J. Mar. biol. Ass. India, 1965, 8 (1): 28-56

EFFECTS OF ANAESTHETICS ON THE BEHAVIOUR OF MULLET FINGERLINGS AND THE SCOPE OF USING THESE IN DIFFERENT FISHERY PROCEDURES-I*

By V. S. DURVE** AND S. K. DHARMA RAJA

Central Marine Fisheries Research Institute, Mandapam Camp

INTRODUCTION

THE use of anaesthetics in fishery work, mainly in the transport of live-fish and also in handling fish for tagging, fin-clipping, weighing and stripping has been recognised by fishery workers everywhere. In live-fish transport, anaesthetics are useful in lowering the metabolic activity of fish, which facilitates the transport of more fish in a given quantity of water for a longer time. It has also been proved that anaesthetics make the otherwise time consuming work of tagging and fin-clipping much easier and also lower the mortality of the fish due to handling. Considerable effort has gone into a search for a proper anaesthetic and its different concentrations to give varying degrees of anaesthesia useful for works from tagging to transport of live-fish.

Several methods have been used to produce anaesthesia. Abramowitz (1937), Parker (1937, 1939), Abramowitz *et al.* (1940) Osborn (1938, 1941) immersed fishes in crushed ice or ice water. Haskell (1940) employed electric shock to produce a paralysis lasting about two minutes. It is doubtful whether the anaesthesia induced by these methods is similar to that induced by chemicals. The disadvantages of such methods have been discussed by Pickford and Atz (1957).

Chemicals used to anaesthetize fishes include Ether, Urethane, Chlorobutanol, MS-222 SANDOZ, Sodium Amytal and Chloral Hydrate, besides 8 to 9 others. McFarland (1959, 1960) has done the most extensive work on anaesthetics, their effects on the behaviour and physiology of fishes and also their use for handling and transport of fishes. He has also reviewed the earlier work considerably. Bell (1964) has prepared a very helpful guide to the properties, characteristics and uses of some general anaesthetics for fish. The most recent attempts to introduce two new anaesthetics for use in fishery procedures are of Howell and Thomas (1964) and Thienpont and Niemegeers (1965). They have suggested the use of 4-Styrylpyridine and Propoxate respectively. Some work on anaesthetics has been done in India by Saha *et al.* (1955), Natarajan & Renganathan (1960) and Sreenivasan (1962).

The aim of the present work was to study the effects of different anaesthetics on the behaviour of the fingerlings of the mullet *Liza tade* and select the most economical anaesthetic suitable for the transport of live-fish, tagging and such other fishery work. This paper, which is first in the series, embodies only the laboratory observations on a few anaesthetics. The planning, experimental work and writing of this

---

* Published with the permission of the Director, Central Marine Fisheries Research Institute.

** Present address : Zoological Survey of India, Central Regional Station, Jabalpur.

paper are done by the senior author while the junior author has done the statistical treatment of the data presented in this paper.

The fingerlings of mullet were selected for this work because, they were readily available around Mandapam and form one of the principal fish used for stocking brackish or fresh water ponds and reservoirs in Madras state. They are thus more subjected to actual transport in live condition. These fingerlings could be readily acclimatised to fresh water and could also be maintained in the laboratory without much difficulty.

Material and Methods

The fingerlings for the experiments were collected mainly from Chinnapalam creek at Pamban and brought to the laboratory in conventional tin carriers. They were gradually acclimatised to fresh water within three days and kept in fresh water for eight to ten days before experiments. They were fed on clam meat blended with equal parts of peptofish, a commercial fish-food which maintained them in a healthy condition.

The fish were taken for experiments mostly in batches of 20 and released individually in one litre fresh water containing the same concentration of the anaesthetic, in twenty different jars. The temperature of the water ranged from 27° to 28°C. and the pH was about 8.1. The behaviour of each fish was noted. The experiment was run for a period of 5 hours. Several concentrations of each chemical were tried in the series of experiments, in order to find out the reactions of fish to these concentrations. In all 2323 fish were employed for this study alone. No fish was experimented on, for more than twice and the interval between two experiments on the same fish was kept at a minimum of 4 days.

Separate experiments were done for determining the rate of oxygen consumption of anaesthetized fish and their tolerance to each anaesthetic. The methods for these have been described in the relevant sections.

Properties of Anaesthetics used and their mode of Action

1. *Tertiary Butyl Alcohol :* It is a hydroxy derivative of a hydrocarbon in the form of a colourless viscid liquid, soluble in water and miscible with alcohol and ether.

2. *Chloral Hydrate :* It is a halogenated alcohol in large monoclinic plates, with peculiar pungent odour and taste. Freely soluble in water. Slowly volatalizes on exposure to air. Active metabolite is Trichloroethanol. Acts as depressant on human heart and lowers blood pressure. Depresses centres of consciousness, motor side of spinal cord and respiratory centre.

3. *Chlorobutanol :* This is also a halogenated alcohol in anhydrous or hydrous crystals with camphor odour and taste. Scarcely soluble in cold water but easily soluble in hot water. Irritant. It is a general nerve sedative and anticonvulsant. Local anaesthesia causes relaxation of involuntary muscles.

4. *Sodium Amytal :* It is a barbiturate in the form of slightly bitter crystals or white powder scarcely soluble in water, freely soluble in Benzene, alkaline solu-

tions and Alcohol. ' Short actor ' anaesthetic, depressant of central nervous system, increases recovery time and the threshold for cerebral neurons esp., of cortex.

5. *Sodium Barbital:* Bitter crystals or white powder soluble in water. Sedative or hypnotic of long duration of action.

6. *Urethane (Ethyl carbamate):* Crystals with cooling saline taste. It is a depressant of the central nervous system. It is reported to produce rapid and profound narcosis with little change in circulation or respiration.

All the above general anaesthetics reversibly depress the sensory centres of the brain to various degrees and finally eliminate reflex action. They first depress the cortex (stage of analgesia), then the basal ganglia and cerebellum (stage of delirium or excitement) and then the spinal cord (stage of surgical anaesthesia). Excessive dosage or prolonged exposure causes the effection of the medulla leading to the paralysis of the vital respiratory and vasomotor centres which ultimately causes death (McFarland, 1959 ; Merck Index, 1960 and Bell, 1964).

Behavioural changes in Mullet Fingerlings during Anaesthesia and the Classification of the Anaesthetic Stages

On using the above mentioned six anaesthetics, it was noticed that the fish show a definite course of behaviour in the anaesthetic. This course is so well marked that it was possible to classify the changing pattern of the behaviour of the fish into seven different recognizable stages. In the stages 2 and 3, two planes of anaesthesia (Goodman and Gilman, 1955—Chap. 3 ; McFarland, 1959) could be recognized. However, these sub-stages were ignored in the present investigation to avoid complication. The stages given below are more or less on a similar pattern as observed by McFarland (1959, 1960).

Stage O (Normal): The fish moves up and down in very co-ordinated movements. The dorsal is incessantly raised and dropped. Movements are rapid and sharp. Pectorals constantly beat, pelvics are spread out and caudal shows lateral movements. Opercular beats are regular. The fish reacts very sharply to the external stimuli. A tap given on the jar makes the fish give a fright reflex and it turns sharply away and moves up or down with co-ordinated swimming movements. Fish even reacts to the shadow of hands on the jar.

Stage 1 (*Light sedation*): The swimming ability slows down. The fish remains stationary in the medium very often and when it swims it does so with slow movements. First dorsal is raised only occasionally and is never fully extended. The fish does not react sharply to the external stimuli like a tap on the jar. Sometimes there is no reaction at all. There is no reaction to the shadow on the jar. However, if touched by a rod, the fish moves away and avoids being touched again. Pectorals beat with slow speed. Pelvics close or rarely open. Opercular beats slow down. Swimming is mainly by the sideward movements of the tail and head portions.

Stage 2 (*Deep sedation*): The fish lies stationary at the bottom or swims very slowly nearer the bottom. The pectoral and caudal movements are extremely slow or sometimes nil. The swimming is by the vigorous sideward movements of head and tail portions. The fish thus exhibits wobbling body movements while swimming,

but maintains its equilibrium. First dorsal and pelvics in collapsed condition. Opercular rate low. The fish does not react either to the external stimuli or even to the glass rod touched to its body.

Stage 3 (*Partial loss of equilibrium*) : The fish loses its equilibrium and then initially stands with its snout touching the floor of the flask at an angle of about 45°. This angle slowly increases and ultimately the fish remains perpendicular to the bottom for some time. It then rolls and swims. In some fish, this rolling and swimming continues for a long time. When at rest, the fish has a tendency to lie on its side. When the fish at rest is disturbed, it swims vigorously for a few seconds with un-coordinated movements, even rolls in the water and then comes to rest perpendicular to the floor. Fin movements are slow and un-coordinated.

Stage 4 (*Loss of equilibrium*) : The fish remains at the bottom with belly upwards. An occasional buoyant individual floats at the surface. The swimming movements stop. Fin movements are absent. Opercular movements are slow but regular. There is a loss of muscle tone. The fish when lifted from the water, is limp and does not react unless pinched or pricked. Sometimes blood accumulates in the rays of pectoral, pelvic and caudal fins and at times also in the outer rim of the opercles.

Stage 5 (*Loss of reflex reactivity*) : The respiratory movements become irregular. The amplitude of each opercular beat becomes large initially and later there are sudden rapid beats of opercles with an interval of a few seconds. Blood accumulates in the bases of the rays of pectoral, pelvic and caudal fins and also in the outer rims of the opercles. Fish remains at the bottom as in the previous stage but an occasional buoyant individual is also seen.

Stage 6 (*Medullary collapse*) : Respiratory movements stop. The opercles are spread. Pectorals are in the extended position perpendicular to the body. This will be followed by the cessation of cardiac movements and death ensues within a minute.

The observed changes in the behaviour of the mullet fingerlings as narrated in the above stages are given in Table I, for easy reference to the changing patterns of the behaviour. There were slight deviations from this pattern of behaviour in the case of some fishes and also in different anaesthetics. The deviations being minor ones, were ignored.

Relationship between dosage and Anaesthesia

Tertiary Butyl Alcohol (Appendix, table IX) : It will be seen from the table that in the lowest concentration of 0.30 ml./100 ml., the majority of fish remained in light sedation (stage 1) while there was some percentage of fish remaining in each stage of sedation. So far as the stage of the total loss of equilibrium (stage 4) is concerned, maximum number of fish were in this stage at the concentration of 0.70 ml./100 ml. The average time required to reach stage 4 at the above concentration was 32.84 minutes. This time increased as the dose was lowered. The average time of recovery from the stage 4 at the dose of 0.70 ml./100 ml., was 11.32 minutes. This recovery time remained more or less the same even at lower dosage. Since 11.25% of the fish remained in the stage 0 (Normal) at the concentration of 0.30 ml./100 ml., no further experiments were performed at the dosage lower than this. The

TABLE I

Synopsis of the stages of anaesthesia for mullet fingerlings

—Observed changes in behaviour—

Anaesthetic stage	External vibrational stimuli	Stimuli of body touch	Fin movements First dorsal (F.D.) Pectoral (Pc) Pelvic (Pv)	Nature of swimming movements	Respiratory movements
1. Normal	Reactive	Reactive	Normal. F.D. raised and dropped incessantly. Pc constantly beat. Pv spread out.	Co-ordinated	Normal. Opercular beats regular.
2. Light Sedation	Slightly reactive to no reaction	Reactive	Slow down. F.D. raised only occasionally and never fully extended. Pc beat with a slow speed. Pv close or rarely open.	Slow down. Swimming mainly by body movements.	Slow. Opercular beats slow but regular.
3. Deep Sedation	No reaction	No reaction	Extremely slow or nil. F.D. collapsed. Pc extremely slow or nil. Pv collapsed	Stationary at the bottom or slow swimming only by body movements	Slow. Opercular beats slow but regular.
4. Partial loss of equilibrium	No reaction	No reaction	Slow and unco-ordinated or nil.	Fish rolls in the water.	Slow. Opercular beats slow but regular.
5. Total loss of equilibrium	No reaction	No reaction	Absent	Nil. Fish lies on the bottom belly upwards.	Slow. Opercular movements slow but regular.
6. Loss of reflex reactivity.	No reaction	No reaction	Absent	Nil. fish lies on the bottom belly upwards.	Irregular. Amplitude of each opercular beat becomes large. Quivering movements of opercles.
7. Medullary Collapse.	No reaction	No reaction	Absent	Nil. fish lies on the bottom belly upwards.	Cease.

number of fish reaching the stage 3 (Partial loss of equilibrium) was appreciable in the concentrations from 0.35 to 0.60 ml./100 ml. Mortality was negligible.

Chloral Hydrate (Appendix, Table X) : The observed lowest concentration was 0.01 g./100 ml., at which the fish reached stages 1 and 2. The fish reaching stage 1 were in overwhelming majority, while there was a small percentage of fish remaining in stage 0. The highest observed concentration was 0.25 g./100 ml., where 73.67% fish reached stage 4. The average time required for reaching this stage was 81.42 minutes and the recovery was within the average time of 33.92 minutes. Negligible number of fish reached or remained in the stage 3. Five per cent of the fish remaining in the stage 0 at the concentration of 0.10 appears to be an observational error. The mortality of fish was low during all the experiments.

Chlorobutanol (Appendix, Table XI) : The lowest observed concentration was 0.002 g./100 ml., in which 85.00% fish reached the stage 1 and 13.33% fish reached the stage 2. The highest concentration was 0.02 g./100 ml., where the mortality was cent per cent. A little lower concentration from 0.01 to 0.019 g./100ml., gave good results so far as stage 4 is concerned, 95.45 to 80.00% fish reaching this stage. The average time of recovery was remarkably short, being only 3.94 minutes at the concentration of 0.019 and 8.50 minutes at the concentration of 0.015. The fish reaching stage 3 were only at the concentration of 0.005 and 0.006. The mortality was also higher at these two concentrations when compared with that at the adjacent concentrations. The reason for this abnormal response of fishes to the concentrations of 0.005 and 0.006 g./100 ml., cannot be said.

Sodium Amytal (Appendix, Table XII) : In this anaesthetic, the highest number of concentrations were tried. The lowest one was 0.0035 g./100 ml., and here 96.67% fish reached the stage 1. The concentrations at which the highest percentage of fish reached the stage 4 were 0.015 and 0.02 g./100 ml., while the concentrations beyond this increased mortality culminating at 90.01% at the concentrations of 0.035. The average time for reaching the stage 4 was 28.6 minutes to 34.47 minutes at the above two best concentrations. The recovery time for these concentrations was from 41.75 to 82.10 minutes. The fish reaching the stage 3 were more at the concentrations of 0.01 and 0.004.

Sodium Barbital (Appendix, Table XIII) : The lowest concentration of 0.1 g./100 ml., induced 91.24% fish to stage 1 and 7.50% fish to stage 2. The concentration of 0.30 g./100 ml., induced 70% of the fish to stage 4. The average time required to reach this stage was excessive, being 216.00 minutes even for the highest concentration. The average time of recovery was also high (195.00 minutes). The fish reaching stage 3 were small in number. The mortality during experiments was less.

Urethane (Appendix, Table XIV) : The lowest concentration was 0.15 g./100 ml., which induced the stage 1 for 71.25% fish and stage 2 for 21.27% fish. Concentrations of 0.40 and 0.70 g./100 ml., induced the stage 4, to 95.01 and 100.00% fish. The average time required to reach this stage was small, being 18.70 minutes for former concentration and 3.00 minutes for the latter. The average recovery time for these concentrations was 14.80 and 20.55 minutes respectively. Fish reaching the stage 3 were negligible.

Table II gives the selected concentrations of each anaesthetic found useful to induce stage 4. This table has been prepared by selecting the suitable concentrations

TABLE II

Concentrations found useful to induce loss of equilibrium (stage 4) in the fingerlings of the mullet Liza tade *with different anaesthetics and the time required for the recovery of the fishes*

Anaesthetic	Concentration per 100 ml. of water g./ml.	Time required to reach the stage 4. (minutes)	Period of exposure to the stage (minutes)	Time required for recovery to stage 0 after the change of water (minutes)	
Tert. Butyl Alcohol	0.50 ml.	97.50	30	8.08	
	0.60	39.36	30	10.82	
	0.70	32.84	30	11.32	
Chloral Hydrate	0.20 g.	116.87	30	20.67	
	0.25	81.42	30	30.92	
Chlorobutanol	0.010 ,,	28.43	60	11.54	
	0.015	5.06	60	8.50	
	0.019	4.25	30	3.94	
	0.020	3.61	—	—	Lethal for immersion for 30 minutes.
Sodium Amytal	0.012 ,,	61.85	38	58.20	
	0.015	28.60	30	41.75	
	0.020	34.47	30	82.10	
	0.030	24.18	30	101.73	
	0.035	14.50	30	145.50	Lethal for majority of the fishes for immersion for 30 minutes.
Sodium Barbital	0.30 ,,	216.00	30	195.00	
Urethane	0.40 ,,	18.70	30	14.80	
	0.70	3.00	30	20.55	
	0.80	3.18	30	30.75	
	0.85	4.00	30	30.34	Lethal for majority of the fishes for immersion for 30 minutes.

of each anaesthetic from its respective table in the Appendix. It will be noticed from this table (Table II) that very low concentrations are required in the case of Chlorobutanol and Sodium Amytal. In respect of other anaesthetics, the concentrations are high and in the case of Tertiary Butyl Alcohol and Urethane, they are the highest. The average time required for the fish to reach the stage 4 is the lowest in the case of Chlorobutanol, next come Urethane and Sodium Amytal. This time interval is the highest in the case of Sodium Barbital. The recovery time is also the shortest in the case of Chlorobutanol and is followed closely by that of Tert. Butyl Alcohol. It is the highest in the case of Sodium Barbital and Sodium Amytal. From this it could be inferred that out of the chemicals studied, Chlorobutanol appears to be the best from the point of view of the time required to induce anaesthesia and also the recovery. It is also economical as the quantity required is considerably low. The results obtained here (Table II) differ from those obtained by McFarland (1960) in the case of *Fundulus parvipinnis* wherein he observed varying concentrations to induce total loss of equilibrium and also more recovery time than observed in the present case.

With reference to the tables in the Appendix, it can be said that increased dosage of anaesthetics decrease the induction time of stage 4. This observation supports that of McFarland (1959). It can also be inferred from the experimental data that the time required for the recovery is proportional to the concentration of the anaesthetic. This is especially so in the case of Chloral Hydrate, Sodium Amytal, Sodium Barbital and Urethane. Whether the duration of exposure also affects the time of recovery or not, could not be tested here as the exposure time for the fish reaching stage 4 was fixed at 30 minutes except for certain concentrations of Chlorobutanol indicated in the Appendix Table XI, where it was 60 minutes. Cope (1953), Muench (1958) and McFarland (*op. cit.*) have indicated that the time required for recovery is proportional to the concentration of the anaesthetic and also to the length of exposure.

Five fish of varying sizes were allowed to reach the stage 5 (loss of reflex reactivity) in Chlorobutanol and then quickly transferred to the fresh water. All the fish recovered fully within 10 minutes.

Table III gives the concentrations suitable for inducing stage 1 or 2 and the period up to which the fish were exposed to these concentrations. This table has also been prepared by selecting the suitable concentrations in respect of each anaesthetic from the tables in the appendix. No induction and recovery time was measured at these concentrations. Here it will be seen that the dosage of Chlorobutanol and Sodium Amytal are the lowest. Chloral Hydrate comes next while Tertiary Butyl Alcohol has the highest dose. In all the cases, fish were exposed for the same time (5 hours) and they tolerated the concentrations well. The mortality was at a considerable low level and this too may not be due to the anaesthetic as, in many cases there was no mortality even at higher dosage as seen from the tables in the Appendix. It is necessary to mention here that even at these lowest dosage, some fish reached the stages 3 and 4, but their percentage was low (Tables IX to XIV appendix) and it is likely that these fish may not be in a good physiological state though apparently they looked normal and healthy.

It could also be presumed that, the anaesthetic in which the induction time for stage 4 is the shortest (Chlorobutanol), will also have the shortest induction time for stages 1 and 2. Similarly, the anaesthetic having the shortest recovery time for

stage 4 (Chlorobutanol and Tertiary Butyl Alcohol), will have the shortest recovery time for the stages 1 and 2. However, it is admitted that no specific experiments were performed to demonstrate this.

TABLE III

Concentrations found useful to induce light or deep sedation (stages 1 and 2) in the fingerlings of the mullet Liza tade *with different Anaesthetics, the period of exposure to these stages and the mortality during and after the experiments*

Anaesthetic	Concentration per 100 ml. of water. g./ml.	Period of Exposure hours	Mortality during the experiment and within next 24 hours % fishes
Tert. Butyl Alcohol ..	0.30 ml.	5 hrs	2.50
	0.35	,,	1.66
Chloral Hydrates ..	0.01 g.	5 hrs	4.99
	0.025	,,	3.34
Chlorobutanol ..	0.002 ,,	5 hrs	1.67
	0.003	,,	nil
Sodium Amytal ..	0.0035 ,,	5 hrs	1.67
	0.0040	,,	6.25
Sodium Barbital ..	0.100 ,,	5 hrs	1.25
	0.125	,,	1.67
	0.150	,,	1.25
Urethane ..	0.150 ,,	5 hrs	5.00
	0.175	,,	3.33
	0.200	,,	2.50

TOLERANCE OF FISH TO ANAESTHETICS AND THE NARCOTIC POTENCY

After determining the minimal dosage of the anaesthetic to induce the stages 1 and 2 for the fish, it was necessary to find out how far the fish can tolerate this concentration though of course, they were found to tolerate it up to 5 hours. For this, separate tolerance tests were performed, in which 20 fish were left in the aquarium jar containing 20 litres of water with the desired concentration of the anaesthetic. The fish were observed at a few hours interval and the experiment was run for full 24 hours wherever possible. At the end of this period, the stage of sedation was noted and the fishes were transferred to a tank of running fresh water. The fish dying during the experimental period were removed and noted. The fish, after the experiment, were kept under observation for a further period of 24 hours to find out the post-experimental mortality. The results of these experiments are tabulated in Table IV. It will be seen from this table that except in Sodium Barbital, the fish were seen in light sedation (stage 1). In some fish, deep sedation (stage 2) was also noticed. In Sodium Barbital, the experiments had to be concluded by about 16 hours as the fish began losing their balance at that period. There was a small percentage of mortality in Sodium Amytal and Urethane during experiments, while mortality within the next 24 hours was noticed in Sodium Barbital, Tertiary Butyl Alcohol and Urethane.

TABLE IV

The results of the tolerance test

No.	Anaesthetics	Concentration g/100 ml.	Total fishes	Avg. exposure. hours	Mortality		Stage reached	Remarks
					During exp. %	Within next 24 hours %		
1.	Chloral Hydrate ..	0.02	60	24	nil	nil	Light Sed.	
2.	Sodium Amytal ..	0.0035	80	24	3.75	nil	Light Sed.	Mortality after 18 hrs.
3.	Sodium Barbital ..	0.10	60	16.67	nil	8.34	Deep Sed.	Fishes began losing balance after 16 hrs.
4.	Chlorobutanol ..	0.002	60	24	nil	nil	Light Sed.	
5.	Urethane ..	0.15	60	24	3.34	8.34	Light Sed.	
6.	Tert. Butyl Alcohol ..	0.30	60	24	nil	1.67	Light Sed.	One fish lost its equilibrium at 17 hrs.

These experiments tend to indicate the suitability of all the anaesthetics except Sodium Barbital, so far as the tolerance of fish to these chemicals is concerned. McFarland (1960) has also not noted any long-term effects of anaesthesia in Opaleye and the woolly Sculpin when these fishes were exposed to anaesthetics. Earlier workers (Johnson, 1954 and Muench, 1958) had also made similar observations on Trout and Green fish. The results of the present experiments also indicate that all the anaesthetic stages from 1 to 5 are controllable and can be maintained for a considerable period (especially upto stage 4) by varying the concentrations of the anaesthetic. This supports the observations of McFarland (1959).

From the concentrations of the anaesthetics useful to induce stages 1 and 2 in the fingerlings of *Liza tade* for a period of more than 12 hours, the narcotic potency of these anaesthetics could be derived. This is calculated by a simple arithmetic equation by allotting a potency value of 1 to the anaesthetic requiring the minimal dose (Tertiary Butyl Alcohol) and calculating the values of other anaesthetics therefrom. It is found that the highest narcotic potency (150.00) is for Chlorobutanol. This is followed by Sodium Amytal (85.70) and Chloral Hydrate (30.00). Sodium Barbital, Urethane and Tertiary Butyl Alcohol have low potencies, being 3.00, 2.00 and 1.00 respectively. From a reference to Table V, it will be seen that the potency of each anaesthetic is somewhat related to the molecular weight of the anaesthetic, except in the case of Sodium Barbital. The anaesthetic having the lowest potency has also the lowest molecular weight. Anaesthetic having higher molecular weights will also have higher potencies which of course, may not be proportional to the increase in the molecular weight. As stated above, Sodium Barbital is the exception. The results of narcotic potencies of the different anaesthetics discussed above are generally in agreement with those of McFarland (1959). It can also be said that a smaller quantity of anaesthetic is required to induce equinarcotic levels as the molecular weight increases. Sodium Barbital is an exception. Führer (1912), Tiffeneau and Dorlencourt (1923), Tiffeneau and Torres (1924), Broun (1928), Lindenberg and Garry-Bobo (1951) and McFarland (1959) all have indicated positive correlation between narcotic potency and increasing molecular weight.

McFarland (*op. cit.*) has also noted a correlation between induction time and the molecular weight. Lesser the molecular weight less is the induction time and *vice versa*. This observation could not be confirmed in the present investigation as Chlorobutanol which has a higher molecular weight was found to be the most quick acting anaesthetic, while Tertiary Butyl Alcohol with its low molecular weight was found to be a slow acting anaesthetic.

Influence of Size on the Depth and the Rate of Induction of Anaesthesia

The size of the fish is one of the most important factors which influence the changes in the rate of induction and the depth of anaesthesia. Goodman and Gilman (1955) have observed the size to influence the rate and depth of anaesthesia in mammals. In fish, it has been reported that larger fish are more susceptible to anaesthetics (Kochman, 1913 ; Keys and Wells, 1930 ; Muench, 1956 ; and McFarland, 1959). However, Adams *et al.* (1926) and Witjens *et al.* (1947) observed no size effect on the induction times of anaesthesia in specimens of *Carassus auratus* that varied between 2 to 10 grams.

In the present investigation, the time required for the induction of stage 4 was alone taken into consideration for finding out the influence of weight on the rate

of induction of anaesthesia. The results tend to indicate that the size of the fish has no relation with the rate of induction. The smaller fish may attain the stage 4 of the anaesthesia sooner than the larger fish or *vice versa* (Table VI). However, the experiments conducted here are for higher concentrations and it is necessary to verify these by experiments at lower concentrations and of longer durations.

TABLE V

Minimal and lethal doses, narcotic potency and molecular weights of different chemicals used in the study

Chemicals (Anaesthetics)	Molecular weight	Narcotic potency	Minimal dose g. % (M.D.)	Lethal dose g. % (L.D.)
1. Sodium Amytal ..	248.26	85.70	0.0035	0.035
2. Sodium Barbital ..	206.18	3.00	0.10	—
3. Chlorobutanol ..	177.47	150.00	0.002	0.020
4. Chloral Hydrate ..	165.42	30.00	0.01	—
5. Urethane ..	89.09	2.00	0.15	0.85
6. Text. Butyl Alcohol ..	74.12	1.00	0.30	—

TABLE VI

Relationship of size and the time required for the induction of the stage 4, in the fingerlings of Liza tade *(Experiments done in Sodium Amytal)*

No.	Concentration of Sodium Amytal g/per cent	No. of fishes	Average size in grammes	Average time required for induction (minutes)	σ_s	σ_t
1.	0.011	6	0.90	58.67	0.18	17.81
2.	,,	13	1.52	59.31	0.34	21.82
3.	,,	7	2.61	61.14	0.31	12.96
4.	,,	2	4.20	50.00	0.10	..
5.	,,	1	5.10	85.00	..	..
6.	,,	1	6.60	95.00	..	..
1.	0.012	1	0.90	55.00	..	..
2.	,,	10	1.58	64.10	0.27	35.57
3.	,,	14	2.50	54.36	0.34	21.26
4.	,,	5	3.38	73.40	0.26	37.63
5.	,,	3	5.43	83.00	0.19	49.44
1.	0.015	9	1.78	28.89	0.25	6.84
2.	,,	17	2.70	27.29	0.27	6.93
3.	,,	10	3.50	31.90	0.28	5.42
4.	,,	3	4.70	26.34	0.22	5.79
5.	,,	1	5.50	22.00	..	..

σ_s—s.d. of size.
σ_t—s.d. of time.

ANAESTHESIA AND METABOLISM

It has been demonstrated by several workers that anaesthetics are helpful in transporting live-fish, as the former reduce the oxygen uptake (Nemato, 1957,

McFarland, 1960). Anaesthetics also lower the rate of production of carbon dioxide and the excretion of nitrogenous wastes, both of which are the limiting factors in fish transport (McFarland and Norris, 1958 ; McFarland, 1960). In the present study, the experiments were performed on individual fish as well as on groups of fish to know the reduction in their rate of oxygen consumption during sedation (stage 1 or 2). The concentrations of anaesthetics selected were those employed in the tolerance tests mentioned earlier. The fish were experimented on, individually by the method of Job (1957) to know their active oxygen consumption. No attempt was made to study the weight dependent metabolism though the weights of the fishes in every experiment were noted. The experimental jars were mounted on a mechanical rocking tray (Fig. 1) to keep the fish active. The experiments were run till

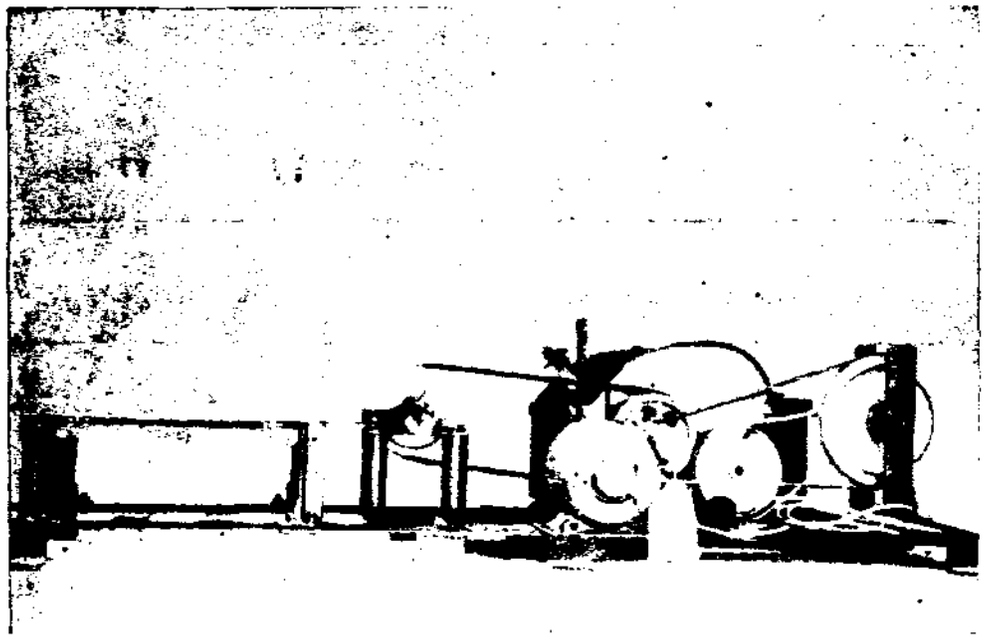

FIG. 1. Rocking tray with electric motor assembly and the gear box. Experimental flasks are mounted on the tray.

the lethal level of oxygen was reached. At this end, the fish were quickly removed and transferred to fresh water and allowed to remain there for the next 24 hours. On the next day, the fish were pre-treated in the experimental concentration of the anaesthetic for a period of one and half hours and the experiment was repeated in the anaesthetic on the same line as on the previous day. The pre-treatment of the fish to anaesthetic is essential as they get enough time to reach the required depth of anaesthesia and mere handling for putting them in the experimental jars does not cause a maximum oxygen uptake. The data obtained by these experiments done on the same fish without and with anaesthetic are plotted in Figs. 2 & 3. For each anaesthetic, six fish were experimented on individually while four experiments were performed in Sodium Barbital alone, with the groups of fish. While the range of initial oxygen level in each set of experiments is given in Table VIII, no data was collected of the changes in *pH* and free carbon dioxide after the addition of anaesthetics. Major changes in these two constituents after the addition of anaesthetics

TABLE VII

The regression equations for the data on oxygen consumption studied without and with anaesthetics

Treatment		Chlorobutanol	Chloral Hydrate	Sodium Amytal	Sodium Barbital	Group data (Sodium Barbital)
Normal	..	y=6.0365-0.0264x	y=6.0365-0.0264x	y=6.0365-0.0264x	y=6.0365-0.0264x	y=6.9762-0.0526x
Anaesthetized	..	y=5.8588-0.0125x	y=6.5234-0.0113x	y=7.2635-0.0160x	y=7.4127-0.0125x	y=7.5190-0.0285x

to the experimental medium (water), so as to affect the fish adversely, does not appear to have been recorded by earlier workers.

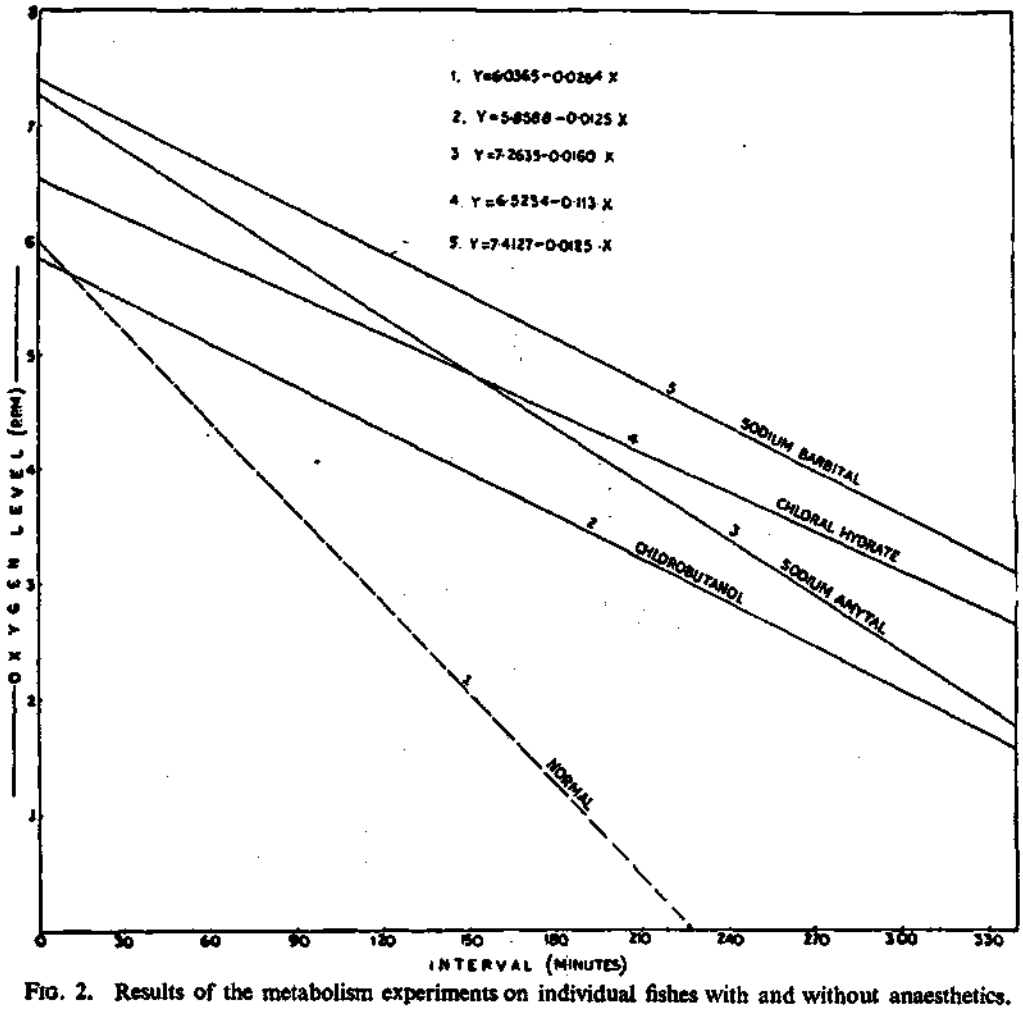

FIG. 2. Results of the metabolism experiments on individual fishes with and without anaesthetics.

The levels of oxygen recorded at intervals of 30 minutes from the start of the experiment were related by the regression equation of the form $y=a+bx$, where x and y represent the time of interval and the level of oxygen respectively. The rate of decrease in oxygen level per minute was calculated by using the formula $dy/dx=b$. The results are shown in Tables VII and VIII and in the graphs (Figs. 2 & 3) for the anaesthetics studied. The percentage of decrease in the level of oxygen in the water with anaesthetic, as compared with that without anaesthetic are, 47.35% in Chlorobutanol, 42.80% in Chloral Hydrate, 60.61% in Sodium Amytal, 47.35% in Sodium Barbital and 54.18% in the group data with Sodium Barbital.

From the above results it is evident that in anaesthetized fishes the survival rate is increased considerably. Thus the weight of the fish in unit volume of water

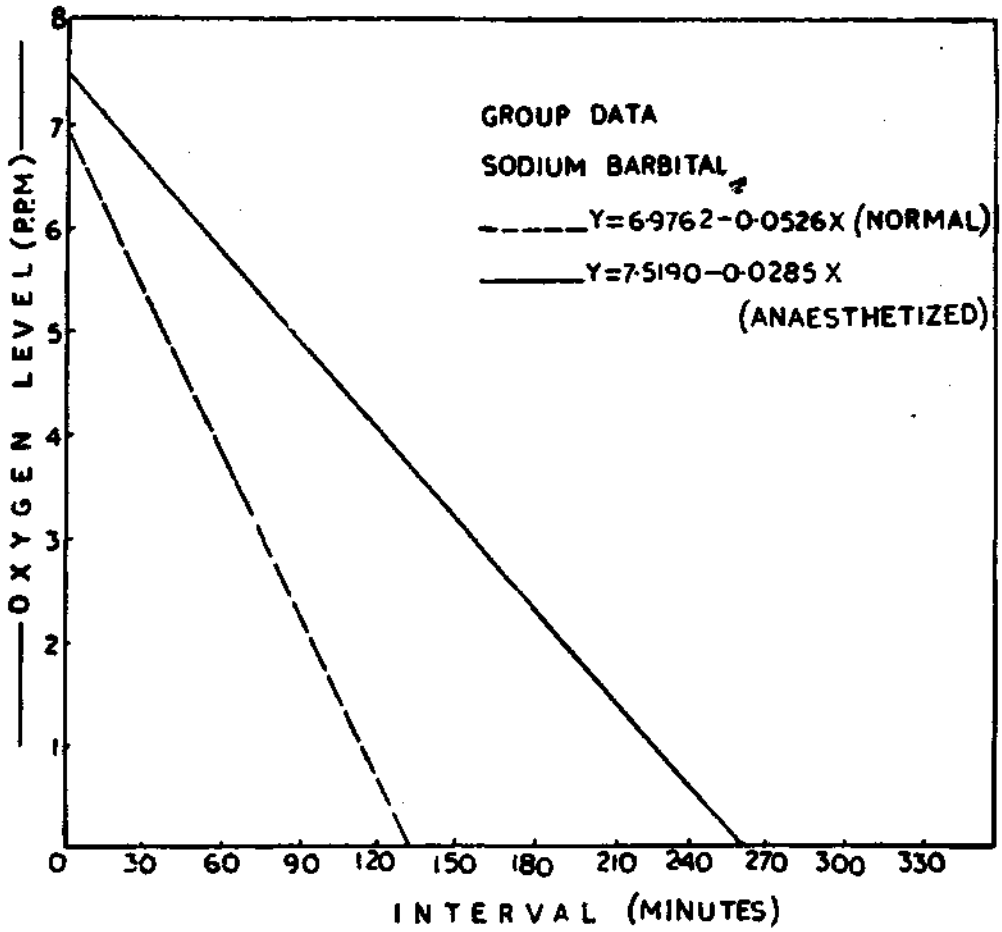

FIG. 3. Results of the metabolism experiments on groups of fishes with and without anaesthetics.

could at least be doubled during transport. Further, among the chemicals used, Sodium Barbital and Chlorobutanol have the same rate of decrease in the level of

TABLE VIII

The rate of decrease in oxygen levels with different anaesthetics used in the Metabolism experiments

Treatment	Chloro-butanol	Chloral Hydrate	Sodium Amytal	Sodium Barbital	Group data (Sodium Barbital)
Normal ..	0.0264	0.0264	0.0264	0.0264	0.0526
Anaesthetized ..	0.0125	0.0113	0.0160	0.0125	0.0285
Decrease % ..	47.35	42.80	60.61	47.35	54.18
Initial Oxygen range p.p.m.	5.95-6.82	5.73-6.90	6.60-7.84	6.83-7.53	6.83-7.76

oxygen (0.0125). Chloral Hydrate has the minimum rate of decrease of oxygen level (0.0113) and Sodium Amytal has the maximum rate of decrease of the oxygen level (0.0160) among the anaesthetics studied.

It appears from Table VIII that Sodium Barbital is more effective on group of fish than individual fish. This is more likely to be due to the 'group effect' in fish where metabolic rates change when fish are in groups, than to the quality of the anaesthetic used. Since the initial oxygen levels were not the same (Table VIII), the optimum rate of survival time of fish could not be compared.

Discussion

Urethane has been most widely used as a general anaesthetic for operative procedures. Young (1935), Hogben (1936) Waring (1940) Hasler and Meyer (1942), Gerking (1949) and Johnson (1954) have used Urethane on fishes to produce immobility at concentrations ranging from 0.5 to 4.0%. McFarland (1960) suggested the dose of 10 to 13 grams per gallon of water to induce the stage of total loss of equilibrium (stage 4). In the present investigation, the dose of 0.40 to 0.80 g./100 ml,, of Urethane is suggested for any operative, tagging or measuring procedures in the fingerlings of mullets where the total exposure of the fish to the anaesthetic is not 30 minutes or more. However, the recovery of fish from Urethane anaesthesia takes a longer time and in view of this, it does not appear to be very useful in operative, tagging or measuring procedures. The use of Urethane in fishery procedures is also not advisable because of its carcinogenic properties (Wood, 1956).

Burrows (1952) and Nelson (1953) have reported that Chlorobutanol causes immobility in 2 to 3 minutes at concentrations from 2.8 to 3.5 mM/L in several Salmonids. Cope (1953) tried Chlorobutanol at the concentration of 1 : 2000 for the tagging of adult cutthroat trout (*Salmo clarkii levisi*) and found that fishes lose equilibrium within 1 or 2 minutes and the recovery is within 2 to 10 minutes. He further indicated that time required for recovery depends upon the time of exposure. Meister and Ritzi (1958) recorded from 1 : 1000 to 1 : 5000 as the useful concentrations of Chlorobutanol. McFarland (1960) observed total loss of equilibrium in *Fundulus parvipinnis* at concentrations of 0.02 to 0.025 g./gallon but also found a very long recovery time even at the exposure of 20 to 30 minutes. The results of the experiments on Chlorobutanol reported here indicate that the concentration from 0.010 to 0.019 g./100 ml. are very useful in immobilising the fish within a short time and keeping it in this condition for a period of at least 30 minutes. As stated earlier, the recovery is also quick, within 4 to 12 minutes.

Unger (1918) and Hara (1924) are the earliest workers using Chloral Hydrate on fish. Aitken (1936) tried Chloral Hydrate for transport at 4% solution. McFarland (1960) observed that Chloral Hydrate at concentrations of 8.5 to 9.0 g./gallon is useful in inducing stage 4. The present studies indicate the usefulness of the anaesthetic at a concentration of 0.20 to 0.25 g./100 ml. The recovery is, however, late and the anaesthetic may perhaps be less useful for tagging or marking.

McFarland (1959) found a very low narcotic potency for Tertiary Butyl Alcohol which has also been confirmed in the present investigation. In his later work (McFarland, 1960), he observed that anaesthesia by Teritiary Butyl Alcohol is not controllable but found 30 ml./gallon as the limit concentration to induce the loss of

reflex reactivity (stage 5). The present work indicates that the stage 4 could be induced by Tertiary Butyl Alcohol at concentrations from 0.50 to 0.70 ml./100 ml. but the induction time is long, though the recovery is comparatively quicker.

Sodium Amytal has not been used for operational purposes though it has found a wider application in transport. McFarland (1960) found concentrations of 0.2 to 0.26 g./gallon useful in giving the fishes total loss of equilibrium. The authors found the concentrations of 0.012 to 0.030 g./100 ml., to induce the same stage in the mullet fingerlings. Sodium Amytal is antagonised by calcium unless chelating agents like Sodium Citrate or Disodium Ethylenediamine Tetraacetate (EDTA) are used. The antagonism of calcium to Sodium Amytal is well seen in the sea water and hence the solution of this anaesthetic prepared in the sea water is found to be less effective (Onkst *et al.*, 1957). McFarland (1954) from some pilot experiments found that barbiturates (Sodium Amytal, Phenobarbital and Barbital) are of limited use as anaesthetics, particularly in the salt water. However, in his later work (McFarland, 1959) he partially removed this calcium antagonism and increased the induction rate and the depth of anaesthesia in fresh water but could not do so in salt water. However, he concludes that ' in spite of its high narcotic potency, Sodium Amytal and other Barbiturates must be considered poor general anaesthetics for fishes because of calcium antagonism. Their use is ineffective where water is not soft or where chelating compounds are not added.' No data are available on the use of Sodium Barbital in tagging or marking work. Aitken (1936) and McFarland (1954) have used this anaesthetic to induce lighter anaesthesia.

Aitken (1936) was perhaps the first to recognize the potential use of anaesthetics in live-fish transport. Gerking (1949) suggested a possibility of using Urethane for transport. So far as the possibility of using the anaesthetics under investigation is concerned, it may be stated that all the anaesthetics could be used for the purpose. Sodium Barbital, of course, has a limit of 16 hours at the lowest concentration already discussed. Both, Sodium Amytal and Sodium Barbital have the disadvantages of calcium antagonism. Urethane has carcinogenic properties and the use of this chemical has, therefore, to be discouraged. Tertiary Butyl Alcohol is useful but has low narcotic potency. Chlorobutanol has a very high narcotic potency but is not readily soluble in plain water. It has, therefore, to be dissolved in hot water or organic solvents. Chloral Hydrate, though a sedative of intermediate potency, is useful as it is readily soluble in fresh water and is also inexpensive.

McFarland (1960) has also indicated the usefulness of Chloral Hydrate. He has advocated the pre-treatment of fishes in anaesthetic prior to transport in order to lessen the mortality. McFarland (*op. cit.*) has tried to fix the theoretical limits for the weight of fish that can be carried during transport by using anaesthetics. However, he has stated later that this limit cannot be realized in practice. Realistic picture will be two to three-fold increase in the weight of fish per unit volume for transport using anaesthetic. Reese (1953) suggested doubling and occasionally the tripling of the normal weights of fishes during transport with anaesthetic. This observation is supported by the metabolism experiments conducted in the present investigation.

SUMMARY

Six different anaesthetics namely, Tertiary Butyl Alcohol, Chloral Hydrate, Chlorobutanol, Sodium Amytal, Sodium Barbital and Urethane have been used to study their effects in the fingerlings of the mullet *Liza tade*, at different concentrations.

The behavioural changes of the mullet fingerlings in anaesthetized condition have been classified.

The dosage of each anaesthetic to give the stages of sedation useful for tagging and transport of live-fish have been determined and discussed.

Tolerance of fishes to anaesthetics and the narcotic potency of each anaesthetic has been determined. Fish tolerate lower concentrations of all the anaesthetics for 24 hours, except in Sodium Barbital where the tolerance limit is 16 hours. The narcotic potency is related to the molecular weight of the anaesthetic with the exception of Sodium Barbital.

Experiments on the metabolism of anaesthetized fish indicate that the metabolism is appreciably reduced by anaesthesia, so that the survival time increases considerably. Thus the weight of fish in unit volume of water could at least be doubled during transport.

Each anaesthetic and its useful doses are discussed in the light of the available literature to determine the suitability of each anaesthetic on merit. Chloral Hydrate appears to be the most suitable of the chemicals studied.

ACKNOWLEDGEMENT

Authors record with pleasure their thanks to Dr. S. Jones, Director, Central Marine Fisheries Research Institute, for his interest in this work and facilities given for its completion. They are also grateful to Dr. R. Raghu Prasad and Shri S. K. Banerji for going through the manuscript critically and suggesting improvements.

APPENDIX TABLES IX TO XIV

Experimental data for finding out the minimal & maximal doses of anaesthetics and also the relationship of dosage with Anaesthesia

APPENDIX TABLE IX

Tertiary Butyl Alcohol

No.	Concentration	No. of fishes	Weight range & Avg. wt.	Mortality (% fishes)	Loss of equilibrium (% fishes)	Avg. time required (minutes)	Avg. time for recovery (minutes)	Partial loss (% fishes)	Deep sedation (% fishes)	Light sedation (% fishes)	Normal (% fishes)
1.	0.30	80	0.6-23.0 (6.50)	2.50	1.25	165.00	5.00	6.25	22.50	56.24	11.25
2.	0.35	60	1.2-4.1 (2.35)	1.66	13.33	204.12	11.75	13.33	28.32	43.33	nil
3.	0.40	60	1.0-24.0 (7.71)	6.67	23.33	97.64	7.57	21.66	15.00	33.33	nil
4.	0.50	20	2.0-25.5 (12.76)	nil	60.00	97.50	8.08	40.00	nil	nil	nil
5.	0.60	20	1.0-13.9 (4.24)	nil	55.00	39.36	10.82	45.00	nil	nil	nil
6.	0.70	20	3.0-27.0 (13.61)	nil	95.00	32.84	11.32	5.00	nil	nil	nil

APPENDIX TABLE X

Chloral Hydrate

No.	Concentration	No. of fishes	Weight range & avg. wt.	Mortality (% fishes)	Loss of equilibrium (% fishes)	Avg. time required (minutes)	Avg. time for recovery (minutes)	Partial loss (% fishes)	Deep sedation (% fishes)	Light sedation (% fishes)	Normal (% fishes)
1.	0.01	60	1.1-4.4 (2.44)	4.99	nil	..	..	nil	8.34	83.33	3.34
2.	0.025	60	1.2-4.0 (2.20)	3.34	nil	..	..	1.67	4.99	89.99	nil
3.	0.05	60	1.0-4.4 (2.47)	6.67	16.66	255.20	7.0	1.67	16.66	21.00	nil
4.	0.075	60	1.0-7.0 (2.63)	3.34	15.00	246.33	11.34	1.67	34.99	45.00	nil
5.	0.10	80	1.0-22.0 (8.19)	2.40	16.25	94.61	17.4	nil	32.50	42.50	5.00
6.	0.15	40	0.7-21.5 (5.73)	10.02	34.99	87.92	16.57	nil	42.49	12.50	nil
7.	0.20	20	1.2-21.0 (6.93)	10.02	40.00	116.87	20.67	nil	50.00	nil	nil
8.	0.25	19	1.1-16.5 (5.77)	10.52	73.67	81.42	33.92	5.26	10.52	nil	nil

APPENDIX TABLE XI

Chlorobutanol

No.	Concentration	No. of fishes	Weight range & avg. wt.	Mortality (% fishes)	Loss of equilibrium (% fishes)	Avg. time required (minutes)	Avg. time for recovery (minutes)	Partial loss (% fishes)	Deep sedation (% fishes)	Light sedation (% fishes)	Normal (% fishes)
1.	0.002	60	1.2-14.0 (3.40)	1.67	nil	—	—	nil	13.33	85.00	nil
2.	0.003	80	1.1-14.2 (3.39)	nil	nil	—	—	nil	8.75	91.24	nil
3.	0.005	80	0.7-5.4 (1.98)	18.75	5.00	231.0	5.66	25.00	8.75	42.50	nil
4.	0.006	80	0.6-14.2 (4.25)	10.00	17.50	54.5	5.86	20.00	32.50	20.00	nil
5.	0.010	66	0.7-25.0 (8.78)	4.54	95.45	28.43	11.54	nil	nil	nil	nil
6.	0.015	20	0.8-4.0 (2.32)	20.00	80.00	5.06	8.50	nil	nil	nil	nil
7.	0.019	20	3.0-11.0 (6.63)	20.00	80.00	4.25	3.94	nil	nil	nil	nil
8.	0.020	18	0.8-16.4 (5.96)	100.00	nil	3.61	—	nil	nil	nil	nil

APPENDIX TABLE XII

Sodium Amytal

No.	Concentration	No. of fishes	Weight range & avg. wt.	Mortality (% fishes)	Loss of equilibrium (% fishes)	Avg. time required (minutes)	Avg. time for recovery (minutes)	Partial loss (% fishes)	Deep sedation (% fishes)	Light sedation (% fishes)	Normal (% fishes)
1.	0.0035	60	1.0-9.8 (3.45)	1.67	nil	—	—	1.67	nil	96.67	nil
2.	0.004	80	1.2-6.2 (3.06)	6.25	16.25	124.76	13.69	18.75	20.00	38.76	nil
3.	0.005	80	1.0-5.4 (2.67)	12.50	32.50	112.96	11.42	6.25	36.24	12.50	nil
4.	0.006	20	2.0-6.0 (3.54)	nil	30.00	129.83	19.16	15.00	50.00	5.00	nil
5.	0.007	20	0.8-7.7 (4.57)	nil	90.10	66.72	40.50	5.00	5.00	nil	nil
6.	0.008	20	1.5-6.2 (3.91)	nil	85.00	72.17	29.88	5.00	10.00	nil	nil
7.	0.009	20	3.0-8.0 (4.68)	nil	85.00	56.76	32.41	10.00	nil	5.00	nil
8.	0.01	40	0.5-6.0 (2.29)	nil	77.50	62.87	48.71	20.00	nil	2.50	nil
9.	0.011	40	0.5-6.6 (2.11)	7.49	79.98	60.25	51.50	12.50	nil	nil	nil
10.	0.012	40	0.9-5.3 (2.51)	17.50	82.49	61.85	58.20	nil	nil	nil	nil
11.	0.015	40	1.5-5.5 (2.91)	nil	100.00	28.60	41.75	nil	nil	nil	nil
12.	0.02	20	2.5-7.3 (4.24)	5.00	95.01	34.47	82.10	nil	nil	nil	nil
13.	0.03	20	0.8-7.0 (3.99)	45.00	55.00	24.18	101.73	nil	nil	nil	nil
14.	0.035	20	1.5-6.4 (3.88)	90.01	10.02	14.50	145.50	nil	nil	nil	nil

APPENDIX TABLE XIII

Sodium Barbital

No.	Concentration	No. of fishes	Weight range & avg. wt.	Mortality (% fishes)	Loss of equilibrium (% fishes)	Avg. time required (minutes)	Avg. time for recovery (minutes)	Partial loss (% fishes)	Deep sedation (% fishes)	Light sedation (% fishes)	Normal (% fishes)
1.	0.1	80	0.8-2.8 (1.41)	1.25	nil	—	—	nil	7.50	91.24	nil
2.	0.125	60	0.9-4.9 (1.94)	1.67	nil	—	—	nil	11.66	86.66	nil
3.	0.15	80	0.8-3.2 (1.29)	1.25	1.25	380.00	150.00	2.50	76.25	18.75	nil
4.	0.20	20	1.0-2.7 (1.66)	nil	25.00	297.00	131.00	15.00	60.01	nil	nil
5.	0.30	20	1.0-18.5 (2.51)	10.00	70.00	216.00	195.00	5.00	15.00	nil	nil

APPENDIX TABLE XIV

Urethane (Ethyl Carbamate)

No.	Concentration	No. of fishes	Weight range & avg. wt.	Mortality (% fishes)	Loss of equilibrium (% fishes)	Avg. time required (minutes)	Avg. time for recovery (minutes)	Partial loss (% fishes)	Deep sedation (% fishes)	Light sedation (% fishes)	Normal (% fishes)
1.	0.150	80	1.45-6.5 (3.33)	5.00	nil	—	—	1.25	21.27	71.25	1.25
2.	0.175	60	1.3-11.9 (2.06)	3.33	nil	—	—	5.00	43.33	45.00	3.33
3.	0.20	80	0.9-10.0 (3.83)	2.50	5.00	92.50	5.50	nil	45.00	47.50	nil
4.	0.225	40	1.5-10.0 (4.07)	nil	4.99	75.00	6.00	nil	70.00	25.00	nil
5.	0.25	80	1.0-6.5 (3.62)	nil	45.00	61.17	13.06	2.50	31.25	21.24	nil
6.	0.30	40	1.0-6.5 (3.21)	nil	79.98	43.28	13.31	10.02	10.02	nil	nil
7.	0.40	20	2.5-10.0 (5.67)	nil	95.01	18.70	14.80	5.00	nil	nil	nil
8.	0.70	20	2.0-9.10 (4.87)	nil	100.00	3.00	20.55	nil	nil	nil	nil
9.	0.80	20	2.0-7.0 (3.98)	20.00	80.00	3.18	30.75	nil	nil	nil	nil
10.	0.85	20	2.5-8.0 (5.06)	70.00	30.00	4.00	30.34	nil	nil	nil	nil

REFERENCES

ABRAMOWITZ, A. A. 1937. The opercular approach to the pituitary. *Science*, **85** : 609.

———, HISAW, F. L., BOETTIGER, F. AND PAPANDREA, D. N. 1940. The origin of the diabetogenic harmone in the dogfish. *Biol. Bull.* Woods Hole, **78** : 189-201.

*ADAMS, R., RIDEAL, E. K., BURNETT, W. B., JENKINS, R. L. AND DREGER, E. E. 1926. Chemical constitution,physiological action and physical properties in a series of alkyl para-aminobenzoates. *J. Am. chem. Soc.*, **48** (1) : 1758-1770.

AITKEN, W. W. 1936. A comparative study of the effects of certain hypnotics on *Lepidosteus osseus* and *Ictalurus punctatus* at different temperatures. *Copeia*, No. 2 : 121-122.

BELL, G. R. 1964. A guide to the properties, characteristics and uses of some general anaesthetics for fish. *Bull. Fish. Res. Bd. Can.*, No. 148.

BROUN, D. 1928. Action narcotique des acides alcoylethyl barbituriques sur les poissons. *C.R. Soc. Biol.*, Paris, **99** : 1792-1794.

BURROWS, R. E. 1952. Chloretone used to anaesthetize salmon fingerlings. *Progr. Fish Cult.*, **14** (2) : 78.

COPE, O. B. 1953. Chloretone as an anaesthetic for adult cutthroat trout. *Ibid.*, **15** (1) : 35.

FÜHNER, H. 1912. Der Wirkungsgrad der einwertigen Alkohole. Ein wergleichend-pharmakologischer Beitrag zur Theorie der Narkose. *Z. Biol.*, **57** : 465-494.

GERKING, S. D. 1949. Urethane (Ethyl Carbamate) in some fishery procedures. *Progr. Fish Cult.*, **11**(1) : 73-74.

GOODMAN, L. S. AND GILMAN, A. 1955. *The Pharmacological Basis of Therapeutics.* 2nd Ed. New York, Macmillan Co., 1831.

*HARA, S. 1924. Über die Wirkung vershiedeener Narkotica auf die atmung des Goldblutt (*Pleuronectes platessa*). *Z. ges. exp. Med.*, **43** : 256-269.

HASKELL, D. G. 1940. Stunning fish by electricity. *Progr. Fish Cult.*, **49** : 33-34.

HASLER, A. D. AND MEYER, R. K. 1942. Respiratory responses of normal and castrated gold fish to teleost and mammalian hormones. *J. exp. Biol.*, **91** (3) : 391-404.

HOGBEN, L. 1936. The pigmentary effector system VII. The chromatic function in elasmobranch fishes. *Proc. Roy. Soc., B*, **120** : 142-158.

HOWELL, J. H. AND THOMAS, P. M. 1964. Anaesthetic effect of 4-styrylpyridine on lamprey and fish. *Trans. Am. Fish. Soc.*, **93** : 206.

JOB, S. V. 1957. The routine-active oxygen consumption of the milk fish. *Proc. Indian Acad. Sci.*, **45** (6) 302-313.

JOHNSON, L. D. 1954. Use of urethane anaesthesia in spawning eastern brook trout. *Progr. Fish Cult.*, **16** (4) : 182-183.

KEYS, A. B. AND WELLS, N. A. 1930. Amytal anaesthesia in fishes. *J. Pharmacol.*, **40** : 115-128.

*KOCHMAN, M. 1913. Beitrage zur Pharmakologic der Mischnarkose. I. Wirkungen von Narcoticage mischen auf poikilotherme Wasserthiere. *Z. exp. Path. Ther.*, **12** : 328-340.

LINDENBERG, B. A. AND GARY-BOBO, C. 1951. Concentration thermodynamique narcotisante des alcools aliphatiques dans l'eau des tissue des poissons. *C.R. Acad. Sci. Paris*, **233** : 212-214.

*MCFARLAND, W. N. 1954. Discussion of selected experiments on effects of drugs on fishes. Appendix, pp. 7-10, in Report of the University of California Salton Sea Research Programme for the period Feb. 1, 1954 to May 20, 1954. 22 pp.+appendix (mimeographed report).

McFARLAND, 1959. A study on the effects of anaesthetics on the behaviour and physiology of fishes. *Publs. Inst. mar. Sci. Univ. Tex.*, 6 : 23-55.

———. 1960. The use of anaesthetics for the handling and the transport of fishes. *Calif. Fish and Game*, 46 (4): 407-432.

———. AND NORRIS, K. S. 1958. Control of *pH* and CO_2 buffers in fish transport. *Ibid.*, 44 (4) : 291-310.

MEISTER, A. L. AND RITZI, C. F. 1958. Effects of Chloretone and MS-222 on eastern brook trout. *Progr. Fish Cult.*, 20 : 104-110.

MERCK INDEX. 1960. *The Merck Index of Chemicals and Drugs*, 7th Edition. Published by Merck & Co., Inc. U.S.A.

*MEUNCH, B. 1956. The screening of compounds to find a drug suitable for use on fish populations. *M.Sc. Thesis, Southern Illinois Univ.*

———. 1958. Quinaldine, a new anaesthetic for fish. *Progr. Fish Cult.*, 20 (1) : 42-44.

NATARAJAN, M. V. AND RENGANATHAN, V. 1960. A note on the possibilities of utilizing quinaldine in transporting live-fish. *Curr. Sci.*, 29 : 393.

NELSON, P. R. 1953. Use of three anaesthetics on juvenile salmon and trout. *Progr. Fish Cult.* 15 (2) : 74.

NEMOTO, C. M. 1957. Experiments with methods for air transport of live-fish. *Ibid.*, 19 (4) : 147-157.

ONKST, H. J., JACOBY AND SCARPELLI, D. G. 1957. Effect of calcium binding substances on rate and duration of narcosis. *Proc. Soc. exp. Biol.*, 96 (2) : 397-399.

OSBORN, C. M. 1938. The role of the melanophore-dispersing principle of the pituitary in the color changes of the catfish. *J. exp. Zool.*, 79 (2) : 309-330.

———. 1941. Studies on the growth of integumentary pigment in the lower vertebrates II. The role of the hypophysis in melanogenesis in the common catfish (*Ameiurus meals*). *Biol. Bull. Woods Hole*, 81 : 352-363.

PARKER, G. H. 1937. Integumentary color changes of elasmobranch fishes especially of *Mustelus*. *Proc. Am. phil. Soc.*, 77 (3) : 223-247.

———. 1939. General anaesthesia by cooling. *Proc. Soc. exp. Biol.*, 42 : 186-187.

PICKFORD, G. E. AND ATZ, J. S. 1957. *The physiology of the pituitary gland of fishes*. New York Zoological Soc., 613 pp.

REESE, A. 1953. Use of hypnotic drugs in transporting trout. *Calif. Dept. Fish. and Game., Mimeo. Report.*, 10 pp.

SAHA, K. C., SEN, B. P. AND BANERJEE, S. M. 1965. Use of Sodium Amytal in transport of fry of Indian major carps. *Ann. Biochemi. exptl. Med.*, 15 (2) : 133-134.

SREENIVASAN, A. 1962. Use of tranquilizing drugs and narcotics in reducing oxygen consumption by fish. *Indian J. Fish.*, 9 (2) : 738-745.

THIENPONT, D. AND NIEMEGEERS, C. J. E. 1965. Propoxate (R 7464) : a new potent anaesthetic agent in cold-blooded vertebrates. *Nature*, 205 : 1018.

TIFFENEAU, M. AND DORLENCOURT, H. 1923. Sur une nouvelle serie d'hypnotiques, les aryldiacoylglycols. *C.R. Acad. Sci. Paris.*, 176 : 1343-1346.

———. AND TORRES, C. 1924. Sur les proprietes hypnotiques de l'hydrobenzoine et de ses homologues alcoyles (diarylglycols symetriques). Relations entre l'activite physiologique et le poids moleculaire. *Ibid.*, 178 : 237-240.

*UNGER, R. 1918. Über den Einfluss der Temperatur auf Wirkungsstarke und Oberflachenaktivitat der Narkotica. *Biochem. Z.*, **89** : 238-278.

WARING, H. 1940. The chromatic behaviour of the eel (*Anguilla vulgaris* L.). *Proc. Roy. Soc., B.*, **128** : 343-353.

*WITJENS, P. H., VAN DIJK, C. P. AND VERKADE, P. 1947. Investigation of the usefulness of the goldfish test for the determination of local anaesthetic activity. *Arch. int. Pharmacodyn.*, **74** : 378-398.

WOOD, E. M. 1956. Urethane as a carcinogen. *Progr. Fish Cult.*, **18** (3) : 135-136.

YOUNG, J. Z. 1935. The photoreceptors of lampreys II. The functions of the pineal complex. *J. exp. Biol.*, **12** (3) : 354-370.