ANAESTHETIZATION OF *LIZA PARSIA* (HAMILTON) FRY FOR OXYGEN - PACKED TRANSPORTATION

By

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THESIS

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Dedicated to my

DADDY AND MUMMY

DECLARATION

I hereby declare that this thesis entitled " ANAESTHETIZATION OF *LIZA PARSIA* (HAMILTON) FRY FOR OXYGEN - PACKED 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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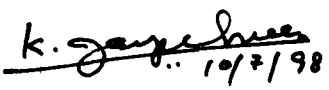

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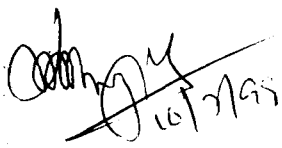

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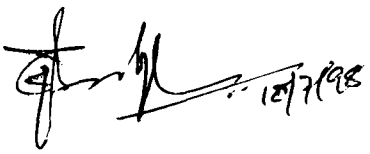

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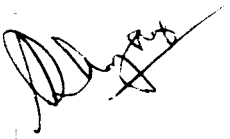

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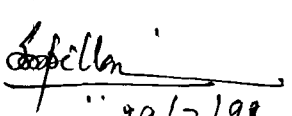

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INTRODUCTION

1. INTRODUCTION

With the advancement of aquaculture techniques, transportation of live fish is gaining importance Transportation of live fish fry from the wild or hatchery to the grow outs or markets is of great economic importance. The main objective of this function is to transport as many fish fry as possible with minimal loss and at economic costs.

Transport of live aquatic organisms perhaps, started in the 1870's (Norris *et al.*, 1960). During the past, several methods were evolved for the transportation of aquatic animals primarily based on empirical knowledge and later on scientific lines. Although many of these methods and devices have proved to be handy and economical, they are not free from the risk of incurring mortality.

Although closed systems were in use (Vaas, 1952), the plastic bag transport method was first introduced by Miller (1956) for carrying and shipping live fish. The primary problems initially encountered in such closed systems were, the low oxygen capacity of the water and accumulation of metabolic end products such as ammonia and carbon dioxide. Other problems included the handling stress and hyperactivity of the fish. Low oxygen capacity has been solved mainly by the use of pure oxygen. However, not only must oxygen levels be adequate, but carbon dioxide and metabolic waste levels must be low enough to allow sufficient oxygen uptake (Mc Farland and Norris, 1958). The first worker to recognise the potential use of anaesthetics for transporting fishes was Aitken (1936). Addition of anaesthetics to the packaging water decreases the metabolic rates of the fish the consumption of

oxygen and the excretion of waste materials (Guo *et al.*, 1995 a.) Anaesthetics have also been reported effective in improving survival rates of some species.

In any aquaculture operation one of the foremost requirements is the availability of seed for stocking as and when required by the farmers. But, the abundance and distribution of seed in the wild show fluctuation from season to season and from year to year. In some cases the sites of seed availability whether wild or hatchery source may not be very near to the farms. Depending on the site of seed availability, long or short distance transportation of seed is required. Transportation of large quantity of seed involving long transit entails severe complex problems. It is in this context that concerted effort is made through this work to investigate the vital problems so as to ensure high survival rate and to minimise economic loss.

Mullets form an important group of fishes in coastal aquaculture. One of the crucial problems in their culture is the high rate of mortality during or after transportation from fry collection centres to farming sites. The major reasons for their mortality are hyperactivity due to claustrophobia, increase in excretion of metabolic products and the resultant exhaustion and injury, and deterioration of water quality in transporting packs. The ideal condition is to carry in closed handy packs as many fish, in as little water as possible, with minimum mortality. Both hyperactivity and metabolic activity could be depressed by the action of anaesthetics which would in turn facilitate the safe and efficient transport of these fishes.

Despite several studies on the use of anaesthetics in the transport of live fish, uncertainty of the behaviour of mullets treated with anaesthetics still exists. Hence, the present work was planned to evaluate the effect of three selected anaesthetics viz chloral hydrate, tertiary butyl alcohol and clove oil on the metabolic rate of mullet fry and also to study the effect of packing density and anaesthetisation on survival during oxygen packed transportation.

Main objectives of the study:-

1. To evaluate the effect of three selected anaesthetics i.e., chloral hydrate, tertiary butyl alcohol and clove oil at optimum doses on the metabolic rate of the mullet *Liza parsia* fry.
2. To determine the effect of different packing densities and anaesthetisation of the mullet fry on survival rate and duration of transport.
3. To find out the important changes in the water quality parameters of the oxygen-packed containers caused by different packing densities and anaesthetisation.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Anaesthetisation and its application in fisheries

Anaesthetisation is the process leading to anaesthesia which is loss of sensation over all or part of the body resulting from pharmacological depression of nerve function. (Williams and Wilkins Company, 1982). Anaesthesia is assumed to be a reversible condition. Anaesthetics are chemical or physical (electricshock, cold) agents that with increasing exposure or concentration, first calm (sedate) an animal, then cause it successively to lose mobility, equilibrium, consciousness and finally reflex action (Summerfelt and Smith, 1990).

Fish anaesthesia and the nature of various anaesthetics have been reviewed many times. Mc Farland (1959), Lamb (1963), Bell (1964), Klontz (1964), Westhumes and Fritsh (1965), Smith and Bell (1967), Klontz and Smith (1968), Mc Farland and Klontz (1969), Randall and Hoar (1971), Jolly *et al.* (1972), Johansson (1978), Stuart (1981). Ross and Ross (1984), Summerfelt and Smith (1990), Iwama and Ackerman (1994) updated the information in various degrees of thoroughness to the date of their respective publications.

Anaesthetisation has many applications in fisheries, primarily to immobilise fishes so that they can be handled faster and less stressfully. Among its principal uses, anaesthesia facilitates operations such as to weigh and measure fishes, to mark and tag them, to study their physiology and behaviour, to perform surgery on them, to collect them in tidepools and in scuba, to photograph them, to prepare them for live shipment and to transport them, to manually spawn them, to inject them with

vaccines and antibiotics and to collect blood and other tissues from them. (Summerfelt and Smith, 1990). In most cases anaesthetics are used in order to sedate or immobilise them, such that any of the procedures can be carried out at a lower stress level than if the fishes were fully conscious and mobile.

2.2 Classification of anaesthesia

According to Summerfelt and Smith (1990) anaesthesia may 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 may involve loss of swimming ability with or without loss of consciousness. Loss of consciousness for a fish is a stage of anaesthesia synonymous with absence of response to stimuli, which usually means loss of some specific reflex action as well. The neurological effects of general anaesthesia are successive depression of sensory centres in the brain cortex, the cerebellum, the spinal chord and finally reflex actions are blocked and the animal will not respond to a skin prick (Bell, 1964). Physiological impairment develops in proportion to the length of exposure to an anaesthetic.

Local anaesthesia occurs when the loss of sensation is limited within a restricted segment of the body by action on sensory nerve endings; the animal remaining conscious. Local anaesthetics such as benzocaine or lidocaine may be injected or applied topically to block nerve conduction from peripheral nerve endings. Activity of local anaesthetic agents in gold fish was studied by Feldman *et*

al. (1975) Benzocaine used as a local anaesthetic in veterinary practice, is used fairly widely as a general anaesthetic on fish (Marking and Meyer, 1985). A local anaesthetic may be administered to produce a temporary block of the olfactory nerve or to immobilise a fin for study of swimming performance.

Regional anaesthesia is accomplished by blocking the sensory innervation to an area with an anaesthetic. Local anaesthetic may be used to block nerve transmission to structures distal to the area of injection (Horrobin, 1968). For example, a local anaesthetic such as hidocaine hydrochloride injected near the spinal cord at midbody can sufficiently infiltrate the nerves of the spinal cord to block innervation of caudal trunk musculature without the loss of consciousness.

Sedation and tranquilisation imply calming or quieting by use of a drug (G.C Merriam Company, 1973 , Williams and Wilkins Company, 1982; Warren, 1983). Pharmacologically, however, there are important differences between the sedative effects of early stages of anaesthesia and the behaviour produced by a tranquilizer (Dorland, 1981; Hampel and Hawley, 1982; Warren, 1983). Tranquilizer drugs do not produce unconsciousness or anaesthesia (Warren, 1983).

2.3. Stages of anaesthesia and related changes

Mc Farland (1959,1960) recognized a sequence of physiological changes that indicated the depth of anaesthesia. A progression of general anaesthesia involves changes in reaction to visual and vibrational stimuli, equilibrium and muscle tone and respiratory rate. Mc Farland (1959) used these changes to describe four stages of anaesthesia and also a stage o for the normal condition, and he designated two

"planes" of anaesthesia for stages I and II. Schoettger and Julin (1967) slightly modified the different stages. According to Schoettger *et al.* (1967) distinction between the stages of anaesthesia varies by species. Mc Farland and Klontz (1969) discarded the planes. A simpler scheme of six stages has been followed by Jolly *et al.* (1972).

Iwama *et al.* (1989) put forward the main stages of anaesthesia and recovery which according to them may suffice for the recognition of the main stages in that transition. The administration of most anaesthetics to stage III will have the Common effects of rendering the animal immobile, and causing the cessation of breathing. This will result in a reduction in gas transfer and a consequent reduction in blood oxygen tension and a concomitant rise in blood CO_2 tension. This has been shown to cause an increase in blood concentration of adrenaline, and a respiratory acidosis in rainbow trout anaesthetized with buffered MS_{222}, 2-Phenoxyethanol, benzocaine, metomidate and CO_2 (Iwama *et al.*, 1989) Unless the gills are artificially irrigated during stage III anaesthesia, death will result. (Graham and Iwama, 1990).

Stages of anaesthesia by Summerfelt and Smith (1990) were modified from Mc Farland (1959) and Jolly *et al.* (1972). Anaesthesia basically begins with a slight loss of reactivity and locomotor activity (Stage 1, light sedation) and progresses to almost complete loss of these functions (stage 2, deep sedation). As anaesthesia further affects the spinal nerves, muscles relax, lose their tone, and cannot be voluntarily controlled (stages 3 and 4, partial and total loss of equilibrium), finally all reflex activity disappears (stage 5) The medullary centre of respiration is affected in

parallel. The respiration rate at first decreases slightly (stage 1-2) due to reduction in physical activity, but it rises as the fish loses equilibrium (stage 3). As anaesthesia deepens (stage 4-5) ventilation becomes very slow, shallow and irregular, and severe hypoxia may develop if the fish initially has a respiratory debt or if deep anaesthesia is prolonged. At the extreme (stage 6), the respiratory centre in the medulla ceases neurostimulation, gill ventilation stops and the fish cannot meet a rapidly accumulating debt unless it is quickly removed to fresh water. Thus, according to Summerfelt and Smith (1990) general anaesthesia causes a progressive depression of central and peripheral nervous system activity.

Although several descriptions of various levels of anaesthesia in fish exist, they differ only in the resolution of the transition stages from the fully conscious and alert state to one of total loss of mobility and reflex functions (Iwama and Ackermann, 1994).

2.4 Quality criteria of anaesthetics

The characteristics of ideal anaesthetics have been listed by Marking and Meyer (1985) and Bell (1987).

An effective anaesthetic should have an induction time of less than 15 minutes preferably less than 3 min, a recovery time after 5 min or less. It should be nontoxic to fish or to humans and should have no lasting physiological or behavioural effects. Furthermore, it should have properties that allow complete clearance from the body tissues after exposure and be biodegradable. It should be inexpensive and engender no cumulative effects or problems from repeated

exposure. A high solubility in freshwater as well as in saltwater and high stability under normal laboratory conditions (light, heat etc.) are also desirable. It is also held beneficial if the anaesthetic does not cause foaming because this can reduce visibility and gas exchange between water and air.

2.5. Procedures for anaesthetisation

2.5.1 Exposure

Fish may be injected with an anaesthetic, but usually they are subjected to general anaesthesia in a dip or bath treatment with static or flowing water. Dip and bath treatments differ only in duration (Herwig, 1979). Apart from the usual dip and bath treatments, spraying also helps anaesthetisation. Kidd and Banks (1990) anaesthetized lake trout with MS_{222} from a spray bottle.

2.5.2 Safety margin

The effective dose (ED 50) is that dose which elicits response in 50% of the animals exposed to it (Drill, 1954). The effective concentration (EC 50) of an anaesthetic is that which produces total loss of equilibrium in 50% of the fish in a specified time (Schoettger and Julin, 1967). The safety margin for an anaesthetic is the difference between the concentration needed for effectiveness and that which is toxic to fish. Toxicity of a chemical is expressed either as lethal concentration-50 (LC 50) or as lethal dose-50 (LD 50). LC 50 is the concentration that results in death of one-half the exposed population within a specified time while LD 50 is the amount administered by injection or feeding that results in death of one-half the treated population within a specified time (Summerfelt and Smith, 1990).

2.5.3. Exposure time

Exposure time is the time for which the fish could be held out of water for working on (Carrasco *et al.*, 1984). Mattson and Riple (1989) defined safe exposure index as the time in minutes for the first fish to reach stage V divided by three. Effective exposure time is the product of anaesthetic concentration and induction time, while exposure time refers to the total time during which the fish is in contact with the anaesthetic solution or the time elapsed between induction of a particular level of anaesthesia and removal from the anaesthetic solution (Summerfelt and Smith, 1990).

2.5.4. Induction and Recovery time

Carrasco *et al.* (1984) described recovery time as the time needed for each individual to recover equilibrium once it is placed in untreated freshwater. 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 anaesthetic solution (Summerfell and Smith 1990).

2.5.5. Efficacy

Efficacy is an anaesthetic's ability to make fish handleable with an induction time of 3 minutes or less, to allow the fish to recover in 10 minutes or less and to cause no mortality after an exposure of 15 minutes (Gildherhus and Marking 1987).

2.5.6. Interactions of anaesthetics

Schoettger and Stencke (1972) proposed a synergistic combination of tricaine and quinaldine or quinaldine sulphonate and found that the concentration of each required for effective anaesthesia was substantially less than that required when either of the compounds was used alone. Elmore (1981) described that the effect of local or regional drugs could be doubled if they were accompanied by 0.5-1.0 ml of a 1 100 solution of epinephrine or by a product containing epinephrine. When an anaesthetic and two or more other drugs are used simultaneously, there is opportunity for chemical interaction; one drug may potentiate a second one nullify its action. Hence, an interaction can be exploited to enhance anaesthesia (Summerfelt and Smith 1990).

2.6. Anaesthetics used for fish transportation.

Inspite of restrictions for use on food fish, many compounds have been and continue to be used as anaesthetics. The major fish anaesthetics like MS-$_{222}$, Carbon dioxide, Quinaldine, 2 phenoxyethanol, Quinaldine sulphate, Benzocaine and Metomidate and some of the rarely used anaesthetics like barbiturates, chloretone, ether, urethane, tertiary amyl alcohol, chloral hydrate, tertiary butyl alcohol and clove oil are included in this review.

2.6.1 Major anaesthetics

2.6.1.1 Tricaine methane sulphonate

Common and proprietary names include tricaine methane sulphonate, MS-$_{222}$, Finquel, and Metacaine (Bove, 1962). Chemical names for tricaine are methane sulphonate salt of ethyl meta-amino benzoate (Schoettger and Steucke, 1972), 3-amino benzoic acid ethyl ester methane sulphonate, ethyl m - amino benzoate methane sulphonate (Merck and Company, 1983). Crystals of MS-$_{222}$ are readily soluble in water 1g/0.8 ml also given as 1:9 (Merck and Company, 1983). MS-$_{222}$ is a lipid soluble drug that moves across the gill by diffusion or by coupling to specific transport systems (Hun and Allen, 1974; Ohr, 1976). Research by Ohr (1976) and Smit *et al.* (1977) drew attention to the acidic nature of tricaine and the effect it has on the pH of weakly buffered fresh water. Tricaine solutions are unstable in sunlight, colour changes to yellow or brown. Although Bove (1962) stated that this did not affect activity in any significant way, he noted that a 10 day old solution with a brownish colour had an activity decrease of about 5%. Stock solutions may be stored frozen, and the crystals are stable when kept cool and dry (Bell, 1967).

Foster (1941) used MS-$_{222}$ to mark trout, while Nelson (1953) used the anaesthetic to reduce stress caused due to various procedures like transport and spawning. Gisler and Backiel (1960) noted that the mortality of Chinook salmon fingerlings was considerably reduced by anaesthetizing them with MS- $_{222}$. Bove (1962) gave the most effective tricaine concentrations as 500-300 mg/l, the

concentration most generally used to immobilise fish as 100mg/l and the effective concentration for producing anaesthesia within 3-4 minutes in 99% of the fish as 40 mg/l Schoettger and Julin (1967) gave concentrations for MS- $_{222}$ that varied with species, induction time and temperature. Reflex action was lost in four species of trout and char in 3 minutes at a concentration of 100 mg/l, a 50-60 mg/l concentration produced anaesthesia that was attained more slowly but could be maintained for approximately 30 minutes. Rainbow trout fingerlings were immobilized by a short exposure to 50 mg MS-$_{222}$/l (Barton and Peter, 1982). Piper *et al.*(1982) noted a concentration of 24.6 mg/l for transport of Salmonids in deep sedation but, warned that anaesthetized salmon had both a high oxygen consumption and a long recovery time. Bell and Blackburn (1984) anaesthetized adult Salmonids at a concentration ranging from 75-100 mg/l. But, for smolts 75 mg/l was found to be optimal. A concentration higher than 75 mg/l was found to be lethal for smolts. The effects of MS-$_{222}$ anaesthesia on salmon behaviour during migration was discussed by Taylor (1988). Quinn *et al.* (1988) reported that anaesthesia of adult Chinook salmon with 100 mg/l of MS-$_{222}$ did not affect their homing response.

Webb (1958) used MS-$_{222}$ as an aid to increase the loads of blue gills carried. But, he stated that though some extremely heavy loads were hauled, the most successful tests could not be duplicated and at times the control fish seemed to haul as well as or better than the drugged ones. Marking (1967) reported a MS-$_{222}$ LC 50 range of 61-39mg/l for bluegill.

Sreenivasan(1962) found MS-222 at a concentration of 40ppm quite effective and harmless in transporting 4 breeders of mirror carp together weighing 10-12-lb in each tin carrier for a period of 14 h. The anesthetic potency of MS_{222} and neutralized MS_{222} was investigated in Cyprinus carpio. Neutralized MS_{222} induced anaesthesia, reduced induction times but increased recovery times. Neutralized MS_{222} resulted in a deeper, more consistent anaesthesia indicating a safer more effective, longer acting anaesthetic. Adult carp anaesthetized with MS_{222} had a faster recovery time compared to eugenol and thiopental sodium (Hikasa et al., 1986), Young Common carp anaesthetized with MS-222 at a low dose of 250-350 ppm induced sedation which was suitable for transport (Jain, 1987). At the following doses the oxygen consumption was reduced by 28.2 and 60.2%/h/g body weight respectively. MS-222 is one of the most popular drug for tranquilising Common, Chinese and Indian major carps. For tranquilising large brood fish the fish are kept in 1:20000 dilution of MS-222 to water for 15-20 minutes. When fishes are fully tranquilised the same solution is diluted 2, $2^1/_2$ and 5 times for species like Common and big head carp, grass carp and silver carp respectively. No dilution at secondary stage for Indian major carps are known. (Peer Mohamed and Devaraj, 1997).

Collins and Hulse (1964) and Piper et al. (1982) reported that 1% salt solution containing 264 mg tricaine/1 reduced shipping mortality of thread fin shad.

A regional but very thorough study of the application of anacsthetics to the fry of the various species of mullets under various climatic conditions and salinities was done by Durve and Dharma Raja (1966) and Durve (1970; 1975). Results

indicate that out of thirteen anaesthetics tried, $MS-_{222}$ was one of the seven reported to be suitable for transportation of live fish. Dick (1975) found that concentrations between 1:75000 and 1:100000 were sufficient to tranquilise fish (*Mugil chelo*) for pretransport handling. An anaesthetic concentration of 1:100000 with a fish biomass of 50 g/l demonstrated that tranquilised fish could safely be held at this density for 48h Sylvester (1975) studied the responses of juvenile striped mullet (*Mugil cephalus*) to the anaesthetic $MS-_{222}$. He reported that induction time to total loss of equilibrium decreased with increasing anaesthetic concentrations over the range of 40-120 ppm. Temperature, pH and oxygen concentrations all significantly affected induction time to total loss of equilibrium in fresh water adapted fish at a concentration of 80 ppm $MS-_{222}$. The light and deep sedation stages of anaesthesia suitable for transportation of post larvae of *Mugil trichodon* were obtained with dosages of 0.02 and 0.03 g/l for $MS-_{222}$ (Alvarez - Lajonchere and Moreno, 1982).

Schoettger (1967) reported that 5-15 cm long Channel cat fish reached full anaesthesia in 2 minutes at concentrations of 100-120 mg/l, but 140-270 mg/l was needed for fish, 18-30cm long. Subadult channel cat fish can be safely exposed to 100 mg $MS-_{222}$/l for 20 minutes (Plumb *et al.*, 1983). Channel cat fish require 25-50 mg/l of $MS-_{222}$ for sedation and 100-250 mg/l for full anaesthesia with 3 minutes induction time; dosage depends on their size and the water temperature (Summerfelt and Smith 1990).

Murai and Catacutan (1981) determined the concentration of MS-$_{222}$ for use in handling of Milkfish fingerlings. They found that the optimum concentration ranged between 100 and 200ppm.

Striped bass (*Morone saxatilis*) weighing about 145 g lost equilibrium in 2.4 -1 8 minutes when exposed to 120-140 mg MS-$_{222}$/l at 20^{0}C in water with 2% sodium chloride; their reflex action was lost in 8.1 - 5.4 minutes (Klar, 1986). Chapman *et al.*(1988) used the anaesthetic MS-$_{222}$ to separate normally developed larvae of Striped bass from larvae with uninflated gas bladders. They noted that the procedure was most successful between 23 and 40d post hatch. Harrell (1992) reported MS-$_{222}$ to be least effective in mitigation of stress of striped bass associated with capture and transport.Henderson *et al.*(1992) used MS-$_{222}$ in the range of 110-123 mg/l to separate striped bass (19-71mm total length) with uninflated gas bladders from normal fish. Fish with inflated gas bladders floated, whereas fish with uninflated gas bladders remained on the bottom. Lemm (1993) reported 150 mg/l of MS-$_{222}$ as the lowest concentration for immobilization of striped bass at 13^{0}C, 18^{0}C and 23^{0}C. Apart from improving production by eliminating striped bass with uninflated gas bladders, MS-$_{222}$ also help to reduce the mortality of the fish hauled from the hatcheries (Mazik and Simco, 1994).

Chatain and Corrao (1992) used MS-$_{222}$ to separate larvae of sea bream with uninflated swim bladders from normal ones. Molinero and Gonzalez (1995) reported that anaesthetisation of sea bream with MS-$_{222}$ produced a stress response greater than that when not anaesthetized.

MS-$_{222}$ at 30ppm was effective in suppressing the oxygen consumption of platy fish. The anaesthetic was able to reduce the excretion of ammonia but not of carbondioxide (Guo *et al.* 1995 a,b).

2.6.1.2. Carbon dioxide

Other names for carbon dioxide are CO_2, carbonic acid, carbonic acid gas and carbonic anhydride. When sodium bicarbonate is the source of CO_2, the resulting anaesthesia is called sodium bicarbonate anaesthesia. Carbon dioxide is a colourless, odourless, noncombustible gas at ordinary temperatures; it is a solid at about -35^{0}C. It is commercially available as a gas over a liquid in steel cylinders under pressure or in solid form as dry ice (-78.5^{0}C), but most commonly in the half bound form as baking soda (Sodium bicarbonate) (Summerfelt and Smith, 1990). Post (1979) recommended mixing a 6.75% (Weight per volume w/v) sodium bicarbonate solution and a 3.95% (w/v) sulphuric acid solution to obtain the desired concentration of carbonic acid. He along with Bell (1987) described CO_2 anaesthesia as safe, effective, soluble in water, inexpensive and nontoxic, but, the user survey by Marking and Meyer (1985) suggested that it was only partly effective, slow acting, stressful and lethal after repeated exposures. Fish hyperactivity was found to be common with CO_2 anaesthesia (Bell, 1987 and MacKinlay *et al.*, 1994).

Carbon dioxide anaesthesia is an old method, first described by Fish (1943), who produced CO_2 concentrations of 150-650 mg/l with sodium bicarbonate and sulphuric acid, and he noted that 200 mg/l was optimum for anaesthetizing fingerling and adult salmon. Booke *et al.* (1978) described a field anaesthetic technique that

used sodium bicarbonate alone and determined that a solution 642 mg/l of sodium bicarbonate at a pH of 6.5 was the most effective medium for Rainbow and Brook trout to cease swimming and to slow respiration within 5 minutes. Prince *et al.*(1995) described a technique for using sodium bicarbonate activated by glacial acetic acid to anaesthetize adult salmon to stage 4 anaesthesia. They determined that 40g of sodium bicarbonate and 15ml of glacial acetic acid in 30l of Fraser River water yielded carbon dioxide concentrations that ranged from 385-195 mg/l.

A solution of sodium bicarbonate at a concentration of 642 mg/l was most effective to cease swimming and slow respiration of Common carp in 5 minutes (Booke *et al.*, 1978). CO_2 was found to be a suitable sedative for Common carp and its behavioural and physiological effects compared favourably with the anaesthetics $MS\text{-}_{222}$ and quinaldine (Mitsuda *et al.* 1980). Mishra *et al.* (1983) using Post's (1979) soda-acid technique, found that 500 mg/l was an optimum concentration of carbonic acid that allowed Rohu (*Labeo rohita*) transportation for 251 h with only 5% mortality. Dupree and Huner (1984) listed carbonic acid as one of the anaesthetics suitable for use in fish culture to sedate and immobilise fish. Kumar *et al.* (1986) reported carbonic acid as an efficient anaesthetic for Indian major carps but observed that at lower concentrations fish were anaesthetized slowly and less deeply whereas at higher concentrations the effect was rapid with greater sedation. The changes in the depth of anaesthesia of carp anaesthetized with a constant level of - CO_2 was described in detail by Yoshikawa *et al.* (1988). The different levels of anaesthesia according to them are level one- partial loss of equilibrium, normal swimming motion; level two - total loss of equilibrium, normal swimming motion;

level three-partial loss of swimming motion, level four-total loss of swimming motion, weak opercular motion and level five -no opercular motion. Zhao and Chen (1994) reported 500 ppm carbonic acid as the optimum concentration for transport of grass carp (*Ctenopharyngodon idella*) fry and the safety survival time as 201 h.

2.6.1.3. Quinaldine

The chemical name for quinaldine is 2-methylquinoline. Quinaldine is a light yellow (Marking, 1969) or colourless (Merck and Company, 1968;1983) liquid but darkens to reddish brown after exposure to air and should be protected from light (Merck and Company, 1983). Its low water solubility may prevent overdoses (Bell, 1967) but its high solubility in organic solvents makes it likely to accumulate in lipid rich areas of the body, such as shark brains (Bradenburger Brown *et al.*, 1972). Jodlbauer and Salvendi (1905) were the first to report use of quinaldine as an anaesthetic for fish according to Bradenburger Brown *et al.*(1972) It was Muench (1958) who published the first report on the use of quinaldine as a fish anaesthetic in the USA. It is more toxic to some fish in hard than in soft water. It is not transformed by the fish, it is excreted in its original form, hence muscle residues are essentially zero, 24h after exposure (Baldridge, 1969).

Natarajan and Renganathan (1960) employed quinaldine at the concentration of 5ppm for the transport of breeders. Small (10-12cm) grass carp lose equilibrium in 5 minutes at 13^0C after exposure to 15 mg/l quinaldine (Jensen *et al.*, 1978). Quinaldine was an effective anaesthetic for grass carp (1.6 - 3.7 kg) at water temperatures less than 26^0C but lethal above 26^0C (Schramm and Black, 1984).

Quinaldine is still one of the most commonly used tranquilizer for the large and brood fish of the Common, Chinese and Indian major carps. The dilution rate of quinaldine to water is 1:40000. But quinaldine treatment sometimes leads to irregular opercular movement (Peer Muhamed and Devaraj, 1997).

Salmonids were anaesthetized in 1-6 minutes by quinaldine of 5-12 mg/l. They took 1-10 minutes to right themselves and maintain equilibrium (Bell, 1967; Locke, 1969). Quinaldine toxicity increased with increasing temperature for Rainbow trout (Marking, 1969). Piper *et al.* (1982) gave a mixture of 40 mg MS_{-222} /l and 10 mg/l quinaldine for anaesthesia of adult Pacific salmon.

Temperature and fish size are important variables for warm water fishes like Channel cat fish. Channel cat fish was found to be very resistant to quinaldine at 12^0C (Marking, 1969). At temperatures above 7^0C channel cat fish exposed to 10-70 mg/l of quinaldine recovered in 1-30 minutes (Schoettger and Julin, 1969).

Durve (1970; 1975) studied the suitability of 13 anaesthetics for transport of live mullet seed. He found quinaldine to be one of the seven chosen for live fish transport, but, indicated that it required a vehicle to go into aqueous solution and had a low induction and recovery time.

The intertidal teleost *Blennius pholis* showed marked reduction in oxygen consumption at quinaldine concentrations of 10-20 ppm. However, at low concentrations, oxygen consumption of smaller specimens was found to be slightly increased (Dixon and Milton, 1978; Milton and Dixon, 1980).

Striped bass broodfish was sufficiently relaxed for egg stripping within 1-2 minutes after their gills were sprayed with a quinaldine solution of 1000 mg/l (Piper *et al.*, 1982).

Onyango (1985) studied the use of quinaldine as a tranquilizer and depressant of metabolism influencing the survival of *Oreochromis niloticus* during transport. Sado (1985) found Tilapia extremely tolerant to quinaldine. A concentration of 25-50 mg/l was required to sedate them, while 50-1000 mg/l was needed for complete anaesthesia with induction times of 2.9 and 1.5 minutes respectively (Sado, 1985).

2.6.1.4. 2 - Phenoxyethanol

Other names of 2-phenoxyethanol are 1-hydroxy -2-phenoxyethane, ethylene glycol monophenyl ether, beta hydroxyethyl phenyl ether, phenyl cellosolve, phenoxethol and phenoxetol. It is a colourless, oily liquid slightly heavier than water, moderately soluble in water, but freely soluble in ethanol (Summerfelt and Smith, 1990). It is used as a topical anaesthetic (Merck and Company, 1983).

Idler *et al.* (1961) used 2-phenoxyethanol for transporting adult Sockeye salmon. 2-Phenoxyethanol at concentrations of 0.11 - 0.22 mg/l immobilized pink salmon fry in 2-4 minutes, the recovery time for which was 3-6 minutes. Its lethal concentration was 0.286 ml/l (Bell, 1967). Barton and Helfrich (1981) suggested a dosage of 0.25 ml/l of 2-phenoxyethanol as appropriate for general use with juvenile Salmonids. Induction times are long and hyperactivity sometimes occurs during

recovery. exposed salmon have exhibited rapid and erratic swimming known as motorboating (Summerfelt and Smith, 1990).

2-phenoxyethanol at concentrations of 0.11 and 0.22 g/l was effective in maintaining a zero mortality rate of guppies. It reduced excretion of carbon dioxide and ammonia to a greater extent than low temperature (Teo *et al.*, 1989). 2 phenoxyethanol suppressed the oxygen consumption of guppies placed individually or in groups in closed vessels. Grouped fish was found to show lower metabolic rates than individual fish (Teo and Chen, 1993).

Broodstocks of silver carp and grass carp were sedated during injection and stripping by adding 0.2 ml/l of 2 phenoxyethanol. It was found to have no effect on sperm motility (Mc Carter, 1992). Effect of 2 phenoxyethanol on adult and fry of Common carp was described by Josa-Serrano *et al.* (1993) and Osanz *et al.* (1993). 2- phenoxyethanol at a dosage of 30-40 ml/100l was found sufficient to anaesthetize large and brood fish of the Common Carp, Chinese and Indian major carps (Peer Mohamed and Devaraj, 1997).

2-phenoxyethanol at a concentration of 0.2 ml/l was found suitable to anaesthetize platy fish for transport (Guo *et al.*, 1993). This anaesthetic was found to be better than anaesthetics like MS-$_{222}$, quinaldine sulphate and metomidate in decreasing the excretion of metabolic wastes and in suppressing the oxygen consumption of Platyfish (Guo *et al.*, 1995 a; 1995 b).

Molinero and Gonzalez (1995) evaluated the effect of 2 phenoxyethanol at three different dosage levels on Gilt head sea bream (*Sparus aurata*) during a

confinement similar to that in transport. The anaesthetic produced a stress response at a dose exceeding 0.075 mg/l.

2.6.1.5. Quinaldine sulphate

Quinaldine sulphate also known as quinate is a light yellow crystalline power, freely soluble in water but lacks the strong odour of quinaldine. But, like quinaldine it is highly irritating to mucous membranes (Merck and Company, 1983). Quinaldine sulphate induces anaesthesia faster than quinaldine and fish recover faster from its effects (Blasiola, 1977). It is only useful as a short term anaesthetic, because it has been shown to be toxic over long periods of anaesthesia (Amend *et al.*, 1982) But, toxicity is less in very soft water than in hardwater because a decreased pH causes a decrease in the concentration of the active, un-inoized form (Marking and Dawson, 1973). The anaesthetic do not completely block involuntary muscular movement and therefore it may not be appropriate for implications such as surgery or marking fish (Gilderhus and Marking, 1987). Its irritating and noxious vapour and high cost make it unfavourable compared to 2-phenoxyethanol (Guo *et al.*, 1994, 1995).

Effective concentrations for warm water species ranged from 15-50 mg/l (Gilderhus *et al.*, 1973).

Concentrations of about 25 mg/l of quinaldine sulphate anaesthetized most Salmonids in less than 4 minutes; the fish recovered in 1-13 minutes. For example 25-40 mg/l was effective for fingerling and adult Rainbow trout, which recovered in 5-6 minutes (Gilderhus and Marking, 1987).

Lemm (1993) reported 5mg/l as the concentration of the anaesthetic for sedation of 300-500g Striped bass. The lowest concentration for immobilization at 13°C, 18°C and 23°C was noted as 55, 25 and 25 mg/l.

Quinaldine sulphate reduced the excretion of waste products, ammonia and carbon dioxide and also suppressed the oxygen consumption of Platyfish (Gnu *et al.*, 1995 a, 1995 b).

2.6.1.6. Benzocaine

Benzocaine is also known as ethyl aminobenzoate, p-aminobenzoic acid ethyl ester, ethyl-p-aminobenzoate and 4 amino benzoic acid ethyl ester. Benzocaine is the most commonly used generic name (Merck and Company, 1983). It is barely water soluble but may be dissolved in acetone (Dawson and Gilderhus, 1979) or ethanol (Ross and Geddes, 1979) and thence carried into water. Ferreira *et al.*(1979) synthesised a water soluble form, benzocaine hydrochloride. Mc Erlean and Kennedy (1968) reported benzocaine more effective than MS_{-222} at lower concentrations. Ross and Geddes (1979) recommended a concentration of 100mg/l at 25°C as a starting point for warm water species whose sensitivity to this anaesthetic is unknown.

Luhning (1973) induced deep anaesthesia in Striped bass by 15 minutes exposure to 63.2 mg benezocaine /l buffered to pH 6.5 at 17.8°C. Concentrations of 55 mg/l at 22°C to 80mg/l at 11°C effectively anaesthetized juvenile and adult Striped bass in about 3 minutes. The recovery was found to be more rapid as the temperature was increased (Gilderhus *et al.*, 1991). The quality of cultured Striped

bass can be assessed by screening them for swim bladder inflation by their response to anaesthesia with benzocaine (Hunn *et al.*, 1992). The concentration of benzocaine for sedation of 300-500g Striped bass was found to be 25 mg/l (Lemm, 1993).

Barham *et al.*(1979) studied the effects of various concentrations of benzocaine hydrochloride on *Sarotherodon mossambicus* and found induction times increasing with increasing anaesthetic concentrations. Ferreira *et al.* (1984 a, 1984 b) induced anaesthesia in Mozambique tilapia with concentrations of 25-100mg/l. The uptake of benzocaine hydrochloride and neutralized benzocaine hydrochloride by gills and skin of Mozambique tilapia was found to be different (Ferreira *et al.*, 1984 c).

Dawson and Gilderhus (1979) provoked total loss of equilibrium in Rainbow and Brown trout in less than 3 minutes with benzocaine concentrations of 50 mg/l. Ferreira *et al.* (1984 a, 1984 b) induced anaesthesia in Rainbow trout with concentrations of 25-100mg/l. Fingerling and adult Rainbow trout maintained at 12°C in soft water could be made handleable in 3 minutes or less with a concentration of 35 mg/l (Gilderhus and Marking, 1987) Allen (1988) found residues of benzocaine near control values for Rainbow trout after the fish was exposed for 15 minutes and then held in flowing anaesthetic free water. Benzocaine concentrations of 25-30 mg/l anaesthetized Chinook salmon and Atlantic salmon in less than 3 minutes and most fish recovered in less than 10 minutes after a 15 minute exposure (Gilderhus, 1989; 1990).

25-100mg/l of benzocaine hydrochloride was used to anaesthetize Common carp (Ferreira *et al.*, 1984 a, 1984 b) But, the uptake of benzocaine hydrochloride and neutralized benzocaine hydrochloride by skin and gills of Common carp was found to be different due to the difference in the degree of ionization and lipid solubility of the anaesthetic (Ferreira *et al.*, 1984 c).

Benzocaine was found second to metomidate for rapid anaesthesia of Cod (*Gadus morhua*). The effective concentration for benzocaine was noted as 40 mg/l at 9.5^{0}C (Maltson and Riple, 1989).

The optimum dose to obtain complete stage of sedation of *Prochlidus lineatus* juveniles was 1:5000 at 20^{0}C and 1:10,000 at 25^{0}C (Parma-de-Croux, 1990).

2.6.1.7 Metomidate

Metomidate (1-(1-phenyl ethyl)-1H- irridazole -5-carboxylic acid methyl ester) is a water soluble powder which has the properties of a hypnotic, or sleep inducing drug (Merck and Company, 1989). Metomidate is an analogue of etomidate and propoxate (Vermeer and Falls, 1988; Iwama and Ackermann, 1994.). Metomidate and etomidate are also known as Methomidate. Hypnomidate or Amidate (Iwama and Ackermann, 1994).

Vermeer and Falls (1988) described metomidate as a safe, effective and low stress anaesthetic for Snook (*Centroponus undecimalis*) They found the induction and recovery time for adult Snook more than twice as long as juveniles.

Maltson and Riple (1989) found metomidate better than anaesthetics like MS-$_{222}$, benzocaine, 2-phenoxyethanol and chlorobutanol for rapid anaesthesia of Cod (*Gadus morhua*); effective concentration was noted as 5 mg/l at 9.6^{0}C.

The unique sedative properties of metomidate was found to have potential benefit to post handling health and survival in sensitive fish such as Chinook salmon (Kreiberg and Powell, 1991). Exposure to this anaesthetic resulted in a reduced plasma corticosteroid response over untreated fish (Kreiberg and Powell, 1991; Kreiberg 1992). Plasma cortisol increase was not obtained in metomidate anaesthetized Atlantic salmon, but did appear in those fish anaesthetized with MS-$_{222}$ (Olsen *et al.*, 1995).

Lemm (1993) noted 0.5 mg/l of metomidate sufficient for sedation of subadult Striped bass (*Morone saxatilis*). But, 7.5, 10 and 10 mg/l of the anaesthetic was required for immobilization of the fish at 13, 18, and 23^{0}C. Metomidate according to Ross *et al.* (1993) appears to be safe for transportation and handling of juvenile American shad (*Alosa sapidissima*). Aggregation, parallel orientation behaviours, precursors of normal schooling though reduced on anaesthetization, normalised on recovery.

Guo *et al.* (1995 a) studied the effects of few anaesthetics on water parameters during stimulated air transport of Platy fish, (*Xiphophorous maculatus*) They reported metomidate to have no effect in the control of waste production. However, at 1ppm was effective in suppressing the oxygen consumption of Platy fish. (Guo *et al.*, 1995 b).

2.6.2. Rarely used anaesthetics

2.6.2.1 Barbiturates

Recognized by a name ending in "al" barbiturates are chemically related to barbituric acid. Barbiturates included in this review are sodium amytal, phenobarbital sodium, pento barbital sodium, sodium barbital, and thiopental sodium Barbiturate drugs are used for general anaesthesia and for their sedative, hypnotic and sleep inducing actions (Warren, 1983) The hypnotic effects of barbiturates are anaesthetic like because they act as central nervous system depressants and this often impair motor skills for several hours after the sedative effect is lost (Considine and Considine, 1984).

2.6.2.1.1 Sodium amytal

Amytal anaesthesia was first used by Key and Wells (1930). The first worker to recognise the potential use of this anaesthetics for transporting fishes was Kitken (1936).

Calhonn (1953) and Reese (1953) reported sodium amytal to be effective in doubling the weight of trout normally transported. Phillips and Brockway (1954) were able to demonstrate reductions in the rates of excretion and of oxygen consumption of Brook trout, (*Salvelinus fontinalis*). Messerby (1959) also used the anaesthetic for the transport of Brook trout at a concentration of 300 ppm.

It was used in the transport of fry of Indian major carps by Saha *et al.* (1955) It was found to reduce oxygen consumption of Common carp by nearly 80% when employed at 40 ppm. Nemoto (1957) indicated that this chemical caused one third reduction in the rate of oxygen consumption of the cichlid *Tilapia mossambica*, sealed in containers. Sodium amytal at a dose of 52-172 mg/l was found sufficient to anaesthetize large and brood fish of the Common, Chinese and Indian major carps (Peer Mohamed and Devaraj, 1997).

Sodium amytal was one of the nine anaesthetics chosen for live mullet transport (Durve and Dharmaraja, 1966; Durve 1975). It reduced the rate of oxygen consumption by 60.61%, but, its major draw back is that it shows calcium antagonism (Durve, 1975).

2.6.2.1.2 Phenobarbital sodium

Phenobarbital sodium has been used by Mc Farland (1959) who reported calcium antagonism in respect of all barbiturates.

This chemical has a prolonged induction and recovery time and hence found unsuitable for transport of mullet seed. Moreover, its narcotic potency was found to be very low (Durve, 1970; 1975).

2.6.2.1.3. Pentobarbital sodium

Walker (1972) used intravenous injections of pentobarbital sodium (10 mg/kg or less) for general surgical anaesthesia of nurse sharks.

Durve (1970) found it unsuitable for transport of live mullet seed though its narcotic potency was very high.

2.6.2.1.4. Sodium barbital

Sodium barbital was not tolerated for a prolonged treatment and should be ruled out for transport of live mullet seed (Durve and Dharmaraja, 1966; Durve, 1975). Sodium barbital was found to decrease the metabolic rate of mullet seed sharply when the fishes were in groups (Durve, 1975). Though not suitable for transport of mullet seed it was found suitable for tranquilising large and brood fish of the Common, Chinese and Indian major carps at a dose of 50 mg/kg of fish (Peer Mohamed and Devaraj, 1997).

2.6.2.1.5 Thiopental sodium

Thiopental sodium at 200-300ppm shortened the time required to induce each anaesthetic stage and delayed recovery of adult Common carp *Cyprinus carpio* (Hikasa *et al.*, 1986). This barbiturate is used as anaesthetic agent, for induction of anaesthesia prior to administration of other anaesthetic agents (Medical Economics Company, 1987).

2.6.2.2. Chloretone

Chloretone, also known as chlorobutanol, methaform or sedaform is a crystalline powder with a camphor odour (Iwama and Ackermann, 1944). Although it can be dissolved in water, it has a high solubility in alcohol (Merck and Company, 1989).

Summer and Wells (1935) and Mc Farland (1960) used chloretone to anaesthetize *Fundulus parvipinnis*. 0.02-0.025gm caused complete loss of equilibrium. However, Mc Farland (1960) described it as an extremely potent anaesthetic that induces deep and prolonged sedation, hence not suitable for fish transport.

Burrows (1952), Cope (1953) and Parkhurst and Smith (1957) used chloretone at concentrations of 400, 500 and 400ppm respectively for immobilising several Salmonids. Meister and Ritzi (1958) used 333ppm of this chemical to anaesthetize Eastern brook trout.

Sreenivasan (1962) reported an aqueons solution of chloretone ineffective upto 100ppm, but, effective when dissolved in alcohol. Even 50ppm chloretone dissolved in alcohol was found toxic to carp fingerlings on long exposure.

Chloretone was one of the anaesthetics selected for live mullet transport, however, it had a low induction and recovery time (Durve and Dharmaraja, 1966, Durve, 1975). It was found to reduce the metabolic rate of mullet fry by 47.35% (Durve 1975).

2.6.2.3 Ether

Ether also known as ethyl ether, diethyl ether, ethoxy ethane, ethyl oxide or sulphuric ether is a very volatile, highly inflammable liquid which when exposed to air, forms explosive peroxides (Iwama and Ackermann, 1944). It is slightly soluble in water, with saturation occurring at 8.43% w/w at 15^{0}C (Merck and Company,

1989) It was the first chemical anaesthetic used on fish in the USA, in about 1939 (Griffiths *et al.*, 1940).

It has been successfully used in various species of salmon and trout at concentrations of one to five percent by Griffiths *et al.*(1940), Foster (1941), Eschmeger (1953), Nelson (1953) and Allison (1954).

Ether was found to induce stage 4 anaesthesia in mullet fingerlings at concentrations of 0.4 and 0.6 ml/100ml (Durve, 1970). It was found unsuitable for transport of live mullet seed because it is difficult to get a thorough solution as it evaporates (Durve, 1970; Durve, 1975).

2.6.2.4. Urethane

Urethane is also known as urethan or ethyl urethan (Iwama and Ackermann 1994). It is a crystalline powder with a water solubility of 2g/ml (Merck and Company, 1989). Its use was discontinued when it was found to be carcinogenic (Wood 1956; Ball and Cowen 1959), though it was a popular fish anaesthetic as it has a wide margin of safety between lethal and effective dosages and has no ill effects to fish with repeated exposures (Mc Farland and Klontz, 1969). Urethane seemed to have been used by Prof. Von Fritsch (Teichman, 1957) as early as 1932 for narcotising fishes.

Urethane was recommended for weighing, measuring and fin-chipping fish (Gerking, 1949) and used in Wisconsin fish hatcheries to facilitate handling of Eastern brook trout for spawning (Johnson, 1954) at concentrations ranging from 0.5 - 4.0%.

100ppm of urethane was needed to narcotise carps, but, lower concentrations were enough to tranquilise fish and reduce oxygen consumption (Sreenivasan, 1962). 100mg/l of this drug was required to tranquilise Common, Chinese and Indian major carps (Peer Mohamed and Devaraj, 1997).

Urethane at a concentration of 0.15g/100ml caused light sedation of mullet fingerlings, but, its use for transport of the fish was discouraged as it proved to be a carcinogen (Durve and Dharmaraja, 1966; Durve, 1975).

2.6.2.5 Tertiary amyl alcohol

Tertiary amyl alcohol has intermediate potency, is readily soluble in fresh and salt water and is compatible with calcium (Mc Farland, 1960). He suggested this anaesthetic as best suited for live fish transport at a concentration of 2ml/gal, while Taylor and Solomon (1979) described it as one of the cheapest of anaesthetics. It was found to reduce the metabolism of fish and thus helped maximal survival (Rothbard, 1988).

A dose of 48 ml/gal of this anaesthetic immobilized Sock eye salmon within 2 minutes while 5.5 -6 ml/gal and 5-6 ml/litre immobilized the fish in 8-12 and 20-25 minutes respectively (Bell, 1964). Saunders as reported by Bell (1964) stated that this anaesthetic as cheap and effective for tagging Atlantic salmon smotts.

Durve (1970, 1975) found 0.08ml of tertiary amyl alcohol 100ml as best suited for transport of mullet fingerlings, as the fish could tolerate this concentration for 24 h without any adverse effects or significant mortality. Alvarez -Lajonchere

and Moreno (1982) noted the concentration of the anaesthetic required for light and deep sedation of *Mugil trichodon* as lower than that reported by Durve (1970;1975).

Tertiary amyl alcohol at 2ml/4.5l was required to tranquilise large fish and brood fish of the Common, Chinese and Indian major carps (Peer Mohamed and Devaraj, 1997).

2.6.2.6 Chloral hydrate

Chloral hydrate is an aromatic acrid smelling powder, with a bitter taste (Iwama and Ackermann 1994). It is also known as Escre, Noctec, Somnos, Lorinal and Chloral durant (Iwama and Ackermann 1994). It can irritate the skin and is a potentially addictive drug which has sedative, narcotic, hypnotic as well as depressant qualities (Merck and Company, 1989). Mc Farland (1960) described it as an anaesthetic with intermediate potency, readily soluble in fresh and salt water and compatible with calcium. Brander *et al.* (1982) reported chloral hydrate anaesthesia to cause fright and struggling due to loss of control before perception ceases.

Carps cannot be anaesthetized by chloral hydrate at high concentrations upto 500ppm (Sreenivasan, 1962). 3-3.5 g/4.5l was required to tranquilise Common, Chinese and Indian major carps (Peer Mohamed and Devaraj. 1997).

Chloral hydrate was one of the seven anaesthetics chosen for live mullet transport by Durve and Dharmaraja, (1966) and Durve (1975). But, it was found to have a prolonged induction time though the recovery was comparatively quicker (Durve, 1975).

2.6.2.7 Tertiary butyl alcohol

Tertiary butyl alcohol is soluble in water but has a prolonged induction time, though recovery of mullet fingerlings from this is comparatively quicker (Durve and Dharmaraja, 1966; Durve, 1975). They found 0.3 and 0.35 ml/100ml of the anaesthetic suitable for light and deep sedation of mullet fingerlings. Alvarez - Lajonchere and Moreno, (1982) too agreed with this concentration for light and deep sedation of *Mugil trichodon.*

Abnormalities in the body curvature was noticed in *Cyprinus carpio* after injection with tertiary butyl alcohol. Abnormalities occurred behind the dorsal fin and progressively developed over a period of four months (Jain and Durve, 1978).

Mary Margaret (1991) reported tertiary butyl alcohol to increase the duration of 100% survival in *Chanos chanos* seed.

2.6.2.8 Clove oil

Clove oil consists primarily of the phenols eugenol (70-90%) eugenol acetate (717%) and karofilen 5 (12%) (Hernani and Tajendjaga, 1988). It has been used for centuries in Indonesia as a topical anaesthetic for toothaches and joint pains in human beings. It is a dark brown liquid resulting from the distillation of flowers, flower stalks and leaves of clove trees *Eugenia aromatica* (Soto and Burhamuddin, 1995).

Burhamuddin *et al.* (1988) demonstrated the potential of clove oil as an anaesthetic for transport of Rabbit fish, *Siganus guttatus*. 10mg/l of the anaesthetic was found to cause the fish lose balance while 15 mg/l caused fish to lose consciousness for 20h before they began to die. Soto and Burhanuddin (1995) found clove oil as a highly effective fish anaesthetic with few or no side effects, suitable for length and weight measurement of *Siganus lineatus*.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Experimental animals

The fry of *Liza parsia* of average weight 0.22g, obtained from the natural collections made at the Kerala Agricultural University Fisheries Station, Puduveypu were used for the experiments. They were transported to the College of Fisheries, Cochin in polythene bags packed with water of 7ppt salinity and oxygen, at ambient temperature of 28 ± 2^0C. The fry were slowly released into 830 l capacity circular fibre glass tanks filled with 750 l water of 7ppt salinity at ambient temperature in groups of 400/tank. Aeration was provided in the tanks. The fry were fed *ad libitum* using rice bran. 25% of the tank water was exchanged every day. The feed remnants and the faecal matter were removed from the tank bottom by siphoning, once daily.

3.2. Experimental containers

Respirometers used for the study consisted of cylindrical flasks of 136 ml capacity, each fitted with a two-holed rubber stopper through which the inlet and outlet tubes were fitted. The inlet tube was fitted with a regulator for adjusting water flow by means of a latex tubing. For collecting final sample, the inlet was disconnected from the reservoir and formed the outlet; and the original outlet served as the airvent. The respirometers were placed inside a trough containing water to avoid temperature variations.

Air tight transparent hard plastic jars of 600ml capacity with screw type lids which were fitted with one way valves similar to those described by Jayasree

Vadhyar *et al.* (1992), were used for the oxygen packing of the *L. parsia* fry under uniform oxygen pressure and water quality conditions.

3.3. Experimental procedures

3.3.1 Determination of optimum dose of anaesthetics.

Three different fish anaesthetics viz., chloral hydrate, tertiary butyl alcohol and clove oil were selected for the study on the basis of their water miscibility and previously reported application on other fishes. An experiment was carried out to determine the optimum dose of each of these three anaesthetics by determining the oxygen consumption rate, by noting the behavioural changes, induction time, recovery time, and survival of the fry after transferring them into anaesthetic free water. Based on previous literature on other mullet species, three different doses of each anaesthetic viz., 0.20, 0.25 and 0.30g/l for chloral hydrate., 2.5, 3.0 and 3.5 ml/l for tertiary butyl alcohol and 8.5, 9.0 and 9.5 mg/l for clove oil were tried in this experiment.

Solutions of chloral hydrate and tertiary butyl alcohol were prepared by mixing the appropriate amounts in water of 7ppt salinity. In the case of clove oil, a stock solution of 10ppt was made by mixing 10ml of clove oil in one litre of boiled fresh water (Burhanuddin *et al.*, 1989). The stock solution was shaken vigorously and 0.85, 0.90 and 0.95ml of this solution was stirred into beakers containing one litre of water of 7ppt salinity.

One hour prior to starting the experiment, the fry maintained in the fibre glass tanks were conditioned in the following manner. Eight numbers of fry were taken at

random from the tank and each fry was initially acclimatised to the restricted space in a beaker of 250 ml with 100ml water of 7ppt salinity. They were then gradually acclimatised to each anaesthetic solution (100ml) of a specific concentration at the same salinity. The behaviour of the fry was observed closely and the induction time as well as recovery time noted.

Each respirometer was filled to the brim with the anaesthetic solution of the same concentration to which the fry were acclimatised. The fry was introduced into the respirometer singly. Later, the same procedure was followed for the different concentrations selected for each of the three anaesthetics.

The respirometer was then stoppered using the rubber stopper and was placed inside a trough containing water of ambient temperature. Each experimental unit was connected to a reservoir containing the anaesthetic solution which was allowed to flow through the respirometer for 15 minutes. At the start of the experiment, initial samples for analysis of dissolved oxygen were collected and the circulation through the respirometer was cut off. Final samples were collected after an interval of 30 minutes. The size of each sample was 47ml. The samples were fixed immediately using winkler solutions for analysis of dissolved oxygen. The fry in each respirometer was weighed at the end using an electronic balance. A control was run with water of 7ppt salinity using unanaesthetized fry in the respirometers. The initial and final samples were taken from the control as in the case of the treatments for analysis of dissolved oxygen. The experiment was conducted at ambient temperature of 28 ± 2^0C. After the experiment the fry was transferred into

fibre glass tanks containing anaesthetic free water and observed for their further survival and activity including feeding for 72h after release.

Completely randomized design (CRD) was used for planning the experiment with four replication for each treatment.

3.3.2. Determination of the effect of anaesthetics on metabolic rate of *L.parsia* fry.

The effect of the three anaesthetics on the metabolic rate of the fry of *L.parsia*, was compared using their optimum dose obtained from 3.2.1.

Completely randomized design (CRD) was used for comparison of the effects and for analysis of the results.

3.3.3. Determination of the effect of packing density and anaesthetisation on survival and duration of the oxygen-packed fry during transportation.

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. The experiment was conducted at ambient temperature (28 ± 2^0C) and at a salinity of 7ppt.

Randomised block design (RBD) was used for planning the experiment and analysis of results.

The fry were conditioned in the following manner prior to oxygen packing. They were taken at random from the fibre glass tanks, the required numbers, counted and introduced into beakers containing 500ml of anaesthetic solution with half the

optimum concentration of each of the anaesthetics. After 15 minutes, the fry were slowly acclimatised to the full optimum concentration of the anaesthetic.

Once the fry got anaesthetised, they were introduced into oxygen packing jars each containing 100ml of anaesthetic solution of the optimum dose, at different packing densities viz., 100, 200, 300, 400 and 500 fry/l. Immediately after transferring them the jars were closed tightly and filled with oxygen from an oxygen cylinder under uniform pressure of 0.2 kg/cm^2. The oxygen pressure in the jars was measured through a pressure gauge with a precision of 0.2 kg/cm^2 (Bourdon type). While filling oxygen, care was taken to displace the air initially present inside the jars with oxygen. To effect this, after filling oxygen initially it was completely released by pressing the valve. This was repeated three times to ensure complete displacement of air with oxygen (see Jayasree Vadhyar *et al.*, 1992). The jars were shaken periodically more or less to simulate transporting conditions.

A control was also run where unanaesthetized fry initially acclimatised in beakers containing 500ml anaesthetic free water at the five different packing densities were packed in the oxygen jars containing 100ml of anaesthetic free water, as mentioned earlier.

Jars filled with 100ml of each anaesthetic solution without the fry, were filled separately with oxygen at 0.2 kg/cm^2, at ambient temperature (28 $\pm$ 2^{0}C) and were opened immediately after filling oxygen to collect the initial water samples of each treatment and control separately.

Time of initial mortality of the oxygen-packed fry was recorded by making hourly observations. This was considered as the duration of 100% survival. There after, the number of survivors was counted at 3 hourly intervals until 70% survival. The jars were them opened and samples of the packing medium were collected for water quality analysis. Initial and final quality of the packing medium was analysed using standard procedures. The parameters analysed were dissolved oxygen, free carbon-dioxide, ammonia -N and pH. Of the replicates of each combination, one was used for analysing dissolved oxygen and the other for free carbon-dioxide and ammonia - N because the determination of both these parameters from the same sample might have yielded erroneous values for the second measured parameter. pH was noted from all the replicate jars.

The survivors from the oxygen-packed jars were released into circular fibre glass tanks of 83 l capacity in anaesthetic free water of 7ppt salinity and observed for their further survival and activity including feeding for 72 hours after release.

3.3.4. Determination of water quality

The following methods were used for analysing the water quality parameters-

Dissolved oxygen - Winkler method (Strickland and Parsons,1972).

Carbondioxide - Alkalimetric titration method (Strickland and Parsons, 1972).

Ammonia-nitrogen - Phenol-hypochlorite spectrophotometric method (Strick land and Parsons,1972).

pH - Using universal PH indicator solution

Temperature - Using mercury bulb thermometer having a precision of $0.1^{0}C$.

Salinity - Using refracto - salinometer.

3.3.5 Statistical analyses.

Data obtained from all experiments were analysed statistically by analysis of variance. Data on oxygen consumption, carbon dioxide and ammonia were analysed using the correlation coefficient.

Pair wise comparisons using critical difference values was made for those treatments which were found statistically significant.

RESULTS

4. RESULTS

4.1. Optimum dose of the three different anaesthetics

4.1.1. Chloral hydrate

The data obtained on behavioural pattern, induction and recovery times, and oxygen consumption of the *L.parsia* fry with chloral hydrate treatment are summarised in table 1, 2 and 3. Of the three different doses of chloral hydrate, the behaviour of the fry at the dose of 0.2 g/l was not found to slow down even after 60 minutes. Tapping on the container caused them to move frantically. At the dose of 0.25 g/l though slow movement was observed after 45 minutes, there was no reduction in their reaction to external stimuli. But, in the case of the highest dose of 0. 3 g/l, the fry showed reduction in movement and reaction to external stimuli within 30 minutes. The recovery time was not clear at 0.2 and 0.25 g/l. But, in the case of 0.3 g/l the recovery time was noted as 3-4 minutes. The analysis of variance done to compare the oxygen consumption levels of the seed under different doses of chloral hydrate, showed significant difference among them (table 4). The critical difference analysis done for pair wise comparison of the effect of different doses, showed that the dose of 0.2 differed significantly from others, while 0.25 g/l and control were not showing any significant difference. Taking note of the behaviour, the induction and recovery times as well as the oxygen consumption, the highest dose of 0.3 g/l was selected. At this dose, both slow movement and reduction to external stimuli were observed within the shortest time (30 minutes), the recovery time (3 to 4 minutes) was clear and the oxygen consumption found to differ from

Table 1. Behavioural pattern of *L. parsia* fry subjected to three different doses of chloral hydrate.

Dose g/l	Behaviour of the fry
0.20	Reaction to external stimuli even after 60 minutes Remains in the water column
0.25	Slow movement after 45 minutes, no reduction in reaction to external stimuli Remains in the water column
0.30	Slow movement in 30 minutes and reduction in reaction to external stimuli Remains in the water column

Table 2. Induction and recovery times of *L. parsia* fry treated with three different doses of chloral hydrate.

Dose G/l	Induction time (minutes)	Recovery time (minutes)
0.20	>60	not clear
0.25	45	not clear
0.30	30	3-4 minutes

Table 3. Oxygen consumption of L. *parsia* fry at three different doses of chloral hydrate and control in four replicates (R1, R2, R3 & R4)

	Oxygen consumption mg/g body wt./h			
	Replicates			
	R1	R2	R3	R4
Control	1.0020	1.0680	1.5420	1.2350
Treated(g/1)				
0.20	2.2400	1.7000	1.7050	1.6230
0.25	1.9300	1.2300	0.7657	0.9521
0.30	0.3165	0.2541	0.2712	0.4543

Table 4. Analysis of variance of the rate of oxygen consumption of *L. parsia* fry under different doses of chloral hydrate and control

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F value computed
Between treatments	4.5423	3	1.5141	14.8441*
Within treatments	1.2241	12	0.102	
Total	5.7664	15		

*Significant at 5% level

Treatment means of different doses of chloral hydrate and control

	Dose (g/l)		
Control (C)	0.2 (T_1)	0.25 (T_2)	0.3 (T_3)
1.21175	1.817	1.21945	0.324

Calculated C.D value = 0.4921

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

Under line indicate 'no significant difference'

that of the control. All the fry treated with different doses of chloral hydrate and control survived for the observed period of 3 days.

4.1.2. Tertiary butyl alcohol

Data obtained on behavioural pattern, induction and recovery times, and oxygen consumption with tertiary butyl alcohol are summarised in table 5, 6 and 7. At the dose of 3.5 ml/l the fry were found to slow down their movement and occupy the bottom after 5 to 6 minutes of treatment; intermittently coming up to the column. Also, no reaction to external stimuli was observed. After 12 minutes, at the same dose the anal peduncle was found to bend slightly. Even at the dose of 3 ml/l the fry were found to exhibit similar behavioural pattern. But, at the lowest dose at 2.5 ml/l, movement was slowed down only after 15 minutes and the fry remained in that manner, in the water column. In the case of all the doses, once slow movement was observed, the fry showed reduction in their reaction to external stimuli. The recovery time was found to be similar for all the doses which was 4 to 5 minutes. The analysis of variance done to test the varition in oxygen consumption with varying tertiary butyl alcohol doses showed significant difference amongst the doses and the control (table 8). Pair wise comparison by critical difference analysis revealed that the three doses of tertiary butyl alcohol gave an oxygen consumption that differed significantly from that of the control. Though all the three doses differed significantly from the control the dose of 2.5 ml/l was selected for further experiments taking into account the best behavioural pattern and the longest induction time. The behaviour at this dose indicated only light sedation as the fry

Table 5. Behavioural pattern of *L. parsia* fry subjected to three different doses of tertiary butyl alcohol.

Dose ml/l	Behaviour of the fry
2.5	Slow movement and reduction in reaction to external stimuli after 15 minutes Remains in the water column
3.0	Slow movement and reduction in reaction to external stimuli after 6 minutes Goes to bottom at intervals
3.5	Slow movement and reduction in reaction to external stimuli after 5-6 minutes Goes to bottom at intervals

Table 6. Induction and recovery times of *L. parsia* fry treated with three different doses of tertiary butyl alcohol

Dose (ml/l)	Induction time (minutes)	Recovery time (minutes)
2.5	15	4 -5
3.0	6	4 -5
3.5	5-6	4 -5

Table7. Oxygen consumption of L. *parsia* fry at three different doses of tertiary butyl alcohol and control in four replicates (R1,R2,R3 & R4)

	Oxygen consumption mg/g body wt./h			
	Replicates			
	R1	R2	R3	R4
Control	1.0020	1.0680	1.5420	1.2350
Treated (ml/l)				
2.5	0.7875	0.8220	0.7830	0.8050
3.0	0.7292	0.7600	0.6820	0.7351
3.5	0.6200	0.6625	0.7051	0.6822

Table 8. Analysis of variance of the rate of oxygen consumption of *L. parsia* fry under different doses of tertiary butyl alcohol and control.

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F value computed
Between treatments	0.7279	3	0.2426	15.9605*
Within treatments	0.1823	12	0.0152	
Total	0.9102	15		

*Significant at 5% level

Treatment means of doses of tertiarybutyl alcohol and control

	Dose (ml/l)		
Control (C)	2.5 (T_1)	3 (T_2)	3.5 (T_3)
1.2118	0.7994	0.7226	0.6675

Calculated C.D value = 0.18996

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

Underline indicate 'no significant difference'

were not resting at the bottom of the container. All the fry treated with different doses of tertiary butyl alcohol and control survived for the observed period of 3 days.

4.1.3. Clove oil

Data obtained on behavioural pattern, the induction and recovery times, and the oxygen consumption are summarised in table 9, 10 and 11. At the highest dose of clove oil, i.e., 9.5 mg/l, the fry were found to go to the bottom of the container after 10 minutes, after which they showed no reaction to external stimuli. For the dose of 9.0 mg/l similar behavioural pattern was observed after 10-12 minutes. For the lowest dose of 8.5 mg/l, the fry were found to remain in the column after 15 minutes and showed reduction in reaction to external stimuli. The recovery time was found to be in the range of 2-5 minutes for all doses tried. The analysis of variance done to analyse the oxygen consumption with clove oil did show significant difference between the doses and also with the control (table 12). Critical difference done for pair wise comparison showed no significant difference between the control and the dose of 9.0 mg/l, and also between the doses of 8.5 and 9.5 mg/l. Taking into account the information obtained from the behavioural pattern, induction and recovery times and oxygen consumption, the lowest dose of 8.5 mg/l was selected for further experiments, as at this dose the fry remained in the column indicating only slight sedation. All the fry treated with different doses of clove oil and control survived for the observed period of 3 days. They were observed to feed actively.

Table 9. Behavioural pattern of *L. parsia* fry subjected to three different doses of clove oil

Dose mg/l	Behaviour of the fry
8.5	Slow movement and reduction in reaction to external stimuli after 15 minutes Remains in the water column
9.0	Slow movement and reduction in reaction to external stimuli after 10-12 minutes Goes to bottom at intervals
9.5	Slow movement and no reduction in reaction to external stimuli after 10 minutes Remains at bottom after 10 minutes

Table 10. Induction and recovery times of *L. parsia* fry treated with three different doses of clove oil

Dose (mg/l)	Induction time (minutes)	Recovery time (minutes)
8.5	15	2 -5
9.0	10-12	2 -5
9.5	10	2 -5

Table 11. Oxygen consumption of L. *parsia* fry at three different doses of clove oil and control in four replicates (R1,R2,R3 & R4)

	Oxygen consumption mg/g body wt./h			
	Replicates			
	R1	R2	R3	R4
Control	1.002	1.068	1.542	1.235
Treated (mgl)				
8.5	0.2733	0.1525	0.3754	0.2853
9.0	1.38	0.556	0.852	0.015
9.5	0.2329	0.4855	0.513	0.6525

Table 12. Analysis of variance of the rate of oxygen consumption of *L. parsia* fry under different doses of clove oil and control.

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F value computed
Between treatments	0.2292	3	0.7431	13.7357*
Within treatments	0.6497	12	0.0541	
Total	0.8789	15		

*Significant at 5% level

Treatment means of doses of clove oil and control

	Dose (mg/l)		
Control (C)	8.5 (T_1)	9.0 (T_2)	9.5 (T_3)
1.2118	0.2716	0.9492	0.47098

Calculated C D value = 0.3584

C T_2 T_3 T_1

Underline indicate 'no significant difference'

4.2. Effect of the three different anaesthetics on the metabolic rate

The oxygen consumption of the *L. parsia* fry at the selected optimum doses of the three different anaesthetics is shown in table 13. Analysis of variance showed significant difference in the oxygen consumption among the different anaesthetics and the control (table-14). Pair wise comparison by critical difference analysis revealed that the three anaesthetics differed significantly from that of the control, while no significant difference occurred between the anaesthetics chloral hydrate and clove oil on oxygen consumption. Significant difference was observed on the oxygen consumption by the fry treated with tertiary butyl alcohol from that of the other two anaesthetics.

4.3. Effect of packing density and anaesthetisation on survival and duration of *L. parsia* fry during oxygen packed transportation

The observations made on the different duration (h) of survival of the fry treated with selected doses of the three anaesthetics and control at 100% and 70% levels were analysed. The analysis of variance of the data at each of these survival levels were carried out.

4.3.1. Duration

The data on the duration of 100% survival referred to as safe duration of transport of oxygen-packed fry at five different packing densities of 100, 200, 300, 400 and 500 fry/l at ambient temperature of 28 ± 2^{0}C and at a salinity of 7 ppt, under three anaesthetics and control are presented in table 15. Analysis of variance showed significant difference amongst the five packing densities tried (table 16). But

Table 13. Oxygen consumption of *L. parsia* fry at the selected doses of the three anaesthetics and control.

Oxygen consumption in mg/g body wt./h			
Control	Chloral hydrate (0.3 g/l)	Tertiary butyl alcohol (2.5 ml/l)	Clove oil (8.5 mg/l)
1.002	0.3165	0.7875	0.2733
1.068	0.2541	0.8220	0.1525
1.542	0.2712	0.7830	0.3754
1.235	0.4543	0.8050	0.2853

Table 14. Analysis of variance of the rate of oxygen consumption of *L. parsia* fry at selected doses of the three anaesthetics and control.

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F value computed
Between treatments	2.3492	3	0.7831	41.76*
Within treatments	0.225	12	0.01875	
Total	2.5742	15		

*Significant at 5% level

Treatment means of the three anaesthetics and control

Control (C)	Chloral hydrate (T_1)	Tertiary butyl alcohol (T_2)	Clove oil (T_3)
1.2118	0.32403	0.79938	0.2716

Calculated C.D value = 0.211

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

Underline indicate 'no significant difference'

Table 15. Duration of 100% survival* of oxygen-packed *L. parsia* fry under the selected doses of the three anaesthetics and control at five packing densities.

	Duration (h)**			
Packing density (Nos./ l)	Chloral hydrate (0.3 g/l)	Tertiary butyl alcohol (2.5 ml/l)	Clove oil (8.5 mg/l)	Control
500	4.0	3.0	3.5	1.5
400	4.5	8.5	6.0	6.0
300	3.5	5.0	3.5	1.0
200	6.5	9.0	8.0	4.5
100	10.0	24.0	8.5	20.0

*Time of initial mortality

** Each value is a mean of duplicates

Table 16. Analysis of variance of the duration of 100% survival of *L. parsia* fry at different packing densities under the selected doses of the three anaesthetics and control.

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F value computed
Between blocks	57.3375	3	19.1125	1.57
Between treatment	420.05	4	105.0125	8.64*
Error	145.8525	12	12.15438	
Total	623.24	19		

*Significant between treatments at 5% level

Block-Anaesthetics + Control ; Treatments - Packing density

Treatment means of packing density

500/l (T_1)	400/l (T_2)	300/l (T_3)	200/l (T_4)	100/l (T_5)
3	6.25	3.25	7	15.625

Calculated C. D. value = 4.8

T_5 $\underline{T_4}$ $\underline{T_2}$ $\underline{T_3}$ $\underline{T_1}$

Underline indicate 'no significant difference'

no significant difference was observed amongst the three anaesthetics and control. Pair wise comparison by critical difference analysis revealed that the packing density of 100 fry/l differed significantly from the other four packing densities (200, 300, 400 and 500 fry/l).

The different durations of 70% survival of the oxygen-packed seed are presented in table 17. Analysis of variance showed no significant difference among the different anaesthetics and control. But, significant difference was observed among the five packing densities (table 18). Pair wise comparison by critical difference analysis showed that the packing densities of 100 and 200 fry/l differed significantly between them and also from those of 300, 400 and 500 fry/l.

4.3.2. Cumulative percentage survival

The cumulative percentage survival of the oxygen-packed fry at five different packing densities, under three anaesthetics and control is summarised in table 19.

The cumulative percentage survival of the oxygen-packed fry at five different packing densities under the anaesthetic chloral hydrate are shown in fig 1. At the packing densities of 100, 200, 300,, 400 and 500 fry/l durations of 90% survival were observed as 13.6 h, 9.0 h, 6.3 h, 7.0 h and 7.2 h ; those of 80% survival were 18.0 h, 12.7 h, 9.0 h, 8.5 h and 8.7 h and those of 70% survival were 22.0 h, 16.0 h, 12.5 h, 10.0 h and 11.0 h respectively. The durations of 90%, 80% and 70% survival of fry treated with chloral hydrate were found to decrease with increase in packing densities of 100, 200 and 300 fry/l.

Table 17. Duration of 70% survival of oxygen-packed *L.parsia* fry at five packing densities under the selected doses of the three anaesthetics and control.

Packing density (Nos of fry/ l)	Duration (h)* Chloral hydrate (0.3 g/l)	Tertiary butyl alcohol (2.5 ml/l)	Clove oil (8.5 mg/l)	Control
500	11	10.5	11	9.5
400	10	19.5	15.5	10.5
300	12.5	12	11	10
200	16	17	22	15
100	22	28	17	22

*Each value is a mean of duplicates

Table 18. Analysis of variance of the duration of 70% survival of *L. parsia* fry at different packing densities under selected doses of the three anaesthetics and control.

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F value computed
Between blocks	44.3	3	14.76670	1.592
Between treatment	373.675	4	93.41875	10.069*
Error	111.325	12	9.2771	
Total	529.3	19		

*Significant between treatments at 5% level

Blocks - Anaesthetics + Control ; Treatments - Packing density

Treatment means of packing density

500/l (T_1)	400/l (T_2)	300/l (T_3)	200/l (T_4)	100/l (T_5)
10.5	13.88	11.38	17.5	22.25

Calculated C. D. value = 4.198

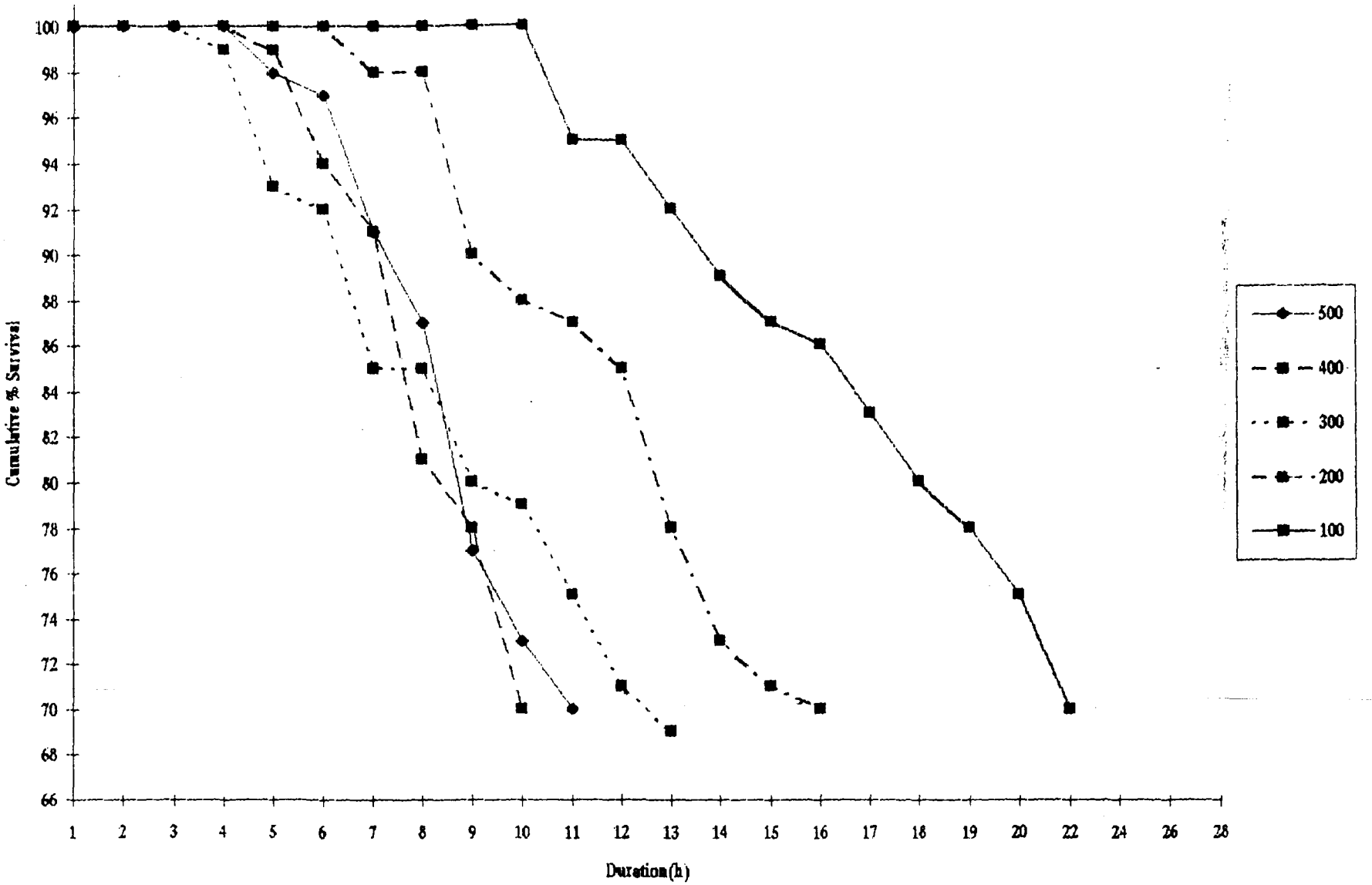
T_5 T_4 $\underline{T_2}$ $\underline{T_3}$ $\underline{T_1}$

Underline indicate 'no significant difference'

Table 19. Cumulative Percentage Survival of oxygen - packed *L. parsia* fry at selected doses of the three anaesthetics and control at five packing densities.

Treatments	Packing density (nos/l)	Duration (h)*																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	22	24	26	28
Chloral hydrate	500	100	100	100	100	98	97	91	87	77	73	70													
	400	100	100	100	100	99	94	91	81	78	70														
	300	100	100	100	99	93	92	85	83	80	79	75	71	69											
	200	100	100	100	100	100	100	98	95	90	88	87	85	78	73	71	70								
	100	100	100	100	100	100	100	100	100	100	100	95	95	92	89	87	86	83	80	78	75	70			
Tertiary butyl alcohol	500	100	100	100	99	95	94	93	90	82	73	68													
	400	100	100	100	100	100	100	100	100	98	95	93	90	86	81	78	76	75	73	71	69				
	300	100	100	100	100	100	97	95	92	85	80	75	70												
	200	100	100	100	100	100	100	100	100	100	95	93	90	87	83	80	76	70							
	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	78	70
Clove oil	500	100	100	100	97	96	92	89	87	79	76	70													
	400	100	100	100	100	100	100	95	93	93	88	85	83	80	78	73	68								
	300	100	100	100	99	95	89	88	85	78	75	70													
	200	100	100	100	100	100	100	100	100	98	97	95	91	89	87	86	85	79	78	75	73	70			
	100	100	100	100	100	100	100	100	100	99	97	93	87	86	83	78	75	70							
Control	500	100	99	98	92	88	81	78	75	72	68														
	400	100	100	100	100	100	100	98	86	75	73	68													
	300	100	98	95	94	87	85	80	78	76	70														
	200	100	100	100	100	99	97	95	93	89	87	85	78	76	70										
	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	70			

* Each value is a mean of duplicates

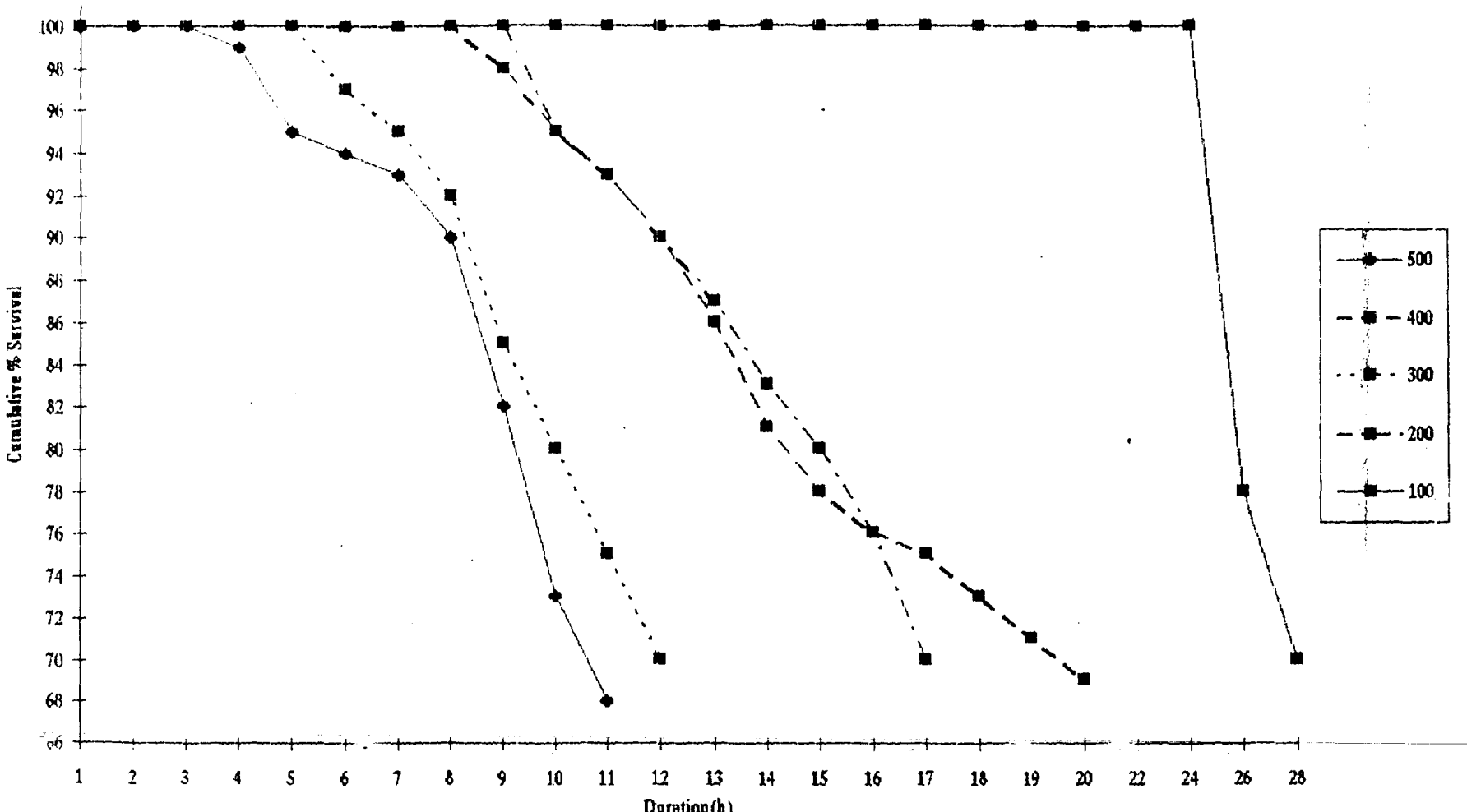


Fig(1) showing cumulative % survival of *Lira parsia* fry at selected doses of chloral hydrate.

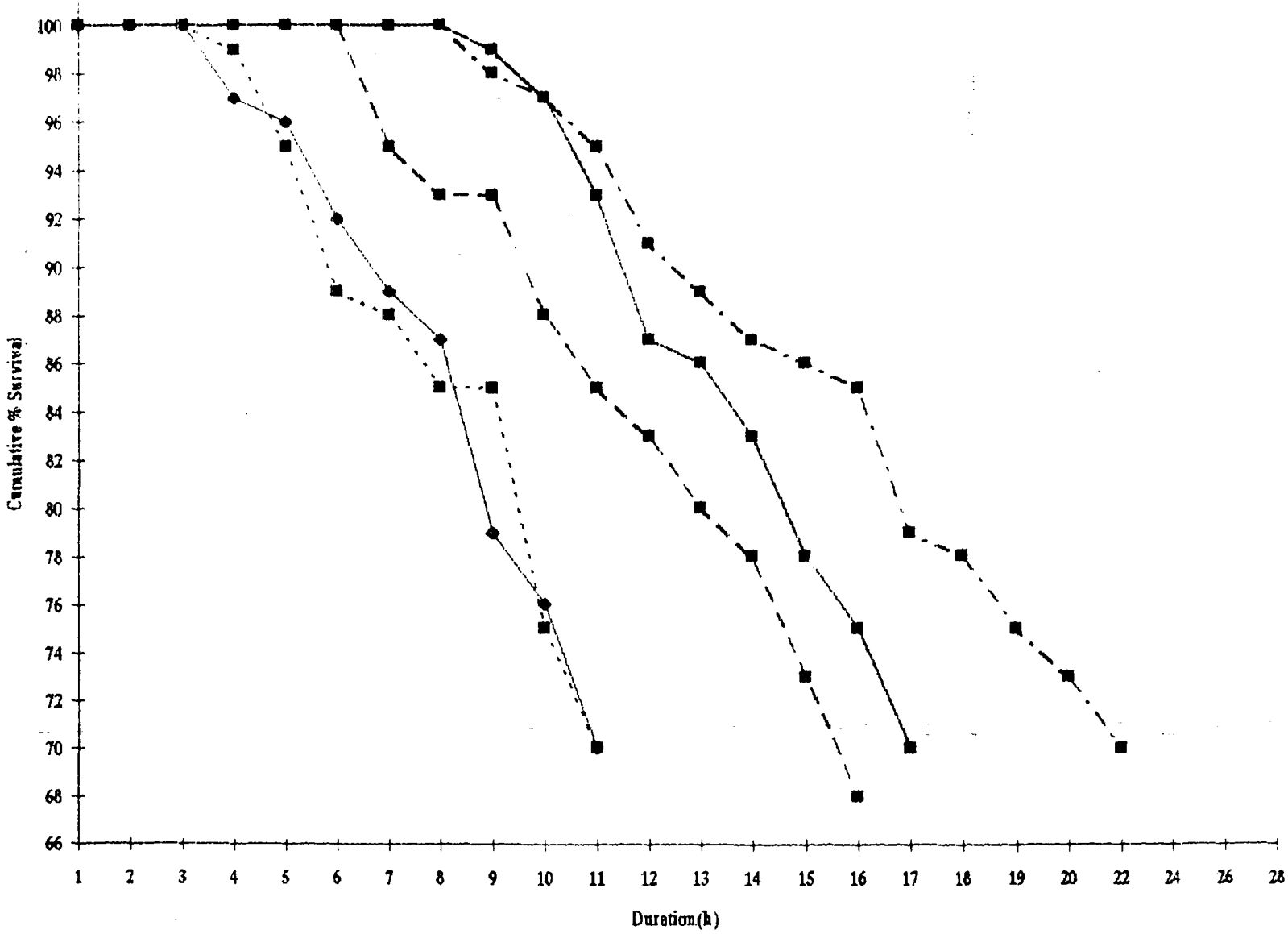
The cumulative percentage survival of the oxygen-packed fry at five packing densities under the anaesthetic tertiary butyl alcohol are shown in fig 2. 24.8 h, 13.0 h, 8.3 h, 13.0 h and 8.0 h were the durations of 90% survival, 25.8 h, 15.0 h, 10.0 h, 14.4 h and 9.3 h of 80% survival and 28.0 h, 18.0 h, 12.0 h, 19.5 h and 10.6 h of 70% survival at packing densities 100, 200, 300, 400 and 500 fry/l respectively. The durations of 90%, 80% and 70% survival of fry treated with tertiary butyl alcohol were found to decrease with increase in packing density.

The cumulative percentage survival of the oxygen packed fry treated with clove oil, at five packing densities are graphically represented in fig 3. Durations of 90% survival were observed as 12.0h, 12.3h, 5.8h, 10.6h and 6.7h; those of 80% survival were 15.6h, 16.8h, 8.7h, 13.0h and 8.9h and those of 70% survival were 18.0h, 22.0h, 11.0h, 15.6h and 11.0h at the packing densties of 100, 200, 300, 400 and 500 fry/l respectively.

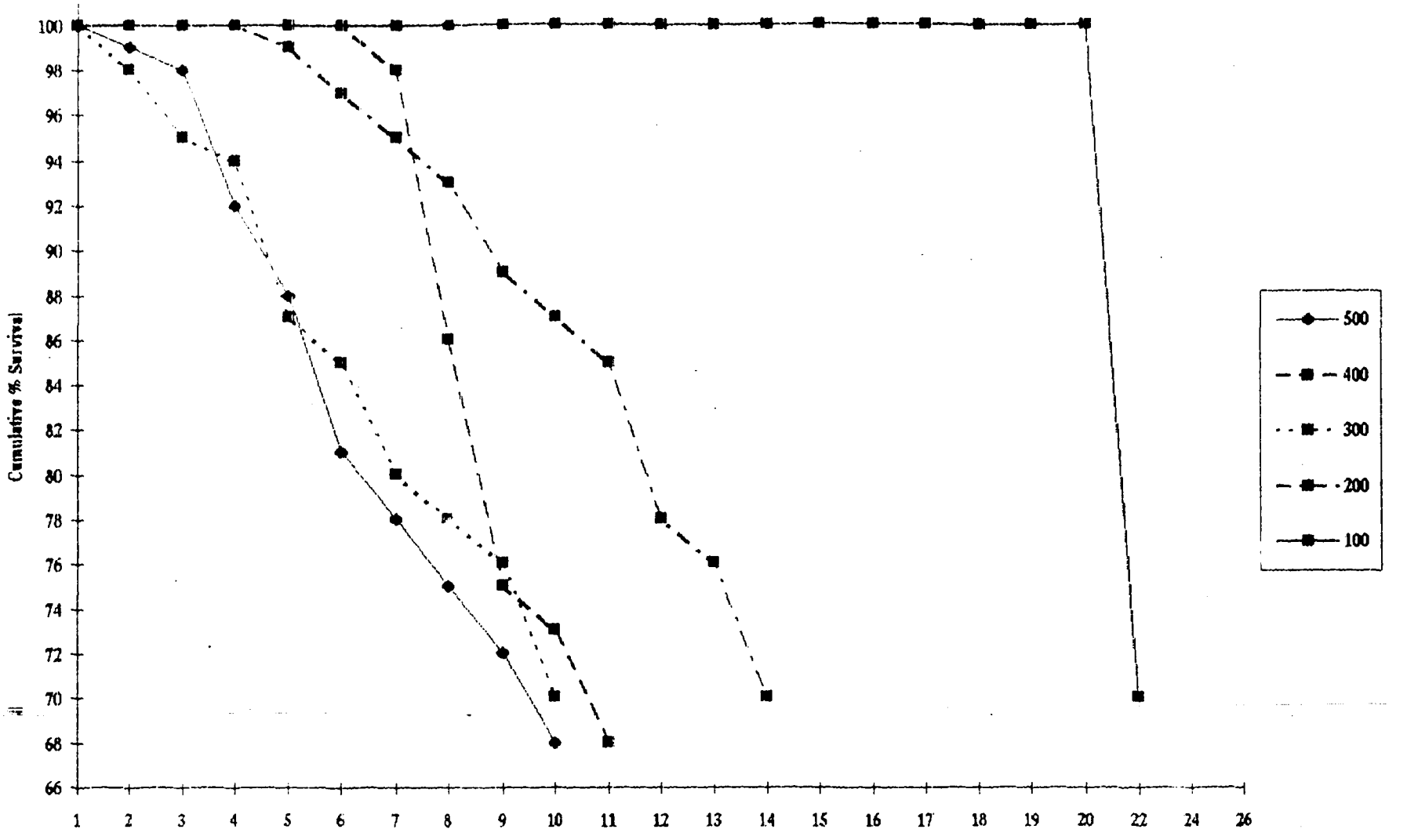
The cumulative percentage survival of the oxygen-packed fry of control at five different packing densities are represented in fig 4. At the packing densities of 100, 200, 300, 400 and 500 fry/l durations of 90% survival were 20.8 h, 8.7 h, 4.6 h, 7.7 h and 4.5 h; those of 80% survival were 21.4 h, 11.7 h, 7.0 h, 8.5 h, 6.3 h and those of 70% survival were 22.0 h, 14.0 h, 11.0 h, 10.6 h and 9.5 h respectively. The durations of survival were found to decrease with increase in packing density of at 70% survival.



Fig(2) showing cumulative % survival of *Liza parsia* fry at selected doses of tertiary butyl alcoholol



Fig(3) showing cumulative % survival of *Liza parsia* fry at selected doses of clove oil



Fig(4) showing cumulative % survival of *Liza parsia* fry under control

4.4. Effect of packing density and anaesthetisation on water quality in the oxygen-packed jars

4.4.1. Dissolved oxygen

The initial dissolved oxygen levels of the packing medium in the oxygen-packed jars, under the three anaesthetics and control at different packing densities were found as 32 ppm. The dissolved oxygen values of the packing medium in these jars at 70% survival are shown in table 20. Correlation coefficient values between the packing densities and the levels of dissolved oxygen under the three anaesthetics, chloral hydrate, tertiary butyl alcohol, clove oil and the control were worked out. The respective values were -0.9907, -0.9568, -0.635 and -0.7225. Out of these, the first two values were seen to be significant at 5% level. Hence the linear equations $y= a+bx$ for the treatments with chloral hydrate and tertiary butyl alcohol were worked out as follows,

for chloral hydrate treatment $y= 14.676-0.155x$

for tertiary butyl alcohol treatment $y= 18.408-0.226x$

where x = packing density ; y= dissolved oxygen in ppm.

Analysis of variance done to analyse the dissolved oxygen in the oxygen-packed jars at selected doses of the three anaesthetics and control at five packing densities at 70% survival (table 21) showed no significant difference between anaesthetics and control.

Table 20. Dissolved oxygen (ppm) in the oxygen-packed jars at selected doses of the three anaesthetics and control at five packing densities at 70% survival of *L. parsia* fry and the correlation coefficients between the packing density and dissolved oxygen under different anaesthetics and control.

Packing density (Nos/l)	Dissolved oxygen (ppm)			
	Chloral hydrate (0.3 g/l)	Tertiary butyl alcohol (2.5 ml/l)	Clove oil (8.5 ml/l)	Control
500	7.00	6.70	10.43	6.47
400	10.00	9.29	8.53	4.56
300	11.04	9.75	13.78	6.70
200	13.60	10.59	9.52	13.32
100	16.50	13.80	16.80	10.00
r	-0.9907	-0.9568	-0.635	-0.7225

$$r = \frac{n\sum xy - \sum x \sum y}{\sqrt{\{n\sum x^2 - (\sum x)^2\} \quad \{n\sum y^2 - (\sum y)^2\}}}$$

x = packing density

y = anaesthetic or control

Table 21. Analysis of variance of the dissolved oxygen (ppm) in the oxygen-packed jars at selected doses of the three anaesthetics and control at five packing densities at 70% survival of *L. parsia* fry.

Source	Sum of squares	Degrees of freedom	Mean of squares	F Value computed
Between treatments	42.1813	3	14.0604	1.3
Within treatments	172.9153	16	10.8072	
Total	215.0966	19		

4.4.2. Carbon dioxide

The initial carbondioxide values of the packing medium in the oxygen-packed jars under the three anaesthetics and control at five packing densities were found to be zero. The carbondioxide values in these jars at 70% survival are given in table 22. Correlation coefficients between the five packing densities and the levels of carbondioxide under the three anaesthetics chloral hydrate, tertiary butyl alcohol, clove oil and control were -0.8839, -0.9594, -0.8504 and -0.8704 respectively. Of the above four values, the first two were found to be significant at 5% level. Therefore, the linear equation $y = a+bx$ for the anaesthetics chloral hydrate and tertiary butyl alcohol were worked out and obtained as

for chloral hydrate treatment $y=6.072-0.044x$

for tertiary butyl alcohol treatment $y= 6.072-0.792x$

where x = packing density ; y= carbondioxide in ppm.

Analysis of variance done to analyse the carbon dioxide in the oxygen-packed jars at five packing densities at 70% survival (table 23) showed significant difference among the three anaesthetics and control. Pair wise comparison by critical difference analysis revealed that the three anaesthetics differed significantly from that of the control.

Table 22. Carbon dioxide (ppm) in the oxygen-packed jars at selected doses of the three anaesthetics and control at five packing densities at 70% survival of *L. parsia* fry, and the correlation coefficients between the packing density and carbon dioxide under different anaesthetics and control.

Packing density (Nos/l)	Carbon dioxide (ppm)			
	Chloral hydrate (0.3 g/l)	Tertiary butyl alcohol (2.5 ml/l)	Clove oil (8.5 ml/l)	Control
500	3.52	1.76	1.76	6.47
400	4.40	3.52	3.52	4.56
300	5.28	3.52	5.28	6.70
200	5.28	4.40	4.40	13.32
100	5.28	5.28	5.28	10.00
r	-0.8839	-0.9594	-0.8504	-0.8704

$$r = \frac{n\sum xy - \sum x \sum y}{\sqrt{\{n\sum x^2 - (\sum x)^2\}\ \{n\sum y^2 - (\sum y)^2\}}}$$

x = packing density

y = anaesthetic or control

Table 23. Analysis of variance of carbon dioxide (ppm) in the oxygen- packed jars at selected doses of the three anaesthetics and control at five packing densities at 70% survival of *L. parsia* fry.

Source	Sum of squares	Degrees of freedom	Mean of squares	F Value computed
Between treatments	71.6707	3	23.8902	13.56*
Within treatments	28.1882	16	1.7618	
Total	99.859	19		

* Significant at 5 % level.

Treatment means of the three anaesthetics and control

Control (C)	Chloral hydrate (T_1)	Tertiary butyl alcohol (T_2)	Clove oil (T_3)
8.448	4.752	3.696	4.048

Calculated C. D. value = 1.8

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

Underline indicate 'no significant difference'

4.4.3. Ammonia-N

The initial ammonia-N values of the packing medium in the oxygen-packed jars, under the three anaesthetics and control at five packing densities were found to be zero. The levels of ammonia-N in these jars at 70% survival are presented in table 24. Correlation coefficients between the five packing densities and the levels of ammonia-N using the three anaesthetics chloral hydrate, tertiary butyl alcohol, clove oil and control were 0.88103, 0.7391, 0.7746 and 0.63. Of the four values cited above only the first one was found to be significant at 5% level. Hence the linear equation Y= a+bx for the anaesthetic chloral hydrate was obtained as follows,

for chloral hydrate treatment y= -0.0011+ 0.0063x

where x = packing density ; y= ammonia-N in ppm.

Analysis of variance done to analyse the ammonia-N in the oxygen-packed jars at selected doses of the three anaesthetics and control, at five packing densities, at 70% survival (table 25) showed no significant difference amongst the three anaesthetics and control.

4.4.4. pH

The initial pH in the treated and control jars at the different packing densities was obtained as 7-7.5. The final pH in the oxygen-packed jars at 70% survival was found to be in the same range (7-7.5).

Table 24. Values of Ammonia - N in the oxygen-packed jars at selected doses of the three anaesthetics and control at five packing densities at 70% survival of *L. parsia* fry, and the correlation coefficients between the packing density and ammonia -N under different anaesthetics and control.

Packing density (Nos/l)	Ammonia - N (ppm)			
	Chloral hydrate (0.3 g/l)	Tertiary butyl alcohol (2.5 ml/l)	Clove oil (8.5 ml/l)	Control
500	0.3650	0.390	0.358	0.320
400	0.2380	0.338	0.335	0.380
300	0.1085	0.080	0.109	0.090
200	0.1180	0.160	0.280	0.290
100	0.1100	0.175	0.030	0.101
r	-0.0011	0.7391	0.7746	0.630

$$r = \frac{n\sum xy - \sum x \sum y}{\sqrt{\{n\sum x^2 - (\sum x)\} \quad \{n\sum y^2 - (\sum y)^2\}}}$$

x = packing density

y = anaesthetic or control

Table 25. Analysis of variance of ammonia - N (ppm) in the oxygen - packed jars at selected doses of the three anaesthetics and control at five packing densities at 70% survival of *L. parsia* fry.

Source	Sum of squares	Degrees of freedom	Mean of squares	F Value computed
Between treatments	0.0068	3	0.0023	0.1332
Within treatments	0.2732	16	0.0171	
Total	0.28	19		

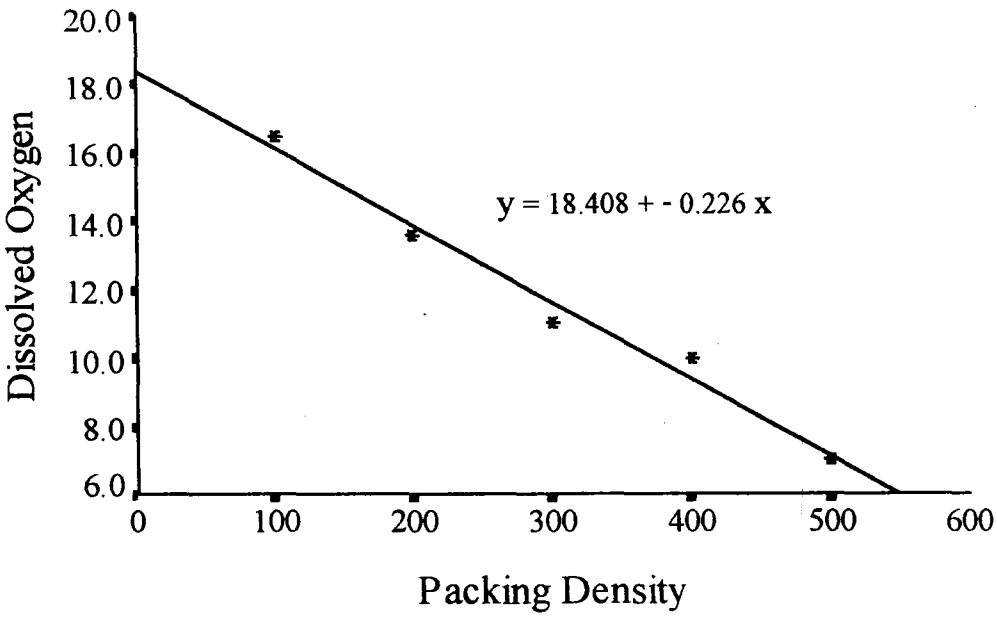


Figure 5 Graph showing the relationship between packing density and dissolved oxygen in the oxygen-packed jars at 70% survival under chloral hydrate treatment

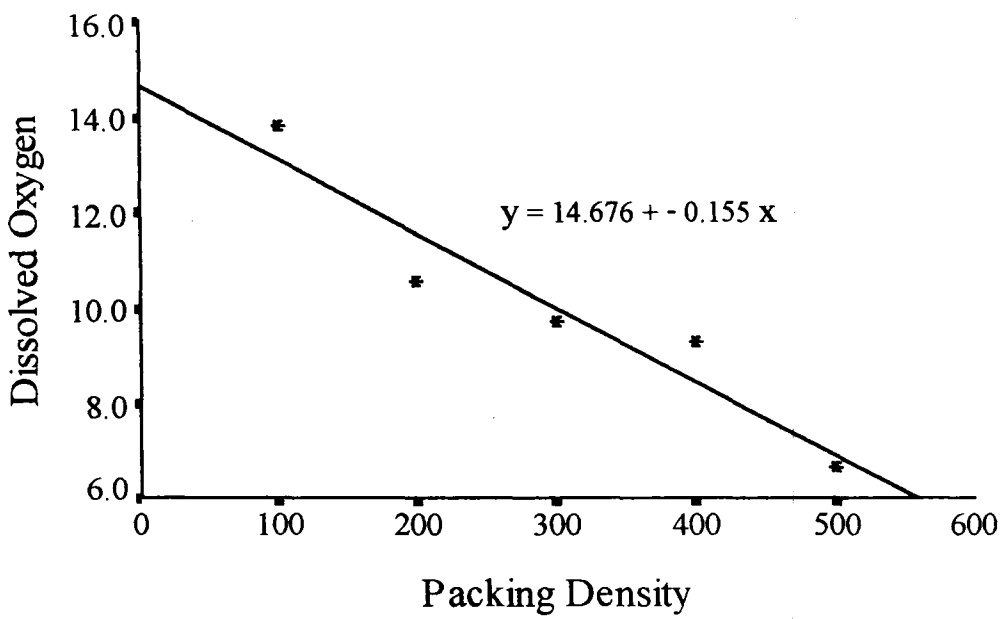


Figure 6 Graph showing the relationship between packing density and dissolved oxygen in the oxygen-packed jars at 70% survival under tertiary butyl alcohol treatment

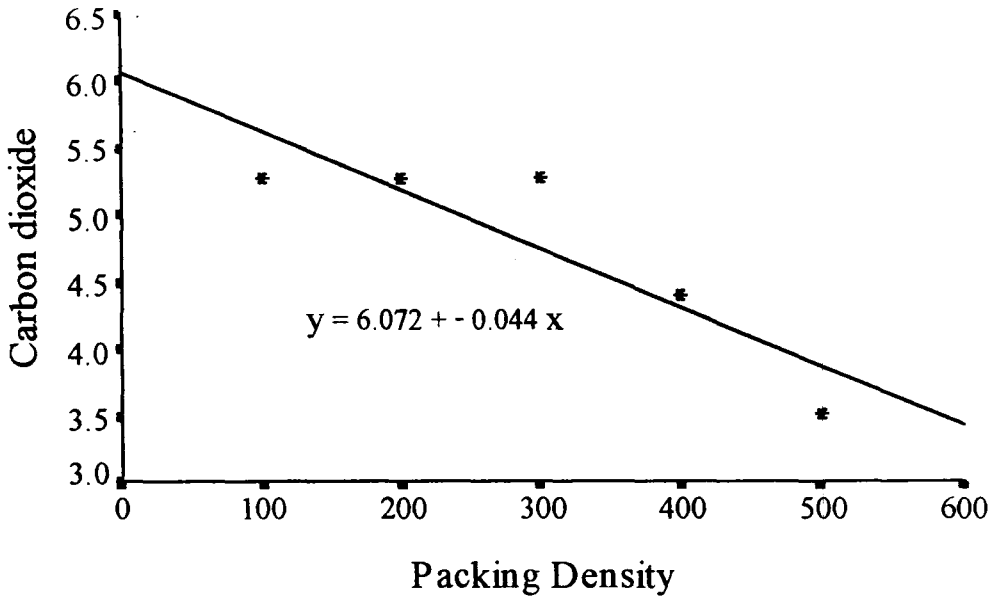


Figure 7 Graph showing the relationship between packing density and carbon dioxide in the oxygen-packed jars at 70% survival under chloral hydrate treatment

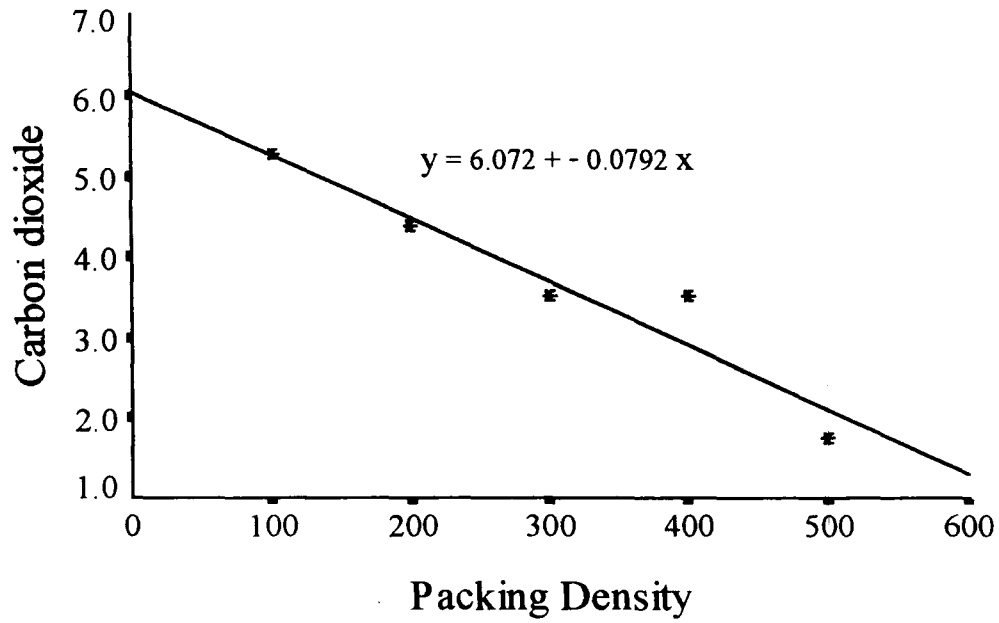


Figure 8 Graph showing the relationship between packing density and carbon dioxide in the oxygen-packed jars at 70% survival under tertiary butyl alcohol treatment

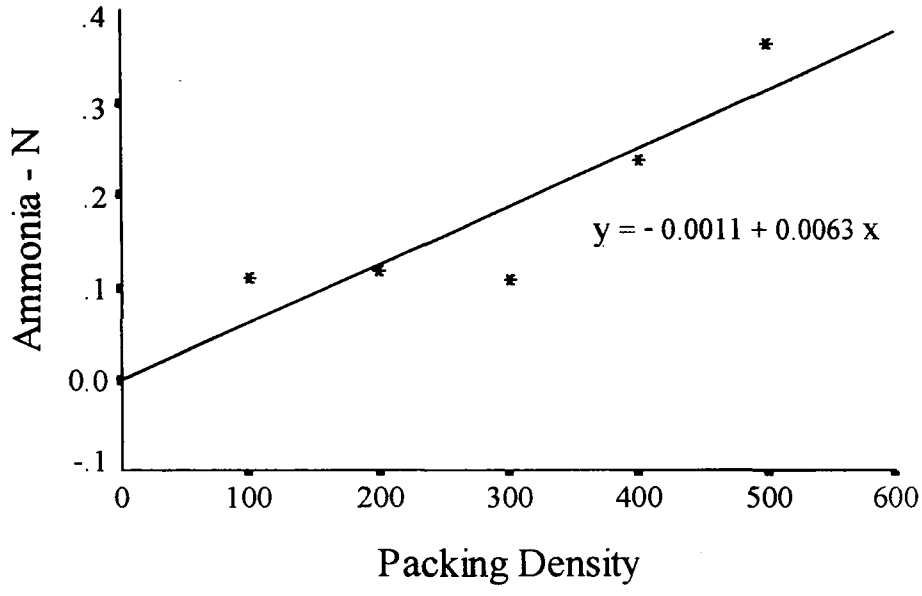


Figure 9 Graph showing the relationship between packing density and ammonia-N in the oxygen-packed jars at 70% survival under chloral hydrate treatment

DISCUSSION

5. DISCUSSION

5.1 Optimum dose of the three anaesthetics.

5.1.1. Chloral hydrate.

The behavioural pattern of the fry treated with three different doses of chloral hydrate (0.2 g/l, 0.25 g/l and 0.3 g/l) is indicative of light sedation as reported by Durve and Dharmaraja (1966) and Durve (1975). Light sedation is preferred for transportation of fish fry (Durve, 1975; Dupree and Huner, 1982 and Ferreira *et al.*, 1984 a). The behaviour of mullet fry during the stage of light sedation was described by Durve (1975). In light sedation, swimming activity slows down and the fish very often remains stationary in the medium. The fish does not react quickly to external stimuli. Swimming is mainly by sideward movements of the tail and head rather than by beating of fins. Instead of the sideward movement described by Durve (1975) in light sedation, in the present study the fry were found to remain in the water column with minimum movement. Of the three dose tried in the present study 0.3 g/l took the least induction time of 30 minutes. The recovery time was also clear (3-4 minutes). The rate of oxygen consumption by the fry treated with this dose was the lowest (0.324 mg/g body wt. /h). No mortality occurred during the observed period of 3 days. The fry were observed to take feed actively. Durve (1975) used the doses of 0.1g/l and 0.25 g/l for light and deep sedation respectively for other mullet species, *Mugil cephalus, Liza tade* and *Liza dussumieri*. The present study revealed that the dose of 0.3 g/l was required by *L. parsia* for light sedation. Chloral hydrate possesses all the basic attributes required by fish

anaesthetics for transportation (McFarland, 1960 ; Durve and Dharmaraja, 1968; Durve, 1975). Durve and Dharmaraja (1966) and Durve (1975) stated that the induction and recovery times were difficult to measure in the case of light and deep sedation. It is also reported that chloral hydrate has a prolonged induction time though recovery from it is comparatively quicker (Durve, 1970; Durve, 1975). Chloral hydrate lowered the rate of oxygen consumption of mullets by 60-61% (Durve, 1975). The percentage decrease of oxygen consumption obtained for the dose of 0.3 g/l was 58.5% less than the control, which is almost in conformity with that obtained by Durve (1975). No mortality was observed in the present study on transferring the treated fry to anaesthetic-free water, which is also in agreement with Durve (1975).

5.1.2. Tertiary butyl alcohol

The behavioural pattern of the fry treated with doses 3.0 and 3.5 ml/l of tertiary butyl alcohol exhibited deep sedation. Induction of deep sedation is undesirable for transportation (McFarland, 1960 ; Ferseira *et al.*, 1984 a). Durve (1975) described deep sedation as; the fish remains stationary at the bottom or moves very slowly at the bottom. Swimming is mainly by vigorous sideward movements of the head and tail. It does not react to external stimuli. But, in the present study, though the behavioural pattern of the fry in deep sedation conforms with that described by the above author, swimming by vigorous sideward movements of the head and tail was not observed. Instead, the fish were found to occupy the bottom and intermittently coming to the water column. Treatment with the dose of

2 5 ml/l indicated light sedation. Durve (1975) used 3ml/l and 3.5 ml/l tertiary butyl alcohol for inducing light and deep sedation respectively in *M.cephalus*, *L. tade* and *L. dussumieri*. Alvarez -Lajonchere and Moreno (1982) too agreed with these doses for light and deep sedation of fry of *M. trichodon*. The induction time for light and deep sedation of fry of *M. trichodon* was 30-40 minutes (Alvarez -Lajonchere and Moreno, 1982). No accurate induction time was noted by Durve (1975), who indicated that tertiary butyl alcohol did have a long induction time though the recovery time was comparatively quicker. But, in the present study the induction time was observed as 6 and 5-6 minutes at the doses 3.0 and 3.5 ml/l respectively. At the lower dose of 2.5 ml/l, the induction time was longer (15 minutes). The recovery time of the fry treated with all the doses was 4 -5 minutes while, Alvarez - Lajonchere and Moreno (1982) had also tried the dose of 2.5 ml/l which they discarded as they noted sedation in only 10% of the *M. trichodon* fry. Though Durve and Dharmaraja (1968) and Durve (1975) reported the depressing action of anaesthetics on oxygen consumption by tertiary butyl alcohol, in this study, oxygen consumption of the fry treated with all doses of tertiary butyl alcohol was significantly different from that of the control. Although Durve (1975) reported mortality of treated fry when transferred to anaesthetic-free water, no mortality occurred for the observed period of 3 days. This is in agreement with Alvarez -Lajonchere and Moreno (1982).

5.1.3. Clove oil

At the doses of 9.0 and 9.5mg/l of clove oil, the behavioural pattern of the fry showed deep sedation. At the lowest dose of 8.5 mg/l the fry exhibited light sedation. The induction time at this dose was 15 minutes. The recovery time for all the doses tried was 2-5 minutes Oxygen consumption of the fry at the dose of 8.5 mg/l was lower than that of the control. No mortality occurred during the observed period of 3 days after, the treated fry where transferred to anaesthetic-free water. Burhanuddin *et al.*, (1989) demonstrated the potential of clove oil as an anaesthetic for transport of Rabbit fish *Siganus guttatus*. A concentration of 10 mg/l caused this fish to lose balance for 12 h while 15 mg/l caused the fish to lose consciousness for 20 h after which they began to die. Soto and Burhanuddin (1995) used clove oil for length and weight measurement of Rabbit fish *S.lineatus*. No mortality was observed after the fish were transferred to anaesthetic-free water. Also the fish were found to feed shortly afterwards. Since there is lack of previous information on the use of clove oil on mullets, the present data could not be compared. However, the doses tried in the present study are very close to those tried by Burhanuddin *et al.*, (1989).

5.2. Effect of the three different anaesthetics on the metabolic rate

The oxygen consumption with the three anaesthetics (chloral hydrate, tertiary butyl alcohol and clove oil) at optimum doses (0.3 g/l, 2.5 ml/l and 8.5 mg/l) was less than that of the control. The rate of oxygen consumption of the fry treated with

chloral hydrate (0.324 mg/g body wt./h) and clove oil (0.2716 mg/g body wt./h) were significantly different from that with tertiary butyl alcohol (0.7994 mg/g body wt. /h) Durve and Dharmaraja (1968) and Durve (1975) showed the depressing action of anaesthetics on *M. cephalus, L. tade* and *L. dussumieri* and claimed that the anaesthetics have the potential for the safe and efficient transport of the mullet fry Chloral hydrate reduced the oxygen consumption of these mullet fry (*M. cephalus, L. tade* and *L. dussumieri*) by 60.61% (Durve, 1975), but reports on oxygen consumption with the anaesthetics tertiary butyl alcohol and clove oil are lacking. It has been reported by several authors that the metabolism under sedation is close to the basal metabolic rate of a particular fish species with subsequent reduced oxygen consumption and with a release of reduced quantities of metabolic breakdown products such as ammonia and carbon dioxide (McFarland, 1959 ; Ferreira *et al.*, 1979, Smit, 1980; Takashima *et al.*, 1983; Desilva *et al.*, 1986 ; Teo *et al.*, 1989; Teo and Chen, 1993 ; Guo *et al.*, 1995 b and Peer Mohammed and Devaraj, 1997). An ideal fish anaesthetic should reduce oxygen consumption and stress during transportation (Marking and Meyer, 1985). Ferreira (1982) and Ferreira *et al.*, (1984 a) reported that light sedation was better than deep sedation for transportation as at the latter stage the fish usually show signs of loss of equilibrium accompanied by active swimming movements to rectify the imbalance. It subsequently results in higher metabolic rates, with subsequent increased oxygen consumption and release of higher concentrations of carbon dioxide and ammonia. Considering these factors the doses which exhibited light sedation were selected for the oxygen-packed experiments.

5.3. Effect of packing density and anaesthetisation on survival and duration of *L. parsia* fry during oxygen-packed transportation

5.3.1. Survival and duration

The percentages of survival from 100% down to 70% of the treated fry at all packing densities tried, showed that they were noticeably better than that of the control. Using chloral hydrate at the packing density of 500 fry /l, the duration of 100% survival was 4h while that of the control was only 1.5 h. The corresponding survival with tertiary butyl alcohol and clove oil were 4.5 h, 8.5 h, 6.0 h at 400 fry/l ; 3.5 h, 5.0 h and 3.5 h at 300 fry/l; 6.5 h, 9.0 h and 8.0 h at 200 fry/l and 10.0 h, 24.0 h and 8.5 h at 100 fry/l respectively, but that of the controls were 6.0 h, 1.0 h, 4.5 h and 20.0 h at 400, 300, 200 and 100 fry/l respectively. The durations of 90% survival with chloral hydrate, tertiary butyl alcohol and clove oil were 7.2 h, 8.0 h, 6.7 h, at 500 fry/l ; 7.0 h, 13.0 h and 10.6 h at 400 fry/l ; 6.3 h, 8.3 h and 5.8 h at 300 fry/l ; 9.0 h, 13.0 h and 12.3 h at 200 fry/l and 13.6 h, 24.8 h and 12.0 h at 100 fry/l. The corresponding durations of the control at the above packing densities were 4.5 h, 7.7 h, 4.6 h, 8.7 h and 20.8 h. The durations of 80% survival with chloral hydrate, tertiary butyl alcohol and clove oil were 8.7 h, 9.3 h, 8.9 h at 500 fry/l ; 8.5 h, 14.4 h and 13.0 h at 400 fry/l ; 9.0 h, 10.0 h and 8.7 h at 300 fry/l ; 12.7 h, 15.0 h and 16.8 h at 200 fry/l and 18.0 h, 25.8 h and 15.6 h at 100 fry/l respectively. But for the control, the duration of 80% survival at the above packing densities were 6.3 h, 8.5 h, 7.0 h, 11.7 h and 21.4 h respectively. Also the durations of 70% survival with the

anaesthetics chloral hydrate, tertiary butyl alcohol and clove oil were 11.0 h, 10.6 h, 11.0 h at 500 fry/l, 10.0 h, 19.5 h and 15.6 h at 400 fry/l ; 12.5 h, 12.0 h and 11.0 h at 300 fry/l ; 16.0 h, 18.0 h and 22.0 h at 200 fry/l and 22.0 h, 28.0 h and 18.0 h at 100 fry/l respectively. The corresponding durations of control at the above packing densities were 9.5 h, 10.6 h, 11.0 h, 14.0 h and 22.0 h.

The above data revealed remarkable difference in durations of survival from that of the control at 500 fry/l, 400 fry/l, 300 fry/l and 200 fry/l. However, analysis of variance did not reveal any significant difference. Present data could not be compared for lack of previous information on these lines. Although the selected doses of the anaesthetics did show significant effect on metabolic rate of the fry, the supersaturated dissolved oxygen levels in the oxygen-packed jars might be playing some role in nullifying the effect at these doses. In-depth study regarding the required levels of these anaesthetics in the oxygen-packed jars is warranted. McFarland (1960) reported that comparison of the different anaesthetised groups with the control groups gave different values for inhibition of mortality for the various anaesthetics. He also mentioned that the differences demonstrated were probably the result of slightly different degrees in the depth of sedation. Though pre-treatment with anaesthetic was done as a measure to reduce stress, the visual indications of loss of reactivity were reported by him not to correspond to immediate decline of the metabolism to basal levels. Black *et al.*, (1939) stated that the mere handling of fish when placing them in respiration chambers caused a maximum oxygen uptake for several hours. In any transport operation the factors that limit the cause of death in fishes were difficult to ascertain (Black, 1958 ; McFarland and

Norris, 1958). Though the factor most often implicated was depletion in the supply of oxygen to the tissues, this could not be overcome by supplying high oxygen tensions in the water (McFarland, 1959). He also emphasised that the same concentration would induce both stages of anaesthesia (light and deep) due to the physiological response of the individual fish to the concentration. Though the durations of survival decrease with increase in packing density, the reason for increased duration obtained at 400 fry/l both in treated and control fry may be due to the cooler temperatures (about 26^0C) during the experiment. The slightly lower durations of survival at 100 fry/l than that of the control may be due to the stress caused by the anaesthetic medium for long duration.

5.4. Effect of packing density and anaesthetisation on water quality in the oxygen-packed jars

Linear relationship could be established between packing density and dissolved oxygen levels in the jars at 70% survival for treatments with chloral hydrate and tertiary butyl alcohol, but not in clove oil treatment. No significant difference was observed in the final dissolved oxygen levels amongst anaesthetic treatments and also with control. Linear relationships could also be established between packing density and carbon dioxide levels in the jars at 70% survival for treatments with chloral hydrate and tertiary butyl alcohol, not with clove oil treatment. Significant difference was observed in the final carbon dioxide levels between the three anaesthetic treatments and control. Linear relationship between packing density and ammonia - N values in the oxygen-packed jars at 70% survival

could be established only for chloral hydrate. But no significant difference was observed in the final ammonia -N levels amongst the anaesthetic treatments and also with control.

Ferreira *et al.*, (1984 a) reported that anaesthetics could slow down deterioration in the water quality over a period of time. This might be due to the reduced stress upon the fish and the consequent decline in metabolic activities of the fish so treated. McFarland (1959) found that the fish under sedation had a metabolic rate close to its basal metabolic rate, which reduced quantities of metabolic breakdown products. Ferreira *et al.*, (1979 b) ; Smit (1980) showed that low production of carbon dioxide resulted in minor changes in the pH of the holding water. But in the absence of anaesthetic, slight increases in the carbon dioxide levels took place accompanied by decrease in pH and alkalinity levels. According to Bouck and Ball (1966), Hattingh and Van Pletzen (1974) and Smit (1980) such environmental conditions may lead to undesirable stress situations. Moreover the anaesthetic themselves might affect the water quality by changing pH, carbon dioxide, alkalinity, calcium hardness and conductivity levels of the water (Ferreira *et al.*, 1979 b). But the use of anaesthetics ensured that the fish stayed calm and prevented drastic changes in water quality, enhancing the successful transport of larger numbers in a given volume of water over longer periods of time (Ferreira *et al.*, 1984 a). Using anaesthetics 2-phenoxyethanol, quinaldine sulphate, MS-222 and metomidate in oxygen-packed experiments with Platyfish, the water pH dropped rapidly in both control and treatment groups indicating massive increase of carbon dioxide in the water without corresponding increase in ammonia during the first 4 h

of the packaging experiment according to Guo *et al.*, 1995 a. They reported that it was probably due to excitement of fish caused by handling and packaging resulting in sudden increase in metabolic rate. But in the next 12 h the fall in pH decelerated. Though the anaesthetics chloral hydrate and tertiary butyl alcohol used in this study were tried in oxygen-packed transportation of mullet seed by Durve (1975), no mention has been made on the water quality parameters.

SUMMARY

6. SUMMARY

The main aims of the study (1) to evaluate the effect of three selected anaesthetics, viz., chloral hydrate, tertiary butyl alcohol and clove oil on the metabolic rate of the *L. parsia* fry, (2) to determine the effect of different packing densities and anaesthetisation of the mullet fry on survival rate and duration of transport and (3) to find out the important changes in the water quality parameters in the oxygen-packed containers.

Fry of *L. parsia* of average weight 0.22 g obtained from natural collections were used for the experiments. They were maintained in brackish water of 7 ppt salinity at a temperature of 28 ± 2^{0}C. Respirometers of 136 ml capacity were used to find the oxygen consumption rate. Each was fitted with a two-holed rubber stopper for the inlet and outlet tubes. They were placed in a trough containing water to minimise temperature fluctuations. Air tight transparent hard plastic jars of 600 ml capacity were used for the oxygen-packed experiments. They were provided with screw type lids with one way valves which facilitated uniform initial oxygen pressure in the jars.

The optimum dose of each of the anaesthetics viz., chloral hydrate, tertiary butyl alcohol and clove oil was determined from the doses tried initially, i.e., 0.2, 0.25 and 0.3 g/l of chloral hydrate ; 2.5, 3.0 and 3.5 ml/l of tertiary butyl alcohol and 8.5, 9.0 and 9.5 mg/l of clove oil. The anaesthetic solutions of the required strength were prepared in water of 7 ppt salinity. The optimum doses were selected taking into account the behavioural pattern of the fry, the induction and recovery

times, the rate of oxygen consumption and post treatment survival. CRD was used for planning the experiment.

Of the three doses tried for chloral hydrate the dose of 0.3 g/l was selected for oxygen-packed experiments. At this dose the induction time was 30 minutes and the recovery time 3 to 4 minutes. The fry exhibited light sedation. Also the rate of oxygen consumption was found to be the least and different from the control. Of the three doses of tertiary butyl alcohol tried, the lowest dose of 2.5 ml/l was used for oxygen-packed experiments. The induction and recovery times were 15 minutes and 4 to 5 minutes respectively. At this dose light sedation was observed and the rate of oxygen consumption differed significantly from the control. Of the three doses of clove oil 8.5 mg/l was selected for oxygen-packed experiments. The induction and recovery times were 15 minutes and 2 to 5 minutes respectively. Light sedation and lowest rate of oxygen consumption was obtained at this dose. The treated and control fry when transferred to anaesthetic free water survived for the observed period of 3 days. The treated fry were found to be as active as the control.

The effect of the three anaesthetics at optimum doses on the metabolic rate of the fry was compared by analysis of variance.

Comparison of the effect of the optimum dose of the three anaesthetics on the metabolic rate showed that all the three anaesthetic treatments differed from the control, while tertiary butyl alcohol treatment differed from the other two. Chloral hydrate and clove oil belonged to one group. They were found to be better than the other anaesthetic as they gave a lower oxygen consumption value.

Determination of the effect of packing density and anaesthetisation on survival and duration of transport of oxygen-packed fry was carried out at 100, 200, 300, 400 and 500 fry/l using fry treated with the selected doses of the three anaesthetics viz., 0.3 g/l of chloral hydrate, 2.5 ml/l of tertiary butyl alcohol and 8.5 mg/l of clove oil at 7 ppt salinity and ambient temperature 28 ± 2^0C. The control was also maintained. The jars were filled with oxygen under uniform pressure of 0.2 kg/cm^2. RBD was used for planning the experiment. The effect of packing density and anaesthetisation on water quality in oxygen-packed jars at 70% survival was studied. The parameters analysed included dissolved oxygen, carbon dioxide, ammonia -N and pH.

Treatment of the fry using the three anaesthetics at the selected doses had no significant effect on the survival and duration in the oxygen-packed jars. Packing density had significant effect on the durations from 100 down to 70% survival. The safe durations with 100% survival was 15.6 h at 100 fry/l and 3 to 7 h at 200, 300, 400 and 500 fry/l.

Analyses of the water quality parameters in the oxygen-packed jars showed that in the case of chloral hydrate treatment linear relationships could be established between packing density and dissolved oxygen, carbon dioxide and ammonia -N values at 70% survival. For tertiary butyl alcohol treatment linear relationships could be established between packing density and dissolved oxygen and carbon dioxide values at 70% survival. No linear relationship between packing density and water quality parameters could be established for treatment with clove oil.

Comparison of the dissolved oxygen levels in the oxygen-packed jars at 70% survival under the three treatments and control showed no significant difference. The carbon dioxide levels in the oxygen-packed jars treated with the anaesthetics differed from the control. No significant difference was obtained in ammonia -N values between the anaesthetic treatments and control at 70% survival. pH variation was only from 7 to 7.5, both in the treatments and control.

ANAESTHETIZATION OF *LIZA PARSIA* (HAMILTON) FRY FOR OXYGEN - PACKED TRANSPORTATION

By

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ABSTRACT OF A THESIS

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7. ABSTRACT

Three fish anaesthetics viz., chloral hydrate at 0.20, 0.25 and 0.30 g/l, tertiary butyl alcohol at 2.5, 3.0 and 3.5 ml/l and clove oil at 8.5, 9.0 and 9.5 mg/l were tried on *L. parsia* fry of average weight 0.22 g, at 7 ppt salinity and at ambient temperature 28 ± 2°C to determine the optimum dose of each. The optimum dose was selected on the basis of behavioural pattern, induction and recovery times, rate of oxygen consumption and post-treatment survival. The optimum doses obtained for chloral hydrate, tertiary butyl alcohol and clove oil were 0.30 g/l, 2.5 ml/l and 8.5 mg/l respectively. The effect of each anaesthetic at the selected dose on the metabolic rate of the fry was compared, and significant difference was observed amongst the three anaesthetics and control at these doses. Chloral hydrate and clove oil treatment resulted in lower oxygen consumption rate than that of tertiary butyl alcohol treatment.

No significant effect was observed on the percentage survival and duration of the fry treated with the three anaesthetics at the selected doses in the oxygen-packed jars. Packing density had significant effect on the survival and duration of oxygen-packed fry. The safe duration with 100% survival was 15.6 h at 100 fry/l and 3 to 7 h at 200, 300, 400 and 500 fry/l.

Linear relationships could be established in the case of (1) chloral hydrate treatment, in the oxygen-packed jars at 70% survival between (a) packing density and dissolved oxygen levels, (b) packing density and carbon dioxide levels and (c) packing density and ammonia -N levels and (2) for tertiary butyl alcohol treatment

between (a) packing density and dissolved oxygen levels and (b) packing density and carbon dioxide levels. Significant difference was observed in the carbon dioxide levels between treated and control jars, while no significant difference was noted in the dissolved oxygen and ammonia -N levels in the treated and control jars.

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