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THE USE OF ELECTRONARCOSIS AS ANAESTHETIC IN THE CICHLID, *OREOCHROMIS MOSSAMBICUS* (PETERS). I. GENERAL EXPERIMENTAL PROCEDURES AND THE ROLE OF FISH LENGTH ON THE NARCOTIZING EFFECTS OF ELECTRIC CURRENTS

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ABSTRACT

BARHAM, W. T., SCHOONBEE, H. J. & VISSER, J. G. J., 1987 The use of electronarcosis as anaesthetic in the cichlid, *Oreochromis mossambicus* (Peters). I. General experimental procedures and the role of fish length on the narcotizing effects of electric currents. *Onderstepoort Journal of Veterinary Research*, 54, 617–622 (1987)

Procedures to narcotize fish by means of alternating or direct electrical currents are described and a method of evaluating the narcotic effects on fish of electrical currents is detailed.

The role of fish length on the narcotizing potential of electrical currents was investigated. The results indicate that there is a positive correlation between fish length and the duration of narcosis.

INTRODUCTION

Fish biologists and aquaculturists have long been aware that the handling of fish for research procedures or for routine examinations in fish farming results in stress (Soivio & Oikari, 1976; Pickering, 1981; Pickering, Pottinger & Christie, 1982). Pickering (1981) describes stress as a stimulus, the reaction of the fish being the stress response. This, however, is a loose description and stress should rather be defined as a stimulus which involves a response adversely disturbing the physiological processes. Nevertheless, responses to stress can vary from minor physiological disturbances to the ultimate response—death. Stress introduces an undesirable variable in experimental work for the fish biologist and, for the fish farmer, stress responses may be reflected in lower fish yields (Pickering, 1981; Wedemeyer & McLeay, 1981).

Biologists studying the effects of stressors on fish, as well as those workers involved in other types of fish studies, are faced with the problem of eliminating, or at least minimizing, the stressful effects of the handling procedures inherent to such investigations. Early efforts to render the fish unconscious involved applying a sharp blow to the head from which the fish may or may not recover (Healey, 1964). Ethyl alcohol, as an anaesthetic, proved equally unsatisfactory (Healey, 1964). Subsequently, a variety of pharmalogical compounds, including barbiturates, were tried, but were never really successful (Abramowitz, 1937; Pickford & Atz, 1957). Today, however, chemical aneasthesia of fish to alleviate handling stress and other types of stress is a common procedure (McFarland 1959, 1960; Smit, Hattingh & Burger, 1979a). Substances used to induce anaesthesia in fishes include quinaldine sulphate (Blasiola, 1977), tricaine methanesulphonate (Houston, Czerwinski & Woods, 1973; Smit, 1980) and benzocaine hydrochloride (Ferreira, 1979; Ferreira, Schoonbee & Smit, 1979). Although these substances effectively anaesthetize the animals and thereby minimize handling stress and other stressful conditions, they also produce their own side-effects on the blood physiology of fish (McFarland, 1959; Smit, 1980). These side-effects, however, are generally less severe than those induced by the experimental procedures. Although in many cases these side-effects may be acceptable to the research worker, in studies on blood physiology they may be of sufficient magnitude to mask or significantly influence experimental results. Thus fish biologists have recently concentrated their energies on evaluating and minimizing anaesthetic-induced stress in fish (Smit *et al.*, 1979a, b; Ferreira *et al.*, 1984). Response to an anaesthetic substance may be immediate or be evident only after a period of time (Soivio & Oikari, 1976; Smit, 1980). Some researchers are only concerned with immediate or short-term effects of anaesthesia, while others are also concerned with long-term effects. In addition to the physiological constraints of recognised fish anaesthetics, cost makes this method of combating stress progressively less attractive. Fish biologists studying stress look, therefore, for an ideal, relatively inexpensive narcotic which would have little or no effect on the physiology of the fish, either immediately or in the long term.

Electronarcosis suggests itself as a possible alternative to chemical anaesthesia, since it appears to be cheap to operate. The few studies to date also suggest that recovery is uneventful and possibly less stressful when compared with that of chemical anaesthesia (Kynard & Lonsdale, 1975; Madden & Houston, 1976). As reported by Meyer-Waarden (1959), Halsband discussed narcotizing pulse thresholds for freshwater fish (1954) and marine fish (1955), while Kreutzer formulated the physiological principles of electric fishing in the sea as early as 1951. No particular criteria appear to have been used, however, in the selection of electrical parameters in the later studies. In addition, a clear distinction between electronarcosis or galvanonarcosis and tetanus was not always made.

From the available literature on electronarcosis in fish and other animals, it is clear that the physiological consequences of this method of inducing anaesthesia are not as yet fully understood. There is also scant information on how the immobilization resulting from electronarcosis occurs.

Electronarcosis can be subdivided into 2 main categories, alternating current (a.c.) electronarcosis and direct current (d.c.) electronarcosis. An evaluation of the anaesthetic potential in fishes of both these categories is essential before a detailed study is made of the physiological effects of electronarcosis. Mammals, including man, have been narcotized over the years by either a.c. or d.c. currents, or combinations of the two. Much information is available regarding suitable wave forms, frequencies, potentials and currents for efficient mammal narcosis (Knutson, 1954; Price & Dornette, 1963; Geddes, 1965). All forms of mammal electronarcosis involve placing electrodes on or in the animal. Such techniques are obviously not feasible with aquatic organisms and use must be made of electrodes immersed in

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water. Previous studies on fish utilized a variety of electrical parameters and values and for this reason it is difficult to compare the findings of different researchers.

As very little information exists regarding suitable a.c. or d.c. narcotizing circuits in contrast to electro-fishing gear, any study of fish electronarcosis must of necessity include the development of suitable a.c. and d.c. circuits which allow for various configurations of electrical parameters, such as wave shape, frequency and potential.

The endemic local tilapiine species, *Oreochromis mossambicus* (Peters), was selected to evaluate electronarcosis in this study, as it is considered a very suitable local candidate for aquaculture. It is also recognised as an excellent fish for warm water culture and as such is expected to be subjected to much research in the near future (Fishelson, 1983). In the present work the narcotic effects of various frequencies, potentials, temperatures and conductivities, as well as wave forms, on *O. mossambicus* were evaluated for both a.c. and d.c. current. The effects of application time were also studied.

The principal aim of this three-part study was, therefore, to evaluate electronarcosis as a tool for reducing handling stress in fish, using as criteria the narcotic effects of electrical currents. Part I deals with experimental methods and the role of fish length in electronarcosis. In Parts II and III the narcotic effects of alternating and direct currents respectively are discussed.

MATERIALS AND METHODS

Experimental fish

Healthy, mature *O. mossambicus* of both sexes were netted in the Midmar and Albert Falls dams near Pietermaritzburg and transported to the university hatchery in 1 000 ℓ fibreglass tanks in well-aerated water. The fish were lightly tranquillized with benzocaine hydrochloride (10 mg/ℓ) and salt (NaC1) a concentration of 10 gℓ^{-1} was added to the transport water to minimize possible osmoregulatory responses resulting from handling and transport stress.

On arrival at the hatchery all the fish were subjected to a malachite green/formalin treatment for 2 h (Leteux & Meyer, 1972). The fish were then held in plastic "port-a-pools" at the hatchery for at least 3 months before being netted rapidly and moved to large, 1 000 ℓ glass holding aquaria in the laboratory. Stocking density was 30 fish per tank. During their time in the hatchery as well as in the laboratory the fish were fed commercial fish pellets.

The fish were held in the large aquaria for at least 4 weeks to acclimate them to laboratory conditions. At no time during the transfer operations at the hatchery or in the laboratory were the fish out of water for more than 10 s.

Electronarcosis apparatus

A unit for a.c. narcosis was designed and constructed in the laboratory and proved to be reliable and satisfactory in operation (Barham, Schoonbee & Visser, 1987).

A d.c. unit based on the fish shocker designed by Moore (1968) was constructed in the laboratory. The main differences from the Moore shocker lay in the values or types of components. A Q4008L type quadrac and a IN5404 diode were used in conjunction with a 3.6 ufd 420 V capacitor. The potential of the d.c. unit was controlled by a variable transformer, and mains power was isolated by a 1:1 transformer. The output was a pulsed half-wave similar to that of the original Moore (1968) device.

The original unit, designed by Moore, employed a half-wave metal rectifier and a thyristor with a high peak inverse voltage (P.I.V.) as a safeguard against a high voltage transient. Output was of the order of 0,3A, with a load resistance of 500 ohm at a potential of 300 Vp. This gave a pulse width of 5,5 ms and a duty cycle of the order of 27 %.

The unit constructed for this study differed somewhat from the original, particularly in that a modern quadrac replaced the thyristor and that a diode was used in place of a metal rectifier to obtain half-wave rectification. This resulted in a pulse time of 7,3 ms and a duty cycle of approximately 36 % at 200 Vp when drawing power from the main supply via a variable transformer. This unit was able to produce a potential of at least 250 Vp. The wave shape of the laboratory unit approximated that of the Moore (1968) shocker.

Electrodes

The electronarcosis unit was attached to stainless steel electrodes which totally covered the ends of the aquarium tanks in order to ensure parallel lines of force in the electric field.

Procedures followed to electronarcotize fish

Unless otherwise stated, experimental groups consisted of 8 fish each. To evaluate the narcotizing effects of various configurations of electrical currents and of various physical factors, including fish lengths, individual, acclimated fish were removed from the holding tank and placed in a glass aquarium provided with stainless steel electrodes connected to the electronarcosis unit. Current was applied for a 30 s period as measured by a stopwatch, except that in studies on the effects of duration of current flow on the fish, when narcotizing times were varied from 15–90 s.

Evaluation of narcotizing effect of a.c. and d.c. current

When evaluating an anaesthetic or narcotic it is necessary not only to consider its efficiency in narcotizing or anaesthetizing the subject but also to determine the ease of induction, and whether recovery is uneventful or otherwise.

In the present study it was necessary to impose somewhat arbitrary criteria upon which electronarcosis would be evaluated. In contrast to chemical anaesthesia, induction of electronarcosis is immediate. Preliminary studies, however, indicated that the duration of narcosis depended on application time. With this in mind, an arbitrary application time of 30 s was selected for all studies except for the studies where the effect of time was evaluated.

A fish can be removed easily from the water, be subjected to cardiac puncture and withdrawal of blood, be weighed and measured and be returned to the water within the space of 2,5 min. This length of time is also sufficient to leave time to spare to mark the animal, if so required. For this reason a narcosis time of the order of 2,5 min was considered to be adequate for most handling purposes. Fish were subjected individually to various combinations of electrical parameters in tanks equipped with electrodes, as already described.

To evaluate the narcotizing effect of a particular electrical parameter, it was essential to establish criteria upon which to base the evaluation. Preliminary investigations showed that gill and opercular movements ceased upon application of a current and only reappeared some time after current had ceased to flow. In view of this it was decided to record: (a) time to onset of opercular movement (opercular recovery time); (b) time to first response to stimulus (narcosis time); and (c) time to commencement of swimming on an even keel (recovery time) as criteria of narcotic effect. All 3 criteria were timed as from cessation of current flow.

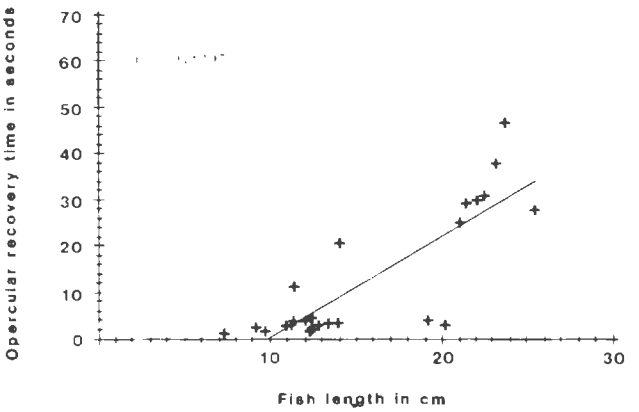

FIG. 1 The relationship between fish length and opercular recovery time in *O. mossambicus* subjected to a.c. electronarcosis at 50 Vp

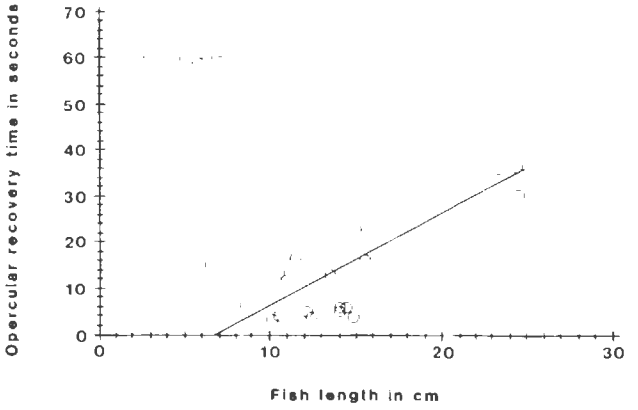

FIG. 2 The relationship between fish length and opercular recovery time in *O. mossambicus* subjected to a.c. electronarcosis at 60 Vp

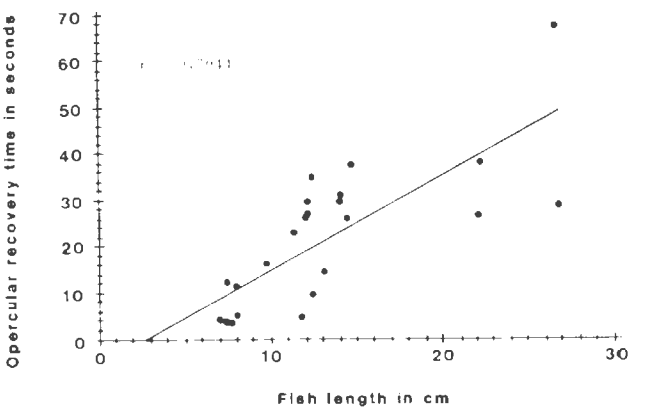

FIG. 3 The relationship between fish length and opercular recovery time in *O. mossambicus* subjected to a.c. electronarcosis at 70 Vp

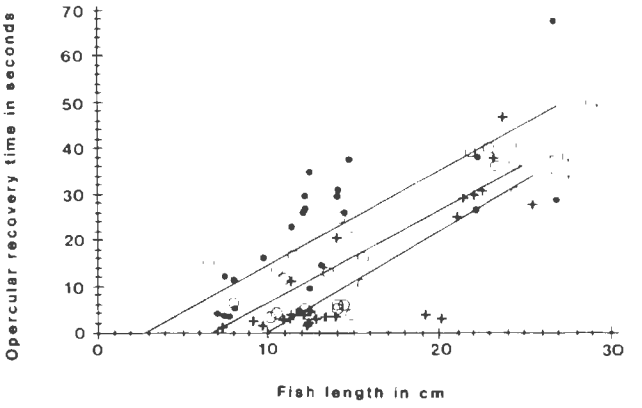

FIG. 4 A comparison of the relationship between fish length and opercular recovery time in *O. mossambicus* subjected to a.c. electronarcosis at three different peak voltages

To obtain comparable results it was essential to standardize the observations of events. This was done in the following manner:

Opercular time: Opercular movement was considered to have commenced when the opercula had beat rhythmically for 3 consecutive beats, since it was observed that initial beats were erratic.

Narcosis time: Immediately the gills started to beat rhythmically the flanks of the fish were stroked with a smooth plastic rod. In order to minimize the possibility of habituation, the strokes were alternated from side to side. Narcosis time was taken to be from induction until the first definite beat of the tail.

Recovery time: After determining narcosis time, stroking continued until the fish, which was invariably upside down or on its side, orientated itself correctly and swam a distance equal to its own length. The time, from the first response to stimulus until this orientation occurred, was taken as the recovery time. This continued stroking was found necessary as it was observed that if this was not done the fish often remained lethargic and did not attempt to right itself for some time after responding to stimuli.

Narcosis coefficient: In view of the possibility that narcosis time may be positively correlated with fish length it was decided to make allowance for the effect of differing fish lengths when comparing treatments. A calculated value relating narcotic potency to fish length can be obtained by dividing the narcosis time (NT) in seconds by the fish length in cm (L). We propose that this value be called the narcosis coefficient. This formula may be abbreviated as follows:

$$\text{Narcosis coefficient (NC)} = \frac{\text{NT}}{\text{L}} \text{ s cm}^{-1}$$

Tests on the effect of fish length on narcosis time were conducted in 46 cm long glass tanks containing 36 ℓ of water. All other tests were conducted in 60 cm long tanks containing 48 ℓ of water. Water temperature was maintained at 20 °C ± 0,5 °C, except when temperature effects were studied when values of 15 °C ± 0,5 °C and 25 °C ± 0,5 °C were achieved. Water conductivity was maintained at 430 μs cm^{-1} except for the conductivity studies, where waters of 100 μs cm^{-1} and 250 μs cm^{-1} were also used. Sample size was 8 fishes except for the fish-length studies, when the sample size was 24 fish.

Statistics

A statistical analysis of experimental data was performed on an Apple II Europlus microcomputer with 64K RAM, using software programmes adapted from Lee & Lee (1982) and Van Tassel (1981). Graphs were drawn using SCIPLOT and CURFIT programmes[1] as well as a modified SCIPLOT programme.

The Role of Fish Length in Electronarcosis

The effect of fish length on electronarcosis of *O. mossambicus* was evaluated at 3 a.c. peak voltages, namely 50 Vp, 60 Vp and 70 Vp. The equivalent rms voltages are 35,4 V, 42,5 V and 49,6 V respectively. The frequency was 50 Hz in each instance.

[1] Interactive Microware, Inc.

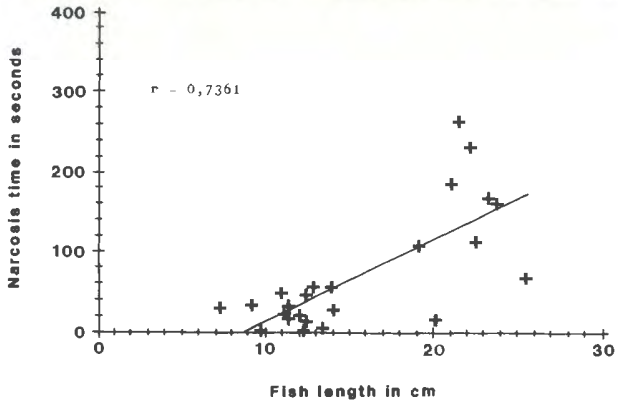

FIG. 5 The relationship between fish length and narcosis time in *O. mossambicus* subjected to a.c. electronarcosis at 50 Vp

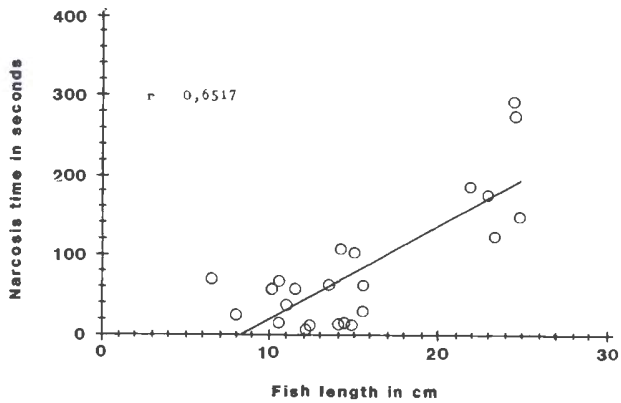

FIG. 6 The relationship between fish length and narcosis time in *O. mossambicus* subjected to a.c. electronarcosis at 60 Vp

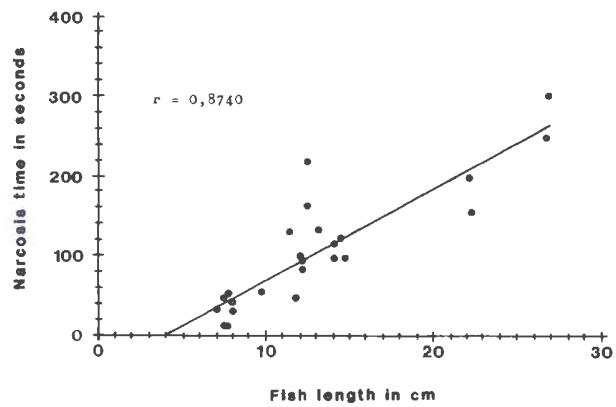

FIG. 7 The relationship between fish length and narcosis time in *O. mossambicus* subjected to a.c. electronarcosis at 70 Vp

The fish used in the 50 V study ranged in length from 7,3 cm–25,5 cm and had masses extending from 6,3–278,9 g. The size ranges for the fish used in the 60 V group were 6,5 cm–24,8 cm and 3,9 g–276,5 g. Those for the 70 V group were 7,0 cm–26,8 cm and 4,84 g–341,6 g respectively. There were no statistically significant differences in the size composition of the 3 groups at the 95 % level of confidence.

From Fig. 1–9 it is clear that both opercular time and narcosis time are positively correlated with the size of the fish. There was no significant correlation between length of fish and recovery time.

A further deduction can be made that an increase in voltage from 50 Vp–70 Vp clearly generally leads to a longer narcosis time for all the size groups investigated

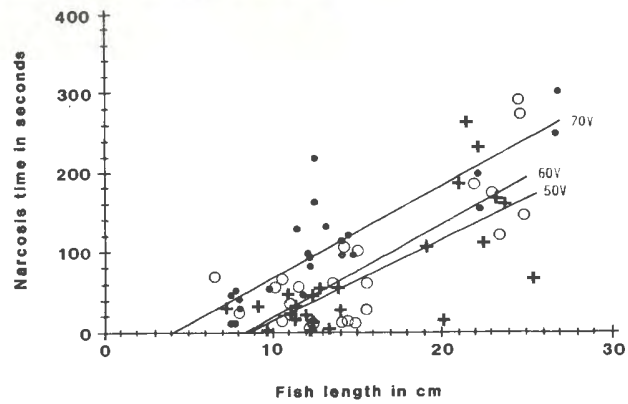

FIG. 8 A comparison of the relationship between fish length and narcosis time in *O. mossambicus* subjected to a.c. electronarcosis at three different peak voltages

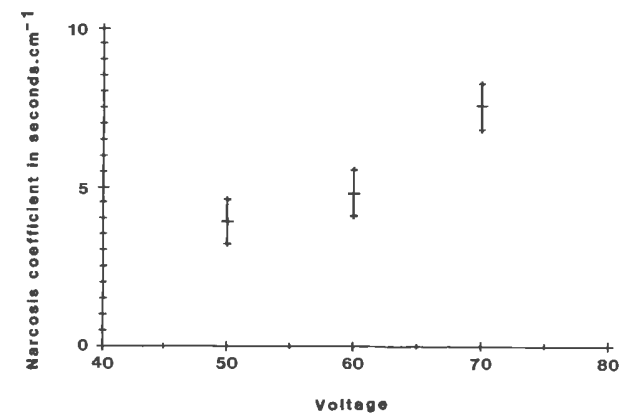

FIG. 9 A comparison of mean narcosis coefficients (± SE) for *O. mossambicus* subjected to a.c. electronarcosis at three different peak voltages

TABLE 1 Analysis of opercular recovery time, narcosis time and recovery time in *O. mossambicus* at 3 narcotizing voltages

	50 Volts		
	Opercular recovery time (s)	Narcosis time (s)	Recovery time (s)
n	24	24	24
Min.	1,0	1,5	0,0
Max.	46,9	265,6	129,5
Range	45,9	264,1	129,5
Mean	12,6	70,8	28,2
Std. dev.	14,1	76,7	31,5
		60 Volts	
	Opercular recovery time (s)	Narcosis time (s)	Recovery time (s)
n	24	24	24
Min.	3,4	6,0	0,0
Max.	40,4	291,2	102,3
Range	37,0	285,2	102,3
Mean	17,1	81,3	39,3
Std. dev.	13,3	81,5	33,2
		70 Volts	
	Opercular recovery time (s)	Narcosis time (s)	Recovery time (s)
n	24	24	24
Min.	3,3	11,0	5,4
Max.	67,3	302,8	169,5
Range	64,0	291,8	164,1
Mean	21,3	107,9	63,4
Std. dev.	15,3	76,5	46,8

(Table 1). Little difference existed between 50 Vp and 60 Vp for the smaller size fish (10 cm–14 cm length, Fig. 8). However, with an increase in fish size the difference in narcosis time between the latter 2 voltages becomes more pronounced.

A similar situation exists for opercular recovery times where, in the case of 70 Vp, a generally longer opercular recovery time is required for all fish sizes (Fig. 4). The difference in opercular recovery times between 50 Vp and 60 Vp is greatest amongst the smaller sized fish. With larger fish, however, the opercular recovery times amongst the fish narcotized at 50 Vp tend to approach the times of fish narcotized at 60 Vp.

To eliminate the effect of fish length on narcosis time the narcosis coefficient (NC) was calculated for each fish in each group (Fig. 9). This figure shows that the standard errors of all 3 groups were remarkably similar and that the mean narcosis coefficients increased exponentially with increasing voltage.

Discussion

The freshwater fish industry in South Africa is in its developing stages. It can only become economically viable if it is supported by specialized technology which enables the production of fish on a large scale under a controlled environment. One of the important aspects in this development is the availability of methods to immobilize fish for routine laboratory or farming practices to overcome problems which may inhibit successful fish production. The use of chemical anaesthetics has thusfar formed part of this specialized technology. Smit (1980) and Ferreira (1982) made in-depth studies of the effective use of tricaine methanesulphonate and benzocaine hydrochloride to overcome stress problems associated with the use of these compounds. These 2 authors solved most of the problems by neutralization of these 2 acidic compounds. It is therefore apparent that the preparative steps taken in the laboratory for the immobilization of fish is a very important aspect when applying a method for routine investigations of fish. Most researchers are only interested in the anaesthetic potency of a particular compound to induce narcosis rather than in the direct and indirect physiological effects thereof. As explained previously, the use of these chemical compounds on fish had 2 negative aspects. First, the relatively long induction times of these compounds when handling large numbers of fish, and second, the cost involved in their preparation. This necessitated an investigation into suitable alternative and equally effective methods for immobilizing fish. The technique considered for this study was electronarcosis. Very little information on this aspect was previously available and, in addition, most of the reports are conflicting. Adequate scope thus exists for the improvements of the design and methodology in the use of electronarcosis for routine fish investigations. This study was therefore aimed at identifying basic laboratory imperfections in techniques which can be used to improve and standardize laboratory procedures whereby a more realistic interpretation of the actual physiological status of the fish can be obtained.

The effects of fish length and, therefore, size, is basically not a biological effect *per se* but a question of applying electrical theory to a biological system. In the case of a freshwater fish the body conductivity of the animal is greater than that of the surrounding water. As a result of this the electrical potential per unit length of fish is less than that of the water.

One may also consider the question from an electrical current aspect. Because the fish has a greater conductivity than the surrounding water, it will allow a greater current flow in a freshwater environment. The greater the bulk of the fish the larger the current flow will be, and therefore the larger the fish the greater the narcotizing effect of a particular current flowing between the 2 electrodes. It can thus be concluded that for any given species the larger the fish the greater the anaesthetic effect of a particular current or potential. This has been clearly demonstrated in this study. This, of course, is according to the laws of electricity, and in a given species, it must obviously, to a large extent, be independent of biological phenomena. Fish as living organisms are composed of various cell and tissue types, and it is conceivable that as they change in size the relationship between these tissues and cell types changes. This would imply that the narcotizing effect/length relationship is not a direct relationship, governed purely by the physical laws of electricity, but is modified by the biological configuration of the organism. This would account for the fact that the correlation between narcosis time and fish length is not unity, although there is a distinct correlation between these parameters. It is clear, therefore, that the relationship between length and electrical potential is modified by biological factors.

In spite of this, however, the narcosis coefficient affords the research worker with an indication of the length of narcosis he may expect from a fish of a given length and a given species. It is clear, however, that although the concept of a coefficient of narcosis appears sound, to eliminate the biological component of electronarcosis, it will be necessary to determine a biological constant to moderate the narcosis coefficient. It is to be expected that this biological constant will, itself, depend on species specificity.

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