UNIVERSITI PUTRA MALAYSIA

# ANAESTHETIC MAINTENANCE OF GRASS CARP, CTENOPHARYNGODON IDELLA, FINGERLINGS PACKED IN DIFFERENT VOLUMES OF WATER WITH VARYING CONCENTRATION OF HALOTHANE/OXYGEN ATMOSPHERES

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by

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To my beloved mum and dad and all at home,

Thank you for your love and encouragement given throughout the period of my study in UPM and also for your support which enabled me to complete my degree course in UPM

my love and gratitude always........................

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## ABSTRACT :

Anaesthetic maintainance of grass carp, Ctenopharyngodon idella, fingerlings packed in different volumes of water with varying concentration of halothane/oxygen atmosphere.

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supervised by Dr. Ian Anderson.

The efficacy and relationship of halothane, as maintenance anaesthetic chemical, in combination with the volume of 0.5% NaCl solution used in transporting grass carp was investigated. It was found that halothane at high concentrations was contraindicated and not suitable for fish packing with a view for transportation in a closed system. A loading density of approximately 62.7g of grass carp per liter of packing solution was achieved using a 0.5% halothane/oxygen mixture.

## ABSTRAK :

Kecekapan dan perhubungan di antara halothane, sebagai ubat bius, berkombinasi dengan isipadu larutan 0.5% NaCl untuk pembungkusan anak ikan kap rumput hidup di selidiki. Adalah di dapati bahawa kepekatan halothane yang tinggi tidak sesuai untuk membungkus kap dalam sistem secara tertutup bertujuan untuk pengangkutan. Kepadatan muatan 62.7g anak kap rumput per liter larutan pembungkusan boleh dicapai dengan menggunakan campuran gas 0.5% halothane/oksigen.

## INTRODUCTION :

Aquaculture is fast becoming a multimillion dollar industry in many parts of the world. Malaysian aquaculture can be divided into two sectors: the production of food fish and the production of aquarium or ornamental fish.

Some of the common problems faced by aquaculturists in obtaining fry and fish marketing are the high cost of transportation and the high mortality during and after transportation (Dick, 1975; Hattingh et al, 1975).

Fishes are commonly transported under two systems, open and closed. In the open system, open drums or tanks filled with continously aerated water are used. The open method is common in overland shipments. The closed system utilises polyethylene bags containing water (approximately 1/3 total volume) to which fishes are added and the remaining two thirds is inflated with oxygen. The bag is sealed by twisting the opening of the bag together, folding the twisted end and binding with rubber bands.

Low densities of fishes is the main factor considered in ensuring high survival of post transported fishes. Low salinities of water and/or anaesthetic chemicals have been used successfully to transport fishes with increased survival rates. Tricaine methanesulphonate (MS 222), benzocaine hydrochloride, quinaldine sulphate, propanidid and halothane are some of the anaesthetic agents used in transportation of fishes (Blasicola, 1977; Stuart, 1981; Siwicki, 1984; Ross and Ross, 1984). Refer to Table I.

The efficacy and relationship of halothane, as a maintenance anaesthetic chemical, in combination with the amount of water used in transporting grass carp, _Ctenopharyngodon idella_, was investigated in this study. The aim of the study was to examine the possibility of reducing transportation costs by reducing packing weight but at the same time maintaining high survival rates.

## LITERATURE REVIEW :

Anaesthesia is the reversible state of insensibility of an organism enabling an uninteruppted manipulation by the operator to be carried out. Anaesthesia is used in fishes during operative procedures eg. surgery, experimental and farm procedures, and transportation (Stuart,1981). Several workers reported that the major route of entry and excretion of drugs was through the gills (Hunn and Allen, 1974 and Houston and Woods, 1976). Ferreira _et al_ (1984) suggested that anaesthetic drugs move across the gills by a diffusion process and indicated that the skin was another possible route of absorption.

Transported fishes can die through transportation stress, inadequate oxygen, toxicity from the accumulation of metabolites ( eg. ammonia ($NH_3$) and carbon dioxide ($CO_2$)), bacteria blooms, pH and temperature stress and excess activity (Stuart, 1981; Amend et al,1982). Weak saline solutions and anaesthetic chemicals can reduce mortalities during transportation (Collins and Hulsey, 1963; Hattingh et al, 1975 Johnson and Metcalf, 1982). They have also been used to achieve loading densities of 50g-90g of fish per liter of water resulting in an increase in the economics of transportation (Dick, 1975; Amend et al, 1982).

Several workers have found that blood osmolarity decreases after capture and transport in certain fishes (Wedemeyer, 1972 ; Nikinmaa et al, 1983). NaCl at a concentration of 0.5% w/v can counter these osmoregulatory problems by inhibiting the decrease in plasma and sodium concentration (Wedemeyer, 1972 and Hattingh et al, 1975) and will also diminish the hyperglycemia that occurs in stress (Wedemeyer, 1972).

Haswell et al, (1982) observed a decrease in blood pH in the mudfish (Labeo umbratus) after capture and transport. Decreased blood pH produces an inteference with normal oxygen transport, increased blood viscosity due to erythrocytic swelling and reduced myocardial contractility (Wood et al, 1977). Introduction of NaCl or, in particular $Na_2SO_4$ or $NaHCO_3$ into the hauling water helped ameliorate the acid-base disturbance associated with hauling and handling (Haswell al,1982).

Anaesthesia can aid handling, loading and transportation operations by inducing quienscence to the fish (Dick, 1975). The state of quiescence and reduced metabolic activity in fishes caused the reduction in excretions of $NH_3$ and $CO_2$ and in oxygen consumption. Consequently the pH values and quality of the transport water remained fairly constant ( Ferreira et al, 1984; Ross and Ross, 1984). The anaesthetic related improvements in quality during transportation enables fishes to be transported at higher loading densities and for longer periods of time with high survival rates (Amend et al, 1982). Anesthesia can also be used to minimise stress and physical damage resulting from capturing, handling and transporting (Ross and Ross, 1984).

Tricaine methanesulphonate, a crystalline water soluble powder, is marketed under the commercial name MS 222. MS 222 is very soluble in water, exerts a prompt response and has a wide margin of safety (Sawyer, 1982). It is widely accepted as being effective for fishes and other cold blooded animals (Lumb and Jones, 1973). It is the only anaesthetic chemical registered for use in food fish by the United States Environmental Protection Agency and the Food and Drug Administration but requires a twenty-one day withdrawal period (Schnick and Meyer, 1978). It has been found to cause physiological consequences such as hypoxia, hypercapnia, hyperglycemia and changes in blood electrolytes, hormones, cholesterol, urea, lactate and interrenal ascorbic acid (Ross and Ross, 1984). In anaesthetising most common fishes (teleosts), concentrations of 0.11g to 0.23g of MS 222 per liter of water at temperatures maintained at 40 °F to 60 °F are effective (Lumb and Jones, 1973).

Halothane is a volatile anaesthetic chemical widely used in small animal practice. Oxygen ($O_2$) gas is used as a vehicle for transporting the vapourised anaesthetic to the receipient animal. Halothane was reported to be good for maintaining anaesthesia at 40 ppm. in fishes (Stuart, 1981). Sawyer (1982) recommended that a 2% halothane oxygen gas mixture could be bubbled into a covered container, with seasoned water, as an alternative anaesthetic agent to MS 222 for inducing and maintaining anaesthesia in fishes. Halothane at a dose of 76.4 mg per liter of water was recommended as inhalant anaesthetic for goldfish (Wallach and Boever, 1983). Ross and Ross (1984) reported that dose levels of 0.5-2.0 ml per liter produces anaesthesia or alternatively the gas could be vapourised and dissolved.

MATERIALS AND METHODS :

Grass carp fingerlings, with a mean weight of 5.83g, were obtained from a local fish importer. The fish were treated with 4 mg/l methylene blue and 1 mg/l Dipterex ( O, O - Dimethyl, 2, 2, 2- Trichloro- 1 - hydroxyethyl phosphonate) in a 24 hour bath for prevention and control of ectoparasitic disease. After 24 hours, the fish were transferred to a holding tank containing 0.5% NaCl and were acclimatised for 7 days prior to the experiment.

Preliminary studies conducted indicated that MS 222, at concentration of 0.05 g/l of water, was suitable to induce anaesthetia in fish to a level of Stage II Plane I (McFarland, 1959). The time requires to reach this stage, after immersing the fish into this concentration, was about 5 minutes. Preliminary studies also indicated that a concentration of more than 2% halothane in the halothane/oxygen mixture killed the fish after 6 hours in a closed container. It was therefore decided to use a concentration of less than 2% halothane for maintenance anaesthesia and an exposure period of not more than 6 hours for this experiment. From the preliminary studies, the volume of air in the polyethylene bag was found to be approximately 7.0 liters.

A sheet of cotton having an area of approximately 324 sq. cm. was packed between two paper towels to ensure the cotton wool would remain intact. This was then stapled with iron staples to plastic mosquito netting to preserved the flat shape. It was later placed inside a polyethylene bag. The various volumes of 0.5% NaCl solution ( packing solution ), were then poured into the bag, soaking the paper towels and the cotton.

The fish were starved for 24 hours prior to the experiment to reduce the fingerlings metabolism. Ten fish were randomly selected from the holding tank and placed in 0.05 g/l MS 222 solution. The fish were observed for the changes in behavior (as described by McFarland, 1959) which indicated that the fish were at Stage II Plane I level of anaesthesia (Table II). The fingerlings were then removed from the anaesthetic solution and placed inside the plastic bag on top of the previously prepared wet paper towels. The polyethylene bag was then immersed in a tank full of water to expel all the air trapped inside. Care was taken to ensure no tank water entered or packing solution left the bag.

With the plastic bag still partially immersed in the water, it was slowly inflated with a mixture of halothane and oxygen. Mixtures with concentrations of 0%, 0.5%, 1.0% and 1.5%. halothane were used. The volumes of packing solution used were 0.41, 0.61 0.81 and 1.01. The different concentrations of halothane were delivered using a recently calibrated Floutec Mark III vapouriser. The vapourised halothane was carried by oxygen gas supplied by a preassurised oxygen gas tank fixed to the vapouriser and via a plastic tube into the plastic bag.

Once fully inflated, the opening of the bag was twisted together, folded and bound with rubber bands. The bags were then floated on a large volume of water having a minimum temperature of 28 °C and a maximum temperature of 29 °C for six hours. Twenty minutes prior to the end of six hours, the bags were floated in recovery tanks filled with aerated 0.5% NaCl solution, to allow the bag temperature to normalise with the recovery tank water temperature. The bags were opened and each batch of fish immersed in water in a different recovery tank. The level of anaesthesia was accessed visually, the time required by the fish to recover to Stage I Plane I was noted, and the number of survivals were counted and recorded. The tanks were covered with black plastic sheets. After 24 hours the fish were accessed visually for swimming abilities, after effects of anaesthesia and number of delayed mortalities. Fish were then released back into the community tank containing conditioned and continously aerated 0.5% NaCl solution.

The results were analysed by a computer software programme, Statgraphic. A multivariate linear regression line was estimated to determine the relationships that existed if any, between halothane concentration, volume of NaCl solution and survival rate. The line was tested with ANOVA and the level of significance determined.

## RESULTS :

Table III The level of anaesthesia after 6 hours of packing, time to recover , survival rate in different halothane concentrations and 0.5% NaCl solution volumes. Ten fingerlings were used for each experiment.

| Halothane conc. (%) | Vol. of 0.5% NaCl soln. added (liters) | Level of anaesthesia | Time recovered | Survival rate (%) |
|---|---|---|---|---|
| 0.0 | 0.4 | | immediate | 100 |
| | 0.6 | | | 100 |
| | 0.8 | | | 100 |
| | 1.0 | | | 100 |
| 0.5 | 0.4 | Stage 0 - Stage I | almost immediate | 40 |
| | 0.6 | | | 100 |
| | 0.8 | | | 100 |
| | 1.0 | | | 100 |
| 1.0 | 0.4 | Stage I, Plane I | approx. 30 seconds | 0 |
| | 0.6 | | | 40 |
| | 0.8 | | | 90 |
| | 1.0 | | | 90 |
| 1.5 | 0.4 | Stage II, Plane II | approx. 120 seconds | 0 |
| | 0.6 | | | 0 |
| | 0.8 | | | 0 |
| | 1.0 | | | 80 |

Mortalities were found to be higher with increasing concentration of halothane in the enclosed atmosphere but decreased with increasing volume of packing solution in the polyethylene bags.

A multivariate linear regression line was estimated (calculations for the model fitting in Appendix I) and found to have an equation :

$$Y = 40.75 - 54X1 + 92.5X2$$

where Y = percentage of survival

X1 = concentration of halothane

and X2 = volume of 0.5% NaCl solution used.

The line plot of predicted against observed values is shown in Figure I.

An ANOVA was calculated (Appendix 2) to test the regression line. The line estimated had a slope significantly different to zero (Appendix 2). The ANOVA indicated that the coefficient of determination, R squared was 0.759752 and was 0.722791 after being adjusted for the degrees of freedom.

Fish in the control group (without halothane ) were observed to be active with rapid opercular movement throughout the packing time and loose scales were seen at the end of the experiment. The water was turbid and foamy and worse in the event where fish had died during the experiment. After the experiment, when fish were placed in the recovery tank water, there was an immediate active swimming which was exaggerated and clumsy.

Fish in the treatment groups (with halothane) were noticed to be in a state of quiescence. The opercular movement was depressed and an increased depression was noted with an increased concentration of halothane. The packing solution was clear and foamy but became turbid when fish had died during the experiment. When placed in the recovery tank water, the fish did not swim immediately. The time required for the fish to start active swimming increased with increased halothane concentrations. Initially the fish movements were slow and sluggish, floating vertically and restricted to the tanks' bottom. Later, horizontal swimming was noticed and then normal movements. The time for recovery varied with the concentration of halothane used (Table III).

No delayed mortalities were observed in the recovery tanks after 24 hours from time of unpacking of fish.

## DISCUSSION :

The results of the experiment indicated that halothane can be used to maintain anaesthesia in grass carp fingerlings. This finding is consistent with reports from Stuart (1981), Sawyer (1982), Wallach and Boever (1983) and Ross and Ross (1984).

Sawyer (1982) stated that 2% halothane can be bubbled into water in a closed container to cause narcosis in fish and maintenance anesthesia can be accomplished by flushing the halothane, dissolved in the water, over the gills. Preliminary experiments conducted however indicated that grass carp fingerlings packed with 2% halothane/oxygen mixture died after six hours.

Ross and Ross (1984) reported that as halothane was relatively insoluble in water, a relatively pure dose of halothane could be found in the water near the air-water interface. Fish near to the surface can die of an overdose of halothane as high concentrations can be absorbed. In this study the adversed effect was somehow counteracted by adding the appropriate amount of packing solution. The packing solution, which was used as an osmoregulatory enhancer (Schnick and Meyer, 1978), probably increased the distance from the gaseous-water interface to the fish. The increased distance could have diluted the halothane as it diffused downwards reducing direct contact of the anaesthetic gas with the fish body surface and also reducing diffusion of anaesthetic gas directly into the blood via the gills.

High mortality was experienced with high concentrations of halothane used. The experimental results indicated that concentrations of more than or equal to 1% halothane in the gas mixture were contraindicated for packing fish for periods of more than six hours. This halothane concentration was in agreement with reports by Ross and Ross (1984) who recommended concentrations of 0.05%-0.964% halothane to be use to induce anaesthesia. It was found that 0.5% halothane in combination with more than or equal to 0.61 of packing solution was suitable for packing fish in closed system, for a period of six hours.

The value of coefficient of determination implied that the line is a reality and that 72.3% of the variation in survival rates was attributed to the independent variables. Of the two independent variables used, the volumes of packing solution were more important in ensuring a higher survival rate in the closed packing system.

Loading densities of about 62.7g of grass carp per liter of packing solution were achieved in halothane but a loading density of 91g/l was obtained in the control (without halothane). This would imply that it would be more economical not to use halothane in packing fishes. However the author has the opinion that the use of low halothane levels in an optimum volume of water would be preferable in packing the fish. This is because of reduced fish activity and thus better water quality during packing resulting in less stress to the fish and ensure a better recovery rate after packing.

CONCLUSIONS :

Halothane at high concentrations is contraindicated for packing fish. Low concentrations, of less than 1% halothane, combined with a corresponding optimal volume of packing solution can ensure less stressful packing, high survival rates and reduce packing weights. A loading density of 62.7g of grass carp per liter of packing solution can be achieved at a 0.5% halothane concentration (in oxygen) with a predicted 100% survival rate. Should this method be considered for transportation, an economical transportation of grass carp fingerlings in closed system is possible.

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## APPENDICES :

## APPENDIX I

```
-------------------------------------------------------------------
                    MODEL FITTING RESULTS
-------------------------------------------------------------------
 VARIABLE   COEFFICIENT    STND.ERROR   T-VALUE   PROB(>T)
-------------------------------------------------------------------
CONSTANT    40.75          20.26        2.0115       .0655
halothane   -54            10.21        -5.2893      .0001
water       92.5           25.52        3.6241       .0031

-------------------------------------------------------------------

 0 CASES WITH MISSING VALUES WERE EXCLUDED.
  RESIDUALS PLACED IN VARIABLE: RESIDUALS
```

APPENDIX 2

```
    ------------------------------------------------  ----------
       ANALYSIS OF VARIANCE FOR THE FULL REGRESSION
-----------------------------------------------  -------------
 SOURCE SUM OF SQUARES DF MEAN SQUARE F-RATIO PROB(>F)
 MODEL   21425.000      2  10712.500   20.555  .000
 ERROR   6775.000       13 521.1538
---------    -------------------------------------------------
  TOTAL (CORR.) 28200.000    15
  R-SQUARE = 0.759752
  R-SQUARE (ADJ. FOR D.F.) = 0.722791
  STND. ERROR OF EST. = 22.8288
```

Table I A compilation of drugs effective in inducing anaesthesia in fish by inhalation. (From Ross and Ross, 1984).

| Name of drugs | Comments |
|---|---|
| Tricaine methanesulphonate (MS 222)<br>Benzocaine<br>Quinaldine (and Quinaldine sulphate)<br>2-Phenoxyethanol | All four drugs used widely. |
| Chloral hydrate<br>Tertiary amyl alcohol<br>Methyl parafynol<br>Chloroform<br>Tribromoethanol<br>Chlorbuthanol | Effective but with side effects, now not very widely used. |
| Sodium amytal<br>Sodium pentobarbitone | Effective and in occasional use. |
| 4-Strylpyridine<br>Diethylether<br>Seccobarbital<br>Piscaine<br>Propoxate<br>Urethane | Have been used in isolated cases. |

Table II Stages of anaesthesia in fish. (From McFarland, 1959)

| | Observed stages of anaesthesia | | | |
|---|---|---|---|---|
| Anaesthetic tages | Reactions to visual stimuli | Reactions to vibrational stimuli | Equilibrium (Eq.) and Muscle Tone (M.T.) | Respiratory rate. |
| Stage 0 | Reactive | Reactive | Normal | Normal |
| tage I | | | | |
| Plane 1 | Slightly reactive | Reactive | Normal | Normal |
| Plane 2 | No reaction | Slight to no reaction | Normal | Normal |
| tage II | | | | |
| Plane 1 | No reaction | Slight to no reaction | Partial loss of Eq. | Increase |
| Plane 2 | No reaction | Slight to no reaction | Complete loss of Eq. & M.T. | Rapid decline |
| Stage III | No reaction | No reaction | Complete loss of Eq. & M.T. | Slow decrease in amplitude |
| Stage IV | No reaction | No reaction | Complete loss of Eq. & M.T | Ceases |