

THE ABILITY OF FOUR SPECIES OF SOUTHERN AFRICAN

CICHLID FISHES TO ENTER DEEP WATER

By

MARK S. CAULTON.

Thesis presented to Rhodes University
for the degree of Master of Science.

Department of Zoology and Entomology,
Rhodes University,
Grahamstown,
South Africa.

October, 1973.

CONTENTS

	<u>Page No.</u>
INTRODUCTION	1

PART 1.

THE TOLERANCE OF CICHLID FISHES TO DEEP WATER

INTRODUCTION	4
MATERIALS AND METHODS	
(i) Construction of the pressure chamber	6
(ii) Operation of the pressure chambers	12
(iii) The fish: species, locality of capture and size groups	16
RESULTS	
(i) Depth equilibrations and descent rates of fry, juveniles, small adult and large adult <u>Tilapia mossambica</u> at a temperature of 22°C	18
(ii) The effect of temperature on the ability of <u>Tilapia mossambica</u> to enter deep water	22
(iii) Depth equilibration and descent rates of adult <u>Tilapia sparrmanii</u> and <u>Tilapia rendalli</u> at 22°C	24
(iv) Depth equilibration and descent rates of <u>Hemihaplochromis philander</u> at 22°C and 30°C	26
DISCUSSION	29

PART 2.

PHYSIOLOGICAL EXPERIMENTAL STUDIES

INTRODUCTION	31
1. <u>DETERMINATION OF THE PARTIAL PRESSURES OF GASES IN THE SWIMBLADDER GAS OF TILAPIA MOSSAMBICA.</u>	
MATERIALS AND METHODS	32
RESULTS	33
DISCUSSION	34

2. THE EFFECT OF TEMPERATURE AND pH ON THE
DISSOCIATION OF OXYGEN FROM THE BLOOD OF ADULT
T. MOSSAMBICA.

MATERIALS AND METHODS	36
RESULTS	41
DISCUSSION	44

3. THE RATE OF OXYGEN LOSS FROM THE SWIMBLADDER OF
T. MOSSAMBICA BY PASSIVE DIFFUSION.

MATERIALS AND METHODS	47
RESULTS	50
DISCUSSION	51
GENERAL DISCUSSION	54
SUMMARY	62
ACKNOWLEDGEMENTS	63
REFERENCES	64

INTRODUCTION

Since the specific gravity of fish is greater than water, they require a mechanism for keeping afloat. Many fish, notably the elasmobranchs, keep themselves up hydrodynamically by means of constant forward motion. Others, notably large pelagic sharks, have large livers consisting of tissue of low specific gravity (Denton 1963). Alexander (1966) has calculated that a constantly moving fish, moving at a velocity of one body length per second, needs sixty percent of its total power output to overcome sinking. The majority of bony fishes have overcome this expenditure of energy by replacing part of their body volume with gas. The function of the swimbladder is to maintain this gas space and to adjust the volume of gas according to the hydrostatic demands and Boyles law.

A consequence of using this floatation device is that the fish is restricted in its vertical movement and is in buoyancy equilibrium at only one depth. A fish with neutral buoyancy at the surface will, on rapidly descending to ten metres or an equivalent hydrostatic pressure of two atmospheres, have a swimbladder volume one half of its original volume. Until the deficit in gas volume can be made up the fish will have to expend additional energy to maintain its position. Once the deficit is restored the fish again regains neutral buoyancy and energy output is minimised. If a neutrally buoyant fish were to ascend rapidly to the surface from a depth of ten metres, the swimbladder volume would double and gas would have to be expelled or absorbed in order for the fish to regain neutral buoyancy.

Movement in a vertical plane is essential to most fish species,

since without this, optimal habitat utilisation and exploitation would be impossible. This ability to move vertically relies on the ability of the fish to secrete and absorb gas from the swimbladder, for it is this ability that governs the rates of descent to and ascent from deep water. Secretion of gas is carried out by the rete mirabilis/gas gland complex while resorption areas are usually areas of the swimbladder having an abundant capillary system into which gas can diffuse passively. The structure and general function of the rete mirabilis/gas gland complex has been well documented and reviewed in recent literature (Jones 1951, 1952; Jones and Marshall 1953; Alexander 1959, 1966; Fange 1966; D'Aoust 1970; Rigg 1970; Steen 1970; McNabb and Mecham 1971 and Denton et al 1972).

Despite this accumulation of generalised knowledge on structure and function, there still remains comparatively little published work on the ecological significance of the ability to move in a vertical plane for individual fish species and communities. This is particularly evident in the African cichlid community as was shown in a recent review of cichlid biology by Fryer and Iles 1972. These authors stated that the presence of a swimbladder in cichlid fishes "was one of the factors that contributed to their ability to colonise lakes and, having done so, probably facilitated their establishment in a diversity of habitats". They further went on to say "when considering which attributes of the cichlids are associated with their remarkable success in the African lakes this is certainly one which has to be taken into account". The importance of depth distribution to the cichlid community thus cannot be over emphasised. The diversity of feeding zones, the breeding habits and the community structure are all functions related to depth neutrality and the ability of these fish

to remain at their equilibrated depths with a minimum of energy expenditure.

The only available work in this field was published by Bishai (1961) and still remains the only comparative reference study. This work however was not entirely satisfactory since the experimental design contained some serious defects. The primary objectives of the present study were to initiate an experimental technique capable of assessing the ability of some Southern African cichlids to enter deep water and to determine some of the limiting factors concerned with this function. The study was divided into two parts. The first deals with the depth tolerances shown by four species of cichlids in a pressure chamber, while the second part deals with some of the physiological mechanisms involved in vertical movement. An important aspect of the study was the correlation of the experimental data with the available field ecology and to determine to what extent depth is restrictive in the success of the cichlid community.

PART 1.THE TOLERANCE OF CICHLID FISHES TO DEEP WATER.INTRODUCTION

Lake Sibaya ($32^{\circ} 40'E : 27^{\circ} 25'S$) is a freshwater lake near the north eastern border of South Africa. The population of cichlids in this lake consists of four species in two genera. The biology of the most important, Tilapia mossambica, has been the topic of a research programme by Bruton (1973). It appears possible from his work on T. mossambica, and the observations of other workers on the remaining species, that these fishes are "depth restricted" and thus are unable to utilise the habitat to its full potential. SCUBA divers records have tentatively indicated that the distribution of cichlids in lake Sibaya was in the littoral and sub littoral regions (Boltt et. al 1969), yet there are no environmental physical factors which prohibit these fish from occupying the entire water column. At its maximum depth of forty metres (Hill 1969) the lake is well oxygenated and has no vertical temperature gradient (Allanson and van Wyk 1969), thus it appeared that hydrostatic pressure might be a factor limiting the distribution of these fishes.

In order to investigate this possibility, an experimental pressure chamber simulating the pressures found at all depths in a lake was required. The chamber should ideally provide the following conditions.

- a) The provision of sufficient space for the fish to swim about.
- b) Water of the correct temperature.
- c) Water flow through the tank at all pressures in order to provide oxygen and remove metabolites.

- d) There should be no increase in dissolved gas with increased pressure,
- and e) It should be possible to simulate the maximum depths of the lake.

On reviewing the current literature a variety of methods became apparent, but were generally regarded as being unsuitable for fulfilling the above requirements. Harden Jones (1952) used an apparatus in which the fish were exposed to increased pressure by means of a water column in a vertical tube. This method is not practical for great depths since the height of the tube has to be equivalent to the required simulated depth. McCutcheon (1966) reproduced depth by introducing compressed air into a pressure chamber. This method did not permit water circulation through the tank and the oxygen deficiency resulting from respiration had to be compensated for by aeration of the water under pressure. This aeration, as well as the exposure of the water surface to high pressure air, would result in an increase in partial pressure of gases in the water, and consequently in the tissues of the fish. This is a most undesirable feature since it reduces the oxygen gradient across the swimbladder wall and is thus not a reflection of physical conditions in natural systems. It must be borne in mind that under natural conditions increased water pressure, associated with increased depth, is NOT accompanied by a rise in partial pressure of dissolved gases.

Similarly, putting an experimental tank into an hyperbaric chamber, as done by Overfield and Kylstra (1971), would result in an increased partial pressure of oxygen in the water. Bishai (1961) used a ten litre tank in which he obtained pressure from the mains water

supply. Once at the required pressure the tank was closed off from the water supply and there was no circulation through the apparatus. He found that adult fish kept in the tank died in ten to twenty hours and he was therefore forced to carry out his experiments with an air space above the water. As stated above, and recognised by Bishai, this causes an increase in gas tension in the water and therefore in the tissue of the fish and would produce confusing results.

The tank designed for the present work enabled these problems to be overcome and fulfilled the conditions set out above. With this apparatus the maximum depth tolerance of the fish, together with their rates of descent, could be determined. Initially a single tank was built and experiments on the depth tolerance of T.mossambica at 22°C were carried out (Caulton 1972). The results of this preliminary work indicated that considerable further work in this field was necessary in order to clarify the depth relations of T.mossambica of different sizes and at other temperatures, and to investigate the depth tolerances of other cichlid fishes.

MATERIALS AND METHODS

(i) Construction of the pressure chamber.

The following description of the construction of the pressure chambers is based upon the single tank which was used during the preliminary part of this study (Caulton 1972). The success of the design led to the construction of four interconnected chambers (Plate 1) which were used in the present study and which enabled observations to proceed more rapidly. The modifications used in this system will be presented after the description of the single chamber as set out below.

The body of the single pressure chamber was constructed of ten millimetre thick steam pipe, thirty centimetres in diameter and forty-five centimetres in length. The endplates were cut from twelve millimetre thick mild steel plate. These end plates had a fifteen

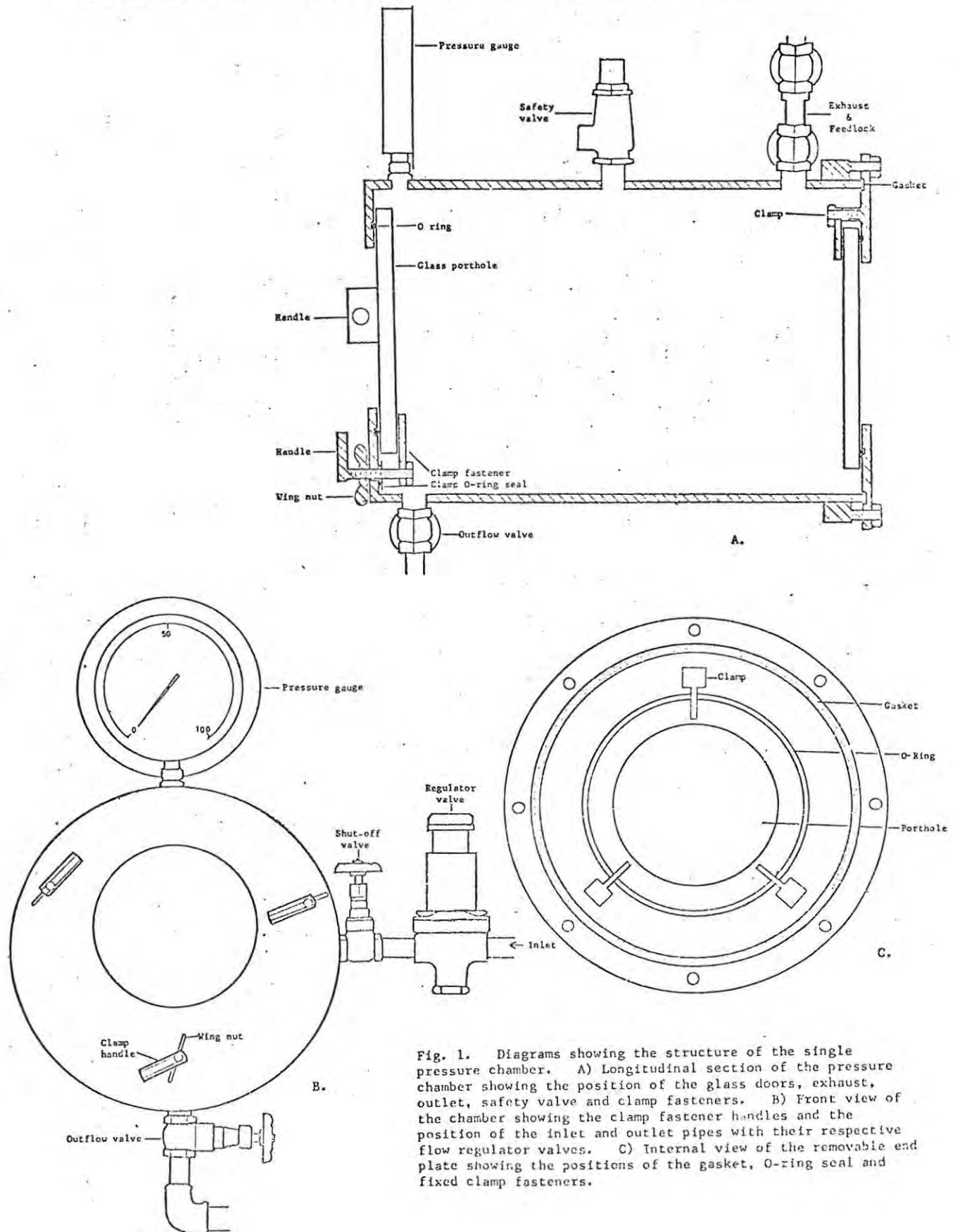


Fig. 1. Diagrams showing the structure of the single pressure chamber. A) Longitudinal section of the pressure chamber showing the position of the glass doors, exhaust, outlet, safety valve and clamp fasteners. B) Front view of the chamber showing the clamp fastener handles and the position of the inlet and outlet pipes with their respective flow regulator valves. C) Internal view of the removable end plate showing the positions of the gasket, O-ring seal and fixed clamp fasteners.

centimetre diameter porthole cut into them. The porthole in the rear end plate (Fig. 1C) was centrally positioned, that in the front plate (Fig. 1B) was off centre so that its top edge was only five centimetres from the edge of the plate. This allowed a ten centimetre deep water level to be maintained in the tank when the porthole door was not in position. On the internal surface of each end plate, two centimetres from the porthole, a four millimetre wide, two millimetre deep circular groove was milled out to accommodate an o-ring. The rear, removable end plate had a ten millimetre wide groove, thirty centimetres in diameter, cut into its inner surface so as to mate with the end of the cylinder (Fig. 1A). A flat rubber gasket glued into the groove ensured an efficient seal. This plate was bolted onto the main chamber by means of eight stainless steel studs welded onto the body of the tank.

The porthole windows were of prestressed armour plate glass, twenty centimetres in diameter and nineteen millimetres thick. The front window of the single tank had a small perspex handle fixed to it with an epoxy glue. The rear porthole was held permanently in position by three rubber covered brass plates bolted onto studs screwed into the rear end plate (Fig. 1A,C). The front porthole door was movable, being held in position by three adjustable clamp fasteners. These clamps were constructed of brass with rubber covering those surfaces in contact with glass. The clamps could be rotated by means of a brass bolt which passed through a hole in the end plate and which had an external handle (Fig. 1A). Wing nuts were used to tension the clamps to hold the glass firmly against the o-ring. When tightened these wing nuts not only pulled the glass forward but simultaneously sealed the hole through which the bolt passed, by means of an o-ring

and a thick brass washer threaded and soldered onto the bolt. The exact position of the glass holder clamps in relation to these metal washers was critical. Initially each was set by screwing up the wing nuts until the o-ring and washer were tight against the inside of the end plate. A clamp was then slipped over the tensioning bolt and tightened against the glass by means of a nut and lock nut (Fig. 1A). Once the correct position was obtained it required no further adjustments. In this design, the internal hydrostatic pressure on the glass increased the pressure on the o-ring, thereby providing an effective water-tight seal. The clamps, therefore, served merely to hold the glass in place and keep the tank sealed under low pressure.

Water inlet and outlet pipes were screwed into threaded holes in the wall of the tank and then welded into place. The water flowed in at the side of the tank and out the bottom. The inlet was fitted with a twelve millimetre gate valve and a sensitive needle pressure regulator valve (Bailey 600 W, type G). A feeding lock, consisting of two gate valves, was fitted at the top and this also served as an air exhaust during filling (Fig. 1A). A safety valve set at 5 kg/cm^2 and a pressure gauge reading to 8 kg/cm^2 were also fitted. The outlet flow was regulated by a twelve millimetre gate valve.

Since this single chamber could only accommodate two fish and most experiments lasted from ten days to two weeks, it was decided to build a multiple system in which a further eight fish could be housed during pressure tolerance work. This allowed a total of ten fish to be tested simultaneously. The multiple chambers differed from the single tank only slightly. Since the rear porthole served

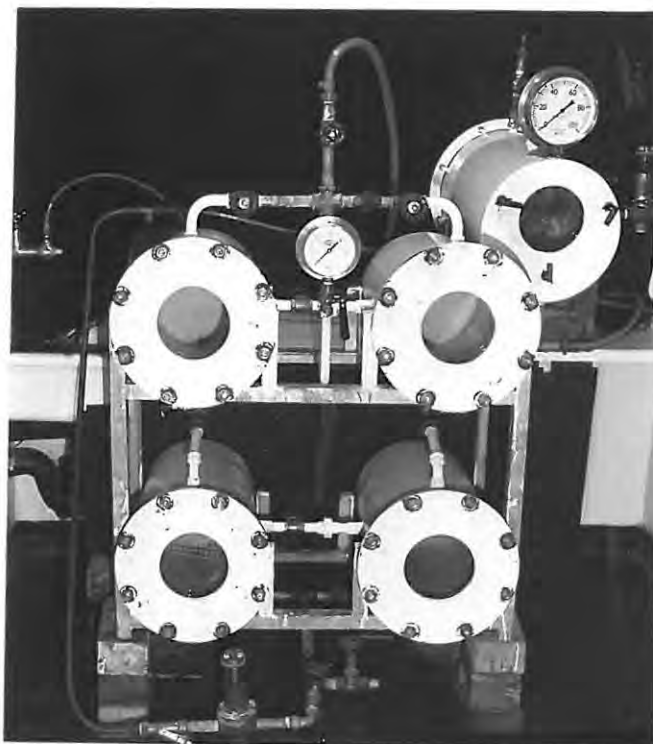


Plate 1. The multiple chambers used to subject fish to simulated depth. The fine regulating valves are visible in the lower foreground and on the single chamber at top right. At left rear is the heater unit which increases the temperature of the inflow water.

no function other than allowing greater internal illumination, this was dispensed with, and the rear end plate was welded onto the cylinder. The front plate, containing the glass window, was removable and held by eight stainless steel studs onto the body of the chamber. The movable clamp fasteners were dispensed with in the multiple tanks and the entire front plate was removed for the introduction of the fish. This method was not as satisfactory as having the clamp fastener system. The tanks were interconnected and the water flowed in at the bottom and out at the top. This was

not as efficient as the system in the single tank, since any waste matter which may collect on the floor of the chamber could not be flushed away. It was decided, however, not to feed the fish on the day prior to use or during the experimental work. This ensured that there was no faecal waste. In this system since the outlet was at the top no air exhaust was necessary.

Internally the chambers had a perforated perspex partition with a central door enabling separation of the two experimental fish. The multiple chambers were mounted in a metal framework and had a slight backward tilt to allow complete expulsion of air on filling. The chambers and fittings were painted inside and out with three coats of white epoxy paint over a coat of an anticorrosion compound. The temperature within the system was monitored by a pressure resistant thermometer, while the tanks were kept in a temperature controlled room to decrease heat loss from the tanks and thus help maintain a constant water temperature.

Inflowing water was heated by passing it through a heat exchanger. This consisted of 4 metres of copper piping, one centimetre in diameter, wound into a fifteen centimetre diameter coil and immersed in a water bath in which the water temperature was thermostatically regulated by a heater stirrer unit. This heating coil was in turn attached by copper piping to the main water supply system. Analysis of the copper content in the water before and after the copper coil showed no measurable increase in copper ions in the water. A value of less than 0.003 ppm copper was found by atomic absorption spectrophotometry, and this level of copper was regarded as being harmless to cichlid fishes.

The maximum pressure obtained in the chamber is dependant on the pressure of the supply system. In Grahamstown this supply pressure was approximately 11 kg/cm^2 , thus a theoretical hydrostatic pressure equivalent to one hundred and ten metres could be generated. Since one of the major requirements of the system used was a constant water flow, the full pressure could not be utilised. With a flow of three to six litres per minute a pressure of 8 kg/cm^2 could be maintained, thus decreasing the theoretical working pressure of the chamber to an equivalent simulated depth of eighty metres. For the purpose of the present study the safety limit was set at a simulated depth of fifty metres. The pressure gauge was checked against a sensitive SCUBA divers depth gauge. During the course of the experiments the fish were subjected to a twelve hour light and twelve hour dark illumination cycle.

(ii) Operation of the pressure chambers.

The water heating exchange unit was set such that the water temperature, after having passed through the coil, corresponded to that to which the experimental fish had been acclimated. In the single chamber the bottom outlet valve was first closed and the top exhaust valve opened. When the water level reached the porthole the inflow was stopped and the fish introduced, generally one on either side of the perspex partition. The porthole door was lifted into place and the clamp fasteners manouvered into position and tightened. Filling continued until water flowed from the exhaust valve. This was then closed while the lower outflow valve was simultaneously opened to maintain a flow of approximately three litres per minute. At this flow rate a drop of three per cent in the oxygen content between water entering and leaving the chamber was recorded when two large

T.mossambica were present (Inflow $PO_2 = 152$ mm Hg; outflow $PO_2 = 145$ mm Hg).

The fish were then allowed a twenty four hour habitat familiarisation period. During this period the chamber was maintained at zero hydrostatic pressure.

In the multiple system the procedure was similar except that the fish were placed in the bottom chambers first, the end plate bolted into position, and then these chambers filled. When the water began to flow into the top chambers the flow was stopped, the fish introduced, and after the end plates had been positioned the flow was continued. This system had a flow of approximately six litres per minute.

The pressure within the chamber was increased by screwing down the regulator needle valve until the correct pressure increment was reached. The single tank and multiple tanks, although drawing water from the same heating system, had independent pressure regulator systems. An important feature of the pressure regulator valve used was that increasing pressure in the tank did not alter the flow rates, and thus very little, if any, regulation of the outflow was required for the duration of each series of experiments.

During the experiments the fish could be seen clearly through the porthole and soon became accustomed to the observer. Increments in pressure were carried out at twelve hour intervals and were designed to keep just ahead of the rate at which the fish could equilibrate. Equilibration was taken as the depth (pressure equivalent) at which the fish just floated off the floor of the chamber. Since the pressure

was kept greater than this equilibration depth the fish were generally "heavy" (a term used to describe a fish which was negatively buoyant i.e. the swimbladder was partially deflated). Thus by decreasing the ambient hydrostatic pressure, the depth of neutral equilibrium of the fish could be accurately determined. After recording this equilibration depth, the hydrostatic pressure was increased to the next level, and the fish became "heavy" once again. At the end of the experiment, curves of equilibrated depths against time were drawn up. These showed the rate of equilibration and the mean maximum equilibrated depth. This was taken as that depth at which no depth increments were shown by fish held for at least thirty-six hours at an ambient hydrostatic pressure greater than the mean maximum equilibrated depth.

Preliminary experiments for the determination of the depth tolerances and descent rates for cichlid fishes showed that at 22°C they equilibrated to depth comparatively slowly. From preliminary experiments for each particular species, suitable experimental regimes for increasing the hydrostatic pressure were devised. The following regime was used for T.mossambica at 22°C.

- a) A habitat familiarisation period of twenty four hours at zero hydrostatic pressure.
- b) The pressure was then increased to 0.5 atmospheres or an equivalent simulated depth of 5 metres. At the end of a twelve hour period the depth at which the fish were neutrally buoyant was determined as previously described.
- c) The pressure was then increased to 1.1 atmospheres (equivalent depth of 11 metres) and after a twelve hour period at this depth the equilibration depth was tested.

d) Thereafter the pressure was raised by 0.2 atmospheres, or an equivalent additional depth of two metres, every twelve hours. Once the fish had apparently attained its maximum equilibrated depth, the hydrostatic pressure was held at 0.5 atmospheres in excess of this depth for a period of 36 hours. If, during the twelve hourly recordings over this 36 hour period, no increase in depth equilibration was noted, that depth was taken as the maximum equilibrated depth.

The results of these recordings were used to construct equilibration curves from which rates of descent and mean maximum equilibrated depths for varying fish sizes at varying temperatures could be compared for different fish species.

Since the rates of equilibration varied at different temperatures and for different fish species, pressure increment regimes suited to each set of conditions had to be developed. Set out below in Table 1 are the increment regimes used in this study.

TABLE 1. Simulated depth increments shown in metres for each 12 hourly increment period for different fish species at different temperatures. *Example described in the text.

Temp. °C Time hrs.	<u>Tilapia</u> <u>mossambica</u>			<u>Hemihaplochromis</u> <u>philander</u>		<u>Tilapia</u> <u>rendalli</u>	<u>Tilapia</u> <u>sparmanii</u>
	15°C	22°C	30°C	22°C	30°C	22°C	22°C
0	0	0*	0	0	0	0	0
12	3	5	5	5	5	5	3
24	6	11	12	10	15	7	6
36	9	13	15	12	18	9	9
48	12	15	20	15	21	11	12
60	12	17	25	17	24	11	15
72	12	19	30	19	27	11	18
84	12	21	30	21	30	11	21
96	12	23	30	23	33		24
108		25	30	25	33		27
120		25		27	33		27
132		25		29	33		27
144				31			
156				33			
168				35			

The regimes of hydrostatic pressure increase such as those described above did not appear to have any effect on the behaviour of the fish. Pairs of Tilapia spp. and Hemihaplochromis philander successfully courted, mated and produced young while undergoing tolerance tests. During the initial rise in hydrostatic pressure, however, Tilapia spp. showed a doubling of their fin beat rate from one hundred beats per minute to two hundred per minute. This increased activity, due to loss of buoyancy, caused the fish to fatigue rapidly and become "heavy". This observation stresses the importance of neutral buoyancy, as proposed by Alexander (1966), in conserving energy otherwise expended in maintaining lift.

(iii) The fish: species, locality of capture and size groups.

The species of fish used in this experimental study were, Tilapia mossambica Peters, Tilapia sparrmanii A. Smith, Tilapia rendalli Boulenger and Hemihaplochromis philander (M. Weber).

T.mossambica were obtained from either Amalinda fish station, East London, or from natural ponds near Kenton-on-sea ($33^{\circ} 40'S$; $26^{\circ} 40'E$). Throughout the experiments these two populations were treated independently and are described as Amalinda fish or Kenton-on-sea fish. T.sparrmanii were obtained from ponds in the vicinity of Grahamstown. T.rendalli and H.philander were caught in Pietermaritzburg, Natal, and road transported to Grahamstown (there were possible indications that air transportation disrupts normal swimbladder function, due to the low pressures present in the cargo compartments of modern aircraft). The fish were held for at least one month prior to use, in one metre deep concrete tanks at a temperature of $22^{\circ}C$ to $25^{\circ}C$ with daylight illumination. Before

being placed in the pressure chambers the fish were temperature acclimated for a period of at least fourteen days.

Sex determinations on Tilapia spp. were generally made by dissection at the end of the experiments or by a method described by Vaas and Hofstede (1952) for live fish. Sex determinations in H.philander were by colour differentiation.

The fishes were divided into size groups and these groups were adhered to for the entire work. The measurements of the size groups in Table 2 are expressed as total length measurements in millimetres.

TABLE 2. The size classes of fish used in this study. All measurements in millimetres.

Size class Species	Large Adults	Small Adults	Juveniles	Fry
<u>T.mossambica</u>	205-246	154-185	40-60	10-15
<u>T.sparrmanii</u>	97-125	60-67	-	-
<u>T.rendalli</u>	-	96-108	-	-
<u>H.philander</u> (male)	50-80	35-40	± 10	< 8
<u>H.philander</u> (female)	40-60	25-30	± 10	< 8

RESULTS

- (i) Depth equilibrations and descent rates of fry, juvenile, small adult and large adult *Tilapia mossambica* at a temperature of 22°C.

Fry of *T. mossambica* (sizes as in Table 2) normally rest on the substrate, making short excursions into mid water, thus it is possible that in this size group the swimbladder is not functional as a hydrostatic organ. The effect of pressure on fry in the pressure chamber was undetectable. Time intervals between resting and swimming were recorded at a variety of pressures but no significant differences were found.

Juvenile *T. mossambica* similarly showed no effect when exposed to the experimental pressure regime. If the hydrostatic pressure, however, was increased by three atmospheres in one minute i.e. a simulated depth increase of thirty metres, a pronounced increase in the fin beat rate was observed. The fish did not fatigue and become "heavy" but tended to congregate towards the bottom of the chamber. After a period of ten to fifteen minutes the normal fin beat rates and random distribution were regained. This increase in fin beat rate and the loss of randomisation indicated that the fish does momentarily lose buoyancy and must compensate for this by added swimming effort. The important feature is, however, the extremely rapid regaining of normal buoyancy. Similar observations on juvenile *Tilapia nilotica* were reported by Bishai (1961). Rapid decompression of deep compensated juveniles showed the same trends, with fish congregating at the roof of the chamber for a

short period of adjustment before regaining a random distribution.

Since juveniles never showed the fatigue patterns of adult fish no quantitative expressions of descent rates or maximum equilibration depths could be given. The fish will seldom encounter depths greater than thirty metres in most natural systems and thus they were not tested beyond this depth. The greatest pressure changes, in terms of Boyles law, are in the upper ten metres and since juvenile fish compensated rapidly for these changes they may indeed be able to penetrate to depths deeper than thirty metres.

The typical behaviour patterns described for adults showed that they spent at least 98% of their time resting on the chamber floor when not equilibrated to the hydrostatic pressure. This enabled accurate equilibration depth readings to be obtained from individual fish every twelve hours using the previously described pressure increment regime.

The equilibration curves constructed from the mean equilibration depths of two Amalinda adult T.mossambica size groups at 22°C are shown in Fig. 2.

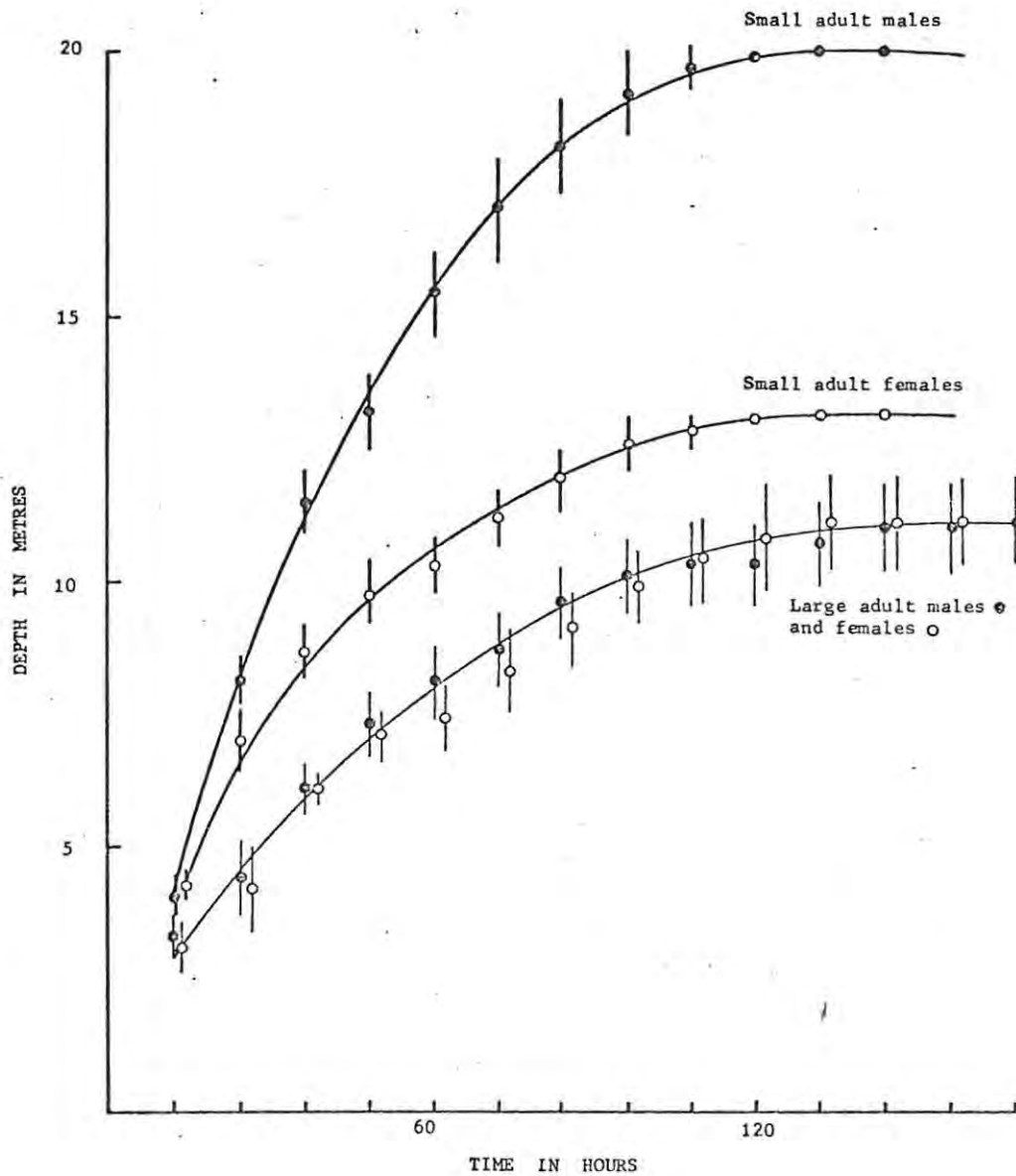


Figure 2. Depth equilibration curves for small and large adult *T. mossambica* at 22°C. Each point represents the mean of the equilibrations of 10 fish, and the vertical line indicates two standard errors about the mean. One curve was given for large adults since males and females showed no significant difference. (Data for small adults from Caulton 1972).

Four striking features emerge from Fig. 2:-

1. Small adults can equilibrate to a greater depth than large adults.

2. A sexual differentiation exists in the maximum equilibrated depths of small adults in this population.

3. There is no sexual differentiation in the maximum depth equilibration of the large adult population.

4. The rates of descent show similar trends i.e. increased rates of descent with increased mean maximum compensated depth.

Large adult males and females and juvenile T.mossambica of both sexes do not show any significant differences in their respective mean maximum equilibration depths or descent rates. Within the small adult group investigated, the fish were of comparable size and originated from the same population (Amalinda fish hatcheries), yet these fish showed a sexual difference in equilibration depths. These fish may, however, not be of the same age class or maturity. Bruton (1973) has found in Lake Sibaya that female T.mossambica are mature from 108 mm in length and males from 130 mm in length. In this rather small sized adult population these length differences represent a years growth, thus it is believed that the females mature a year before male fish. Thus in the experimental Amalinda population of small adults, although the males and females were of the same size, they were not necessarily of the same age or maturity.

Since it has been demonstrated that there is a marked decline in the ability of fish to enter deep water from juvenile to adult, it is proposed that the small adults used in this work may represent a section of the population which is in a transitory period between juvenile and adult equilibrium. Since the small adult female equilibration curve resembles more closely the large adult equilibration curve than does that of the small and possibly less

mature males, it may be proposed that the ability to enter deep water is related to maturity.

Although the experimental data indicated maximum equilibration depths, the fish can move about this plane of equilibration by active swimming. Bishai (1961) showed that Tilapia nilotica could move out of an equilibrium plane and tolerate a 16 to 22 per cent expansion of the swimbladder. Since a T.mossambica equilibrated to 2.5 metres can be immediately returned to surface pressure without undue stress, unlike fish equilibrated to greater depths, it is proposed that these fish can withstand a swimbladder expansion of approximately 25 per cent. This tolerance would thus enable a large adult fish to move out of its plane of equilibration at 11 metres and move up to seven metres. The energy cost of this movement would be large and thus possibly only occurs under stress from, for example, predators.

(ii) The effect of temperature on the ability of Tilapia mossambica to enter deep water.

Small adult T.mossambica from the population collected from the ponds near Kenton-on-sea were used in this series of experiments. The retested equilibration of these fish at 22°C showed no sexual differentiation in maximum equilibration depths and followed the curves of the Amalinda large adult population. Thus, as previously discussed, it was believed that the small adults of the Amalinda population were not fully mature, although gonad development had commenced, while the Kenton-on-sea population of the same size was mature since they followed the equilibration curve of large mature fish as shown in Figure 4.

In addition to the retesting of these fish at 22°C ten fish of each sex were acclimated to 15°C and 30°C. For each temperature a new experimental increment regime was developed. These regimes largely followed the regime as described under methods, except that the pressure increments were smaller for the cooler temperature and larger for the warm temperature but were still carried out at 12 hour intervals as shown in Table 1.

The equilibration curves for these fish are shown in Fig. 3.

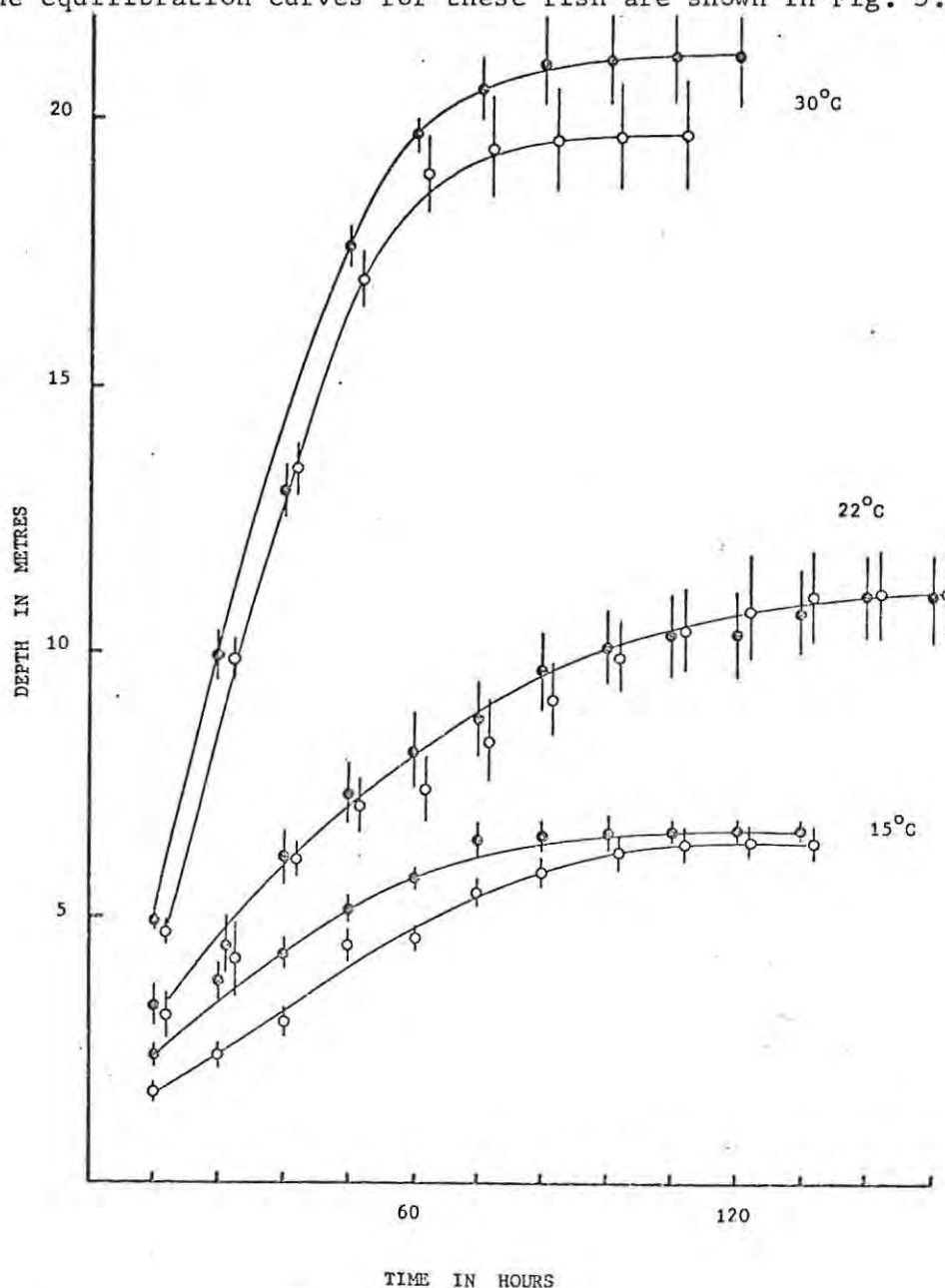


Figure 3. Depth equilibration curves for mature adult *T. mossambica* at 15°C, 22°C and 30°C. Each point represents the mean of the equilibrations of 10 fish and the vertical line indicates two standard errors about the mean. One curve was given for fish at 22°C since males and females showed no significant differences. (Males = closed circles; females = open circles).

The data shown in Fig. 3 indicates the importance of temperature as a parameter affecting both the rate of equilibration and the maximum equilibration depth. At 30°C male fish equilibrated to a mean depth of 21.2 metres while female fish equilibrated to a mean depth of 19.7 metres. Similarly at the lower temperatures the male fish had a slightly higher mean maximum equilibration of 6.7 metres as against the female mean maximum of 6.6 metres. However, at none of the three temperature ranges were the maximum depths of males and females significantly different. Corresponding to the trend of increased depth equilibration with increased temperature were the rates of equilibration. At 30°C male fish required 13 hours to equilibrate to 6 metres, while at 22°C they required 35 hours and at 15°C they required 65 hours. Thus temperature not only affects the maximum equilibration depth, but simultaneously affects the rates of equilibration.

(iii) Depth equilibration and descent rates of adult *Tilapia sparrmanii* and *Tilapia rendalli* at 22°C.

These cichlids differ from *T. mossambica* since they belong to the group of substrate spawners and non mouth brooders. *Tilapia sparrmanii* is adapted to living in cooler temperatures but is widely distributed in rivers and reservoirs south of the Zambezi river (Jubb 1967). It feeds largely on benthic crustacea and aquatic insect larvae while *T. rendalli* feeds on macrophytes and periphyton (le Roux 1956).

At 22°C mature adult *T. rendalli* showed a very shallow maximum equilibration depth of 8.5 metres, attained after about three days, which was the same as the initial rate of descent shown by mature adult *T. mossambica*.

The depth equilibration curve for mature adult T.sparrmanii showed a mean maximum depth equilibration of 15 metres for both males and females at 22°C (Fig. 4). The initial rate of descent to six metres was similar to that of T.mossambica. A further experiment on smaller sized sub adult fish (60-67 mm) showed the same maximum depth equilibration as the larger fish but this depth was attained at a rate twice that of the larger fish.

No sexual difference was obtained in the equilibration rates of either T.sparrmanii or T.rendalli.

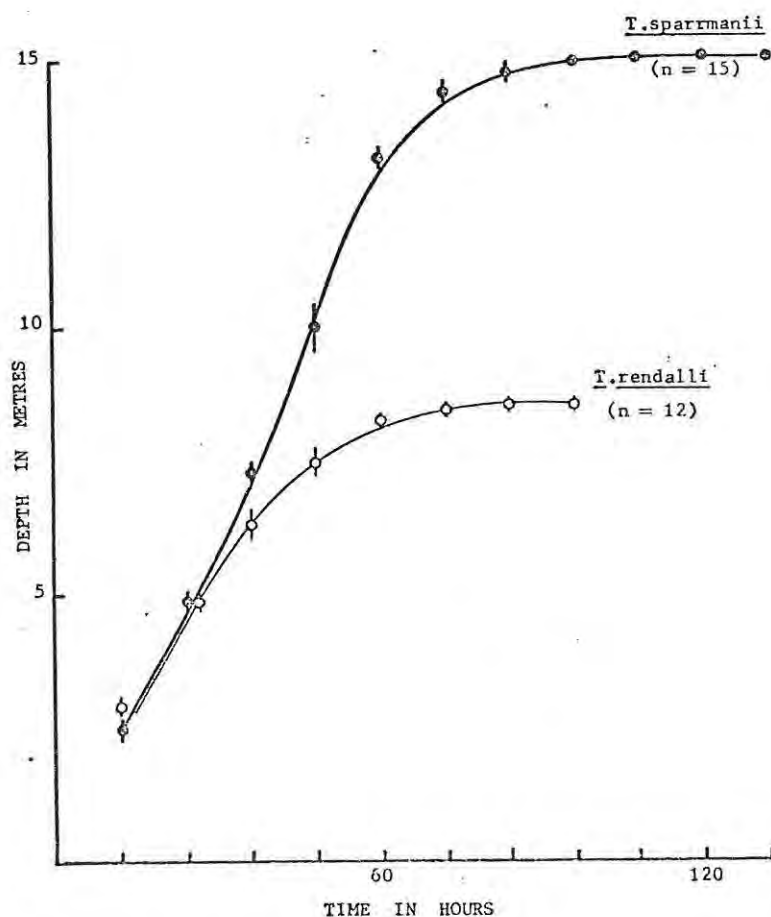


Figure 4. Depth equilibrium curves for T.sparrmanii and T.rendalli adults at 22°C. Each point is the mean of n fish and the vertical line indicates two standard errors about the mean. Sexes are not differentiated although approximately equal numbers of each sex were used.

- (iv) Depth equilibration and descent rates of *Hemihaplochromis philander* at 22°C and 30°C.

H.philander, like T.mossambica, is a member of the mouth brooding group of cichlids and is found widely distributed throughout southern Africa except in the extreme south where it is generally restricted to isolated warm water systems.

During the experiments in the pressure chambers these fish were extremely active and very sensitive to movement by the observer. Frequently during periods of recording, the changes in pressure caused the fish to swim up before equilibrium was attained. This made accurate recordings of equilibration depths extremely difficult and this was probably one of the contributing reasons for the comparatively large standard errors as shown in Figure 5.

Pressure experiments were carried out on young and old fry as well as juveniles of three and four months old at 22°C. Six day old fry were introduced into the chamber in a glass beaker with a gauze top and the chamber was filled in the normal manner. These fish showed no effects with increased pressure, but no quantitative data could be obtained due to their almost constant activity. Fry of this age divide their time between resting on the floor of the container and swimming into mid water. This activity was unaffected by pressure changes since the time spent on the container floor or in mid water was not statistically variable with changes in pressure. Eight week old fry treated in the same manner and showing a similar behaviour pattern did show a definite increase in swimming effort and longer resting periods if the pressure was increased to four atmospheres. Normal behaviour and distribution was regained within fifteen minutes. At lower pressures no change in behaviour was observed.

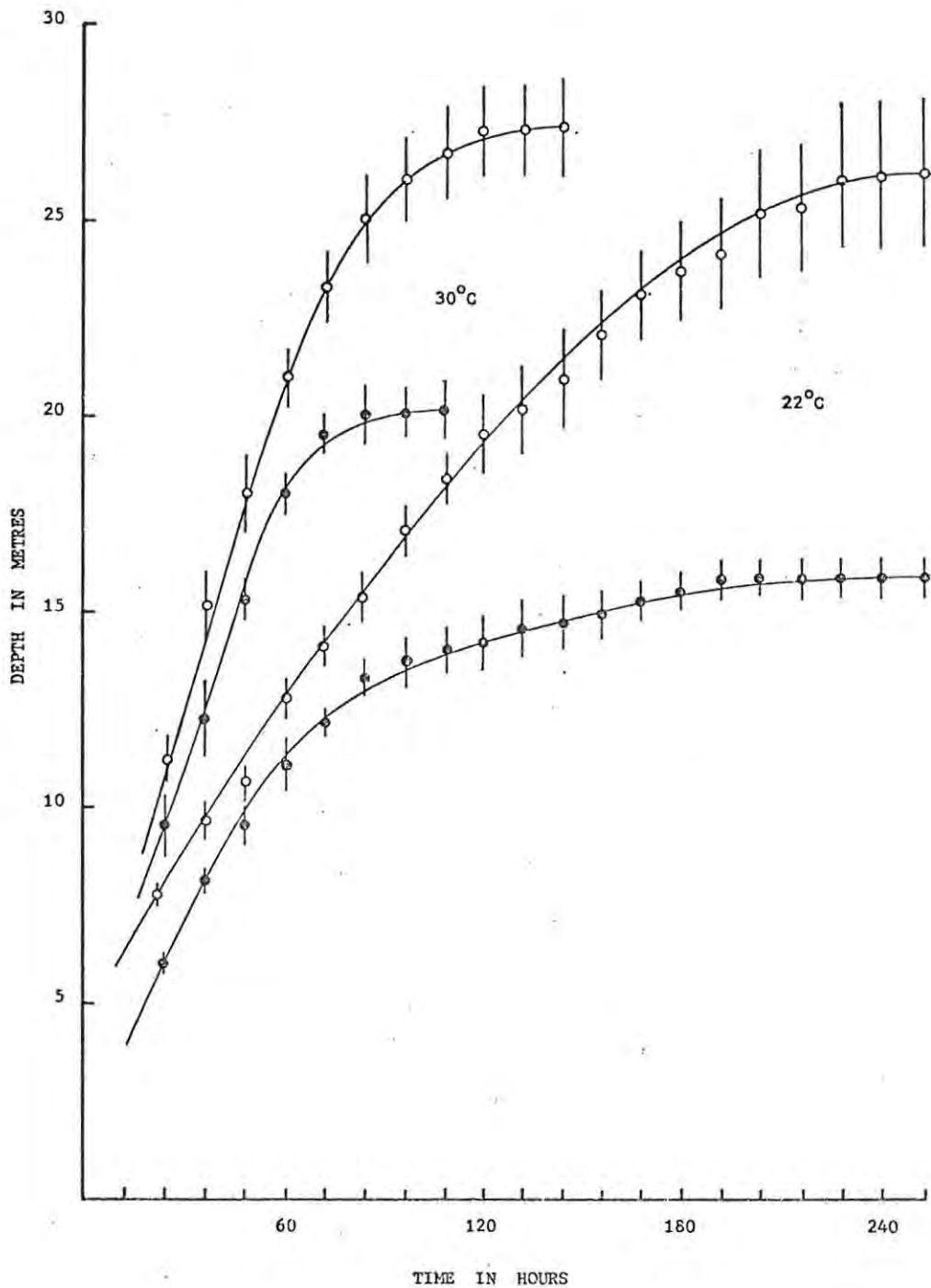


Figure 5. Depth equilibration curves for mature adult *H. philander* at 22°C and 30°C. Each point represents the mean of the equilibrations of 15 fish and the vertical line indicates two standard errors about the mean. (Males = closed circles; females = open circles).

Three month old juvenile fish showed depth equilibration to at least forty metres - the maximum depth tested. These fish did become "heavy" and rest on the floor of the container, but due to their extreme activity and sensitivity to movement and pressure

changes, no accurate readings were obtained. However, at lower hydrostatic pressures (down to an equivalent depth of ten metres) the fish showed increased swimming effort but never fatigued. They did, however, congregate towards the lower portion of the container, but equilibration was apparently regained within 15 minutes after which swimming effort was normalised and a random distribution regained. Juveniles, four months old, showed the adult pattern of equilibration but at a faster rate, attaining a maximum depth of between 15 and 25 metres in five days, whereas the adults required about 10 days.

At 22°C adult male H.philander equilibrated to 15.8 metres (range 14 to 17 metres) while females equilibrated to a mean maximum depth of 26.2 metres (range 22 to 30 metres). Adult fish acclimated to 30°C showed a marked increase in their rates of descent, but while male fish did show an increase in their mean maximum depth equilibration from 15.8 metres at 22°C to 20.3 metres at 30°C, the females showed no significant increase. Thus temperature, although having a marked effect on the rate of descent, does not have the same marked effect on the maximum equilibration shown by T.mossambica.

DISCUSSION

A common feature throughout the results of Part 1 of this study was the finding that the four cichlid fishes used were all restricted in their ability to enter deep water. This restriction, when considered in relation to the habitats occupied by these fishes may have been expected, since very few natural water systems south of the Zambezi river have a depth greater than 20 metres. It is also significant that three of these fish are largely herbivorous, the exception being H.philander, and thus their depth limitation may be related to the depth of the photosynthetic zone. Adult T.rendalli feed almost exclusively on macrophytes and since macrophyte growth is largely restricted to water less than 8 metres deep, the maximum depth attained by this fish (7.5 m) closely corresponds to this depth. T.mossambica, and to a certain extent T.sparrmanii, feed on benthic algae and diatoms. Since the production of this food source generally occurs to a much greater depth than the macrophytic production, the ability of these fish to penetrate to at least 11.5 metres (even in relatively cool temperatures of 22°C) would ensure access to this food supply. The rate of descent to deep water is, however, slow and thus no diurnal vertical movement would be expected from adult fish. (Adult T.mossambica required 9 days to descend to 11.5 m and then return to the surface at 22°C). Juvenile fish of the species investigated would be capable of diurnal movements to and from deep water.

In habitats largely devoid of fast moving fish predators e.g. lake Sibaya, juvenile cichlids have been recorded as inhabiting

deep water (Bruton 1973, Boltt et al 1969, Hart pers.comm.) and in so doing can extend their feeding areas and utilise the benthic food sources more efficiently than adults. In many systems, however, the ability of juveniles to inhabit deep water is not apparently utilised. This has largely been attributed to the effect of predation (Donnelly 1969). Since most fish predators are fast moving physostomes (Matthes 1968), for example Hydrocynus spp., Lates spp., and Hepsetus sp., the juvenile cichlids would only find relative safety in the densely vegetated margins and not in open or deep water.

Temperature plays an important role in the vertical distribution of cichlids and would thus be expected to have an important bearing on the ecology of those species subjected to large seasonal temperature differences. These fish will obviously be able to penetrate to a greater depth in summer than in winter. These and other ecological problems relating to vertical movements in natural systems will be analysed in more detail in the final discussion.

PART 2.

PHYSIOLOGICAL EXPERIMENTAL STUDIES.

INTRODUCTION

The experimental results of Part 1 of this study have revealed several physiological problems. The mechanism of depth equilibration obviously involves the secretion of gas into the swimbladder. Although oxygen is generally regarded as being the most common gas used in swimbladder filling Abernethy (1972) has demonstrated that nitrogen could be accumulated in a similar manner. Since both nitrogen and oxygen have been found in the swimbladders of freshwater fishes, it was therefore of interest to establish what gases were actively secreted by T.mossambica. The well known pH controlled Root effect shown by most teleosts with swimbladders is a blood function related to efficient gas secretion (both oxygen and nitrogen - Abernethy 1972) and since this effect has not been demonstrated for T.mossambica haemoglobin, it was investigated in the following section of this study. The total pressure maintained by the swimbladder at any depth should be balanced by the rate of active secretion of gases and the rate of passive gas loss by diffusion. Recent work has shown that passive gas loss from the swimbladder is an important aspect of swimbladder function in some deep sea fishes (Denton et al 1972). The role of passive loss by diffusion in shallow water fishes has not been documented and was therefore investigated in T.mossambica. Since temperature played an important part in the depth distributions of cichlids, the effect of temperature on both the secretory mechanisms as well as on the passive loss by diffusion was investigated.

1. DETERMINATION OF THE PARTIAL PRESSURES OF GASES IN THE
SWIMBLADDER GAS OF TILAPIA MOSSAMBICA.

MATERIALS AND METHODS

All fish were obtained from the Amalinda population and held at 22°C. The swimbladders of unanaesthetised fish were exposed by ventral dissection. A ten millilitre glass syringe fitted with a fine bore needle was used to withdraw the gas sample. The dead space in the needle and syringe was previously filled with a saturated sodium chloride solution so as to prevent contamination of the gas sample with residual air. The volume of gas extracted from adult fish was generally 3 to 4 mls leaving about 2 mls of gas in the swimbladder.

The gas sample was injected directly into a micro blood-gas analyser (Radiometer BMS-3) capable of determining the partial pressure of oxygen (PO_2) and carbon dioxide (PCO_2) in a gas sample to an accuracy of one percent. The determinations were carried out at 37°C (the most efficient analytical temperature) but the results were corrected to the acclimation temperature of the fish. Calibration of the oxygen electrode was carried out using the prevailing atmospheric pressure as measured with a Fortinette mercury barometer. In Grahamstown, due to its altitude, the atmospheric pressure was usually around 720 mm Hg.

Swimbladder gas pressure determinations were conducted on a variety of size groups and from fish equilibrated to both shallow and deep water. The analysis of the swimbladder gas from deep equilibrated fish was carried out on rapidly decompressed fish. These were removed from the pressure chamber following instantaneous decompression, and

gas analysis was completed within five minutes of decompression. These fish generally died, probably due to asphyxiation since the expanded swimbladder constricts both the heart and dorsal aorta, but in no instances did the swimbladder rupture. The PO_2 values from these deep equilibrated fish were corrected to the acclimation temperature and pressure to which the fish had been equilibrated. The total pressure exerted by the swimbladder gas would be expected to be 720 mm Hg at the surface and 720 mm Hg plus 760 mm Hg hydrostatic pressure for each 10 metres to which the fish had equilibrated while under pressure.

RESULTS

The PCO_2 of the swimbladder gas was found to be below 8 mm Hg (the lower limit of the electrode used) and was therefore ignored in all calculations.

The results of the oxygen analysis are shown in Table 3.

TABLE 3. Partial pressures of oxygen (PO_2) in swimbladder gas obtained from *T. mossambica*. Gas corrected to 22°C and atmospheric pressure. The sex of large sized adults is indicated by f for females, and m for males.

Juvenile 0 m		Small adult males 0 m		Small adult females 0 m		Small adult males 20 m		Small adult females 12.8 m		Large adults at their individual maximum equilibrated depths	
length mm	PO_2 mm Hg	length mm	PO_2 mm Hg	length mm	PO_2 mm Hg	length mm	PO_2 mm Hg	length mm	PO_2 mm Hg	length mm	PO_2 mm Hg
65	234	158	347	137	192	162	1865	128	1357	205 f	968
67	203	170	388	140	302	172	1486	136	1274	205 m	1184
69	198	174	347	170	329	180	1554	145	1108	215 f	1203
70	302	177	347	170	397	185	1770	145	1254	225 m	945
79	201	181	374	188	347	185	1256	150	1346	226 m	1284
80	187	190	297					170	1357	231 m	1077
		193	311							232 m	1256
										241 f	1240
										246 f	1359
Mean PO_2	220		344		313		1586		1283		1168
Std Dev.	43		33		76		240		96		142
2x Std Err.	35		25		68		214		79		95
Total Pressure	720		720		720		2240		1708		1556

Juvenile fish showed a lower PO_2 than did adults equilibrated to surface pressures ($P < 0.01$). There was no significant difference in oxygen content between small adult males and females equilibrated to surface pressures ($P > 0.1$). The small adults showed a significant difference in PO_2 between males and females at depth ($P < 0.05$) and as expected the deeper equilibrated males showed a higher PO_2 value. Similarly the PO_2 in small adults was significantly higher, in the case of both males and females, than the PO_2 found in the swimbladders of large mature adults from their respective maximum equilibrated depths.

DISCUSSION

Since the PO_2 of the blood of fish cannot exceed the partial pressure of oxygen of the ambient water (160 mmHg at sea level) and is generally less than 120 mm Hg (Satchell 1971), small adult T. mossambica have a two to threefold increase in swimbladder PO_2 , relative to arterial PO_2 , when at the surface pressures. The same fish show at least a tenfold increase in swimbladder PO_2 , relative to arterial PO_2 , in order to equilibrate to their maximum depths. Thus it is certain that oxygen is secreted and accumulated in the swimbladder, probably in the manner described by Kuhn et al (1963) since the swimbladder structure of T. mossambica conforms to the typical euphysoclistis plan (Dehardrai 1959, Caulton 1972).

The PN_2 values can be calculated from the available data since, as pointed out above, the remaining gas pressure would be expected to be exerted almost entirely by nitrogen. The total pressure in the swimbladder was 720 mm Hg from surface equilibrated fish and at 22°C 20 mm Hg of this total pressure would be exerted by the partial

pressure of water vapour. Thus the total gas pressure would be 700 mm Hg. Since in small adult females at the surface, 313 mm Hg of this total pressure was exerted by oxygen, it would leave approximately 387 mm Hg to be exerted by nitrogen (PCO_2 disregarded). Although these fish had never been subjected to depths greater than one metre, the swimbladder gas was not in equilibrium with the blood and tissue since the PO_2 was at least two to three times the expected blood value. When these fish had equilibrated to their maximum depth of 12.8 metres the total pressure within the swimbladder would be 1708 mm Hg (720 mm Hg plus 978 mm Hg hydrostatic pressure) and since the PH_2O would be 46 mm Hg the total pressure exerted by oxygen and nitrogen would be 1662 mm Hg. The PO_2 recorded from these fish was 1283 mm Hg (Table 3) thus leaving approximately 379 mm Hg to be exerted by nitrogen. Since the swimbladder volume returns to its original volume on equilibration, and since the partial pressures of the gas components act independently, it would be expected that the PN_2 would remain at the original pressure of 387 mm Hg. Thus nitrogen does not appear to be actively secreted in T. mossambica although nitrogen has been shown to be secreted in many other fish (Abernethy 1972).

Similarly the total gas pressure in the swimbladders of large mature fish was 1516 mm Hg of which 1168 mm Hg was exerted by oxygen and thus 348 mm Hg by nitrogen. Since the initial PO_2 was 331 mm Hg (combined males and females at surface) the expected PN_2 would be 369 mm Hg, again indicating no significant movement of nitrogen during depth equilibration. The small adult males did, however, show a PN_2 increase but this was not accepted as being significant due to the large standard deviations shown by the PO_2 values (Table 3).

2. THE EFFECT OF TEMPERATURE AND pH ON THE DISSOCIATION OF OXYGEN FROM THE BLOOD OF ADULT T. MOSSAMBICA.

The mechanism of gas secretion into the swimbladders of teleost fishes has been demonstrated as a function of pH change in the counter current multipliers of the rete mirabilis. The pH of the blood entering the rete complex drops by a value approaching 1 pH unit (Hochachaka and Somero 1971), and this decrease in pH facilitates the release of oxygen through the Root effect. Lactic acid is a byproduct of localised swimbladder epithelial glycolysis (Steen 1963, D'Aoust 1970), and since the Pasteur effect (the inhibition of glycolysis by high oxygen pressures) does not function in this tissue (Ball et al 1955), the Root effect is generally believed to be the most important mechanism of oxygen secretion. No data is available on oxygen dissociation from the blood of T. mossambica and in view of its importance in swimbladder physiology the effect of temperature and pH on oxygen dissociation was determined.

MATERIALS AND METHODS

All fish used in this section were small mature adults obtained from the Kenton population. A comparative spectrophotometric analysis of blood haemoglobin solutions was devised in order to determine the oxygen dissociation from T. mossambica blood. Preliminary experiments using whole blood cells were unsatisfactory due mainly to excessive light scattering by the erythrocytes (Kay 1964). Thus with the instrumentation available, whole cell analysis was abandoned in favour of haemolysate solutions. The pH measurements were obtained using a micro pH unit (Radiometer BMS-3) capable of

reading to an accuracy of 0.002 pH units. The pH electrode was calibrated with two precision buffers whose temperature coefficients were known and accounted for during calibration.

The pH of T.mossambica blood from fish acclimated to 22°C was found to vary between 7.30 and 7.45 (range of samples from five fish). A suitable basic buffered fish saline for freshwater fish was prepared as described by Kerkut (1968) and the pH of this solution was 7.36.

Fish, very lightly anaesthetised with sandoz MS.222, were bled from the heart by exposing the heart through the floor of the pharynx after removing the operculum and deflecting the gills. The blood was drawn up into a heparinised glass pipette and immediately mixed with five millilitres of buffered saline at 10°C and containing 2 to 3 drops of heparin solution. Generally one to two millilitres of blood were obtained. The blood/saline mixture was centrifuged for five minutes at 3000 r.p.m. and the compacted erythrocytes collected while the supernatant was discarded. A second and third wash and centrifugation of the compacted erythrocytes ensured a plasma free sample of blood cells. These cells were then lysed by the addition of 2 mls of distilled water and the resulting haemolysate made up to 12 mls with buffered saline. This solution was then centrifuged for 15 minutes and the supernatant collected while the ghost cells and other compacted debris were rejected. The haemolysate was subdivided into 3 equal portions which were heated in a water bath to the required temperature and used immediately.

Gas mixtures, made up in cylinders using oxygen free nitrogen and filtered compressed air, were used to equilibrate the haemolysate

to a variety of PO_2 mixtures. Prior to use, the PO_2 of the mixtures was determined using the gas analyser (Radiometer BMS-3). The spectrophotometric analysis procedure used is set out below.

1. A Beckman DB (double beam) spectrophotometer was calibrated to 100% transmission using two haemolysate subsamples which had been aerated by passing air ($PO_2 = 152$ mm Hg) through them at a rate of 2 litres per minute for 15 minutes. One of the matched pairs of cuvettes remained full of haemolysate and this remained as the reference sample throughout each series of analyses. Thus all readings were compared to this aerated reference sample.

2. A gas mixture containing the highest PO_2 was bubbled through the working haemolysate subsample at 2 litres per minute for 15 minutes.

3. This sample of haemolysate was then rapidly decanted into the empty cuvette using a pipette and the difference in light transmittance between the reference ($PO_2 = 152$ mm Hg) and this working subsample was recorded.

4. The working sample was then removed from the cuvette, subjected to successively lower PO_2 gas mixtures and the comparative light transmittance noted for each PO_2 value.

5. Full deoxygenation was obtained using nitrogen. The comparative transmission of this sample gave the percentage light transmission range, from oxygenated to deoxygenated haemolysate, and from this value the intermediate transmission readings were calculated to show the proportionate percentage saturations for the various PO_2 values.

In this method of comparative transmission it was important to obtain as wide a difference in transmittance between oxygenated and deoxygenated blood as possible. This is largely governed by the wavelength used. In order to determine the most suitable wavelength a comparative scan through the entire visible light spectrum was undertaken. The wavelength showing the maximal light transmission difference between oxygenated and deoxygenated haemolysate was taken as the most satisfactory. This was 600 nm (Fig. 6).

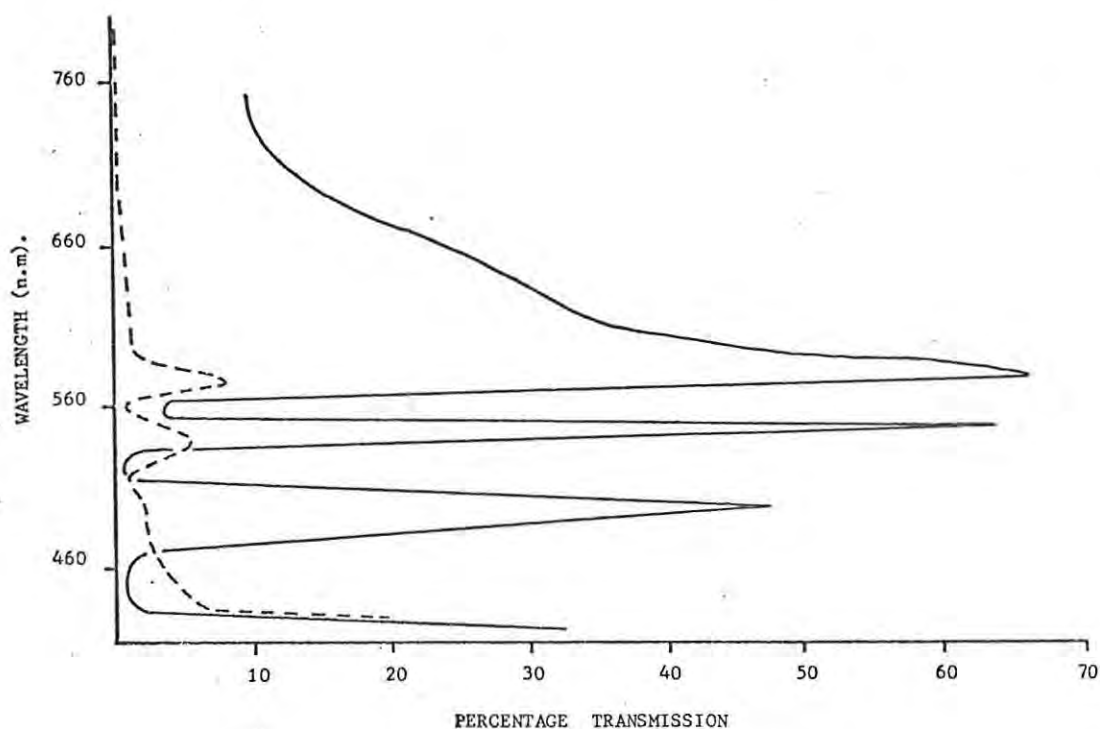


Figure 6. A light wavelength transmission scan of oxygenated and deoxygenated T.mossambica haemolysate solution at pH 7.36 and 22°C. (Solid line = deoxygenated haemolysate, broken line = oxygenated haemolysate).

Using the same procedure outlined above, the effect of temperature on the dissociation of oxygen was determined. The reference sample remained at a temperature of 22°C while the working solution was

maintained at either 15°C or 30°C. Thus the effect of temperature was shown relative to 22°C and the difference in oxygen content between samples exposed to the same PO_2 could be regarded as being entirely due to the effect of temperature.

Investigation of the effect of pH change on oxygen dissociation was determined by comparing the standard sample (pH 7.36 at 22°C) with a sample in which the pH had been lowered by the addition of 1.5 N hydrochloric acid. This acid was used in preference to a weak acid such as lactic acid because being a strong acid only very small volumes are required to change the pH of the sample. To ensure that the volume added did not cause a comparative light transmission difference due to the dilution effect, an equal volume of saline was added to the reference sample. This data was used to construct a dissociation curve. In order to establish the pH range most effective in the Root effect, it was necessary to examine the oxygen content of blood exposed to a range of pH changes. Exact replication of the pH values was difficult using the method outlined above. To overcome this problem samples of haemolysate were subjected to a number of small pH changes, through the sequential addition of approximately 5 microlitres of 1.5 N HCl. After aeration at a PO_2 of 152 mm Hg the sample of acidified haemolysate was compared to a suitably diluted reference sample at pH 7.36. The addition of acid continued, on the same haemolysate sample, until the pH had dropped below 6. Thus through replicating this procedure a Root effect curve was constructed at PO_2 152 mm Hg. The effect of temperature was determined by comparing a sequentially acidified sample at 30°C to a 22°C reference sample.

Hematocrits were determined on samples of fish blood. These were analysed by centrifuging for 15 minutes on a MSE hematocrit centrifuge at 12,000 r.p.m. at a temperature of 22°C.

RESULTS

The haemolysate studies showed that the blood of T.mossambica has a high affinity for oxygen (Fig. 7). At 22°C the P_{50} value was below 10 mm Hg. Fish (1956) found a P_{50} value of 6 mm Hg in Tilapia esculenta. The effect of temperature follows the classical shift in dissociation as described by Brown 1957, namely that the haemoglobin has a higher affinity for oxygen at lower temperatures (Fig. 7).

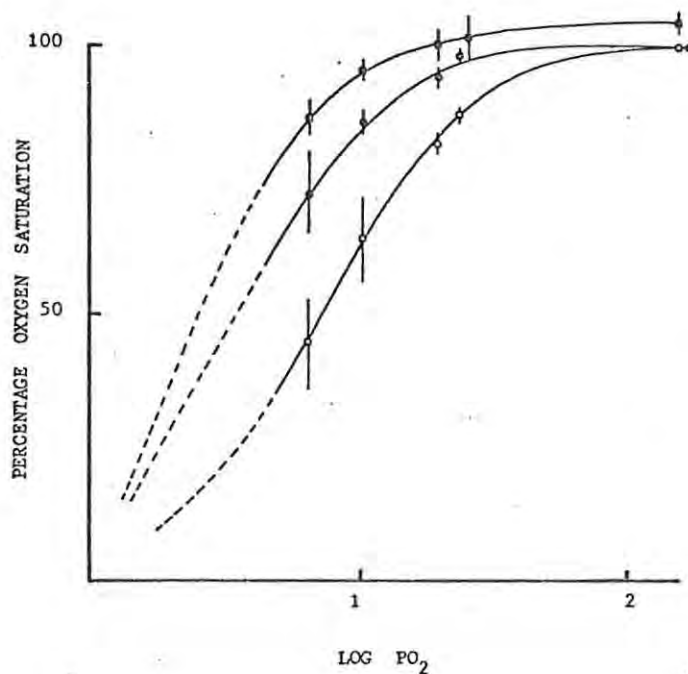


Figure 7. Oxygen dissociation curves for T.mossambica haemoglobin. Solid circles represent twelve replications at 22°C, open circles represent five replications at 30°C and squares represent five replications at 15°C. The vertical line indicates two standard errors about the mean.

It is of interest to note that at saturation (PO_2 152 mm Hg) the effect of temperature shows no difference in the affinity of oxygen in the haemolysate at 30°C and 22°C , but that an increased affinity is found in the 15°C solution. There was no significant difference between the oxygen affinity of the male and female haemoglobin at any temperature.

The effect of pH change on the dissociation curve is shown in Fig. 8. The Bohr effect and the Root effect are both clearly shown by T.mossambica blood.

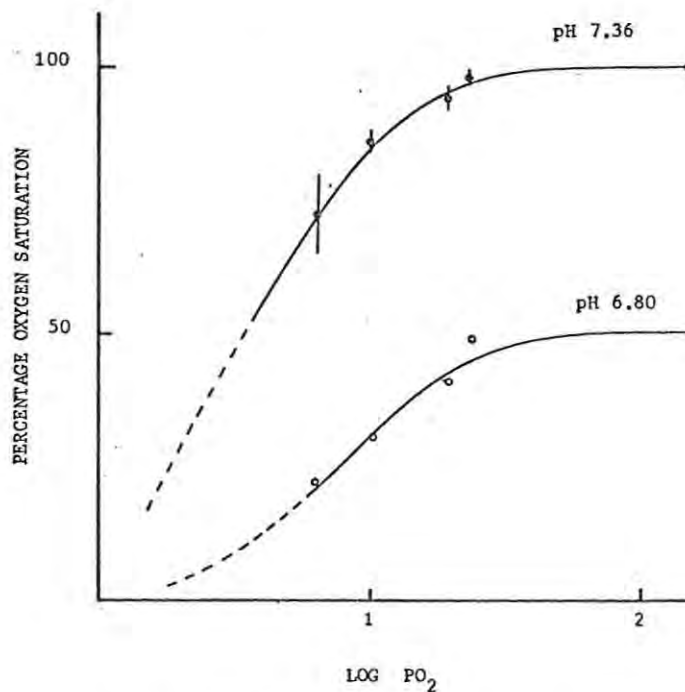


Figure 8. The effect of pH on the oxygen dissociation of T.mossambica haemoglobin. The solid circles represent twelve replications at pH 7.36 and open circles represent a single determination at pH 6.80. The vertical line indicates two standard errors about the mean. Temperature 22°C .

The Root effect was investigated in greater detail at two temperatures and the results are shown in Fig. 9. The Root effect was marked in the range pH 6.6 to 7.36 (normal blood pH).

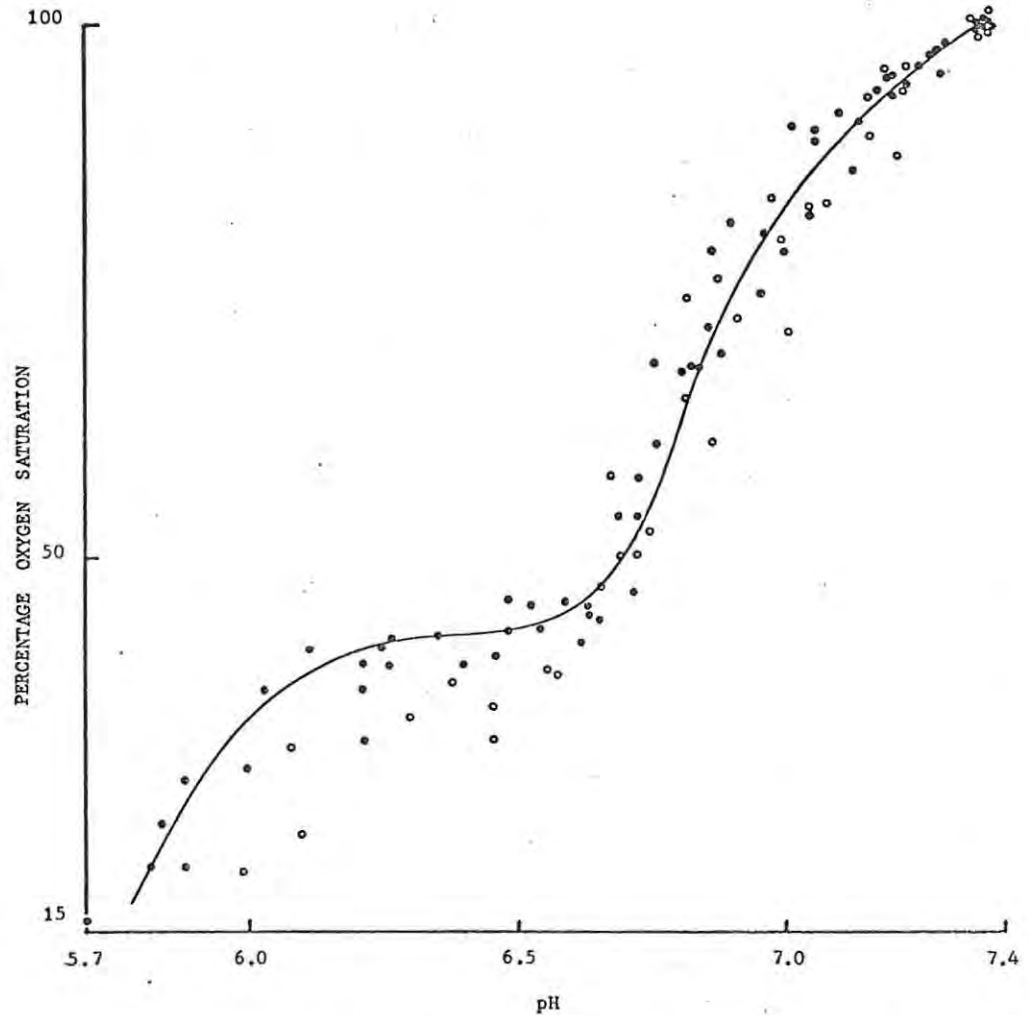


Figure 9. The Root effect as shown by *T. mossambica* haemoglobin at PO_2 150 mm Hg. Closed circles represent determinations at 22°C and open circles at 30°C. The line of best fit was constructed for the 22°C determinations.

Within this pH range it was possible to return the blood to full saturation by readjustment of the pH by addition of a strong base. Below forty percent saturation (pH 6.4) however, the

haemolysate showed signs of deterioration and some degree of precipitation occurred. It is possible that the isoelectric point of T.mossambica haemoglobin lies between pH 6.3 and 6.6.

The results of the hematocrit determinations are given in Table 4.

	Female	Male
Number of determinations	11	11
Mean % packed cell volume	40.818	36.090
Std. Deviation	5.547	6.625

TABLE 4. Hematocrit values for male and female mature adult T.mossambica at 22°C.

No significant difference was found between the P.C.V. of males and females ($P > 0.07$). The mean value of 38.46% is considerably higher than that of 24.2% recorded by Hattingh (1972) for T.mossambica. Since the latter author gave no indications of method, number of replications, fish size, state of maturity or acclimation temperature, no reasons for this difference can be suggested.

DISCUSSION

The effects of temperature on the oxygen affinity of T.mossambica haemoglobin are in general of the same nature as the effects observed for trout and tench (Eddy 1973). Decreasing temperatures caused an increase in the oxygen affinity of the

haemoglobin. In trout and tench haemoglobin it has been demonstrated that pH is directly linked to temperature. At low temperatures arterial blood has relatively higher pH values. Thus it would be oxygen saturated at relatively low oxygen tensions, and its oxygen unloading tensions will be lower (Eddy 1973). Whether or not this phenomenon is important in the functioning of oxygen secretion into the swimbladder could not be demonstrated, but since secretion of oxygen is relatively slower at low temperatures, it may be expected that the relatively lower unloading tensions would possibly be of some importance.

The effect of temperature, however, on gross physiological factors, such as heart beat rate, respiratory rate etc., may be more important than the direct effect on blood chemistry in proposing a mechanism responsible for the increased rates of descent. Josman (1972) has shown that T. mossambica has a five fold increase in heart beat, and presumably cardiac output, with a temperature increase of 15°C (from 15°C to 30°C). A three fold increase in the opercular/buccal respiratory rate has also been shown for the same temperature range (Josman 1972) and these differences agree with the large increase in oxygen uptake over the same temperature range (Job 1969). Thus it may be expected that an increase in the rate of blood passing the rete/gas gland complex would facilitate an increase in oxygen release into the swimbladder. McNabb and Mecham (1971) analysed the secretion rates of oxygen into the swimbladder of the blue gill sunfish (Lepomis macrochirus) and showed an increase in the rate of oxygen secretion with increased temperature. These authors did not expect these results, arguing that increased cellular metabolism at higher temperatures would leave less oxygen available for secretion to the swimbladder. In discussing their results McNabb and Mecham suggested that their

findings may be due to gross physiological functions such as those mentioned above, and in addition a possible increased activity of the lactate dehydrogenase, resulting in an increased production of lactic acid. This, in terms of the generalised role of lactic acid and the Root effect, may increase the rates of oxygen secretion to the swimbladder. As shown by the work of McNabb and Mecham and the data presented in this section, no single factor can, at this stage, be proposed as being responsible for increased oxygen secretion with increased temperature, but it would appear rather to be related to a number of integrated physiological functions.

3. THE RATE OF OXYGEN LOSS FROM THE SWIMBLADDER OF
T. MOSSAMBICA BY PASSIVE DIFFUSION.

Denton et al (1970), Kutchai and Steen (1971) and Denton et al (1972) have all reported loss of oxygen from the swimbladder of eels by diffusion through the swimbladder wall. If the loss of oxygen from the swimbladder of T. mossambica was relatively large, it may be expected that a state of equilibrium would be reached when, at a relatively high PO_2 , the loss of oxygen by passive diffusion would equal active secretion by the rete/gas gland complex. This balance would be expected to restrict the final depth equilibration of the fish. Although higher temperatures may result in increased rates of oxygen transport and secretion, the rate of loss of oxygen by passive diffusion from the swimbladder may not follow the same trend. At high temperatures, if the loss of gas by passive diffusion did not alter at the same rate as the increased oxygen secretion, it may be expected that the fish would have either greater or lesser mean maximum equilibration depths. Thus with the increased secretion at warmer temperatures and the greater maximum equilibration depths, it may be proposed that the loss of gas from the swimbladder was not proportionately increased at higher temperatures. This hypothesis was tested by determining the effect of temperature on the rate of oxygen diffusion across the swimbladder wall.

MATERIALS AND METHODS

Oxygen diffusion across the swimbladder wall was measured using a modified version of the apparatus described by Kutchai and Steen (1971). The apparatus (Fig. 10) was constructed of perspex and fitted with a Beckman model 777 oxygen probe capable of recording to a 1% accuracy.

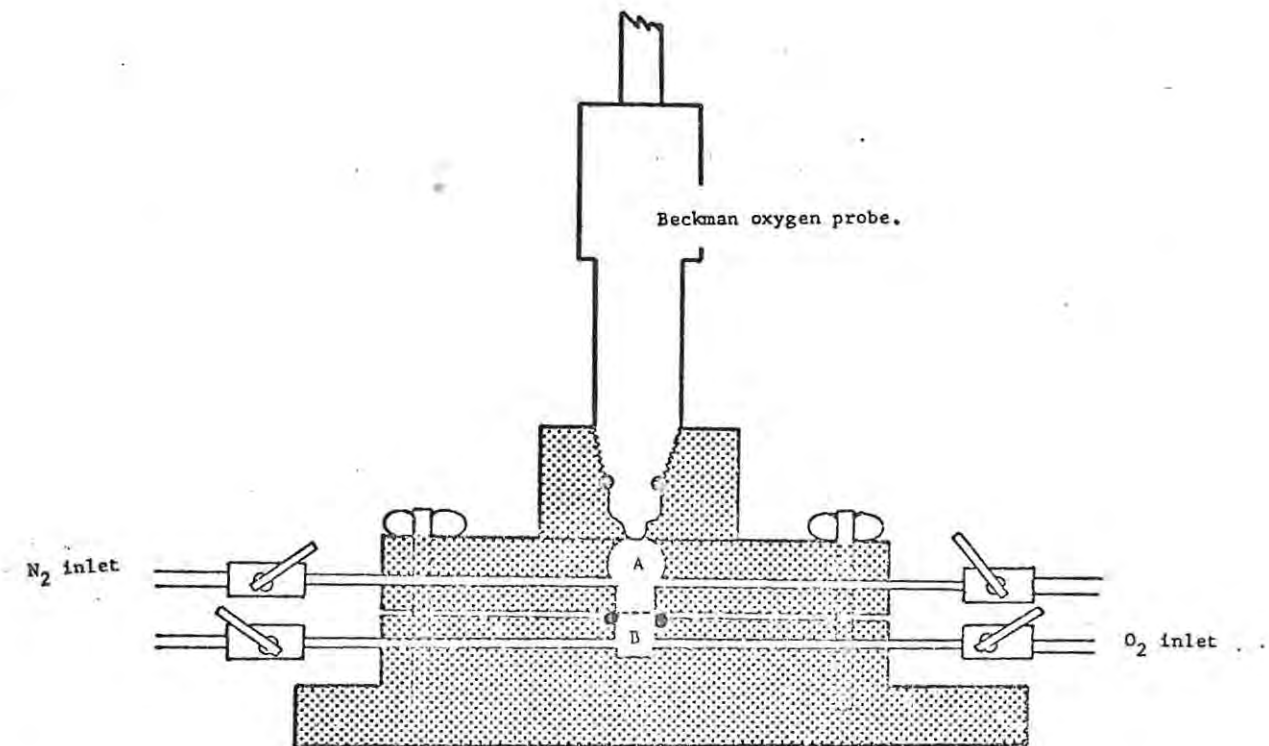


Figure 10. Apparatus used for determining the oxygen permeability of the swimbladder wall. The tissue (broken line) separates chamber A and B. The whole unit was immersed in a constant temperature bath.

The swimbladder membrane was removed from lightly anaesthetised fish, generally from the anterior chamber of the swimbladder. The dorsal surface of the bladder was used as well as the ventral surface, this latter membrane was usually attached to the thick peritoneal membrane separating the visceral cavity from the swimbladder. The membrane was positioned across the o-ring between chambers A and B. The two halves of the apparatus were closed and held tightly by brass bolts. Chamber A was flushed with humidified nitrogen and the oxygen probe zeroed, the probe was then calibrated by flushing humidified air through the chamber. After calibration, humidified nitrogen was passed through chamber A and humidified oxygen through chamber B. When the oxygen probe registered zero PO_2 in chamber A it was sealed by closing the inlet and outlet taps. The flow of oxygen continued through chamber B at about 1 litre per minute. The PO_2 in chamber A

was measured every 15 minutes for 90 minutes indicating the rate of diffusion of oxygen from chamber B to chamber A through the membrane. The rate of diffusion of oxygen in $\mu\text{l/hr/cm}^2$ for a difference of 1 atmosphere of oxygen was calculated from the following formula

$$k = \frac{(PO_2f - PO_2i) (V)}{(720) (a) (T)}$$

where PO_2f was the final PO_2 (derived by regression analysis) after time T (hours). PO_2i was the initial PO_2 and V was the volume of chamber A in microlitres and the area of the membrane (a) was given in square centimetres.

A control experiment in which a non permeable membrane (aluminium foil) replaced the swimbladder membrane and chamber A was filled with a gas mixture of $PO_2 = 20$ mm Hg showed that the oxygen probe itself used no measurable oxygen after 4 hours. Thus the PO_2 values monitored during the experiments were attributed to oxygen diffusion through the swimbladder from chamber B to chamber A. These rates of diffusion were investigated at 15°C , 22°C and 30°C by placing the apparatus in a temperature controlled water bath.

The thickness of the swimbladder wall of mature adult fish was determined by two methods. The first was by direct recordings from a mechanical measuring device (Mercer gauge) capable of reading to 0.0005 mm. The second method involved calculating the thickness from the weight of a known area of tissue. The specific gravity of T.mossambica tissue was taken as 1.066 (Caulton 1972). Fifteen measurements were made using the Mercer gauge and five by the area-weight technique.

RESULTS

There was no measurable diffusion across the ventral swimbladder wall when attached to the peritoneal membrane. All the results reported below therefore refer solely to the swimbladder wall.

The results of the diffusion analysis are shown in Table 5. Since no significant difference was found between mature adult fish ($P > 0.2$) these values were not treated independently. In the small sub adult fish, however, there was a significant difference in the rate of oxygen diffusion ($P < 0.01$) with the deeper equilibrated males having a larger rate of diffusion.

Sex and Size	n	Temp. °C	Equilibration depth m.	Rate of diffusion $\mu\text{l/hr/cm}^2/\text{atmos.}$	Std. deviation
Small adult male	9	22	20	81.303	14.683
Small adult female	10	22	13	35.200	8.6013
Mature male and female	8	15	7.5	24.286	9.849
Mature male and female	8	22	11	20.684	5.188
Mature male and female	10	30	20	21.562	5.866

TABLE 5. Rates of oxygen diffusion through the swimbladder wall of T. mossambica.
(n = number of replicates).

Although the experiments were all carried out with the same pressure differences across the membrane, those fish that equilibrated to the deepest maximum depth i.e. small sub adult males, showed the greatest loss of oxygen by diffusion at 22°C. Thus there was a

marked tendency for decreasing rates of passive diffusion with increased maturity and equilibration to shallower depths. The effect of temperature on the rates of oxygen diffusion across the swimbladder wall showed no significant differences in mature adult fish at either 15°C, 22°C or 30°C.

The swimbladder of T.mossambica is extremely thin varying from 1.6 μ m to 5.0 μ m. The swimbladder was not of a uniform thickness and varied from one area to another. The mean thickness as determined by the area/weight method was 2.8 μ m.

DISCUSSION

T.mossambica loses relatively large volumes of oxygen per unit area of swimbladder in comparison to the loss of oxygen from the swimbladders of eels (Denton et al 1972, Kutchai and Steen 1971).

Species	Swimbladder thickness (μ m)	Max. depth recorded m	loss of O ₂ by passive diffusion μ l/cm ² /hr/atmos	Author
<u>Conger conger</u>	182	1000	0.26	Denton <u>et al</u> (1972)
<u>Anguilla anguilla</u>	300	250	1.9	Kutchai & Steen (1971)
<u>T.mossambica</u>	2.8	11.2	20.7	This study

TABLE 6. Swimbladder wall thickness and comparative rates of oxygen loss by passive diffusion from three species of fish.



The rate of oxygen loss will be dependant on the relative thickness of the swimbladder and since the swimbladder of T.mossambica is extremely thin it would be expected to lose a large amount of gas by passive diffusion. From the data above, Anguilla anguilla, having a swimbladder 100 times as thick as that of T.mossambica, loses about 10 times less oxygen by diffusion. It is also noted from Table 6 that the deeper the maximum depth to which the fish penetrates the lower the loss of oxygen. However it would be expected that at depth the eels would lose greater absolute amounts of oxygen. If the rate of loss is related to pressure, the loss of oxygen in Conger conger at 100 atmospheres would be considerably larger than $0.26 \mu\text{l}/\text{cm}^2/\text{hr}/\text{atmos}$. Similarly the loss of oxygen in T.mossambica sub adults may be expected to be greater at their maximum equilibration depths than are reported in Table 5. Since a greater diffusion rate was found in sub adults it may be suggested that on attaining maturity the swimbladder undergoes a structural change, so decreasing the loss by gaseous diffusion. Denton et al (1972) have demonstrated the crystalline structure of the silvery layer to be an important factor in preventing diffusion and this crystalline component may, in T.mossambica, be only fully developed at maturity. Thus the rate of gaseous diffusion from the swimbladder could be a limiting factor governing the maximum depth to which these fish can descend. If the rate of active secretion equalled the loss by passive diffusion the fish would remain in equilibrium. Thus in T.mossambica the increased equilibration depth shown by juveniles and sub adults would therefore necessitate an even greater secretory rate in order that these fish can maintain a deeper equilibration. A further important fact in this function may be the relatively small volume of gas required to recharge the swimbladder of juveniles at depth and

similarly the smaller area of the bladder would be expected to have a relatively smaller total loss of gas by diffusion. Thus it would appear that a number of integrated factors relating to secretion rates and diffusion rates would be important in determining the mechanisms enabling juvenile fish to penetrate to deeper depths at faster rates than adults. Since the rate of secretion is affected by temperature while the rate of passive loss is not, it would be expected that the adult fish would be able to descend at a faster rate and to a deeper depth in warmer temperatures. This has been demonstrated in the tolerance experiments in Part 1 and the results shown in this section have indicated a proposed mechanism for this function.

GENERAL DISCUSSION

Of particular interest to this study is the correlation of the presented experimental facts with the available field data on the biology and distribution of the respective cichlids. The physiological effects of temperature have been shown to be an important factor in the tolerance and rate of descent to deep water. This should have important implications in the biology of these fishes. Bruton (1973), working on the biology of T. mossambica in lake Sibaya, reported that these fish showed both a vertical and horizontal seasonal movement. The trend was for breeding adults to occupy the shallow 2 metre deep marginal terraces in the summer. During the transitional seasons and when not actively breeding, the majority of adults occupied water to a depth of 13 metres, with a maximum of 18 metres having been recorded. During the winter adult fish occupied the upper 7 metres of the water column but generally away from the shore and over deep water. The juveniles during most seasons of the year occupied the shallow terrace but according to Bruton also occupied the shelf and deep water. These juveniles were seen to move diurnally from the terraces to deep water during winter, only occupying the terrace during midday when water temperatures were relatively high.

The horizontal seasonal movement of adult T. mossambica has been largely attributed to the marked temperature preferendum shown by these fish. During winter the water temperature of the lake varies between 17°C and 18°C which is diurnally constant in deep water but not on the marginal terrace, where water temperature may change by 15°C over a 24 hour period (Bruton 1973). It is generally accepted

that diurnal temperature fluctuations of this magnitude would prohibit the adults from inhabiting this area during winter and thus they inhabit the homothermal open waters. During the transitional seasons, the fish feed almost exclusively on the abundant benthic diatoms, while the few winter fish analysed indicated that these fish were feeding on terrestrial flying insects that had fallen into the water as well as on phytoplankton extracted from the water (Bruton 1973), but not apparently on the benthic diatoms although these were abundant on the substrate during winter. Thus the problem arises as to exactly why the winter adult population was inhabiting these relatively poor feeding areas of open water and not feeding on the bottom, as were the juveniles.

From the results of the experimental and physiological data presented in this study it may be suggested that these fish have restricted vertical movements coinciding with the seasonal water temperature of the environment. The maximum equilibration depths of mature adult T. mossambica at the experimentally tested temperatures are summarised in Fig. 11.

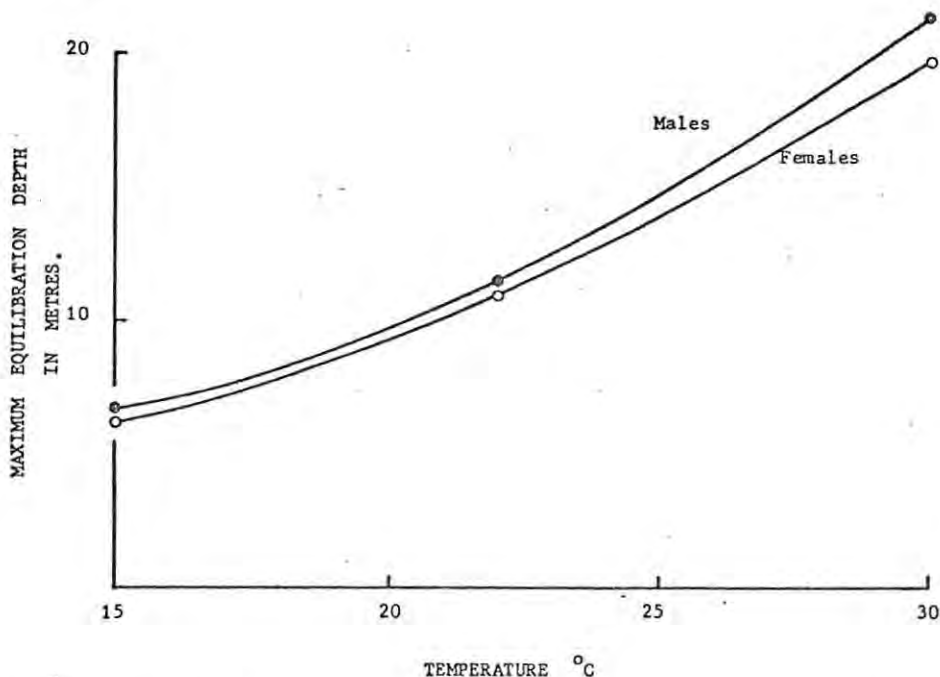


Figure 11. The effect of temperature on the maximum equilibration depth of mature adult T. mossambica. (Summary of Figure 3).

If these maximum depths are replotted to correspond with the mean monthly temperatures found in lake Sibaya, an expected seasonal maximum depth distribution for any particular year can be shown as for example 1968/1969 (Fig. 12).

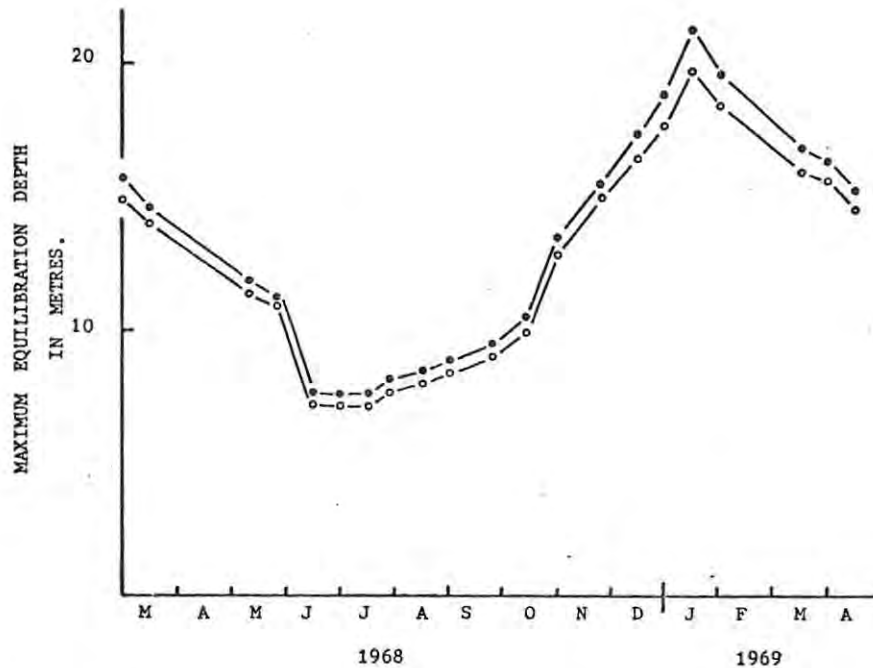


Figure 12. The expected maximum depth equilibrations of mature adult *T. mossambica* in Lake Sibaya for 1968/69. Solid circles = male fish; open circles = female fish.

From the data presented above, it may be expected that during winter adult *T. mossambica* would be restricted to water less than 7 metres deep, and since they are not found on the marginal terraces, their bottom distribution would be restricted to a relatively narrow area of the steep slope where the water is relatively homothermal. The bottom substrate area available to the summer population would be greatly expanded since they can occupy, in addition to the terraces,

bottom areas down to a depth of 20 metres. The distribution of immature sub adults and juveniles would not be expected to show the same seasonal variations since they are capable of inhabiting deep water throughout the year and only with the onset of maturity will they become restricted. The restriction of adults during winter and their non feeding summer habits would probably result in poor feeding for a large part of the year. Needham (1964) and Martin (1966) have both shown that poor feeding results in early gonad development, ovaries in particular, and consequently a population of small sized fish which breed at a young age. In lake Sibaya the male T.mossambica mature after two years while the females after one year and both at a small size. The maturity age reported for the Sibaya population and the small adult sizes could be due to a poor food supply, not necessarily through the abundance of food but rather the accessibility of the food source to the fish.

In other T.mossambica habitats where the bathymetry of the lake follows a more gently sloping regime, unlike the steep slope of lake Sibaya, and where the winter diurnal temperature does not prohibit habitation of this gently sloping area, it would be expected that feeding areas would not be inaccessible and a larger mature fish of a greater age would be produced. In lake Kariba for example marginal slopes generally extend for long distances, up to 5 km, from the shore, and thus would be accessible as feeding grounds throughout the year and would probably thus be a factor contributing to the fish attaining maturity only after 3 years at a length of 30 cms. (Donnelly pers.comm.).

It has been shown that T.rendalli, which feeds on rooted macrophytes,

is restricted to water less than 8.5 metres in depth at 22°C. Like T.mossambica, it would be expected that this depth would be extended in water of a higher temperature. In lake Kariba T.rendalli has been recorded from 7.5 metres (Coke 1967) which corresponds to the maximum depth of macrophyte distribution (Donnelly 1969). Tilapia sparrmanii adults are shown to penetrate to 15 metres at 22°C and would similarly be expected to increase this depth with increasing temperature, however no field data on vertical distribution is available for this species.

Hemihaplochromis philander fry have been caught in the deepest parts of lake Sibaya. Very young fry have been trapped at 38 metres in zooplankton traps designed to sample substrate zooplankton populations (Hart pers.comm.). Adult fish have been recorded courting at 15 metres (Hill pers.comm. SCUBA record 25-1-'71, water temperature 26°C) while nest sites and territorial males have been observed to 20 metres (Ribbink pers. comm.).

Ribbink (pers.comm.) has suggested that in lake Sibaya H.philander mates during early summer at 15 to 20 metres. This conforms to the expected equilibration depths of both sexes at a temperature of 26°C to 28°C. After mating the females migrate to deep water and release their fry. Both fry and female fish show a tolerance to depths in excess of 20 metres under experimental conditions. This behaviour pattern would allow the fry maximum protection from the predatory males, which would be limited to water less than 20 metres deep. The fry and juveniles feed predominantly on zooplankton and small benthic crustacea (Jubb 1967) in which the deeper waters of lake Sibaya abound (Boltt 1969, Hart 1973), and since they are relatively

free from predators, would be expected to face few factors that would impede their survival and growth in deep water.

In addition to the advances in the understanding of the ecology of the species, the data presented may have some value in the efficient design and development of commercial fisheries in newly formed lakes such as Kariba and Cabora Bassa.

In lake Kariba prior to inundation, Jackson (1961), in conjunction with the Joint Fisheries Research Organisation of Northern Rhodesia, suggested the clearing of all trees and bush to a depth of 60 feet (19 metres) below the lowest proposed water level. Since the fishing industry at Kariba was to be based largely on Tilapia mossambica (= T. mortimeri), this action was undertaken in certain areas to facilitate commercial gill net fishing of this species. An annual draw-down of at least 30 ft. (9 metres) was expected, thus the clearing limit from the high water mark was to be a total of 90 feet (28 metres). These levels were not based on any factual data but rather "in view of the fact that nearly all African freshwater fisheries catches were taken in less than 80 feet in depth" Jackson (1961).

Once the dam had filled and had attained equilibrium, it was found that the maximum annual draw was less than expected, somewhere in the region of 1 to 3 metres below high water level (Begg 1970). Secondly, it was noted that fish catches were relatively poor in cleared areas as compared with the better returns obtained among the submerged trees in the non cleared areas (Mortimer 1965). This trend has generally been followed in the succeeding years, but with a steady decline in the fish population in the cleared areas. This

decrease is shown in commercial catches which decreased from 5200 tons in 1964 to 1300 tons in 1970 (Minshull 1973).

During the initial period of productivity decline, it was suggested by Mortimer (1965) that this was due to the fish moving into water deeper than the 28 metre cleared limit. Joeris (in Jackson 1966) stated that T.mossambica (T?.mortimeri) were present from 90 feet (28 metres) to 160 feet (49 metres). These records, obtained both by SCUBA observations and netting records, are extremely suspect. The inexperience of the early data collectors, especially in the art of setting deep set nets could have produced misleading results. The setting of deep gill nets requires a special skill and in areas of extremely high fish production, such as was present at that time, the chances of snaring fish at shallow depths whilst lowering and/or raising these nets cannot be overlooked. Although he gives no actual figures, Joeris does state that catches were poor, and thus unless the net setting technique had the required precision to ensure trapping at the desired depth only, these results may be misleading. Similarly, possible misidentification with fish such as Serranochromis or non cichlid fish by the non biologist divers in generally poor visibility and low light levels may have been responsible for the added confusion. This data has never been substantiated, and the comprehensive survey by Coke (1967) showed T.mossambica (T?.mortimeri) only to a maximum depth of 15 metres. Mr P.B.N. Jackson (pers.comm.) has agreed on the probable unreliability of these early records.

Winter temperatures of lake Kariba above the 20 metre thermocline are from 22°C to 24°C (Begg 1970, McLachlan 1970). The experimental data presented in this work suggests a maximum equilibration depth of

11 to 13 metres at this temperature. The mid summer temperatures of 28°C to 31°C would suggest a depth tolerance of 17 to 22 metres.

In the light of these results, tree clearing to a maximum depth of 15 metres would probably have been adequate, and in fact clearance to this level would probably have been beneficial to fish production. Mortimer (1965) has indicated a trend for greater fish yields to be obtained from non cleared areas. These fish are probably utilising the periphyton that grows abundantly on the submerged trees. Since macrophyte growth only extends down to 7.5 metres (Donnelly 1969), the cleared areas would be relatively barren, in terms of periphyton growth, below this depth. The macrophyte zones vary in extent from a few metres to nearly 5 kms. Thus the areas of non macrophyte growth to the submerged tree line may be relatively large. In the Sanyati east cleared area, for example, only 7 sq.km of the total 33 sq.km area had a macrophyte cover (December 1969). If the submerged trees, however, had been available below the 15 metre contour the fish population would have accessible periphyton feeding areas while sufficient space for commercial netting would still be available whereas at the present level the submerged trees may be largely inaccessible to the adult population.

The statement by Fryer and Iles (1972) on the importance of the swimbladder in the success of the cichlid community has been confirmed by the data presented in this study, and many formerly unexplained activities in the cichlid community of lake Sibaya and probably other systems as well may possibly be related directly to swimbladder function. It is hoped that future workers engaged in ecological studies on cichlid biology will consider this aspect.

SUMMARY

1. Using an experimental pressure chamber the rates of descent and the maximum depths of buoyancy equilibrium of some cichlid fishes were tested.
2. Tilapia mossambica juveniles could descend to depths greater than 30 metres very rapidly. At 22°C small sub adult male fish (length $\pm$ 160 mm) could descend to a maximum buoyancy depth of 20 metres, small sub adult females to 13 metres while large adults (length $\pm$ 200 mm) of both sexes could descend to 11 metres. The fish generally take 4 to 5 days to reach their maximum equilibration depth.
3. At 30°C mature adult male and female T.mossambica increased their maximum equilibration depth to 20 metres and at 15°C showed a decrease to a maximum depth of 6.7 metres. Temperature affects the rates of descent in a similar manner.
4. T.rendalli can descend to 7.5 metres at 22°C.
5. T.sparmanii can descend to 15 metres at 22°C.
6. Hemihaplochromis philander fry and young juveniles are not affected by pressure. Adult males can descend to 16 metres at 22°C and 20 metres at 30°C. Adult females can descend to 26 metres at 22°C and 27 metres at 30°C.
7. In T.mossambica oxygen appears to be the only gas secreted into the swimbladder to compensate for buoyancy loss.

8. The haemoglobin of T.mossambica showed a marked Bohr and Root effect.
9. Increased rates of descent with increased temperature are believed to be due to increased O₂ uptake, heart output etc. rather than as a direct effect of blood chemistry.
10. The swimbladder wall of T.mossambica is extremely thin (2.8 μ m) and consequently a large amount of gas is lost through passive diffusion.
11. Diffusion is not affected by temperature, consequently the increased secretory rates at higher temperatures are not balanced by a proportionately higher diffusion loss, thus enabling the fish to maintain a deeper buoyancy equilibrium at higher temperatures.

ACKNOWLEDGEMENTS

The counsel and suggestions of Dr B.J. Hill in his supervision of this work and the financial grant given by the South African Council for Scientific and Industrial Research are greatly appreciated.

REFERENCES

- ABERNETHY, J.D. (1972). The mechanism of secretion of inert gases into the fish swimbladder. *Aust.J.exp.Biol.med.Sci.*, 50:365-374.
- ALEXANDER, R. Mc N. (1959). The physical properties of the swimbladder in intact cypriformes. *J.exp.Biol.*, 36: 315-332.
- ALEXANDER, R. Mc N. (1966). Physical aspects of swimbladder function. *Biol.Rev.*, 41:141-176.
- ALLANSON, B.R. & J.D. VAN WYK. (1969). An introduction to the physics and chemistry of some lakes in Northern Zululand. *Trans.R.Soc.S.Afr.*, 38:217-240.
- BALL, E.Q., C.S. STRITTMATTER AND O. COOPER. (1955). Metabolic studies on the gas gland of the swimbladder. *Biol.Bull. mar.biol.Lab., Woods Hole.*, 108:1-17.
- BEGG, G.W. (1970). Limnological observations on lake Kariba during 1967 with emphasis on some special features. *Limnol.Oceanogr.*, 15:776-788.
- BISHAI, H.M. (1961). The effect of pressure on the distribution of some Nile fish. *J.exp.Zool.*, 147:113-124.
- BOLTT, R.E. (1969). The benthos of some southern African lakes. Part II: The epifauna and infauna of the benthos of lake Sibaya. *Trans.R.Soc.S.Afr.*, 38:249-269.
- BOLTT, R.E., B.J. HILL, AND A.T. FORBES. (1969). The benthos of some southern African lakes. Part I: Distribution of aquatic macrophytes and fish in lake Sibaya. *Trans.R. Soc.S.Afr.*, 38:241-248.

- BROWN, M.E. (1957). The physiology of fishes. Vols. 1 and 2. Academic Press, New York.
- BRUTON, M.N. (1973). A contribution to the biology of Tilapia mossambica Peters in lake Sibaya, South Africa. M.Sc. thesis. Rhodes University.
- CAULTON, M.S. (1972). The swimbladder and its role in vertical movements in the cichlid fish, Tilapia mossambica Peters. Honours project report. Rhodes University, South Africa.
- COKE, M. (1967). Fish biology in Lake Kariba. Part I. The distribution of fish on a bush-cleared area in Lake Kariba. Roneod report. Natal Parks, Game and Fish preservation Board, South Africa.
- D'AOUST, B.G. (1970). The role of lactic acid in gas secretion in the teleost swimbladder. *Comp.Biochem.Physiol.*, 32:637-668.
- DEHARDRAI, P.V. (1959). On the swimbladder and its connection with the internal ear in the family cichlidae. *Proc.Nat.Inst. Sci.India*: 25:254-261.
- DENTON, E.J., J.D. LIDDICOAT AND D.W. TAYLOR. (1970). Impermeable "silvery" layers in fish. *J.Physiol.Lond.*, 207:64-65.
- DENTON, E.J., J.D. LIDDICOAT AND D.W. TAYLOR. (1972). The permeability to gases of the swimbladder of the conger eel (Conger conger). *J.mar.biol.Ass.U.K.*, 52:727-746.
- DONNELLY, B.G. (1969). A preliminary survey of the Tilapia nurseries on Lake Kariba during 1967/68. *Hydrobiologia*, 34(2):195-206.
- EDDY, F.B. (1973). Oxygen dissociation curves of the blood of tench Tinca tinca. *J.exp.Biol.*, 55(2):281-294.
- FANGE, R. (1966). Physiology of the swimbladder. *Physiol.Rev.*, 46:299-322.

- FISH, G.R. (1956). Respiration of six species of fish from Uganda. *J.exp.Biol.*, 33:186-195.
- FRYER, G. AND T.D. ILES. (1972). The cichlid fishes of the Great lakes of Africa. Their biology and evolution. Oliver and Boyd. Edinburgh.
- HART, R.C. (1973). A contribution to the biology of Pseudodiaptomous hessei (Mrazek) (Copepoda:calanoida) in Lake Sibaya, South Africa. Ph.D. thesis. Rhodes University, South Africa.
- HATTINGH, J. (1972). Observations on the blood physiology of five South African freshwater fish. *J.Fish Biol.*, 4: 555-564.
- HILL, B.J. (1969). The bathymetry and possible origins of lakes Sibayi, Nhlangwe and Sifungwe in Zululand. *Trans.R.Soc.S. Afr.*, 38:205-216.
- HOCHACHAKA, P.W. AND G.N. SOMERO. (1971). Biochemical adaptations to the environment, in: Fish Physiology vol. 6. ed. Hoar, W.S. and D.J. Randall. Academic Press, New York and London.
- JACKSON, P.B.N. (1961). "Kariba Studies: Ichthyology, the fish of the middle Zambezi". Manchester University press.
- JACKSON, P.B.N. (1966). in: Man-Make Lakes. ed., Lowe-McConnell, R.H. Academic Press, London and New York.
- JOB, S.V. (1969). The respiratory metabolism of Tilapia mossambica (Teleostei). II. The effect of size, temperature, salinity and partial pressure of oxygen. *J.mar.Biol.*, 3: 222-226.
- JONES, F.R.H. (1951). The swimbladder and the vertical movements of teleostean fishes. I. Physical facts. *J.exp.Biol.*, 28:553-566.

- JONES, F.R.H. (1952). The swimbladder and the vertical movements of teleostean fishes. 2. The restriction to rapid and slow movement. *J.exp.Biol.*, 29:95-109.
- JONES, F.R.H., AND N.B. MARSHALL. (1953). The structures and functions of the teleostean swimbladder. *Biol.Rev.*, 28: 16-83.
- JOSMAN, V. (1971). Some aspects of the effect of temperature on the respiratory and cardiac activities of the cichlid teleost Tilapia mossambica. M.Sc. thesis. Rhodes University, South Africa.
- JUBB, R.A. (1967). Freshwater fishes of southern Africa. A.A. Balkema. Cape Town and Amsterdam.
- KAY, R.H. (1964). Experimental Biology. Measurement and analysis. Chapman and Hall, Ltd., London.
- KERKUT, G.A. (1968). Experiments in physiology and biochemistry. Academic press. London and New York.
- KUHN, W., A. RAMEL, H.J. KUHN AND E. MARTI. (1963). The filling mechanism of the swimbladder. *Experientia*, 19: 497-511.
- KUTCHAI, H., AND J.B. STEEN. (1971). The permeability of the swimbladder. *Comp.Biochem.Physiol.*, 39:199-123.
- LE ROUX, P.J. (1956). Feeding habits of the young of four species of Tilapia. *S.Afr.J.Sci.*, 53:33.
- McCUTCHEON, F.H. (1958). Swimbladder volume, buoyancy and behaviour in the pinfish, Lagodon rhomboides (Linn.). *J.cell.comp. Physiol.*, 52:453-480.
- McLACHLAN, S.H. (1970). The influence of lake level fluctuation and the thermocline on water chemistry in two gradually shelving areas in lake Kariba, Central Africa. *Arch.Hydrobiol.*, 66: 499-510.

- McNABB, R.A. AND J.A. MECHAM. (1971). The effects of different acclimation temperatures on gas secretion in the swimbladder of the blue gill sunfish, Lepomis macrochirus. Comp. Biochem. Physiol., 40:609-616.
- MARTIN, N.V. (1966). The significance of food habits in the biology, exploitation and management of Algonguin park, Ontario, Lake Trout. Trans. Am. Fish. Soc., 95(4):415-422.
- MATTHES, H. (1968). The food and feeding habits of the tiger fish Hydrocynon vittatus (Cast, 1861) in lake Kariba. Beaufortia, 15:143-153.
- MINSHULL, J.L. (1973). The establishment of a fishery among the resettled Batonka people at lake Kariba, Rhodesia. Commission Internationale des Grandes Barrages. Madrid. Q40. R28. p. 413-419.
- MORTIMER, M.A.E. (1965). Lake Kariba - Fisheries Research Symposium - Roneod Report p. 69-72.
- NEEDHAM, A.E. (1964). The growth processes in Animals. Pitman, London.
- OVERFIELD, E.M. AND J.A. KYLSTRA. (1971). The volume and rate of volume change of the swimbladder of the goldfish. Respiratory Physiology, 13:283-291.
- RIGG, A. (1970). Properties of fish haemoglobin. in: Fish Physiology vol. 4. ed. Hoar, W.S. and D.J. Randall. Academic Press. New York and London.
- SATCHELL, G.H. (1971). Circulation in fishes. Cambridge University Press.
- STEEN, J.B. (1963). The physiology of the swimbladder in the eel Anguilla vulgaris. III. The mechanism of gas secretion. Acta physiol. scand. 59:221-241.

- STEEN, J.B. (1970). The swimbladder as a hydrostatic organ. in:
Fish Physiology vol. 4. ed. Hoar, W.S. and D.J. Randall.
Academic Press. New York and London.
- VAAS, K.F. AND A.E. HOFSTEDE. (1952). Studies on Tilapia
mossambica Peters in Indonesia. Contr.Ind.Fish Res.St.
No. 1:1-88.