	Lay on their sides at the surface immediately after adding the chemicals and returned to normal up – right condition within 4 – 5 minutes, but did not respond to external stimuli. Remained in the water column with slow movement

4.1.2. Induction time and recovery time

Table 2 shows the induction time and recovery time of the fry under the three treatments (T_1 , T_2 and T_3) at the five packing densities. In the first treatment (T_1), the induction time was 15, 14, 10, 10 and 10 minutes at 100, 200, 300, 400 and 500 fry / l respectively. The recovery time was not clear at 100, 200 and 300 fry/l and it was 3 – 4 minutes at 400 and 500 fry/l.

In the case of the second treatment (T_2) , the induction time was 5, 3, 2, 2 and 1 minutes at 100, 200, 300, 400 and 500 fry/l respectively. The recovery time was 8 minutes at 100, 200 and 300 fry/l and 10 minutes at 400 and 500 fry/l.

But , in the third treatment (T_3) , the induction of anaesthesia was immediate and the induction time was recorded as less than 1 minute at all the five packing densities. The recovery time was long and it was observed as 12 minutes at 100 and 200 fry/l and 15 minutes at 300, 400 and 500 fry/l.

Table 2. Induction time and recovery time of *L. rohita* fry under three treatments of carbon dioxide anaesthesia using sodium bicarbonate at 0.2 g/l (T_1), 0.4 g/l (T_2) and 0.6 g/l (T_3) mixed with acetic acid (ml/l) in the ratio 2:1 at five packing densities.

Treatment	Packing density (No. / l)	Induction time (Minutes)	Recovery time (Minutes)
T_1	100	15	Difference not clear
	200	14	Difference not clear
	300	10	Difference not clear
	400	10	3 – 4
	500	10	3 – 4
T_2	100	5	8
	200	3	8
	300	2	8
	400	2	10
	500	1	10
T_3	100	<1	12
	200	<1	12
	300	<1	15
	400	<1	15
	500	<1	15

4.1.3. Safe duration of survival

Safe duration of survival in the oxygen – packed fry under three treatments (T_1, T_2 and T_3) and control at the five packing densities are presented in Table 3. This duration is referred as safe duration of survival for transportation. The graphical representation of the safe duration of survival is shown in Fig.1 and the results of the analysis of variance (ANOVA) in Table 4. Analysis of variance showed significant difference among the treatments and among the packing densities. Pair – wise comparison by critical difference analysis revealed that there was no significant difference between T_1 and control and also between T_2 and T_3. It was also found that the packing density of 100 fry / l differed significantly from the other four packing densities . Linear relation between the packing densities and duration of transport at 100% survival could be established for T_1, T_2 and control as follows and is presented in the Fig. 2, 3 and 4.

T_1 $Y = 39.9 - 8.3\ x$

T_2 $Y = 15.9 - 2.9x$

C $Y = 26.2 - 5.8x$

Table 3. Safe duration of survival * of oxygen – packed *L. rohita* fry under three treatments of carbon dioxide anaesthesia using sodium bicarbonate at 0.2 g/l (T_1), 0.4 g/l (T_2) and 0.6g/l (T_3) mixed with acetic acid (ml/l) in the ratio 2:1, and control at five packing densities.

Duration (h) * *				
Packing density (No./l)	T_1	T_2	T_3	Control
100	39.0	15.5	11.5	25.0
200	17.0	7.0	1.0	11.0
300	10.0	6.5	1.0	6.0
400	6.0	5.0	1.0	1.0
500	3.0	2.0	1.0	1.0

*Time of initial mortality.

** Each value is a mean of triplicates.

Table 4. Results of the analysis of variance of the safe duration of survival of oxygen – packed *L. rohita* fry under three treaments of carbon dioxide anaesthesia using sodium bicarbonate at 0.2 g/l (T_1), 0.4 g/l (T_2) and 0.6 g/l (T_3) mixed with acetic acid (ml/l) in the ratio 2:1, and control at five packing densities.

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F value computed
Treatment	365.94	3	121.98	5.17*
Packing density	1133.3	4	283.33	12.01*
Error	283.0	12	23.58	
Total	1782.24	19		

* Significant at 5% level.

Treatment means of different doses of anaesthetic and control

T_1	T_2	T_3	Control (C)
15.0	7.2	3.1	8.8

Computed critical difference value = 6.69

T_1 C T_2 T_3

Block means of different packing densities.

B_1	B_2	B_3	B_4	B_5
22.75	9.0	5.875	3.25	1.75

Computed Critical difference value = 7.48

B_1 B_2 B_3 B_4 B_5

Underline indicate 'no significant difference'.

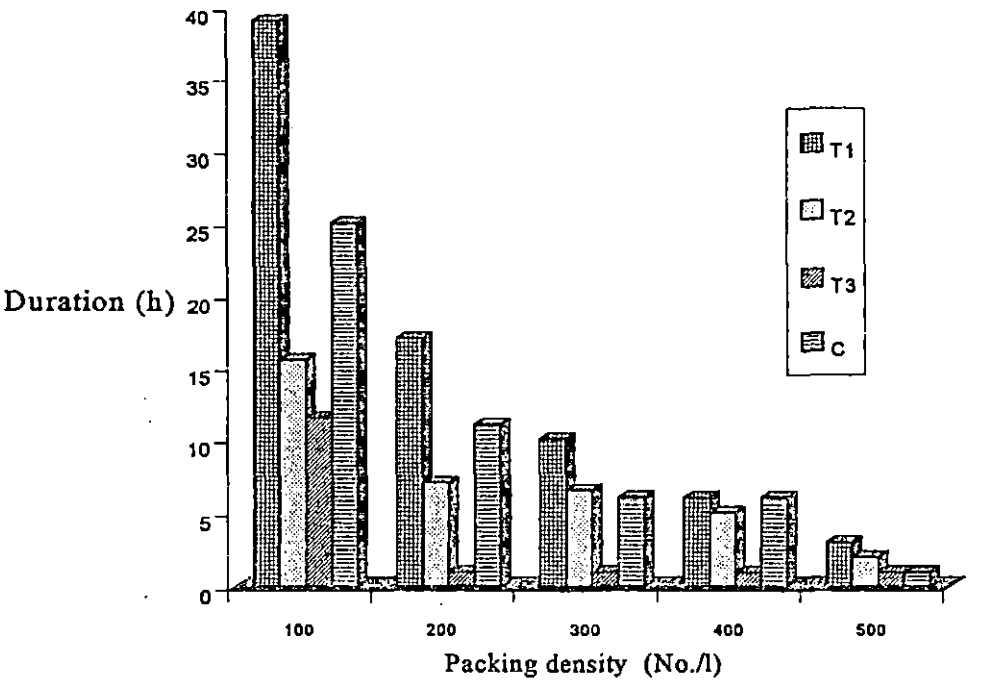

Figure 1. Duration of 100% survival of oxygen - packed L. rohita fry under three treatments of carbon dioxide anaesthesia using sodium bicarbonate at 0.2 g/l (T_1), 0.4 g/l (T_2) and 0.6 g/l (T_3) mixed with acetic acid (ml/l) in the ratio 2:1, and control

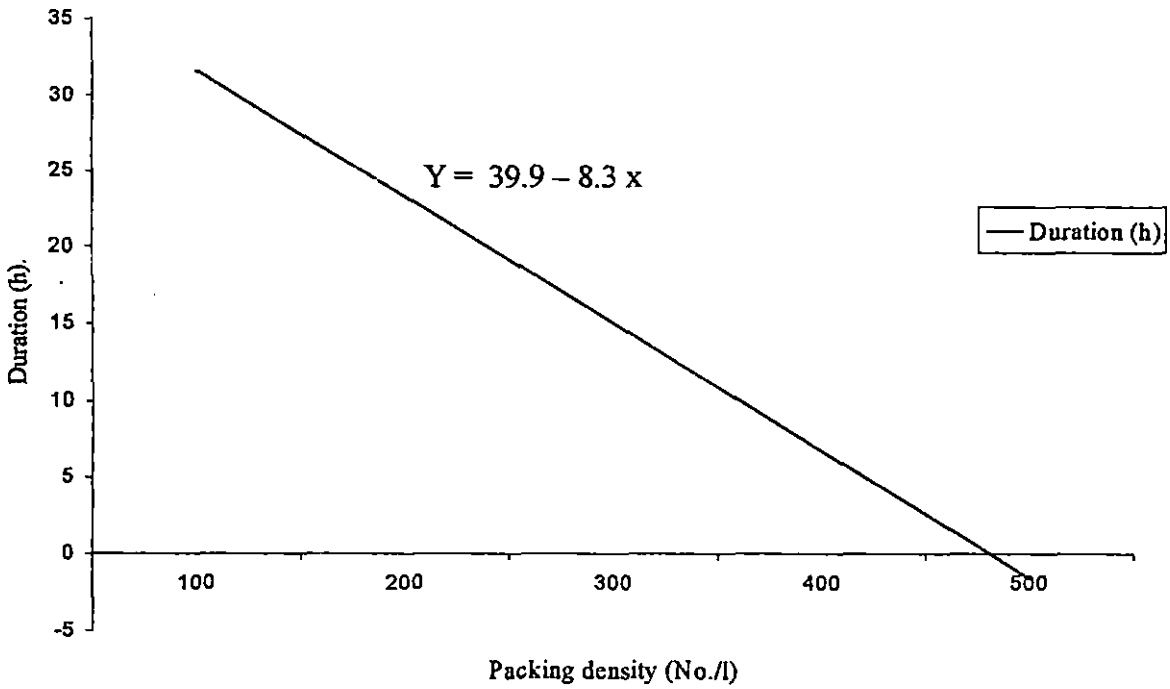

Figure 2. Graph showing the relationship between packing density and duration of transport of oxygen - packed *L. rohita* fry under T_1 (sodium bicarbonate 0.2 g/l and acetic acid 0.1 ml/l) at 100% survival.

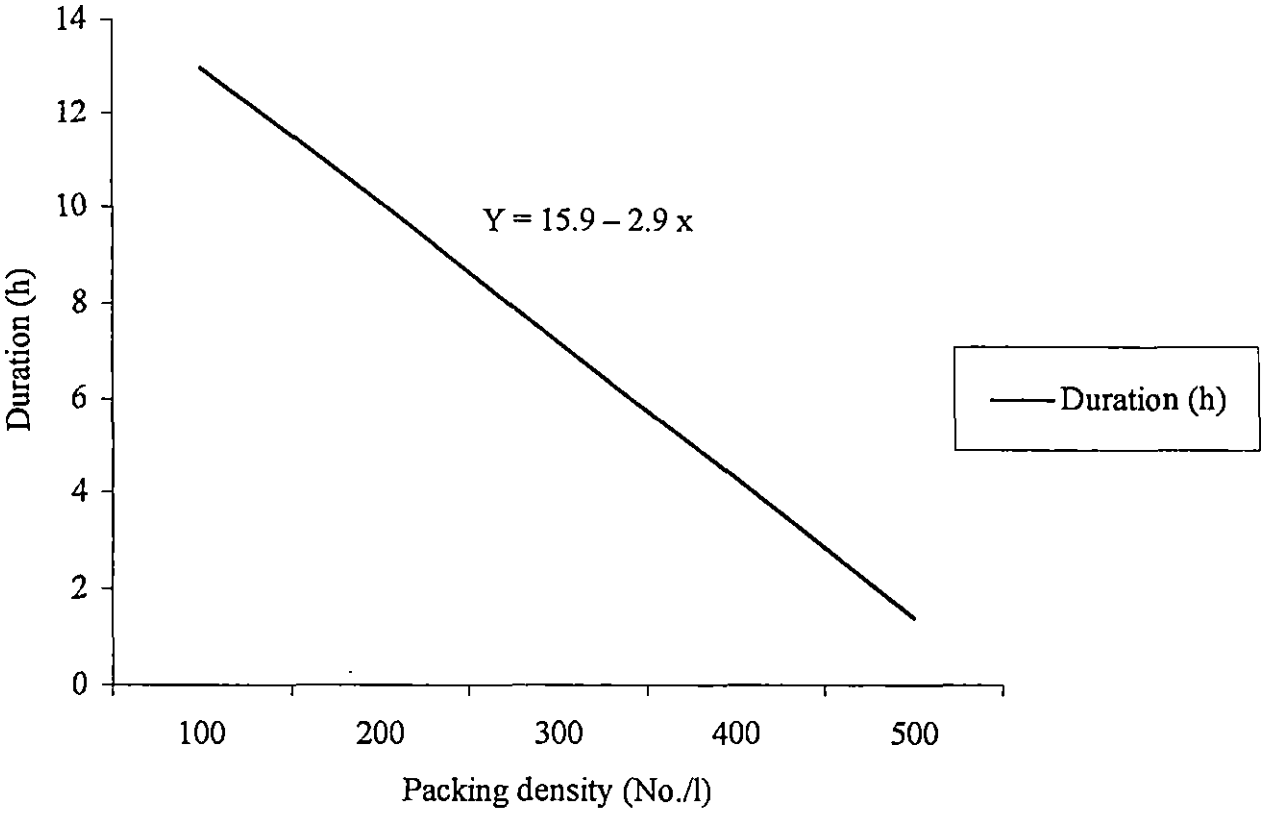

Figure 3. Graph showing the relationship between packing density and duration of transport of oxygen - packed *L. rohita* fry under T_2 (sodium bicarbonate 0.4 g/l and acetic acid 0.2 ml/l) at 100% survival.

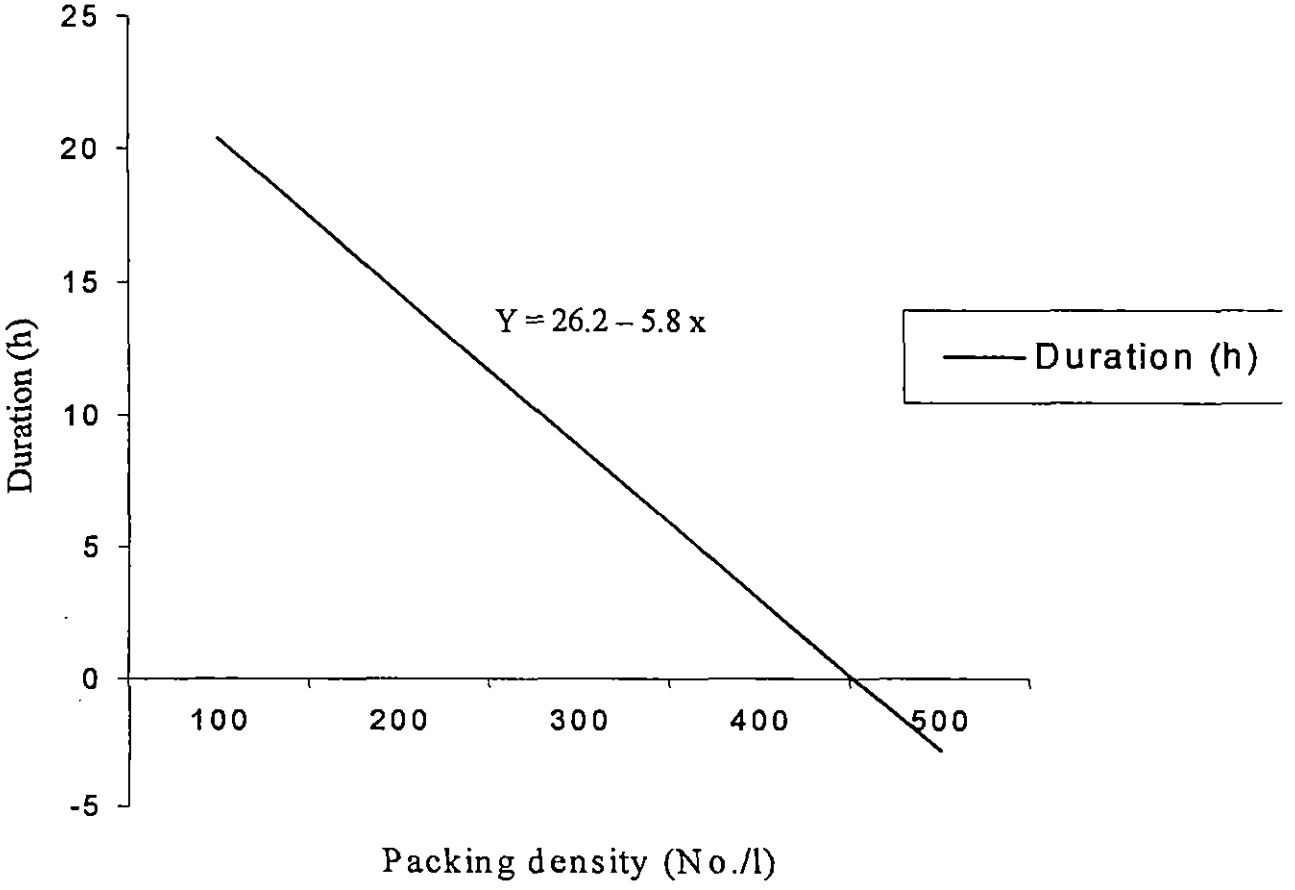

Figure 4. Graph showing the relationship between packing density and duration of transport of oxygen - packed *L. rohita* fry under control (C) at 100% survival.

4.1.4. Duration of 70% survival

The data on the duration of 70% survival of oxygen – packed fry under the three treatments (T_1, T_2, T_3) at the five different packing densities (100, 200, 300, 400 and 500 fry/l) are presented in Table 5 and Fig. 5 shows its graphical representation. Analysis of variance of the data (Table 6) showed significant difference among the packing densities tried. But, no significant difference was observed among the treatments. Pair – wise comparison by critical difference analysis revealed that the packing density of 100 fry / l differed significantly from the other four packing densities. Linear relationship between the packing densities and the duration of transport at 70% survival could be established for T_1, T_2 and control as follows and is presented in the Fig. 6,7 and 8.

T_1 $Y = 42.6 - 7.8x$

T_2 $Y = 21.05 - 2.85x$

C $Y = 29.0 - 5.1x$

Table 5. Duration of 70% survival of oxygen – packed *L. rohita* fry under three treatments of carbon dioxide anaesthesia using sodium bicarbonate at 0.2 g/l (T_1), 0.4 g/l (T_2) and 0.6 g/l (T_3) mixed with acetic acid (ml/l) in the ratio 2:1, and control at five packing densities.

	Duration (h) *			
Packing density (No./ l)	T_1	T_2	T_3	Control
100	43.0	19.0	14.0	29.0
200	20.0	14.0	13.0	15.0
300	14.0	13.0	11.0	9.5
400	10.0	9.5	9.0	8.0
500	9.0	7.0	7.0	7.0

* Each value is a mean of triplicates.

Table 6. Results of the analysis of variance of the duration of 70% survival of oxygen – packed *L. rohita* fry under three treatments of carbon dioxide anaesthesia using sodium bicarbonate at 0.2 g/l (T_1), 0.4g/l (T_2) and 0.6 g/l (T_3) mixed with acetic acid (ml/l) in the ratio 2:1 , and control at five packing densities .

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F value computed
Treatment	198.05	3	66.02	2.34
Packing density	891.33	4	222.83	7.89*
Error	339.07	12	28.25	
Total	1428.45	19		

* Significant at 5% level .

Block means of different packing densities

B_1	B_2	B_3	B_4	B_5
26.25	15.5	11.87	9.12	7.5

Calculated critical difference value = 8.19

B_1 $\underline{B_2 \quad B_3 \quad B_4 \quad B_5}$

Under line indicates 'no significant difference.'

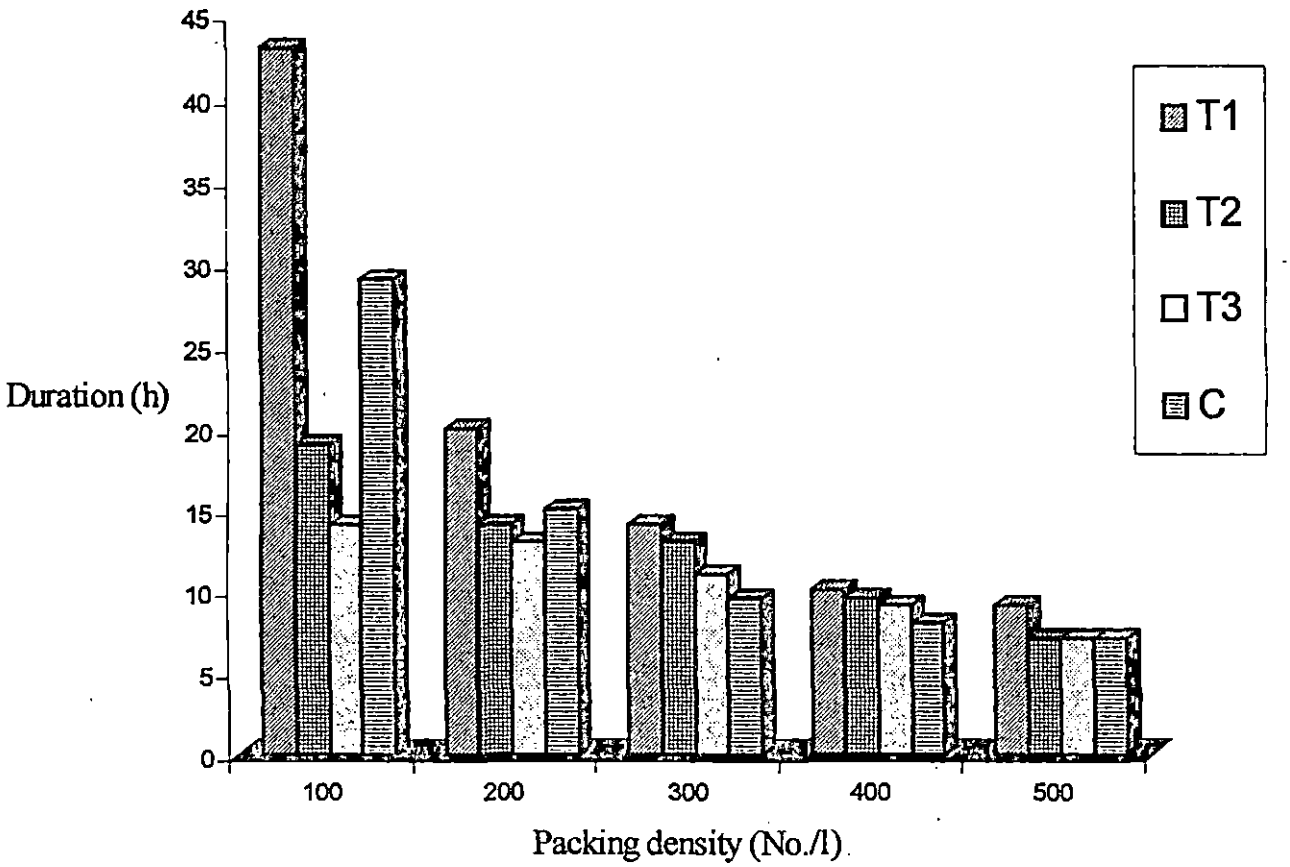

Figure 5. Duration of 70% survival of oxygen - packed *L. rohita* fry under three treatments of carbon dioxide anaesthesia using sodium bicarbonate at 0.2 g/l (T_1), 0.4 g/l (T_2) and 0.6 g/l (T_3) mixed with acetic acid (ml/l) in the ratio 2:1, and control.

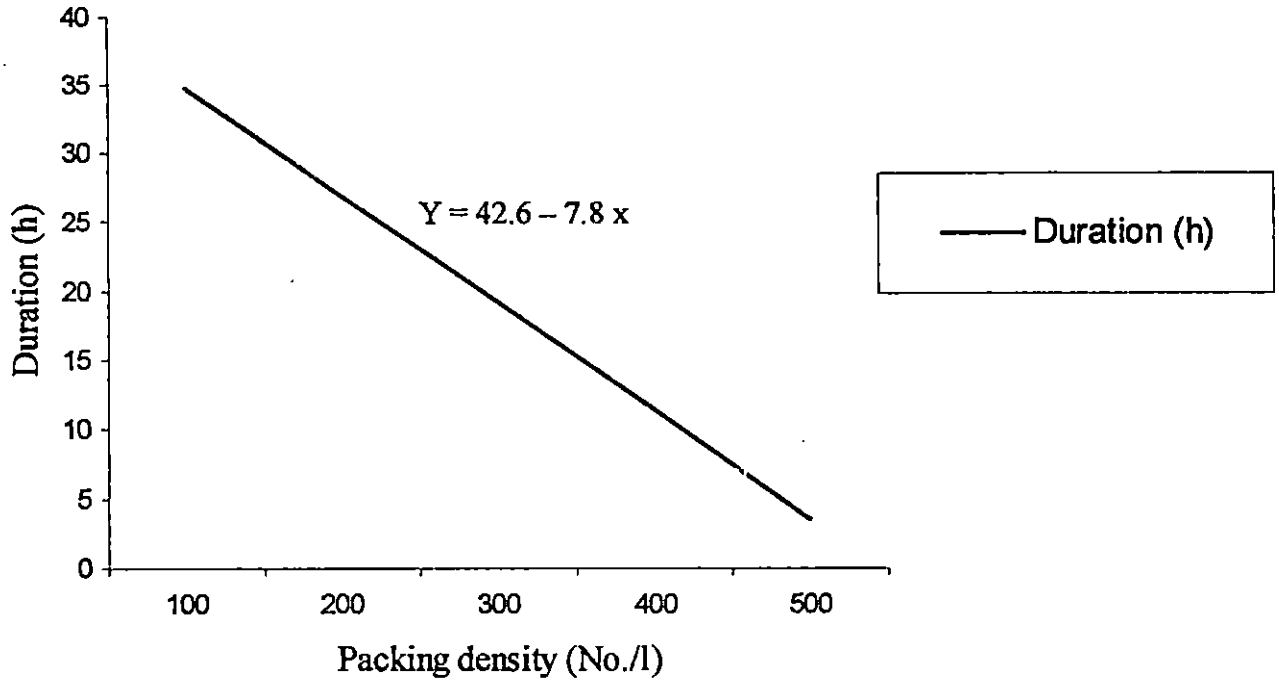

Figure 6. Graph showing the relationship between packing density and duration of transport of oxygen - packed *L. rohita* fry under T_1 (sodium bicarbonate 0.2 g/l and acetic acid 0.1 ml/l) at 70% survival.

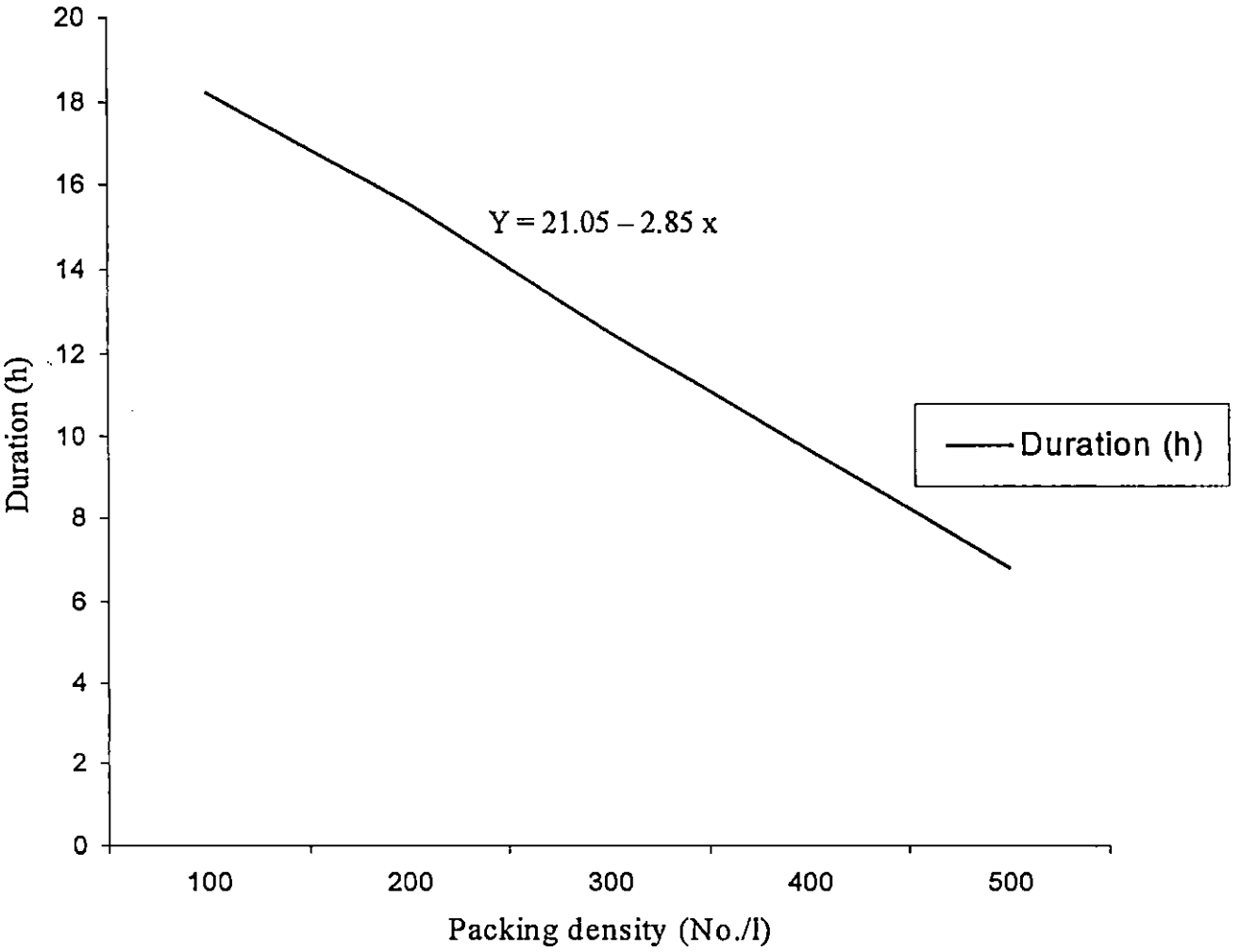

Figure 7. Graph showing the relationship between packing density and duration of transport of oxygen - packed *L. rohita* fry under T_2 (sodium bicarbonate 0.4 g/l and acetic acid 0.2 ml/l) at 70% survival.

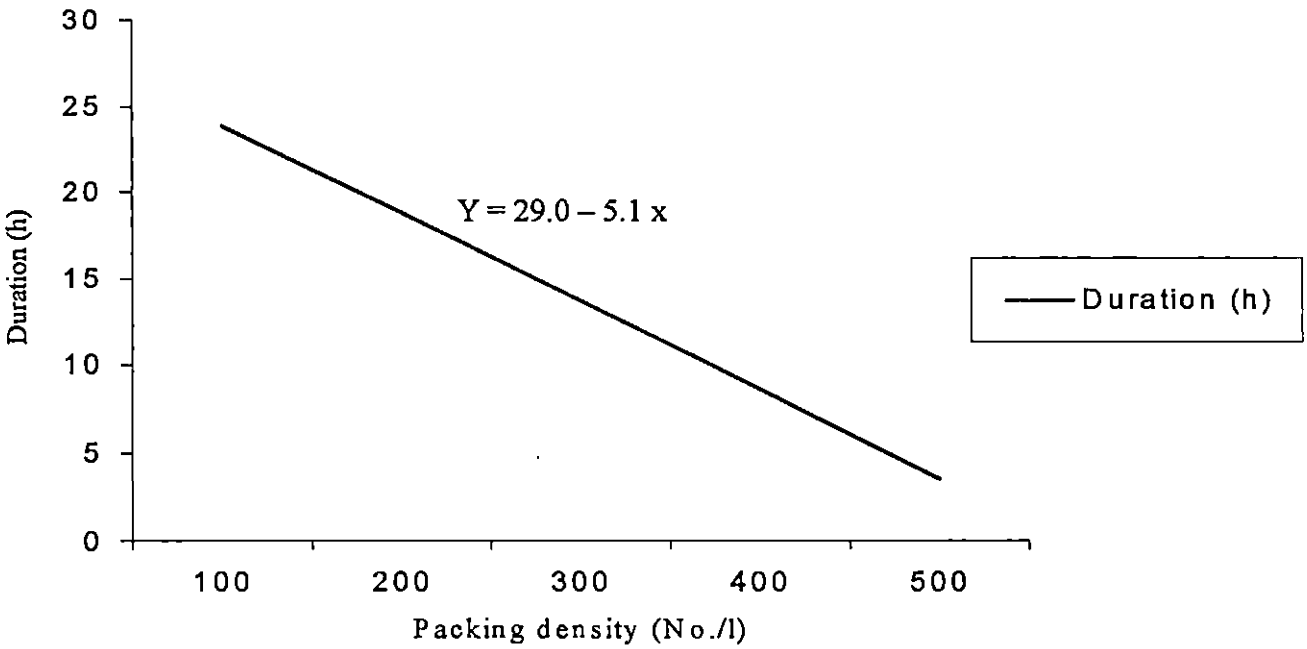

Figure 8. Graph showing the relationship between packing density and duration of transport of oxygen - packed *L. rohita* fry under control (C) at 70% survival.

4. 1. 5. Cumulative percentage survival

Table 7 and Fig. 9,10,11 and 12 shows the cumulative percentage survival of the oxygen – packed fry at the five packing densities, under the three treatments and control.

Under the first treatment, at 100, 200, 300, 400 and 500 fry / l, duration of 90% survival was observed as 41.5 h, 19.5 h, 11.5 h, 8.7 h and 5 h respectively. The corresponding duration of 80% survival were 42 h , 20 h, 13 h , 9.3 h and 6.5 h and that of 70% survival were 43 h, 20 h, 14 h, 10.3 h and 8.3 h respectively. The duration of 90 %, 80% and 70 % survival of fry under T_1 was found to decrease with increase in packing densities.

In the second treatment, 90% survival was observed as 4.8 h, 7.3 h,8.5 h,11.0 h and 16.0 h at 100, 200, 300, 400 and 500 fry/l respectively. The corresponding duration of 80% survival was 5.8 h, 8.7 h,11.0 h,12.5 h, 17.0 h and those of 70% survival was 6.8 h, 9.8 h,12.9 h, 12.8 h, 19.0 h respectively.

In the T3, 90% survival was observed as 3.2 h, 3.6 h, 5.3 h, 3.0 h and 12.0 h, 80% survival was noted as 5.3 h,6.0 h, 9.2 h, 10.3 h 13.0 h and that of 70% survival were 6.4 h, 8.8 h,10.7 h,12.5 h and 14.0 hat the packing densities of 100, 200, 300, 400 and 500 fry/l respectively.

In the control, the duration of 90% survival was recorded as 3.0 h, 4.6 h, 7.6 h,13.5 h, 26.0 h, that of 80% survival was 5.7 h, 6.7 h, 8.5 h, 14.2 h, 28.0 h and that of 70% survival was 6.8 h, 7.3 h, 9.8 h, 15.0 h and 29.0h at 100, 200, 300,400 and 500 fry/l respectively.

Table 7. Cumulative percentage survival of oxygen - packed *L. rohita* fry under three treatments of carbon dioxide anaesthesia using sodium bicarbonate at 0.2 g/l (T_1),0.4 g/l (T_2) and 0.6 g/l (T_3), mixed with acetic acid (ml/l) in the ratio 2:1 ,and control at five packing densities.

Treatments	Packing density (No. /l)	Duration (h)*
T_1	500	100 100 100 96 90 80 78 73 68
	400	100 100 100 100 100 100 97 93 75 70
	300	100 100 100 100 100 100 100 100 100 100 90 83 77 67
	200	100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 95 90 70
	100	100 100 100 100 100 100 100 100 100100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 90 90 80 70
T_2	500	100 100 96 94 88 74 66
	400	100 100 100 100 100 95 92 88 74 68
	300	100 100 100 100 100 100 94 92 87 85 80 76 69
	200	100 100 100 100 100 100 100 95 95 95 90 85 65
	100	100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 90 80 80 70
T_3	500	100 98 94 86 83 74 65
	400	100 98 95 87 84 80 78 75 68
	300	100 97 95 95 93 85 82 82 82 73 66
	200	100 97 90 90 90 85 83 82 82 82 74 72 68
	100	100 100 100 100 100 100 100 100 100 100 100 90 80 70
Control	500	100 98 90 87 82 79 65
	400	100 98 98 92 87 85 72 65
	300	100 100 100 100 100 100 97 86 75 68
	200	100 100 100 100 100 100 100 100 100 100 100 97 97 82 70
	100	100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 90 90 80 70

* Each value is a mean of triplicates.

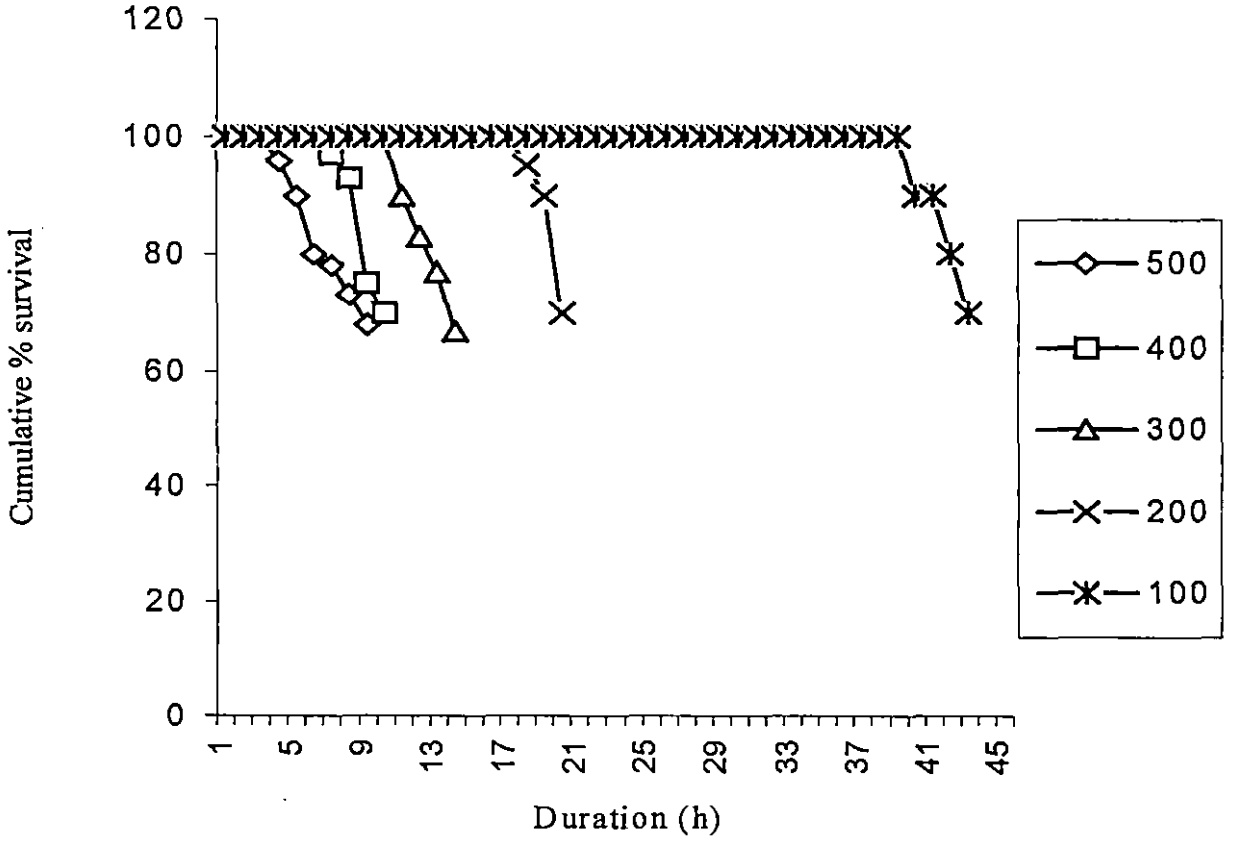

Figure 9. Cumulative % survival of oxygen - packed *L.rohita* fry under T_1 (Sodium bicarbonate 0.2 g/l and acetic acid 0.1ml/l) at five packing densities .

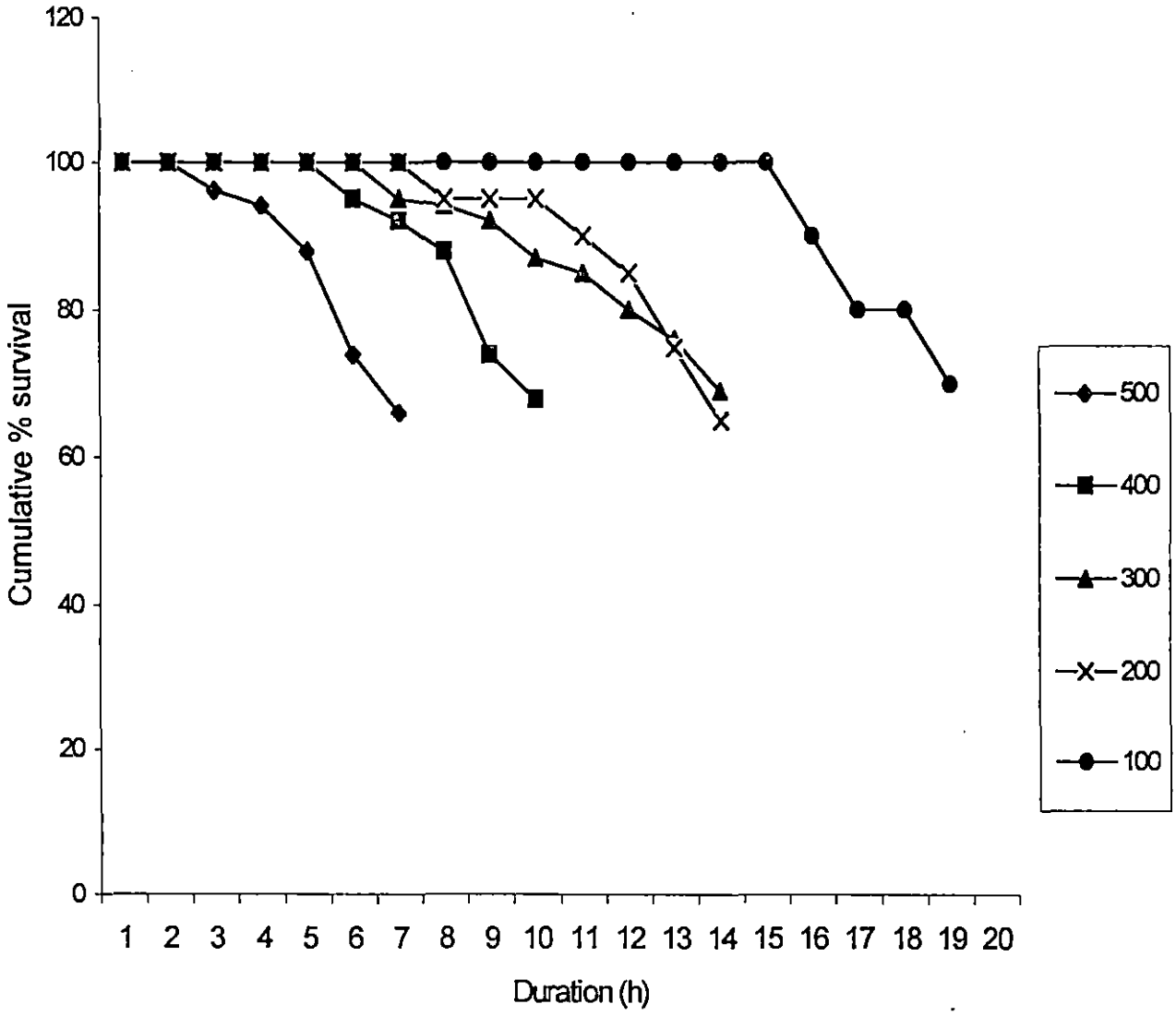

Figure 10. Cumulative % survival of oxygen - -packed *L. rohita* fry under T_2 (sodium bicarbonate 0.4 g/l and acetic acid 0.2 ml/l) at five packing densities.

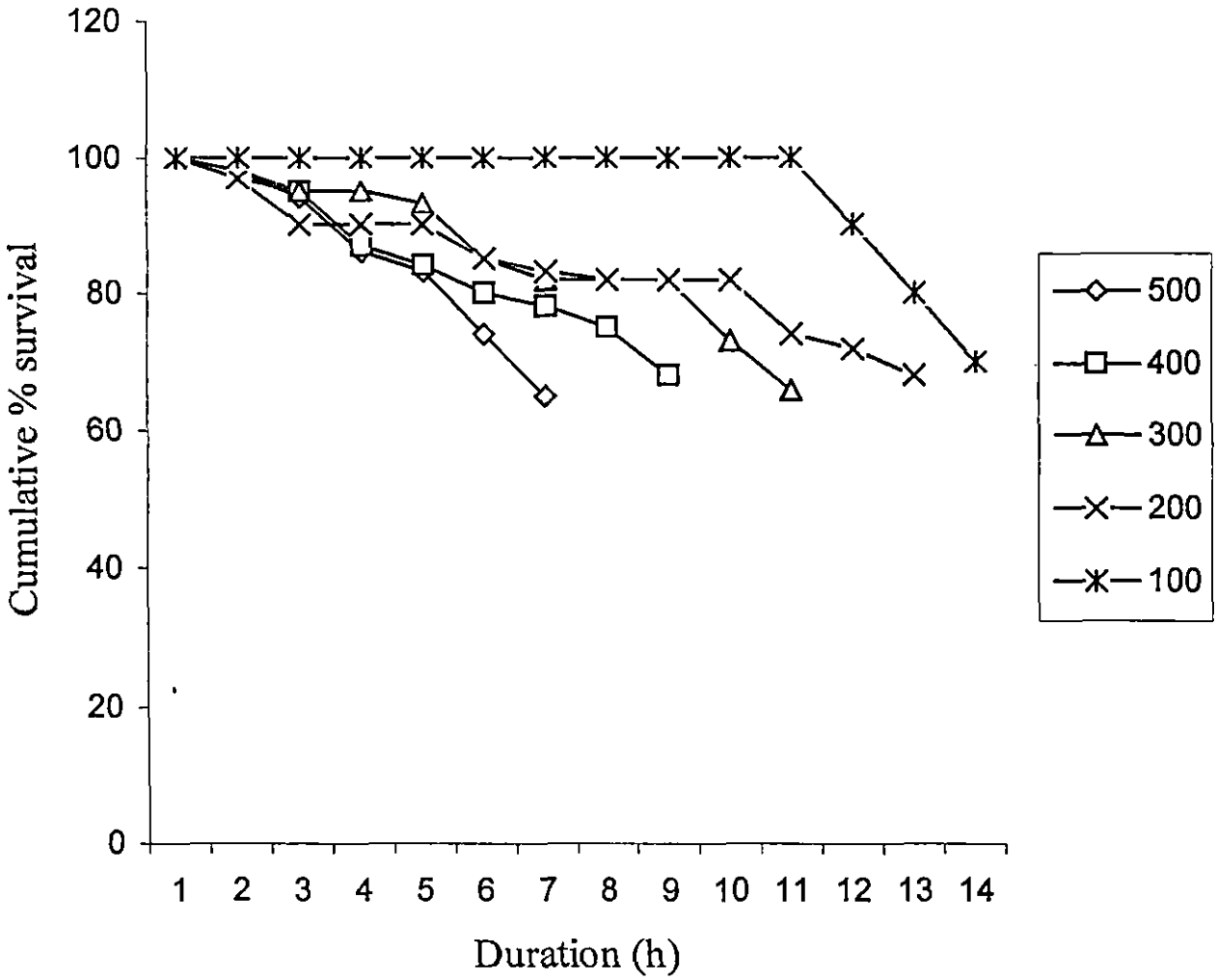

Figure 11.Cumulative % survival of *L.rohita* fry under T_3 (sodium bicarbonate 0.6 ml/l and acetic acid 0.3 ml/l) at the five packing densities.

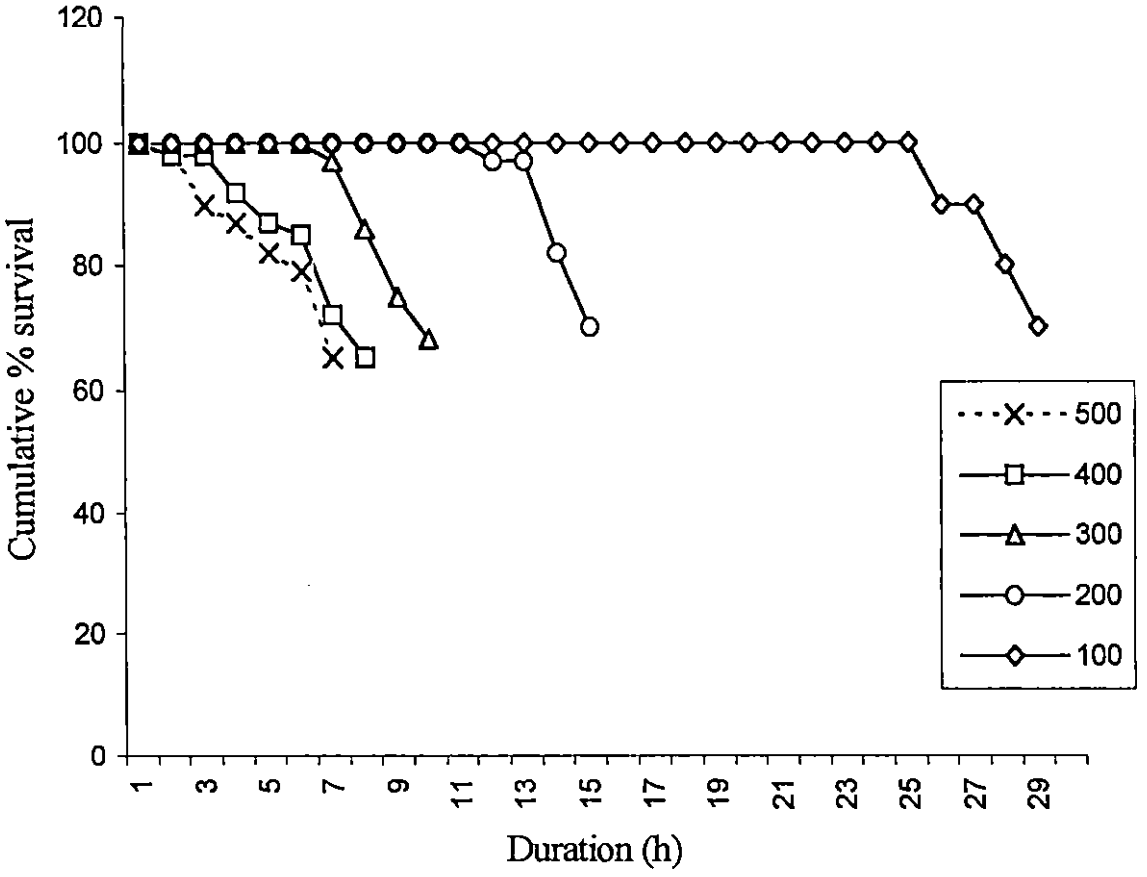

Figure 12. Cumulative % survival of oxygen - packed *L.rohita* fry under control (C) at five packing densities.

4.2. Effect of anaesthetization and packing density on water quality in the oxygen – packed jars

4.2.1. Dissolved oxygen.

The initial dissolved oxygen levels of the packing medium in the oxygen – packed jars, under the three treatments and control at the five packing densities were found as 30 ppm. The dissolved oxygen values of the packing medium in these jars at 70% survival are shown in Table 8 and Fig. 13.

Results of the analysis of variance of dissolved oxygen in the oxygen – packed jars at three treatments of the anaesthetic and control at the five packing densities at 70% survival is shown in Table 9. No significant difference was found among the treatments and control. But, there was significant difference among the packing densities. Pair – wise comparison by critical difference analysis showed that the packing densities of 100, 200 and 300 fry / l differed significantly from the other two (400 and 500 fry/l).

Table 8. Dissolved oxygen (ppm) in the oxygen – packed jars at three treatments of carbon dioxide anaesthesia using sodium bicarbonate at 0.2 g/l (T_1) , 0.4 g/l (T_2) and 0.6 g/l (T_3) mixed with acetic acid (ml /l) in the ratio 2:1 , and control at five packing densities at 70% survival of *L. rohita* fry.

	Dissolved oxygen (ppm)			
Packing density (No./ l)	T_1	T_2	T_3	Control
100	4.32	2.592	2.592	2.21
200	4.41	2.16	2.16	2.21
300	2.21	2.16	2.16	0
400	0	0	0	0
500	0	0	0	0

Table 9. Results of the analysis of variance of the dissolved oxygen (ppm) in the oxygen – packed jars at three treatments of carbon dioxide anaesthesia using sodium bicarbonate at 0.2 g/l (T_1) , 0.4 g/l (T_2) and 0.6 g/l (T_3) mixed with acetic acid (ml/l) in the ratio 2:1 , and control at five packing densities at 70% survival of *L . rohita* fry.

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F value computed
Treatment	4.37	3	1.56	3.1*
Packing density	32.30	4	8.07	17.3*
Error	5.61	12	0.47	
Total	42.28	19		

* Significant at 5% level.

Treatment means of the three doses of anaesthetic and control

T_1	T_2	T_3	Control (C)
4.188	3.88	3.88	2.884

Calculated critical difference value = 0.942

$\underline{T_1 \quad T_2 \quad T_3} \quad C$

Block means of the different packing densities

B_1	B_2	B_3	B_4	B_5
4.93	4.74	3.63	2.0	2.0

Calculated Critical difference value = 1.053

$\underline{B_1 \quad B_2} \quad B3 \quad \underline{B_4 \quad B_5}$

Underline indicates 'no significant difference.'

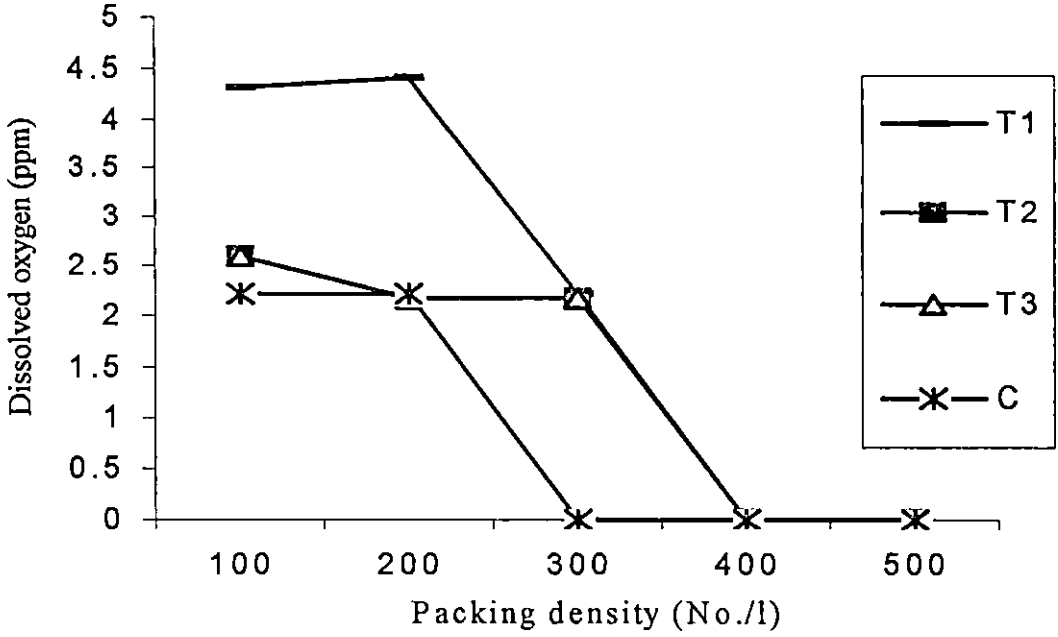

Figure 13. Dissolved oxygen (ppm) in the oxygen - packed jars at three treatments of sodium bicarbonate viz.,0.2 g/l(T_1) 0.4 g/l (T_2) ,0.6 g/l (T_3) mixed with acetic acid in the ratio 2:1, and control at 70% survival of *L.rohita* fry.

4.2.2.Carbon dioxide

Initially, carbon dioxide values of the packing medium without filling oxygen in the jars was 44 ppm, 70.4 ppm and 88 ppm at T_1, T_2 and T_3 respectively and after filling oxygen it was zero. In the control jars it was zero before and after filling oxygen. The carbon dioxide values in these jars at 70% survival are given in Table 10 and Fig. 14.

Results of the analysis of variance of carbon dioxide n the oxygen – packed jars under the three treatments and control at the five packing densities at 70% survival is presented in Table 11. There was significant difference among the treatments and among the packing densities. Pair – wise comparison by critical difference analysis showed that T_1 differed significantly from T_2 and T_3 as well as control. The packing density 100 fry/l differed significantly from the other four packing densities.

Table 10. Carbon dioxide (ppm) in the oxygen – packed jars at three treatments of carbon dioxide anaesthesia using sodium bicarbonate at 0.2 g/l (T1), 0.4 g/l (T2) and 0.6 g/l (T3) mixed with acetic acid (ml/l) in the ratio 2:1, and control at five packing densities at 70% survival of *L. rohita* fry.

Carbon dioxide (ppm)				
Packing density (No./ l)	T_1	T_2	T_3	Control
100	44.0	105.6	61.6	96.8
200	88.0	114.4	96.8	105.6
300	132.0	220.0	132.0	132.0
400	176.0	211.2	202.4	220.0
500	176.0	290.4	299.2	290.4

Table 11. Results of the analysis of variance of carbon dioxide (ppm) in the oxygen – packed jars at three treatments of carbon dioxide anaesthesia using sodium bicarbanate at 0.2 g/l (T_1) , 0.4g/l (T_2) and 0.6 g/l(T_3), mixed with acetic acid (ml/ l) in the ratio 2:1 and control at five packing densities at 70% of survival of *L. rohita* fry.

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F value computed
Treatment	11193.95	3	3731.32	4.97*
Packing density	91983.23	4	22995.81	30.67*
Error	8998.53	12	749.77	
Total	112175.71	19		

*Significant at 5% level.

Treatment means of the three doses of anaesthetic and control

T_1	T_2	T_3	Control (C)
123.2	188.32	158.4	168.96

Calculated critical difference value = 37.74

$\underline{T_2 \quad C \quad T_3} \quad T_1$

Block means of different packing densities.

B_1	B_2	B_3	B_4	B_5
77.0	101.2	154.0	202.4	264.0

Calculated critical difference value = 42.19

$B_5 \quad B_4 \quad B_3 \quad \underline{B_2 \quad B_1}$

Underline indicates 'no significant difference.'

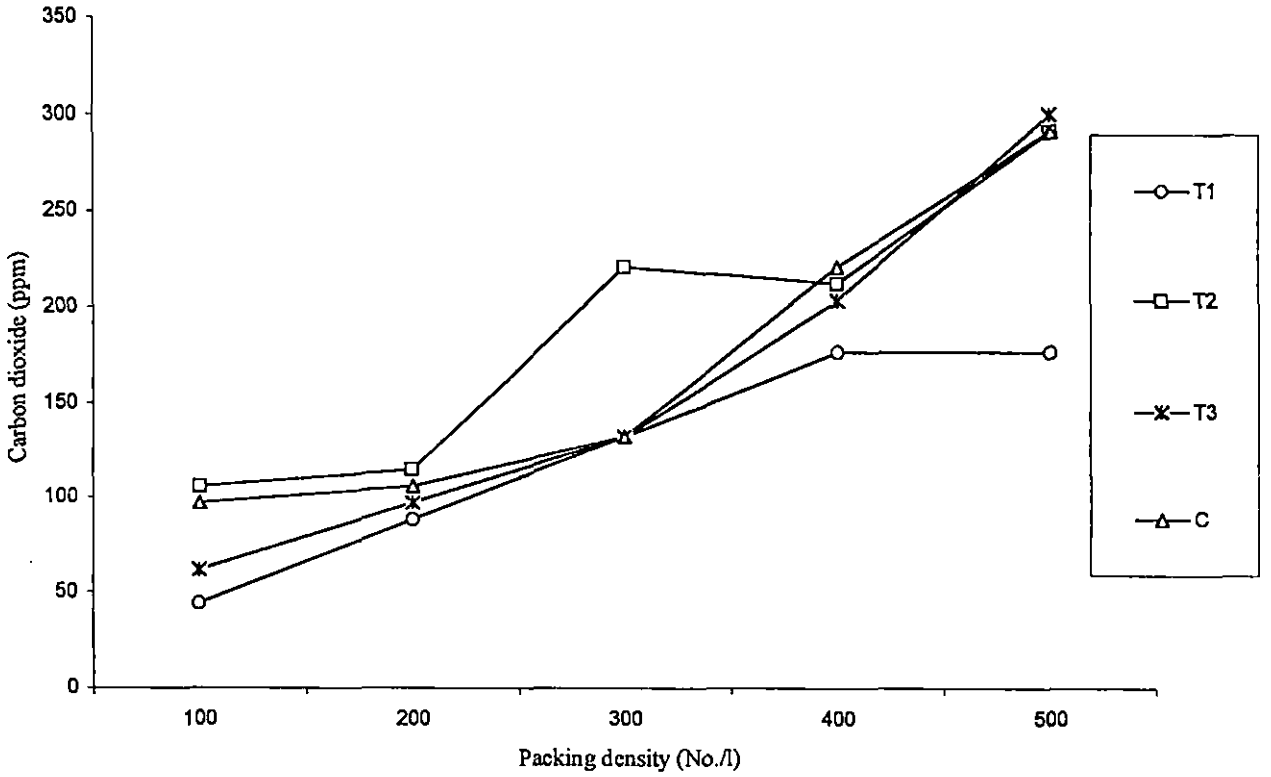

Figure 14. Carbon dioxide (ppm) in the oxygen - packed jars at three treatments of sodium bicarbonate viz., at 0.2g/l (T_1), 0.4g/l (T_2) and 0.6g/l (T_3) mixed with acetic acid (ml/l) in the ratio 2:1,and control at 70% survival of *L.rohita* fry.

4.2.3. Ammonia – N

The ammonia – N concentration at the initial stage in the oxygen – packed jars under the three treatments and control at the five packing densities were found as zero. The values of the ammonia – nitrogen concentration in these jars at 70% survival are presented in Table 12 and Fig. 15.

Analysis of variance of ammonia – nitrogen in the oxygen – packed jars under the three treatments and control at the five packing densities at 70% survival is given in Table 13. There was no significant difference among treatments; but significant difference was observed among the packing densities. Pair – wise comparison by critical difference analysis revealed that the packing density 500 fry/l differed significantly from the other four packing densities.

4.2.4. Total alkalinity

The initial values of the total alkalinity of the packing medium in the oxygen – packed jars under the three treatments and control at the five packing densities were found as zero. The total alkalinity values of the packing medium in these jars at 70% survival are shown in Table 14 and Fig. 16.

The Results of the analysis of variance of total alkalinity in the oxygen – packed jars at three treatments of anaesthetic and control at the five packing densities at 70% survival is shown in Table 15. There was significant difference among the treatments and

also among the packing densities. Pair – wise comparison by the critical difference analysis showed that T_1 significantly differed from T_2 and T_3 as well as control. The packing density 100 fry / l was significantly different from the other packing densities.

4.2.5. pH

Initially, the pH of the packing medium soon after adding the chemicals in the treated jars was 4.0 and that of control jars was 7.0 at different packing densities. The final pH in the oxygen – packed jars under T_1, T_2 and T_3 at 70% survival was 7.5 and that of control was 9.0.

Table 12. Ammonia – N (ppm) in the oxygen – packed jars at three treatments of carbon dioxide anaesthesia using sodium bicarbonate at 0.2g/l (T_1), 0.4 g/l (T_2) and 0.6 g/l (T_3), mixed with acetic acid (ml/l) in the ratio 2:1, and control at five packing densities at 70% survival of *L. rohita* fry.

Ammonia - N (ppm)				
Packing density (No./ l)	T_1	T_2	T_3	Control
100	0.1757	0.2668	0.2797	0.1941
200	0.2107	0.2935	0.3386	0.4766
300	0.4241	0.5621	0.6099	0.7857
400	0.7489	0.6799	0.8868	0.8133
500	0.9191	1.3423	0.9650	0.8868

Table 13. Results of the analysis of variance of ammonia – N (ppm) in the oxygen – packed jars at three treatments of carbon dioxide anaesthesia using sodium bi-carbonate at 0.2 g/l (T_1) , 0.4g/l (T_2) and 0.6 g/l (T_3), mixed with acetic acid (ml/l) in the ratio 2:1 and control at five packing densities at 70% of survival of *L. rohita* fry.

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F value computed
Treatment	0.064	3	0.02	1.24*
Packing density	1.71	4	0.43	24.8*
Error	0.21	12	0.02	
Total	1.98	19		

* Significant at 5% level.

Block means of different packing densities.

B_1	B_2	B_3	B_4	B_5
0.23	0.33	0.59	0.78	1.03

Calculated critical difference value = 0.2021

B_5 B_4 B_3 B_2 B_1

Underline indicates 'no significant difference.'

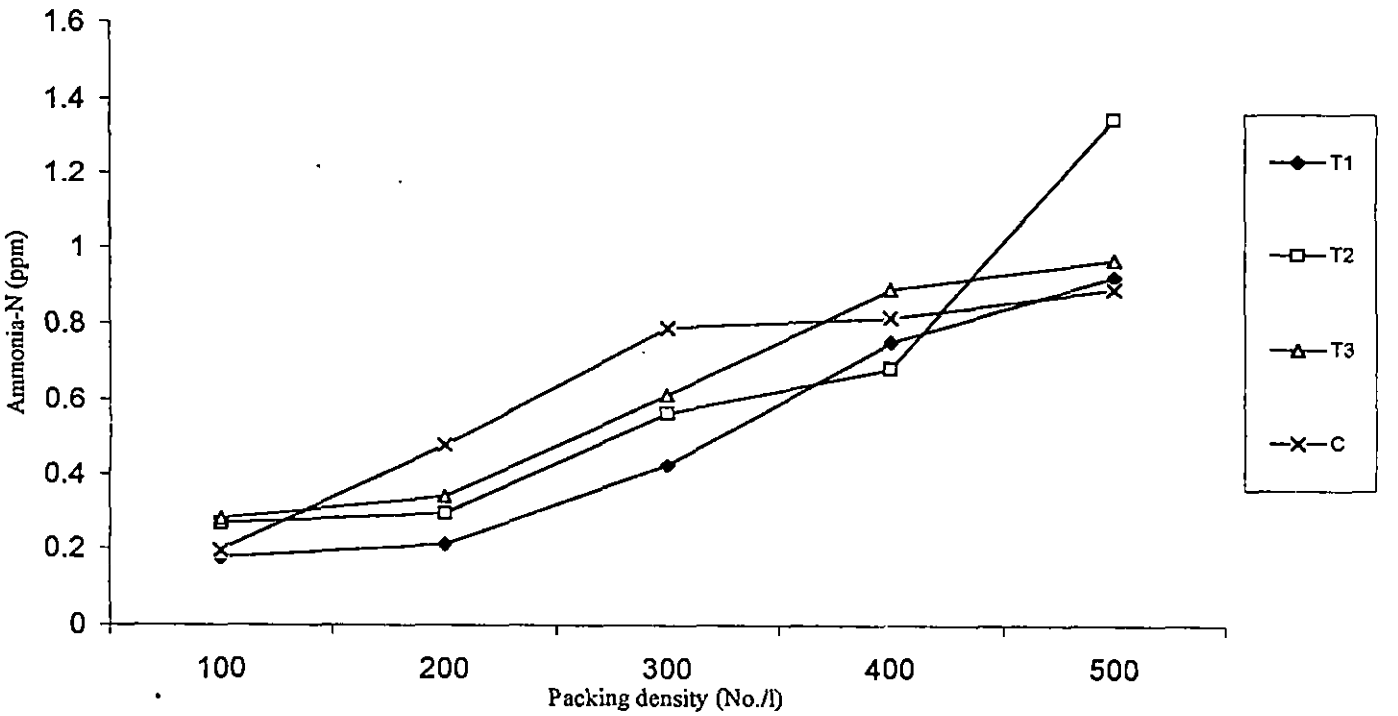

Figure 15. .Ammonia-N (ppm) in the oxygen- packed jars at three treatments of sodium bicarbonate viz., 0.2g/l(T_1), 0.4g/l (T_2), 0.6 g/l (T_3) mixed with acetic acid (ml/l) in the ratio 2:1, and control at 70% survival of *L.rohita* fry

Table 14. Total alkalinity (ppm) in the oxygen – packed jars at three treatments of carbon dioxide anaesthesia using sodium bicarbonate at 0.2g/l (T_1), 0.4 g/l (T_2) and 0.6 g/l (T_3) , mixed with acetic acid (ml/l) in the ratio 2:1, and control at five packing densities at 70% survival of *L. rohita* fry.

Total alkalinity (ppm)				
Packing density (No./ l)	T_1	T_2	T_3	Control
100	3.0	6.5	7.0	6.0
200	5.5	7.0	7.5	7.0
300	6.0	8.5	8.5	8.5
400	7.0	7.5	12.0	11.0
500	7.5	11.0	12.5	12.0

Table 15. Results of the analysis of variance of Total alkalinity (ppm) in the oxygen – packed jars at three treatments of carbon dioxide anaesthesia using sodium bicarbonate at 0.2 g/l (T_1), 0.4g/l (T_2) and 0.6 g/l (T_3), mixed with acetic acid (ml/l) in the ratio 2:1 and control at five packing densities at 70% of survival of *L. rohita* fry.

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F value computed
Treatment	39.44	3	13.14	14.18*
Packing density	66.57	4	16.64	17.95*
Error	11.12	12	0.93	
Total	117.14	19		

* Significant at 5% level.

Treatment means of the three doses of anaesthetic and control.

T_1	T_2	T_3	Control (C)
5.8	8.1	9.5	8.9

Calculated critical difference value- 1.33.

T_3 C T_2 T_1

Block means of different packing densities.

B_1	B_2	B_3	B_4	B_5
5.62	6.75	7.87	9.37	10.75

Calculated critical difference value = 1.48.

B_5 B_4 B_3 B_2 B_1

Underline indicates 'no significant difference.'

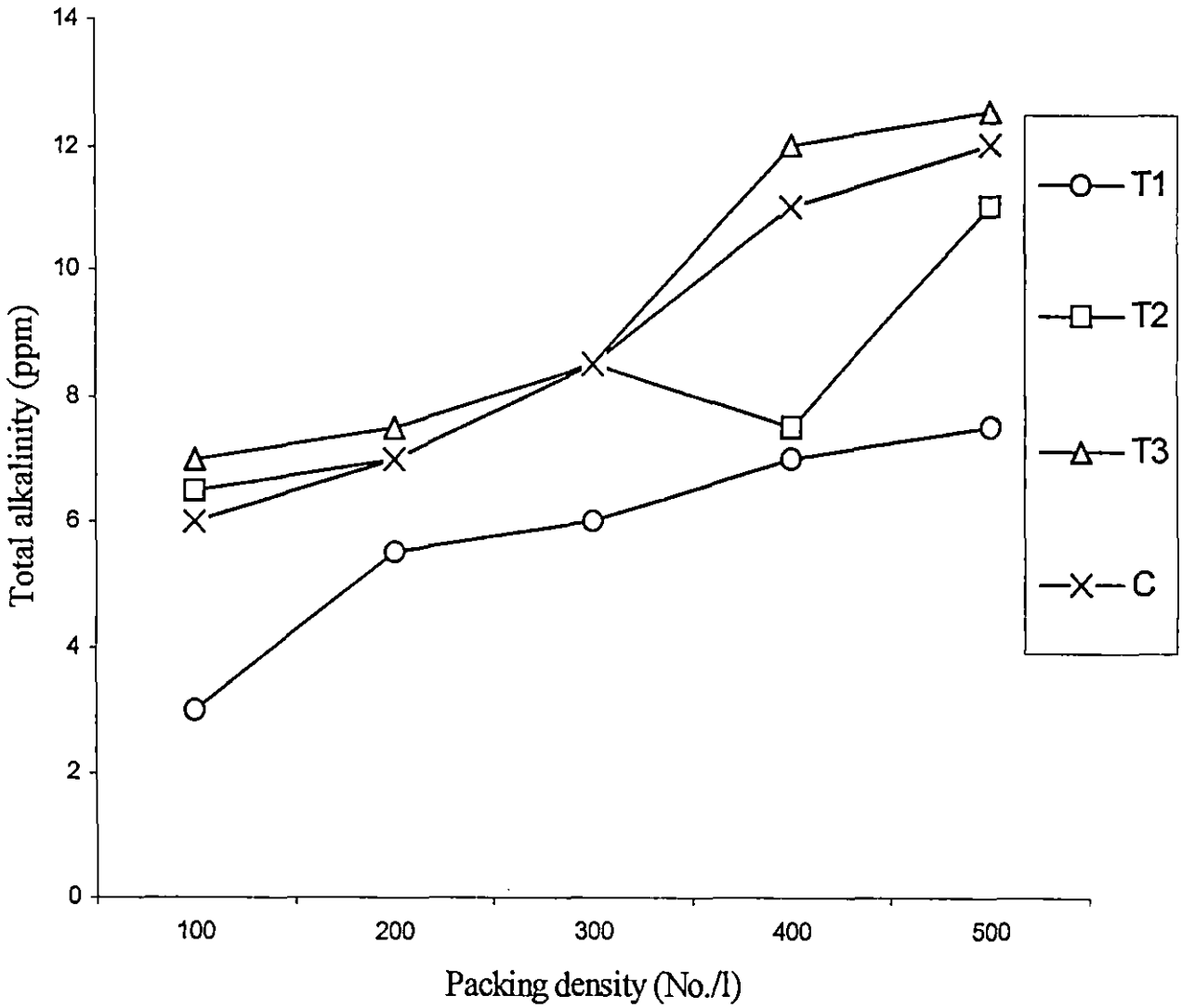

Figure 16. Total alkalinity (ppm) in the oxygen - packed jars at three treatments of sodium bicarbonate viz., 0.2 g/l (T_1), 0.4 g/l (T_2) , 0.6 g/l (T_3) mixed with acetic acid (ml/l) in the ratio 2:1, and control at 70% survival of *L.rohita* fry.

DISCUSSION

5. DISCUSSION

5.1. Effect of carbon dioxide anaesthesia and packing density on *L. rohita* fry

5.1.1. Behavioural pattern

Light sedation and deep sedation are the only stages of anaesthesia suitable for live transportation of fish fry (Durve, 1975). In the present study, the behavioural pattern of *L. rohita* fry subjected to the first treatment – T_1(sodium bicarbonate 0.2g/l and acetic acid 0.1ml/l) - of carbon dioxide anaesthesia is indicative of light sedation . This stage is characterized by slow movement without losing equilibrium and with response to external stimuli (Summerfelt and Smith, 1990). In the case of the second treatment – T_2 (sodium bicarbonate 0.4 g/l and acetic acid 0.2 ml/l) - and the third treatment – T_3 (sodium bicarbonate 0.6 g/l and acetic acid 0.3 ml/l) , the fry showed deep sedation. The characteristics of deep sedation are slow movement without losing equilibrium and with no response to external stimuli (Summerfelt and Smith, 1990). In T_2, the fry were observed to lie on their sides after 2 minutes and in T_3 immediately after adding the chemicals. Effectively there was no loss of equilibrium because the fry returned to normal upright condition within 2 minutes and 4 – 5 minutes in T_2 and T_3 respectively. The behavioural pattern observed in the present study conforms with that reported by Mishra *et al.* (1983). They had used solutions of sodium carbonate and sulphuric acid to anaesthetize rohu fry for transportation purpose.

5.1.2. Induction time and recovery time

The induction time was the longest in the case of T_1 followed by T_2 and T_3. At 100 fry/l, the for T_1 it was 15 minutes, for T_2 it was 5 minutes and for T_3 it was less than 1 minute. The corresponding time at 200 fry/l was 14 minutes, 3 minutes and less than 1 minute respectively . At 300, 400 and 500 fry/l it got reduced to 10 minutes, 2 minutes and less than 1 minute for T_1, T_2 and T_3 respectively. According to Marking and Meyer (1985), the induction time of an ideal anaesthetic should be less than 15 minutes and preferably less than 3 minutes. In all the three treatments of carbon dioxide anaesthesia tried in the present study, the induction time varied from less than 1 minute to 15 minutes, indicative of an ideal anaesthetic.

The recovery time was the shortest for T_1 followed by T_2 and T_3. At 100, 200 and 300 fry/l the recovery time for T_1 could not be clearly differentiated; at 400 and 500 fry/l it was 3 – 4 minutes. For T_2 it was 8 minutes at 100, 200 and 300 fry/l and 10 minutes at 400 and 500 fry/l. The recovery time for T_3 at 100 and 200 fry/l was 12 minutes and that at 300, 400 and 500 fry/l was 15 minutes.

Marking and Meyer (1985) reported that the recovery time for an ideal anaesthetic was 5 minutes or less. However, unlike other anaesthetics, carbon dioxide is reported to leave no residues in the tissues, requires no withdrawal period and is considered as safe (Gilderhus and Marking, 1987) although it takes longer recovery time.

5.1.3. Safe duration and survival

The safe duration (duration of 100% survival) was significantly different among the treatment and among the packing densitises. The longest safe duration of 39.0 h, 15.5 h, 11.5 h for T_1, T_2 and T_3 respectively could be observed at 100 fry/l. The corresponding shortest safe duration was 1 h, 2 h and 3 h at 500 fry/l . In T_2 and T_3, since the safe duration was shorter than that of the control at 100, 200 and 300 fry/l these two treatments could not be practicable at the lower packing densities. At the higher packing densities of 400 and 500 fry/l, T_1 and T_2 showed longer duration of 100% survival than that of the control. This indicates that T_1 and T_2 can be applied for enhancing the safe duration of transport of the rohu fry at these higher packing densities. Similar trend was observed at the duration of 90% survival also. At 70% and 80% survival only T_1 was found to show longer duration than the control at all the packing densities tried . By using this dose the safe duration could be enhanced 1.5 to 6 hold compared to the control depending on the packing densities. T_2 enhanced the duration of transport only at 300 and 400 fry/l. The highest dose of carbon dioxide anaesthesia (T_3) tried in the study is not applicable to enhance the safe duration and also the subsequent duration at 70, 80 and 90% survival.

5.2. Effect of anaesthetization and packing density on water quality in the oxygen-packed jars

Ferreira *et al.*(1984) reported that anaesthetics could slow down deterioration in the water quality over a period of time. This might be due to the reduced stress up on the fish and the consequent decline in metabolic activity of the fish so treated. The dissolved oxygen levels in the oxygen- packed jars at 70% survival under the three treatments and

control were below the normal level of 5 ppm. No significant difference was observed among the treatments and control, but significant difference was observed among the packing densities. At 300fry/l the dissolved oxygen level in the control jars reached to zero levels when there was trace levels in the treatment jars. At 400 and 500 fry/l there was absolutely no dissolved oxygen in any of the jars. The high levels of carbon dioxide that existed in the jars might be the reason for the lack of dissolved oxygen. Carbon dioxide values in the oxygen - packed jars at 70% survival showed significant difference among the treatments and also among the packing densities tried. T_1 was significantly different from the T_2, T_3 and control. Packing density of 100 fry/l was significantly different from the other four packing densities and control and showed the lowest values of carbon dioxide. Ammonia- N levels in the oxygen-packed jars differed significantly among packing densities, but not among treatments. The values of total alkalinity in the oxygen-packed jars at 70% survival differed significantly among the treatments and also among the packing densities. The pH in the treated jars increased from 4.0 to 7.5 and that of the control increased from 7.0 to 9.0. Published information on the effect of carbon dioxide anaesthesia and packing densities on water quality parameters is lacking and hence no comparison could be made in this aspect.

SUMMARY

6. SUMMARY

The main objectives of the study were (1) to evaluate the effect of carbon dioxide anaesthesia on *Labeo rohita* fry, (2) to determine the effect of anaesthetization and different packing densities on their survival rate and duration of transport and (3) to find out the important changes in the water quality parameters in the oxygen – packed containers caused by anaesthetization and different packing densities.

Fry of *L. rohita* of average length 25 mm and weight 750 mg were used for the experiments. Air tight, transparent, hard plastic jars of 600 ml capacity were used for oxygen – packing. Each jar was provided with a screw – type lid with one – way valve, which facilitated uniform initial oxygen pressure in the jars.

Sodium bicarbonate and glacial acetic acid were used for *in situ* production of carbon dioxide in freshwater as the packing medium. Three different concentrations of sodium bicarbonate i.e., 0.2, 0.4 and 0.6g/l of water mixed with acetic acid (ml/l) in the ratio 2:1 (considered as T_1, T_2 and T_3 respectively) were tested for carbon dioxide anaesthesia on rohu fry. Five different packing densities of 100, 200, 300, 400 and 500 fry/l under the three treatments and control (without adding chemicals) were tried at uniform oxygen pressure of 0.2kg/cm^2 and at ambient temperature of 28 ± 2^0c. The experimental jars were shaken periodically to simulate transport conditions. The duration of 100% survival was noted by making hourly observations and the subsequent survival down to 70% was noted at 3 hourly interval. Samples of the packing medium were collected from the jars, initially and at 70% survival of the fry, for water quality analyses. The experiment was designed statistically using Randomized Block Design (RBD)with three repli-

cations for each treatments. Effect of anaesthetization and packing density on the fry and on the water quality parameters viz., dissolved oxygen, free carbon dioxide, ammonia – N, total alkalinity and pH were investigated. The survivors were transferred to aerated freshwater without the chemicals and were observed for 96 hours for subsequent survival.

In T_1, the fry showed slow movement after 10 minutes of adding the chemicals without losing equilibrium and with response to external stimuli, indicative of light sedation. The fry subjected to T_2 lay on their sides at the water surface after 2 minutes of adding the chemicals but returned to normal upright condition within another 2 minutes. The fry remained in the water column with slow movement without response to external stimuli indicating deep sedation. Similar behavioural pattern was observed in T_3 except that the fry lay on their sides at the water surface immediately after adding the chemicals and returned to normal upright condition within 4 – 5 minutes.

The induction time for T_1 at 100 and 200 fry/l was 15 and 14 minutes respectively, while at 300, 400 and 500 fry /l it was 10 minutes. For T_2, it was 5, 3, 2, 2 and 1 minute at 100, 200, 300, 400 and 500 fry/l respectively. In the case of T_3, the induction time was less than 1 minute at all the packing densities.

The recovery time could not be clearly differentiated in T_1 at the lower packing densities of 100, 200 and 300 fry/l, while it was 3 – 4 minutes at the higher packing densities of 400 and 500 fry/l. For T_2, it was 8 minutes at 100, 200 and 300 fry/l and 10

minutes at 400 and 500 fry/l. The recovery time for T_3 was 12 minutes at 100 and 200fry/l and 15 minutes at 300, 400 and 500 fry/l. The recovered fry survived for the observed period of 96 hours.

The safe duration (duration of 100% survival) was significantly different among the treatments and among the packing densities. The longest safe duration of 39.0 h, 15.5h, 11.5h for T_1, T_2 and T_3 respectively could be observed at 100 fry/l. The corresponding shortest safe duration was 1h, 2h and 3h at 500 fry/l. In T_2 and T_3, since the safe duration was shorter than that of the control at 100, 200 and 300 fry/l, these two treatments could not be practicable at the lower packing densities. At the higher packing densities of 400 and 500 fry/l, T_1 and T_2 showed longer duration of 100% survival than that of the control. This indicates that T_1 and T_2 can be applied for enhancing the safe duration of transport of rohu fry at these higher packing densities. Similar trend was observed in the duration of 90% survival also. At 70% and 80% survival, only T_1 was found to show longer duration than the control at all the packing densities tried. By using this treatment, the safe duration could be enhanced 1.5 to 6 fold compared to the control, depending on the packing densities. T_2 enhanced the duration of transport only at 300 and 400 fry/l. The highest dose of carbon dioxide anaesthesia (T_3) tried in the study could not enhance the safe duration and also the subsequent duration at 70, 80 and 90% survival, and hence can not be recommended.

Linear relation between the packing densities and the duration of transport at 100% and 70% survival could be established for T_1, T_2 and control (C) as follows:

100% survival

T_1 $Y = 39.9 - 8.3\ x$

T_2 $Y = 15.9 - 2.9\ x$

C $Y = 26.2 - 5.8\ x$

70% survival

T_1 $Y = 42.6 - 7.8\ x$

T_2 $Y = 21.05 - 2.85\ x$

C $Y = 29.0 - 5.1\ x$

Where, Y = Duration, x = Packing density .

Initial dissolved oxygen concentration was 30 ppm in the oxygen – packed jars under the three treatments and control. At 70% survival the dissolved oxygen levels in all the oxygen – packed jars was below the normal level. No significant difference was observed in the dissolved oxygen concentration among the treatments and control, but significant difference was observed among the packing densities. At 300 fry/l, the dissolved oxygen in the control jars reached zero levels, when it was trace levels in the treatment jars. At 400 and 500 fry/l there was absolutely no dissolved oxygen in any of the jars. Initially, carbon dioxide values of the packing medium without filling oxygen in the jars was 44 ppm ,70.4 ppm and 88 ppm at T_1, T_2 and T_3 respectively and after filling oxygen it was zero. Carbon dioxide values in the oxygen – packed jars at 70% survival showed significant difference among the treatments and also among the packing densities. T_1 was significantly different from the T_2, T_3 and control. Packing density of 100 fry/l was significantly different from the other four packing densities and control; and it showed lowest values of carbon dioxide. The initial ammonia – N level in the oxygen –

packed jars was zero. The final levels differed significantly among packing densities, but not among treatments. The values of total alkalinity in the oxygen – packed jars was initially zero and at 70% survival the values differed significantly among the treatments and also among the packing densities. The pH in the treated jars increased from 4.0 to 7.5 and that of the control increased from 7.0 to 9.0.

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ABSTRACT

EFFECT OF CARBON DIOXIDE ANAESTHESIA ON *LABEO ROHITA* (HAMILTON) FRY DURING TRANSPORTATION.

By

LEESHA O.S., *B.F.Sc.*

ABSTRACT OF A THESIS

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Faculty of Fisheries
Kerala Agricultural University

DEPARTMENT OF AQUACULTURE,
COLLEGE OF FISHERIES,
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ABSTRACT

The present work was undertaken to study the effect of carbon dioxide anaesthesia and packing density on *Labeo rohita* fry in oxygen – packed transportation. Hatchery produced rohu fry of 25 mm and 750 mg average length and weight were used for the study. Sodium bicarbonate at three different concentrations viz., 0.2g /l, 0.4g /l and 0.6g /l mixed with glacial acetic acid (ml/l) in the ratio 2:1 in freshwater as the packing medium was tried for *in situ* production of carbon dioxide anaesthesia. The fry were packed at five packing densities viz., 100, 200, 300, 400 and 500 fry/l under uniform initial oxygen pressure of 0.2 Kg/cm^2. The behavioral pattern of the fry, induction time, recovery time, safe duration of 100% survival and the subsequent duration down to 70% survival were studied. The surviors were transferred to aerated freshwater without the chemicals and were observed for 96 hours for subsequent survival.

Light sedation of the fry characterized by slow movement without losing equilibrium and with response to external stimuli was observed in the first dose of carbon dioxide anaesthesia with an induction time of 10 – 15 minutes. The recovery time was not clear in the lower packing densities of 100, 200 and 300 fry/l, while it was 3 – 4 minutes at the higher packing densities of 400 and 500 fry/l. The safe duration was maximum under this dose i.e., 39.0 h, 17.0 h, 10.0 h, 6.0 h and 3.0 h at 100, 200, 300, 400 and 500 fry/l respectively. This was significantly different from the other two treatments as well as control. By using this dose the safe duration could be enhanced 1.5 to 6 fold compared to the control depending on the packing densitity. Similar trend was noticed at 90, 80 and 70% survival. The second and third doses resulted in deep sedation characterized by slow movement without losing equilibrium and with no response to

external stimuli. In the former dose the safe duration was more than that of the control only at the higher packing densities of 400 and 500 fry/l. In the latter case it was shorter than that of the control and hence not at all practicable at any of the packing densities tried. The recovered fry survived for the observed period of 96 hours. Linear relation could be established between the packing densities and duration of transport for the first and second dose as well as the control.

The water quality parameters of the oxygen – packed jars viz., dissolved oxygen, free carbon dioxide, ammonia – N, total alkalinity and pH were analyzed initially, before packing the fry, and finally at 70% survival of the fry. The final water quality in the jars treated with the first dose was the least stressful for the fry, compared to the other doses and control.