THE SALINITY TOLERANCE OF SOME EASTERN PROVINCE FISH IN RELATION TO THEIR KNOWN DISTRIBUTION

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ABSTRACT

The tolerances to sea water, sodium chloride and sodium sulphate of various cichlid, cyprinid and anabantid species which occur naturally in or near the Eastern Province region of Southern Africa, were determined, and correlated with their distribution ranges. Of the nine freshwater species tested, only *Tilapia mossambica* is able to disperse through the sea and is therefore the only member of the secondary division (Myers 1937) present in this region. The remaining species are possibly all limited to the use of freshwater links for dispersal between river systems. Death of fish in sea water and in sodium chloride is chiefly due to loss of osmotic control, but in solutions of sodium sulphate a pronounced toxic effect is responsible for death. In all the solutions used, an inverse relation between survival time and concentration is present. No evidence of any cells specialised for salt excretion was found in the gills of fish exposed to various salt concentrations.

1. INTRODUCTION

In the study of South African freshwater fishes, sufficient information is now available on the systematics to allow attention to be paid to the zoogeography of the various species. Many of the early travellers, like Andrew Smith and Burchell, provided the initial impetus for systematic work, and further descriptions of species were given by Cuvier and Valenciennes, Castelnau, Boulenger, and Gilchrist and Thompson, and by the turn of the century a large number of species were known. Barnard (1943), Groenewald (1958), Jubb (1959, 1963) and other authors have put the classification of the freshwater fishes of Southern Africa on a sound basis by careful revision of various groups.

Consequent on the careful systematic separation of valid species, it has been possible to study the distribution of these species and attempt a synthesis of the local fauna and its relations to the freshwater fish faunas of the neighbouring zoogeographical regions, the Congo basin and East Africa. This work was initiated by Barnard (1943), but the most significant advances were made by Farquharson (1962), Jubb (1964), and Jubb and Farquharson (1965). Details of the distributions of individual species are also given by Groenewald (1958), du Plessis (1963), and Crass (1964) as well as in Jubb (1965).

According to the evidence presented by Jubb and Farquharson, the South African cyprinids, anabantids, clariids and bagrids not only show affinities with the Congo region, but appear to have utilised various freshwater links in their dispersal both into and within Southern Africa. On the other hand, at least some of the South African cichlids have apparently used the sea as a dispersal route, although they also show affinities with the Congo and East Africa faunas like the other groups.

These interpretations are based in the first instance on the distribution ranges which the various species exhibit at present. These conclusions on the dispersal routes followed are also supported by the hypothesis formulated by Myers (1937), which states that freshwater fishes can be separated into at least two categories, as far as their distributionary possibilities are concerned. These are -

(a) the primary division which cannot enter the sea under normal circumstances and therefore depend on various freshwater links, caused by physiographic changes, for dispersal,

(b) the secondary division which can enter the sea freely and can use it as a dispersal route therefore.

Myers' conclusions on the ability of various freshwater fishes to tolerate sea water or not, are based mainly on records of occurence in high salinities and on some experimental evidence of the tolerance of a few species. Experimental determination of the salinity tolerance of individual species and correlation of these results with the distribution routes available to these species have been undertaken only by Renfro (1959) and Armitage and Olund (1962). Further work on this aspect of the hypothesis is therefore urgently required, as has also been emphasised by Ewer in the discussion of Farquharson's (1962) paper, particularly with respect to the distribution of fish fauna in the rivers of the Eastern Cape. The present study is intended to close this gap in our knowledge by careful correlation of experimentally determined salinity tolerances with the distribution of a number of local freshwater fishes. An examination of the role of osmoregulation in the tolerance of various species towards different ionic environments was also carried out, in addition to an examination of the gill histology of an intolerant species.

2. LITERATURE

For the purposes of the present study, the Eastern Province region is arbitrarily limited to all the river basins between and including the Buffalo River in the east and the Gamtoos basin on the western side. The escarpment, as defined by Wellington (1955), is taken as the northern boundary. All the rivers in this region flow in a general north to south direction and open in the Indian Ocean. The arrangement of the various rivers within the region is indicated in figure 1.

Possible methods of distribution

Two main dispersal methods are of special importance in the distribution of freshwater fishes, and these are freshwater links and dispersal through the sea. Other possible methods, such as transport of fish eggs on birds' legs and by wind, could also extend the distribution range of a species, but Jubb (1964b, and personal communication) thinks that they have not played any role in the dispersal of freshwater fish in Southern Africa.

Freshwater links: Freshwater links, consisting mainly of river captures and coastal freshwater lakes, are always the results of physiographic changes of varying magnitudes.

Darlington (1957) and Myers (1963) consider river capture to be the most important type of freshwater link in the dispersal process. Among the list of undoubted river captures known today, examples which are known to have assisted in the dispersal of fish are rare. Kuehne and Bailey (1961) have discussed the role of a very small capture which enabled *Etheostoma* sp. to reach the headstreams of the Kentucky River from the Cumberland system. This capture involved two small streams which were connected by the eroding

KEY TO FIGURE 1 :

1 :	Coastal river systems
2 :	Gouritz system
3 :	Orange system
4 :	Gamtoos system
5 :	Swartkops River
6 :	Coega River
7 :	Sundays system
8 :	Bushmans River
9 :	Kariega River
10 :	Kowie River
11 :	Fish system
12 :	Keiskamma River
13 :	Chalumna River
14 :	Buffalo River

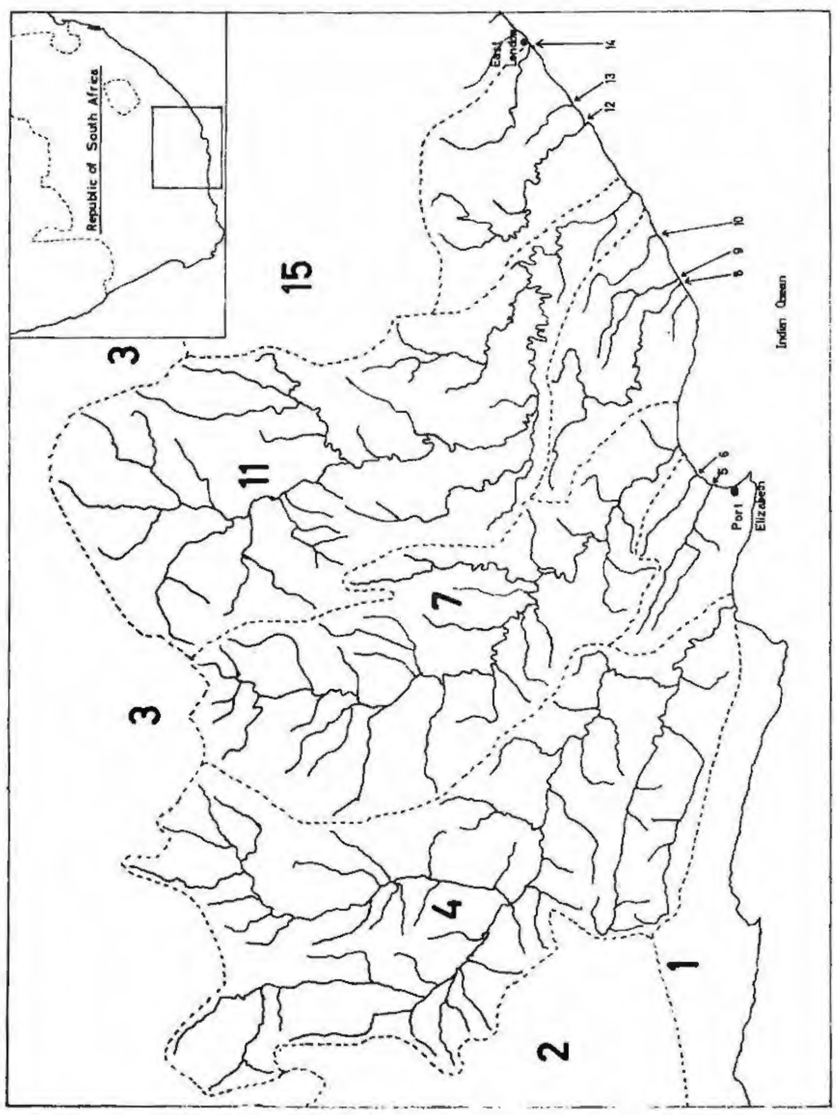


FIGURE 1: The river systems of the Eastern Province

down of a watershed of only a few feet in height. Barnard (1943) has also discussed the role of river capture in the distribution of various cyprinids between the Berg and Breede systems. The river capture as suggested by him, occurred in the Tulbagh Kloof and allowed the dispersal of Barbus andrewi into the Breede system. Barnard (1943), Jubb (1965) and Gabie (1965) have all suggested that a connection between the Olifants and Orange Rivers occurred in the vicinity of Verneukpan and was responsible for the entrance of Barbus capensis ancestors in the Olifants River. Very often, all geological evidence of river captures has been obliterated and in these instances fish distribution patterns then provide the only indication that such a process has occurred. Bond (1964), for instance, postulates that such a link between the Zambesi and Limpopo Rivers occurred on the basis of Hydrocyon distribution.

Only a few instances of river capture, some very doubtful, are known as yet from the Eastern Province. Schwartz (1926) has discussed a possible capture between the Great Fish and Kowie Rivers. Mountain (1962) has discussed a few small captures which all involved the Keiskamma and Buffalo systems. Mountain (personal communication) has further suggested that a capture could have occurred between the Bushmans and Great Fish Rivers. Whether these captures occurred recently enough (geologically speaking) to have allowed dispersal of the local species is not known. Since river captures occur sooner or later in most river systems, it is presumed that they also occurred between those river basins for which no specific examples have yet been described. The very soft strata of the Karroo Series which cover most of the Eastern Province, and the folded beds of the Cape system near the coast, both provide favourable conditions for captures, according to King (1964).

Incipient river captures, consisting of the flooding of low watersheds, as proposed by Barnard (1943) and the sharing of common sources by different river systems, for example the Congo and Zambesi systems, or the Orange and Tugela systems, as discussed by de Beaufort (1951), King (1964), and Jubb (1965b), could provide adequate freshwater links for the dispersal process. These links are often, however, accompanied by severe physical conditions, such as waterfalls or oxygen deficient marshes, which can limit the free dispersal of fish, as indicated by de Beaufort (1951) and Darlington (1957).

After a heavy rain, Jubb (personal communication) has observed individuals of Tilapia sparrmanii swimming up a small stream of water in a footpath, and Farquharson (personal communication) has also noted large Clarias, Barbus trimaculatus, B. paludinosus and indigeneous cyprinodonts swimming up a stream of water so small that the large Clarias had the dorsal half of the body exposed to air. Under favourable conditions, very tenuous freshwater links would therefore allow dispersal, so that even small physiographic changes could produce the desired effect without leaving any geological evidence that a freshwater link had been established briefly.

Farquharson (1962) and Crass (1964) both suggest that freshwater links between adjacent river systems can also be brought about by heavy flooding in conjunction with the presence of an extensive series of freshwater lakes on the coastal plain. In the Eastern Cape the coastal plain is so narrow that no such links could have been formed at all within recent geological history.

Distribution through the sea: Unlike the freshwater links, which can be used by all freshwater fishes, dispersal through the sea is only possible for those groups of freshwater fish which can tolerate sea water. This difference in sea water tolerance between various freshwater fishes, was first clearly recognised by Myers (1937) and has been elaborated by Myers (1949, 1951 and 1963) and by Darlington (1957). At present a primary or obligatory freshwater division, a secondary division which can tolerate sea water for limited periods, and a peripheral division which can tolerate sea water for indefinite periods, are recognised (Darlington, 1957).

Determination of salinity tolerance

Evidence on the ability of freshwater fishes to tolerate sea water or various dilutions of sea water has been obtained in a number of ways. Of these the most important are records of actual occurrence of a species in various salinities and survival experiments.

Actual occurrence of the fish in water of various salinities: Such studies have been undertaken by Chipman (1959), Renfro (1960), Carpelan (1961), Ganapati (1964) and Keup and Bayless (1964) in estuaries or in brine polluted streams and consist of a comparison of the numbers and species of fish collected at localities with different salinity levels. Further information can be obtained for some species from incidental observations during studies of a general biological nature. Myers' formulation of the tolerance categories was mainly based on this type of evidence. The chief objection against the use of this evidence, is that the occurrence of a species in any given salinity is a function of complex interacting environmental factors and chance, and does not depend on the salinity tolerance of the species alone (Pearse and Gunter, 1957). The fish may therefore conceivably tolerate higher salinity levels, than those they are recorded from.

Survival experiments: Under carefully chosen conditions the survival of fish in various salinities provides the best estimate of salinity tolerance.

The most frequently used method consists of exposing a population of fish to gradually increasing salinities and noting the concentration at which the last of them dies. Wiebe et al. (1934) , Renfro (1959), Mortimer (1960) and Sasaki and Ito (1961) have all used this method. A better measure of salinity tolerance is an estimate of the concentration at which 50% of the sample just survive, since a sample may include some individuals with exceptional tolerance which would provide very high values with the former method. This concentration parameter, known as the median tolerance limit (TLm), is also very suitable for comparative purposes, since with the small samples normally used in this procedure, the midpoint of the survival curve is statistically the most accurate point and can be used to characterise the sample, because the survival values for the population can be expected to range about this estimate. Since any toxic agent also affects the length of survival of an animal, the median time of survival (MTS), which is an estimate of the exposure time required to reduce survival to 50% of a sample in any given concentration, is another useful measure of toxicity. The 50% value is once again used since it is statistically the most reliable estimate on the time-survival curve. For zoogeographical purposes the median time of survival is very useful, since long survival periods in high concentrations or in undiluted sea water would allow dispersal through the sea, while shorter lengths of survival may be inadequate. Graphical estimates of both the

TLm and the MTS can be obtained by the use of probit transformation methods, as developed by Bliss (1937) and Finney (1952), and will be used in the present study to indicate the concentrations a species will tolerate and the time it will survive in these concentrations.

Evidence on the salinity tolerance of various freshwater fish groups.

Some evidence is available in the literature on the salinity tolerances of various members of the three families which are represented in the Eastern Province.

Cyprinids: From occurrence records most cyprinids appear to have a low salinity tolerance. Berg (1959) and Zenkevich (1957) have found various cyprinids in the Caspian Sea with an average salinity of 12 to 13‰ (parts per thousand). Anadromous cyprinids also occur in the Aral Sea with a salinity of 10‰ according to Zenkevich (1957). They are also found in the Black Sea and the Sea of Azov, at a maximum salinity of 20‰ (Kosswig, 1955; Caspers, 1957; Slastenenko, 1959). The ionic proportions of the Aral and Black Sea waters differ somewhat from the normal sea water proportions, in that sodium and chloride are more predominant. Keup and Bayless (1964) have found cyprinids in the Neuse River estuary in salinities up to 4‰. In the Cooum River in Southern India, Ganapati (1964) has found Barbus ticto in polyhaline conditions (18 to 30 ‰) and other Barbus and Rasbora species in lower salinities. Renfro (1960) has reported a cyprinid from a salinity of 1 ‰ in the Aransas River, Texas. Tribolodon hakonensis is found in sea water although it does not breed there (Darlington, 1957; Myers, 1963). Darlington (1957) also mentions another cyprinid which sometimes occur in the sea off British Columbia, Canada. Various exceptional cyprinids, with a suggested tolerance of sea water, which belong to the genera Rasbora, Puntius and Nematalestes, occur on the more distant islands of the East Indian archipelago, and on Mindanao in the Phillipines (Myers, 1951).

Duval (1925, quoted in Black, 1957) has found that Cyprinus carpio can osmoregulate up to salinities of 11 ‰, but can live up to a level of 19‰. In survival experiments, Pora (1939) found that Carassius auratus can live indefinitely in salinities up to 15‰. Herbert and Mann (1958) reported that Rutilus rutilus has a maximum tolerance of 60% sea water (approximately 20 ‰) with a median survival time of less than 2 hours. Garrey (1916) has concluded that Notropis blennius can survive 2 to 5 weeks in 35% sea water (about 12 ‰) but only for 4 to 20 hours in 40 % sea water (about 14 ‰). Other Notropis species have been demonstrated to have a maximal tolerance of 12 ‰ of a sea salt solution (Renfro, 1959).

Cichlids: The cichlids are far more tolerant of high salinities than the cyprinids and on occasion they are actually found in the sea. Myers (1949) has cited the example of a Tilapia that was caught in brack water and lived in sea water in an aquarium for 7 years. Aronson (1949) has indicated that Tilapia macrocephala inhabit brackish lagoons (salinity unknown) in West Africa. El-Zarka (1956) has found T. zilli in Lake Qarum at a salinity of 29 ‰ and also found that T. nilotica and T. galilea did not survive this salinity although they can live at slightly lower concentrations. Renfro (1960) has reported specimens of Cichlasoma cyannoguttatum in a salinity of 17.4 ‰. Crass (1964) has found Tilapia mossambica in Lake St. Lucia where salinities in excess of that of sea water are reached on occasion, and calls it a "... remarkable adaptable species that can live in both fresh and saline water." Tilapia mossambica can complete its entire life cycle in sea water, as has been demonstrated by Reid, Townsley and Ego (1959). Tilapia sparrmanii and T. melanopleura are less resistant of high salinities (Crass, 1964).

The only survival experiments with cichlids have been undertaken by Mortimer (1960). Tilapia mossambica and T. andersonii could tolerate higher concentrations of sodium chloride after gradual increases in salinity than T. melanopleura and T. macrochir could tolerate under similar conditions.

Anabantids: Forselius (1957) has summarised the occurrence records of various anabantids in waters of high salinity. Anabas testudineus thrive in brackish water according to Forselius, and Colisa fasciata occur in the Ganges estuary. Macropodus cupanus and M. opercularis often occur in brackish water. Unfortunately Forselius does not give any salinity values for these conditions.

From these references it must therefore be concluded that most cyprinids appear to have a low salinity tolerance while the anabantids are perhaps slightly more tolerant. Both groups, therefore, with a few exceptions, are apparently not sufficiently tolerant to use the sea as a dispersal route. The cichlids are in general more tolerant and some, but not all, of them will be able to disperse through the sea. But it is also clear that salinity tolerance varies widely within all three families and the tolerance of any given species, such as our indigenous species, has to be determined experimentally as has been attempted in the present study.

Osmoregulatory physiology.

In the case of the Salmonidae and the Anguillidae intensive work has been done on osmoregulatory processes

(Black, 1957, and Jullien et al., 1959), but in the case of Ostariophysi very little is known. Most of the studies summarised by the above two authors have dealt with the efficiency of the osmoregulatory processes in various species and under varying external salinity levels. This efficiency is usually expressed in terms of the osmotic pressure of the blood and of the external environment. A useful estimate of both these osmotic pressures is provided by the use of the freezing-point depression technique. Since determinations of the freezing-point depression of various solutions can be made on very small volumes of fluid by the use of the Ramsay apparatus (designed by Ramsay and Brown, 1955), it is the method of choice in the study of fish osmoregulation. Determinations of the osmotic pressures of fish serum after different salinity exposures have been made by Duval (1925, quoted in Black, 1957), Parry (1960, 1961), Gordon (1959) and other workers.

In the case of cyprinids, Duval (1925, quoted in Black, 1957), has found that at an external salinity of 11 ‰, the freezing-point depression of carp blood starts increasing gradually above the freshwater level and this process continues till a salinity of 19 ‰ is reached where the osmotic pressure of the blood is vastly increased and death occurs. Initially the blood is hyperosmotic to the environment, but from 11 ‰ upwards the blood becomes isosmotic to the environment, unlike the blood of various euryhaline fishes, which become hyposmotic at these salinities and the fish therefore survive, since the osmotic pressure of the serum is not increased. Ernst (personal communication) has determined freezing-point depressions in <u>Tilapia mossambica</u> from various sodium chloride concentrations and he has found that the freezing-point depression varied only slightly in all the concentrations tested up to the upper limit of 15 ‰ used by him, which is already hyperosmotic to the blood. This species can therefore be considered as an hyposmotic regulator in high salt concentrations.

The role of the gills in osmotic regulation

Smith (1930) and Keys (1931) have demonstrated the extrarenal excretion of electrolytes, observed in euryhaline fish in hyperosmotic media, to be localised in the cephalic portion of the body. In *Anguilla* Keys and Willmer (1932) and other later authors (reviewed in Straus, 1963) have found specialised eosinophilic cells, situated on the gill filaments between the bases of the gill lamellae, which they consider are responsible for the excretion of electrolytes. Morphologically these cells resemble other cells with a known ion-secretory activity, as noted by Ito (1961). According to most authors, the difference in structure between the cells of fish exposed to fresh water and those of fish exposed to sea water indicate their involvement in the processes of ionic regulation. Datta-Munshi (1964) has, however, found typical "sea water" cells in a strictly freshwater species as well. Bevelander (1936), on the basis of his histological work, is of opinion that the entire gill epithelium has a salt-excretory function and that no specialised cells occur in the gills for this purpose. The latest evidence has been summarised and reviewed by Doyle and Gorecki (1961) and by Straus (1963), from whose conclusions it is clear that morphological investigations will not solve this interesting controversy on the function of these specialised cells. Fleming and Kamemoto (1963) could not correlate increased secretory activity of the gills (as demonstrated by radioactive ions) with histological changes of the specialised cells, which only occurred some time after the peak of the secretory activity.

As far as the families represented in the Eastern Province are concerned, Datta-Munshi (1964) has found these specialised cells in cyprinids from fresh water. Among the cichlids, such cells have been found in Tilapia macrocephala by Doyle and Gorecki (1961), but no information is available for the anabantids.

These specialised cells have been generally known as "chloride excretory" cells, but the term "S-cells" (short for specialised cells) as adopted by Doyle and Gorecki (1961) is preferred, since the former term implies a function which has not yet been demonstrated conclusively.

3. MATERIALS AND METHODS

Materials

The origin of and other details about the different species used in the present experiments are summarised in Table 1.

Except for Barbus holubi, all the species were obtained by means of electrofishing (the method of choice) or by netting, which often injured the fish. Immediately after capture the fish were transported to the laboratory in cans or in plastic bags. The same methods of transport were used for B. holubi, which survived a 800-mile train journey with hardly any casualties. On arrival in the laboratory, the fish were immediately transferred to clean water of approximately the same temperature as that of the water in which the fish were transported. The fish were kept for at least two weeks before use in experimental work, to allow complete adjustment to laboratory conditions. Tank covers were provided during this period to prevent the fish from jumping out of the tanks but were not necessary afterwards because the fish rapidly grew accustomed to the confined space.

Daily feedings of a simple mealie-meal and fishmeal mixture were given. Temperature control was provided by bi-metallic thermostats in conjunction with heating elements. Aeration was provided by passing compressed air through diffuser blocks or corner filters. The experimental tanks measured 2 feet by one by one, and were always topped up to 30 liters. The tanks were cleaned, when necessary, after the fish had been transferred to tanks with clean water of the same salt concentration and the same temperature. For experimental purposes ten fish were kept in each tank. In the sea water experiments the fish were kept in 5 liters of solution in plastic

TABLE 1

THE ORIGIN AND USE OF THE EXPERIMENTAL SPECIES

Experimental Species	Number of batches used	Source of each batch	Experiments performed on each batch
Barbus holubi	2	a) Lydenburg hatchery, Transvaal	Preliminary and final chloride and sulphate experiments
		b) - do -	Final sea water experiments
B. pallidus	2	a) Kariega River	Preliminary chloride and sulphate experiments
		b) - do -	Preliminary sea water experiments.
B. trevelyani	1	Buffalo River	Preliminary chloride and sulphate experiments
B. asper	1	Klein Berg River (Gamtoos system)	Preliminary chloride experiments
B. afer	1	Witte River	Preliminary chloride experiments
Labeo umbratus	1	Lake Mentz (Sundays River system)	Preliminary chloride, sulphate and sea water experiments
Tilapia sparrmanii	3	a) Brickfields Grahamstown	Preliminary chloride experiment
		b) Pirie hatchery	Preliminary sulphate and final chloride and sea water (partially) experiments
		c) Kariega River	Final sea water experiments (partially)
T. mossambica	3	a) Kowie River estuary	Preliminary chloride and sulphate experiments
		b) Bushmans River	Final sea water experiment (partially)
		c) Port Alfred Pond	Final sea water experiment (partially)
Sandelia capensis	2	a) Klein Berg River (Gamtoos system)	Preliminary chloride and sulphate experiments
		b) - do -	Final chloride experiments

basins, due to the difficulty experienced in transporting glass aquarium tanks for long distances.

The water used for all stockkeeping and all experimental purposes, except the sea water experiments, was Grahamstown tap water, as supplied by the local municipality. An analysis of this water is given in Table 2. The water is treated with chlorine and with lime at the municipal waterworks. For the sea water experiments rain water was used for dilution purposes.

During the adjustment period hardly any mortalities occurred in the stock tanks. In two instances, however, very large mortalities were experienced due to a parasite infection of Barbus trevelyani and excess chlorine in the water in the case of a second B. asper batch, which prevented the use of these species for anything other than sighting experiments.

In the determination of the relation between various concentrations of sodium sulphate or sea water and the freezingpoint depression of serum only Labeo umbratus and a few fish from the second batch of B. holubi were used. A few Barbus holubi obtained in Pretoria were used for the histological examination of the gills.

Tolerance determination: experimental procedure

The survival of various species was determined in solutions of sodium chloride and of sodium sulphate and in various dilutions of sea water, where possible. Sodium chloride was used since it forms such a large proportion of the dissolved salts of sea water, and it therefore served as a convenient solute for increasing the osmotic pressure of the environment of the fish. Sodium sulphate is an important constituent of the waters of brack rivers in the area. Due to the unfavourable ionic proportions produced by both these salts, in comparison with the more balanced composition of sea water as demonstrated by Jullien et al. (1959), sea water experiments were undertaken to provide an estimate of the specific tolerance to sea water itself, which is the most important parameter for zoogeographical interpretation. The sodium chloride and sodium sulphate experiments were completed in Grahamstown, but the sea water experiments were undertaken at the F.W. Armstrong Field Station of Rhodes University at Port Alfred, where sea water is freely available.

Three different experimental procedures, which can be designated as initial, preliminary and final procedures, were used in the tolerance estimation. The initial procedure provides data on what occurs with very rapid increases in salinity while the preliminary and final procedures indicate the effects of longer exposures to high salinities. During each experiment the stock tanks served as control groups. In no case were any mortalities reported from the control groups during the duration of an experiment.

Initial procedure: This procedure and the next served chiefly as sighting experiments. In the initial method rapid increases of 3 ‰ of sodium chloride at a time were made every hour, until all the fish succumbed. Observations were made of the number of fish surviving at the end of each hour.

Preliminary procedure: The very rapid increases in salinity of the initial series produced very high estimates of tolerance, since the fish died in concentrations far in excess of those in which they were initially affected. The preliminary procedure was therefore designed to indicate long-term survival of the fish subjected to small daily increases in salinity. Daily increases of 1 ‰ in the concentration of sodium

TABLE 2

THE CHEMICAL COMPOSITION OF GRAHAMSTOWN TAP WATER (INFORMATION SUPPLIED BY THE GRAHAMSTOWN CITY COUNCIL)

Date of analysis: unknown

pH	8.4
Total solids, p.p.m.	150
Loss on ignition, p.p.m.	50
Soluble solids, p.p.m.	150
Insoluble solids, p.p.m.	0
Chlorides (as NaCl), p.p.m.	52
Free chlorine, p.p.m.	0
Alkalinity (to methyl orange as NaOH), p.p.m.	20
Acidity (to phenol phthalein as H_2SO_4) p.p.m.	1.0
Temporary hardness p.p.m.	0
Permanent hardness, p.p.m.	17

TABLE 3

THE CHEMICAL COMPOSITION OF "SALNOVA" SALT (AS SUPPLIED BY CEREBOS, LIMITED)

Date of analysis : January, 1963

Moisture, %	0.033
Calcium, as Ca, %	0.018
Magnesium, as Mg, %	0.027
Sulphate, as SO_4, %	0.031
Insolubles, %	0.027
Starch, %	0.5
Sodium Chloride, %	99.38

chloride, sodium sulphate or sea water were used, except in some sodium sulphate experiments where a daily increase of 0.44 ‰ was used, due to the use of hydrated sodium sulphate. "Salnova" brand household salt was used to obtain the required sodium chloride concentrations. The chemical composition of this salt is given in Table 3. For the sodium sulphate solutions, analytical quality salt with low impurities was used in the unhydrated form, except in specified instances. Sea water was obtained at high tide from the estuary of the Kowie River and had an average salinity of 35.29 ‰ (according to the simplified titration technique of Barnes, 1959).

Ten fish were used for each experiment, except where otherwise stated, and were selected at random from the stock tanks. The number of fish surviving in each concentration was recorded before increasing the concentration by another step. The analysis of these results is discussed in a later section.

Final procedure : In this procedure an estimate of the median time of survival was obtained for various concentrations of the solutions being tested. The range of concentrations was chosen in most cases from the range of concentrations suggested to be lethal by the preliminary experiments. Within this range, the concentrations to be tested were arranged at 1 ‰ intervals.

After random selection from the stock, the fish to be used in the experiments were gradually acclimated by daily increases in concentration of 1 ‰ until the testing range of concentrations is reached. The fish were then randomised into groups of ten fish each, by the use of numbered cards drawn from a container. These groups were then assigned to the various testing concentrations by a similar randomisation technique. The groups were then transferred as simultaneously as possible to their respective concentrations, which were at the same temperature as the acclimation solution. Observations were made continuously for the first thousand minutes after transfer, and thereafter at logarithmically spaced intervals, until a period of 5,000 minutes elapsed. The logarithmic spacing of observation times was adopted, firstly because the survival probit transformation and the logarithm of the time instances when the percentage survival changes are very frequently linearly related, as was also found in the first experiments of the present study. Secondly it provided evenly spaced points on the graph of the survival probit against the logarithmically arranged time intervals to improve the accuracy of curve-fitting. The usual length of the observation period, 5,000 minutes, was arbitrarily chosen, but was also used by Allanson and Noble (1964) who found that with longer exposure periods external factors obscure the cold-death relation. In the present study no such factors were observed when the length of exposure was increased. This length of exposure provided a tolerance estimate for medium-term exposure to the test solutions. In addition the experimental results of Barbus holubi, summarised in figure 4, indicate that for the 5,000 minute period tolerance is nearly maximal but drops rapidly for longer periods of exposure.

In a few instances the chosen range of concentrations did not prove lethal enough to provide a reliable estimate of the median time of survival, due to various reasons. This condition was remedied in one of two possible ways, as discussed below. These remedies had to be adopted because in none of the cases were sufficient fish available to start a new series of experiments at the higher concentrations. If a number of deaths occurred in most of the concentrations used initially, the period of observation was prolonged to 10,000 minutes in order to obtain an estimate of the median time of survival for a given concentration selected at the start of the experiment. Where no deaths occurred at all in at least two or three of the concentrations during the initial 5,000 minute period, these groups of fish in which no deaths were recorded were transferred to the range of concentrations immediately higher than that selected initially. The group from the lowest test concentration was transferred to the lowest concentration of the new range of concentrations, and similarly with the rest of the unaffected groups. This procedure was followed in order to allow maximum acclimation, and to minimise any possible shock effects which could affect the results.

The initial, preliminary and final experiments were all undertaken at a temperature of 20°C. While the possible effects of temperatures other than the ambient (20°C) on the salinity tolerance are duly recognised, lack of experimental material did not allow an investigation of these effects.

Analysis of results.

The rapid rate of death in the initial experiments does not allow the use of the probit transformation since at the most only one probit point can be obtained. In those cases where all the fish died at one concentration no probit points could be obtained and no median tolerance limits can be estimated for this procedure.

The survival figures of the preliminary experiments were calculated as the percentage fish surviving in each concentration. For each percentage the corresponding probit value was determined from tables in Finney (1952). These probit values were then plotted on a linear scale against the concentrations at which they were obtained, which were arranged on a logarithmic scale. A straight line was fitted by eye to these points. From this line the concentration which corresponds to a probit value of 5 was read off. This concentration is the median tolerance limit as defined earlier since a probit of 5 corresponds to 50% survival. A procedure similar to this one is illustrated in figure 3.

In the final procedure the number of survivors recorded in a given concentration after each observation interval, during which a change in the number of survivors occurred, was also converted to a percentage and the corresponding probit then read off. This probit value was then plotted on a linear scale against the length of time which had elapsed since the start of the experiment, arranged on a logarithmic scale. A straight line was fitted to the series of points obtained thus, and the length of survival which corresponds to the probit value of 5 was then read of from this line. This length of survival is the median time of survival, as defined previously. This procedure is then repeated for each concentration tested, and a median time of survival is estimated for each concentration. An example of this procedure is illustrated in figure 2. The results of the final experimental procedure also provided estimates of the median tolerance limits for various exposure periods. The number of survivors in each concentration at the end of the selected exposure period was also converted to a percentage basis and transformed to probit values. The median tolerance limits were then obtained as described for the preliminary procedure. An example of this procedure is given in figure 3.

The MTS values obtained were further plotted on a linear scale against the logarithm of concentration.

The semi-logarithmic method of plotting was adopted to indicate the presence or not of a threshold concentration below which the fish can apparently withstand unlimited exposure.

Criteria other than the Tlm and MTS, such as the LD_{90}, are undoubtedly of greater value in the zoogeographical application of tolerance studies since if only a few very resistant fish could traverse a given barrier the species could become established in new territory. Limitations of the present experimental procedure invalidate comparisons between LD_{90}'s of the various species since so few fish were used in most cases. Furthermore as so many environmental factors can affect the salinity tolerance of any species and especially such extreme criteria as the LD_{50}, the TLm and MTS are retained as the best comparative indicators of tolerance in the present study.

Freezing-point depression of blood: experimental procedure

Blood samples were collected by removing the peduncle of the fish under liquid paraffin and the blood droplets were left to clot in liquid paraffin. After one hour the serum which separated from the clot was drawn up as fine drops into a very thin capillary tube of Vitreosil glass. The drops were separated from one another by drops of liquid paraffin. After sealing both ends of the capillaries with plasticine, they were stored in a vial of liquid paraffin at a temperature of -15 °C, till the freezing-point depression measurements could be made.

The depression of freezing-point was measured in an apparatus similar to the one described by Ramsay and Brown (1955). The method differed slightly from the recommended one in so far that a slower rate of heating was used in the present study. The freezing-point was recorded as the temperature at which the last ice crystal contained within the serum droplet under observation disappeared. Five replicates of this reading were taken to provide an accurate average. The freezing-point of glass distilled water, determined by the same method, served as reference point for freezing-point depression estimates.

Histological methods

Bouin's fluid (Pantin, 1962) was used as fixative and the fish were killed by dropping directly into the fixative, and the gills were directly exposed to the action of the fixative by removal of the operculum. The usual series of increasing alcohol concentrations was used for dehydration. Xylene was used for clearing the gill tissue and the gills were vacuum-embedded in filtered paraffin wax with a melting point of 55°C. Sectioning was done on rotary microtome at a thickness of 8 μ. Staining with Heidenhain's hematoxylin and counter-staining with eosin proved reasonably satisfactory. Better control of staining intensity was achieved by the use of Mayer's hematoxylin and erythrosin.

4. RESULTS

THE SYMPTOM SEQUENCE IN LETHAL CONCENTRATIONS.

The symptom sequence is of great importance in survival experiments since the criterion for death must be rigorously defined. While the symptom sequence differs slightly between species, and in different solutions, a basic pattern can be recognised in all the experiments. The behaviour of Barbus holubi in sodium chloride provides a useful basis for comparison. Soon after the transfer of the fish to the experimental solutions, the fish and the bottom of the tank become covered with a whitish precipitate, which also clings to the sides of the tank. This is followed by the performance of rapid darting movements by disturbed fish, completely unlike the movements of normal fish when they are disturbed. The fish also keep near the surface of the water during this stage and gradually enter a stage of periodic loss of equilibrium, interspersed with short bursts of darting movements. The loss of equilibrium periods gradually increase in length and eventually result in permanent loss of equilibrium. The respiration rate increases at first and then becomes slower and intermittent. On cessation of breathing the fish is considered dead for the purpose of the experiment, since at this stage the fish do not react to any stimuli.

In B. holubi kept in sodium sulphate solutions, the sequence is similar to that described above, except that no evidence of a precipitate on the tank bottom is found. In sea water dilutions the sequence was exactly similar to that found in sodium chloride. Here the rapid darting movements always terminated with a rolling movement of the body, probably due to very sudden loss of equilibrium. In the first stages of permanent loss of equilibrium, the respiration rate increased from normal 96 per minute to 120 per minute.

B. pallidus exhibits darkening of the skin in sub-lethal concentrations of sea water and a brownish precipitate is produced in sublethal and lethal concentrations.

Labeo umbratus also undergoes loss of equilibrium and increased rates of respiration in sodium chloride and sodium sulphate solutions. In sea water dilutions, in addition, a darkening of the skin appears in sub-lethal concentrations and persists till death in lethal concentrations.

Tilapia sparrmanii initially exhibits dark horizontal and vertical lines on the lateral portions of the body, but this does not necessarily occur in all the affected fish. The next stage is characterised by the tendency of the fish to stay near the surface while swimming continuously - if it stops swimming the fish drops to the bottom, unlike unaffected fish which maintain their position under these conditions. The affected fish also perform rapid movements through the water with a "corkscrew" motion. The fish eventually comes to rest on the bottom and gradually leans over sideways, with occasional interruptions of further "corkscrew" movements. This is followed by complete loss of equilibrium and intermittent respiration and eventually death. In sodium chloride this last stage is characterised by spasmodic movement of the pectoral fins and in some cases by tetanic contraction of body muscles. In both sea water and sodium chloride the overall colour of the fish is much enhanced.

In 9 ‰ and higher concentrations of sodium chloride, Sandelia capensis also shows evidence of a white precipitate and from 11 ‰ upwards the smaller fish become very dark while the larger fish are unaffected. In temporary loss of equilibrium, the periods of loss of equilibrium are alternated with swimming at the surface with the head nearly vertically above the tail.

In the initial experiment most fish died in apparent acute respiratory distress with the mouths wide open. In none of the other experiments did this occur, with the exception of some Tilapia sparrmanii in which muscular spasms ccasionally caused a similar condition.

Small transfer experiments have given some indication of the ability of fish to recover from some of these stages in the death sequence. Transfer of B. holubi, at the first signs of permanent loss of equilibrium in 14 ‰ diluted sea water, to fresh water did not lead to recovery. T. sparrmanii transferred from undiluted sea water to fresh water on the first signs of temporary loss of equilibrium survived for periods up to 3,000 minutes, but then succumbed. It therefore appears that as soon as evidence of loss of equilibrium, however slightly, occurs, recovery is severely impaired. On the other hand, the changes in salinity which these fish had to undergo in this transfer process may have been too drastic to allow recovery, in which case a slower reduction of salinity may produce complete recovery.

Due to lack of experimental material no further experiments on recovery of fish could be undertaken. Only carefully designed experiments which reduce the shock effect of direct transfer as far as possible could indi-

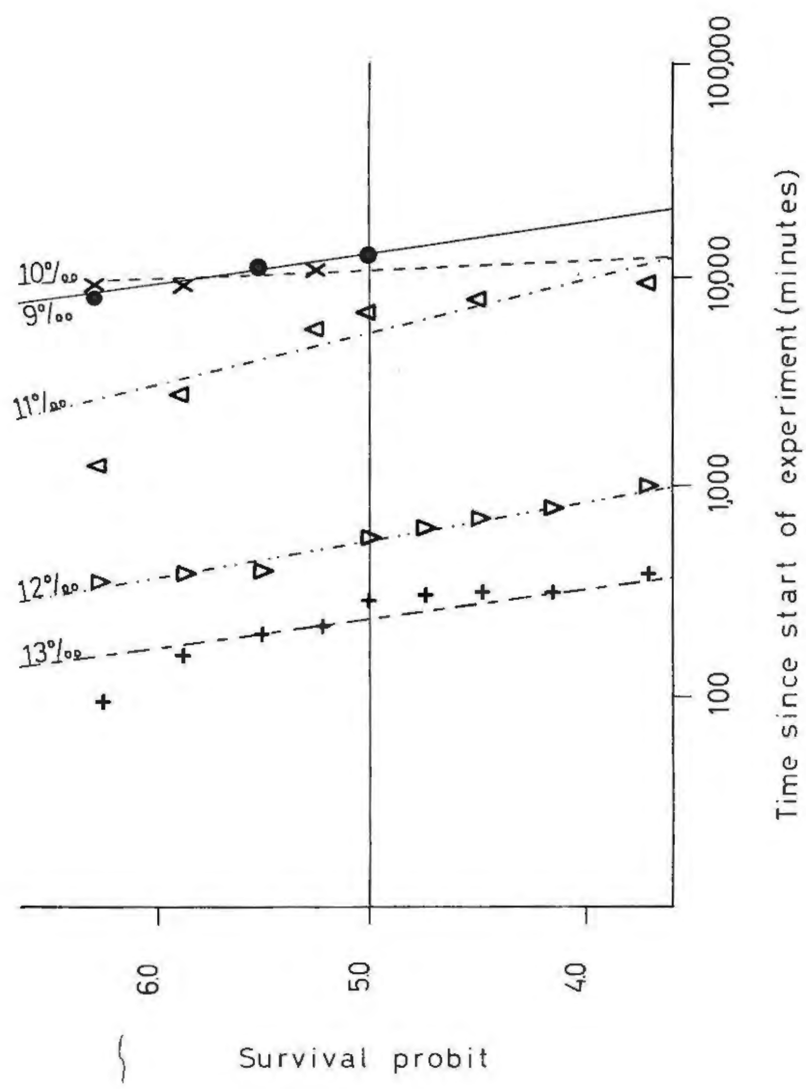


FIGURE 2: The graphical estimation of median times of survival of *Barbus holubi* in sodium chloride.

cate conclusively at exactly what stage recovery becomes impossible. Further work on the recovery aspects is therefore of great importance in zoogeographical study, especially in those species which are just sufficiently tolerant to allow sea dispersal under favourable conditions.

SURVIVAL EXPERIMENTS.

The most useful data for this purpose were yielded by the final experimental procedure, but in many cases not sufficient fish were available and conclusions had to be based on the results of initial and preliminary experiments.

Barbus holubi Steindachner, 1894.

This species was tested the most exhaustively of all in the present series of experiments, since it was the only cyprinid which could be obtained in sufficient numbers, although it is not indigenous to the Eastern Province.

Sodium chloride tolerance: The data from the preliminary experiment are summarised in Table 4. The final procedure results of Table 5 were used in the graphical estimation of the median times of survival, which is illustrated in figure 2. Figure 5 indicates the relation between die median times of survival and concentraion. The graphical estimation of the median tolerance limit is illustrated in figure 3 and the resulting values are given in Table 6 and in figure 4. The long exposure and observation period of 12,500 minutes adopted for this experiment was also accompanied by the transfer of two groups of fish from 7 and 8 ‰ to 12 and 13 ‰, respectively.

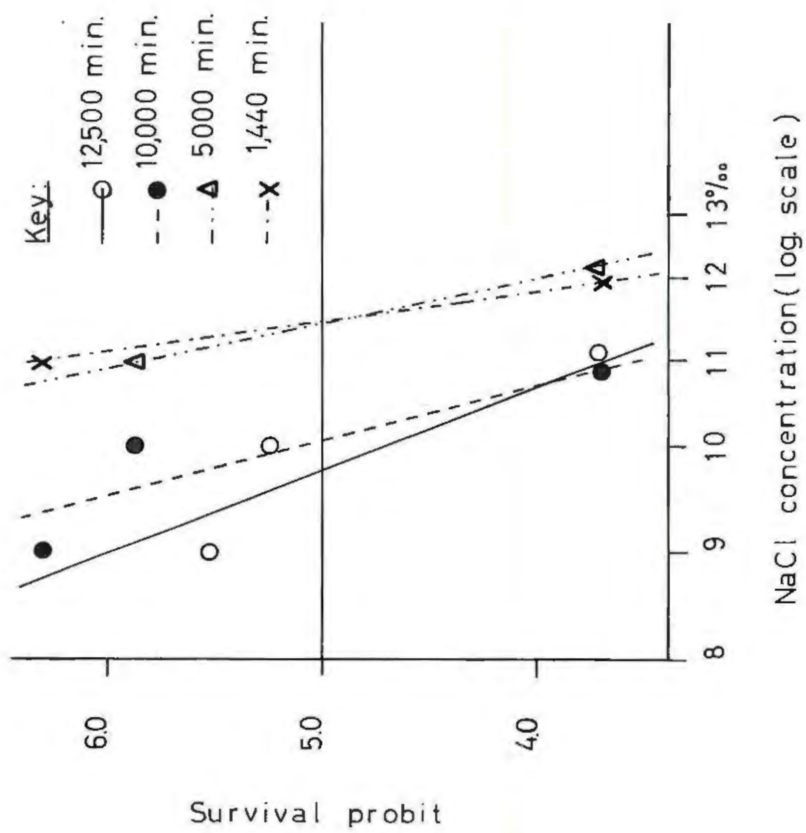


FIGURE 3: The graphical estimation of median tolerance limits of Barbus holubi to sodium chloride.

TABLE 4.

PRELIMINARY PROCEDURE: THE SURVIVAL OF Barbus holubi IN GRADUALLY INCREASED SODIUM CHLORIDE CONCENTRATIONS.

Number of fish used: 10

Date: September, 1964.

TLm estimate: not possible.

Rate of concentration increase: 1‰ per day.

Average temperature: 19.4°C.

Concentrations at which death occurred.	Number of survivors.	Percentage survival.
8 ‰	2	20
9 ‰	0	0

From results it is apparent that Barbus holubi has survived slightly higher concentrations in the final procedure than in the preliminary experiment. Figure 5 indicates that a hyperbolic relation between the median times of survival and the logarithmically arranged concentrations exists. Figure 4 provides an indication of the length of exposure's influence on tolerance figures, the latter which drops rapidly as soon as the exposure length exceeds about 5,000 minutes.

Sodium sulphate tolerance: The preliminary procedure yielded the data summarised in Table 7. The time-survival of this species in different sodium sulphate concentrations is given in Table 8, and is derived from the final procedure experiment. The graphical estimates of the median times of survival are given in Table 9. From these results produced by the final experimental procedure estimates of the median tolerance limits were also made and they appear in Table 10.

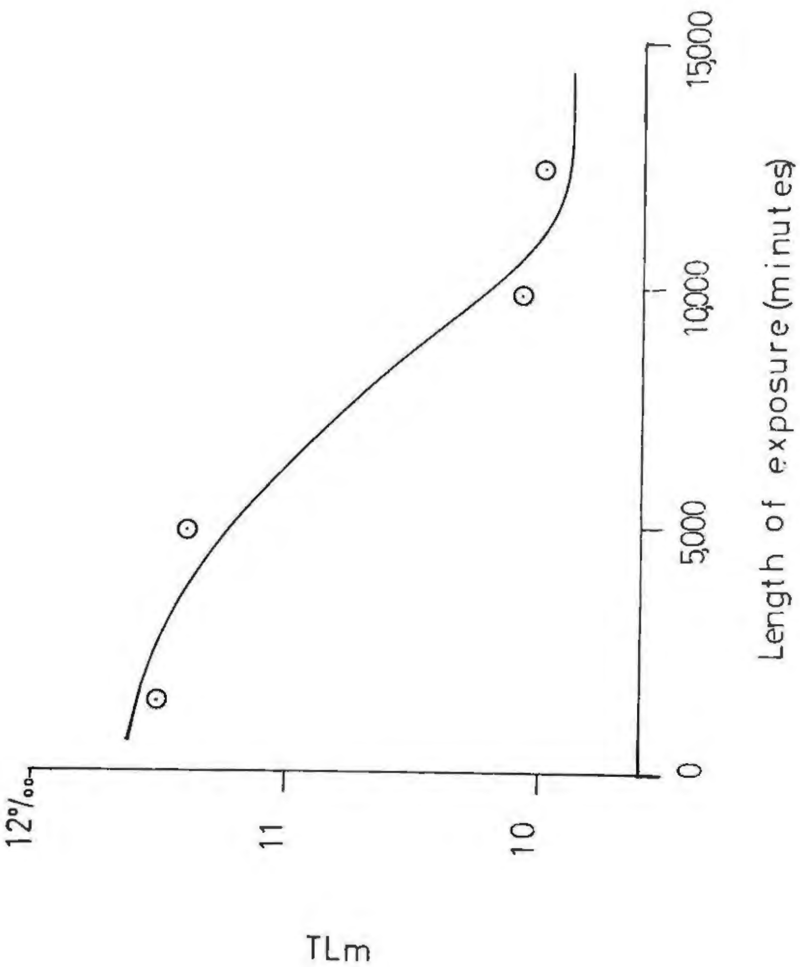


FIGURE 4: The relationship between median tolerance limits and length of exposure of *Barbus holubi* to sodium chloride.

TABLE 5.

FINAL PROCEDURE: THE SURVIVAL WITH TIME OF *Barbus holubi* IN DIFFERENT SODIUM CHLORIDE CONCENTRATIONS.

Date: September, 1964. Acclimation: to 6 ‰ NaCl.

Concentration. (‰)	Number of fish used	Average temperature. (°C)	Time since start of experiment. (minutes)	Number of live fish.	Percentage survival.	Survival probit.
7	10	19.9	12,500	10	100	-
8	10	20.2	12,500	10	100	-
9	10	19.9	8,390	9	90	6.28
			11,270	7	70	5.52
			11,890	5	50	5.00
10	10	20.1	8,510	9	90	6.28
			9,830	8	80	5.84
			11,270	6	60	5.25
11	10	20.1	1,305	9	90	6.28
			2,739	8	80	5.84
			5,720	6	60	5.25
			6,950	5	50	5.00
			8,390	3	30	4.48
			9,830	1	10	3.72
12	10	20.1	340	9	90	6.28
			390	8	80	5.84
			400	7	70	5.52
			560	5	50	5.00
			620	4	40	4.75
			730	3	30	4.48
			800	2	20	4.16
			1,010	1	10	3.72
			11,000	0	0	-
13	10	20.5	95	9	90	6.28
			160	8	80	5.84
			200	7	70	5.52
			210	6	60	5.25
			280	5	50	5.00
			300	4	40	4.75
			310	3	30	4.48
			320	2	20	4.16
			370	1	10	3.72
			560	0	0	-

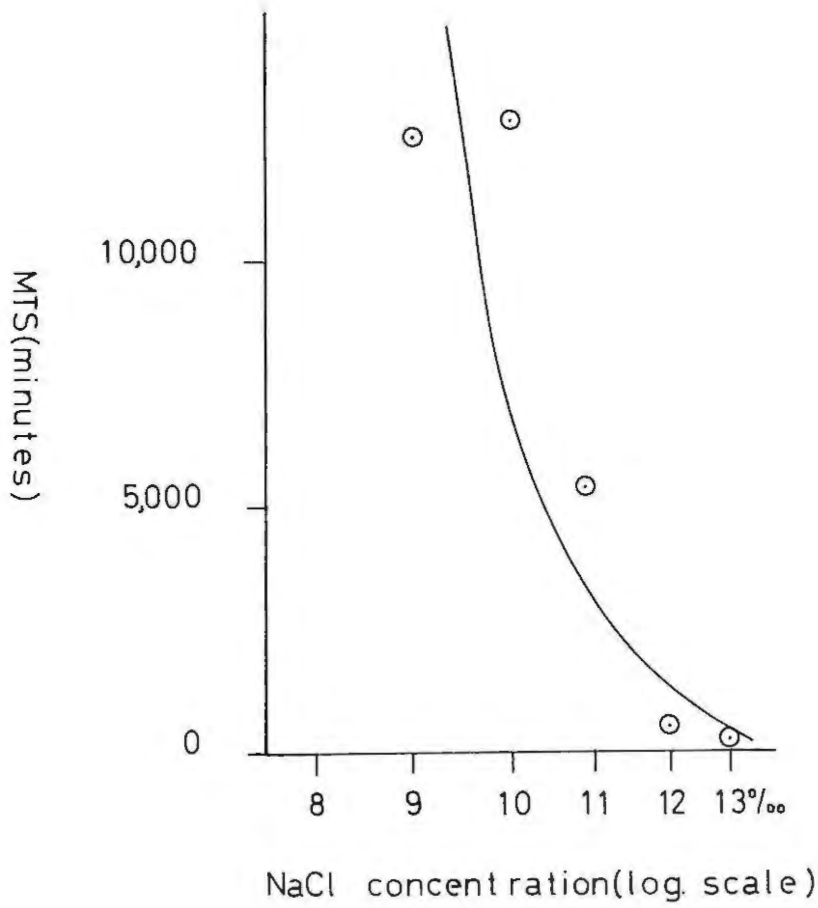


FIGURE 5: The relationship between the median time of survival and sodium chloride concentration for *Barbus holubi*.

TABLE 6.

FINAL PROCEDURE: THE ESTIMATED MEDIAN TOLERANCE LIMITS OF *Barbus holubi* TO SODIUM CHLORIDE FOR DIFFERENT LENGTHS OF EXPOSURE.

Length of exposure (minutes).	Median tolerance limit (‰ NaCl).
12,500	10.0
10,000	10.1
5,000	11.4
1,440	11.5

Comparison of the tolerance values produced by the final and preliminary procedures indicate that lower values were obtained in the final experiment. A possible explanation for this difference is the non-randomly selected group of fish on which the experiment was performed, since a number of fish died during the acclimation period due to unknown causes (possibly due to an increase in the free chlorine content of the tap water, which is known to have occurred at this time and affected other stocks of fish as well) which lowered the physiological tolerance of the fish in the final experiment. In the comparison of the median times of survival with the concentrations at which they were determined, very little difference between the two MTS estimates for 5 and 6 ‰ was found.

Sea water tolerance: Only the final procedure was used in the estimation of the tolerance of *B. holubi* to sea water. Since hardly any deaths occurred in the range of concentrations (suggested by die sodium chloride experiments) which was tested initially in this experiment, the groups of fish from 7, 8, 9, 10 and 11 ‰ were respectively transferred to sea water dilutions with salinities of 14, 15, 16, 17 and 18 ‰, and were observed for a further 5,000 minutes. The survival in the various concentrations tested is summarised in Table 11. From these data estimates of the median times of survival were made, which are indicated in Table 12, while figure 6 indicates that once again a hyperbolic relation exists between the median time of survival, where it can be estimated, and concentration, if the latter is arranged on a logarithmic scale. Comparison of the median tolerance limits obtained in sea water, as summarised in Table 13, and the values from the sodium chloride experiments, demonstrate that this species is far more tolerant to sea water than to sodium chloride.

TABLE 7.

PRELIMINARY PROCEDURE: THE SURVIVAL OF *Barbus holubi* IN GRADUALLY INCREASING CONCENTRATIONS OF SODIUM SULPHATE.

Number of fish used: 10 Rate of concentration increase: 0.44‰ per day.

Date: October, 1964. Average temperature: 19.9°C.

Concentrations at which deaths occurred (‰ Na_2SO_4).	Number of survivors	Percentage survival	Survival probit
6.22	6	60	5.25
8.00	3	30	4.48
8.44	2	20	4.16
10.66	1	10	3.72
11.11	0	0	-

TLm estimate: 6.8‰.

TABLE 8.

FINAL PROCEDURE: THE SURVIVAL WITH TIME OF Barbus holubi IN DIFFERENT SODIUM SULPHATE CONCENTRATIONS.

Date: November, 1964. Acclimation: to 3 ‰.

Concentration. (‰)	Number of fish used.	Average temperature. (°C).	Time since start of experiment (minutes)	Number of live fish.	Percentage survival.	Survival probit.
4	10	19.4	230	9	90	6.28
			5,000	9	90	
5	10	19.9	720	9	90	6.28
			1,380	8	80	5.84
			2,280	6	60	5.25
			2,880	5	50	5.00
			5,000	5	50	
6	10	20.2	1,380	8	80	5.84
			2,280	7	70	5.52
			2,880	5	50	5.00
			3,420	4	40	4.75
			5,000	4	40	

TABLE 9.

FINAL PROCEDURE: THE ESTIMATED MEDIAN TIMES OF SURVIVAL OF Barbus holubi IN DIFFERENT CONCENTRATIONS OF SODIUM SULPHATE.

Sodium sulphate concentration (‰ Na_2SO_4).	Median time of suvival (minutes).
4	No estimate possible
5	2,884
6	2,754

TABLE 10.

FINAL PROCEDURE: THE ESTIMATED MEDIAN TOLERANCE LIMITS OF Barbus holubi TO SODIUM SULPHATE FOR DIFFERENT LENGTHS OF EXPOSURE.

Length of exposure (minutes).	Median tolerance limit (‰ Na_2SO_4)
5,000	5.3
1,440	No estimate possible

TABLE 11.

FINAL PROCEDURE: THE SURVIVAL WITH TIME OF *Barbus holubi* IN DIFFERENT DILUTIONS OF SEA WATER.

Date: April, 1965. Acclimation: to 6 ‰.

Concentration (‰).	Number of fish used.	Average temperature (°C).	Time since start of experiment (minutes).	Number of live fish.	Percentage survival.	Survival probit.
7	10	21.6	5,000	10	100	-
8	10	21.4	5,000	10	100	-
9	10	21.3	5,000	10	100	-
10	10	21.6	5,000	10	100	-
11	10	21.6	5,000	10	100	-
12	10	21.6	5,000	10	100	-
13	10	21.6	3,675 5,000	9 9	90 90	6.28
14	10	20.3	3,763 4,483 5,000	8 7 7	80 70 70	5.84 5.52
15	10	20.3	358 613 823 2,983 4,003 4,243 4,723 5,000	9 8 7 6 5 2 1 1	90 80 70 60 50 20 10 10	6.28 5.84 5.52 5.25 5.00 4.16 3.72
16	10	20.6	473 1,023 1,903 2,023 2,803 4,903 4,963	9 7 5 4 2 1 0	90 70 50 40 20 10 0	6.28 5.52 5.00 4.75 4.16 3.72 -
17	10	20.7	283 403 443 718 823 1,003 1,543	9 8 7 4 3 2 0	90 80 70 40 30 20 0	6.28 5.84 5.52 4.75 4.48 4.16 -
18	9	20.7	228 268 328 373 583 943 1,363 1,423	8 7 6 4 3 2 1 0	89 78 67 44 33 22 11 0	6.23 5.77 5.44 4.85 4.56 4.23 3.77 -

TABLE 12.

FINAL PROCEDURE: THE ESTIMATED MEDIAN TIMES OF SURVIVAL OF Barbus holubi IN DIFFERENT DILUTIONS OF SEA WATER.

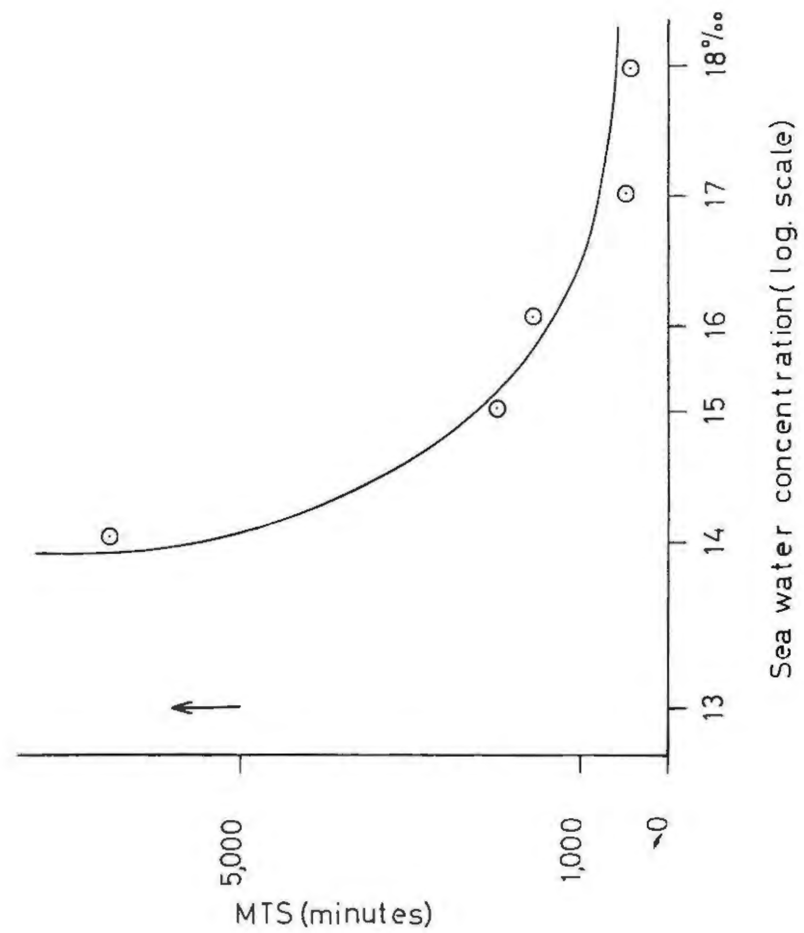
Sea water dilutions (‰).	Median times of survival (minutes).
7	No estimate possible
8	do.
9	do.
10	do.
11	do.
12	do.
13	do.
14	6,761
15	1,950
16	1,479
17	616
18	490

TABLE 13.

FINAL PROCEDURE: THE ESTIMATED MEDIAN TOLERANCE LIMITS OF Barbus holubi TO DILUTIONS OF SEA WATER FOR DIFFERENT LENGTHS OF EXPOSURE.

Length of exposure (minutes).	Median tolerance limits (‰).
5,000	14.1
1,440	16.1

Individuals of Barbus holubi that were transferred directly from 13 ‰, in which they had spent about 5,000 minutes, to undiluted sea water suvived for a maximum of



<u>FIGURE 6</u>: The relationship between median time of survival and sea water concentration for <u>Barbus holubi</u>.

75 minutes, but after 15 minutes had elapsed they were all in a state of permanent loss of equilibrium. It can therefore be concluded that the survival time of this species in undiluted sea water is negligible, as far as zoogeographical work is concerned.

In this rapid transfer to undiluted sea water severe physiological shock is unavoidable. Less rapid transfer by gradual increasing of the concentration may result in slightly longer survival periods. No significant increase in survival time is expected, however, since an increase of only a few parts per thousand in concentration leads to death within a few hours.

Barbus trevelyani Günther, 1877.

This rare species was subjected to preliminary experiments to determine its tolerance of sodium chloride and sodium sulphate solutions respectively.

Sodium chloride tolerance: For the preliminary experiment only five fish were used, which provided the survival values summarised in Table 14. This was the only experiment performed to determine the sodium chloride tolerance of this species, and the tolerance values appear to be of the same order as that of *B. holubi*.

TABLE 14.

PRELIMINARY PROCEDURE: THE SURVIVAL OF *Barbus trevelyani* IN GRADUALLY INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

Number of fish used: 5

Date: June, 1964.

Rate of concentration increase: 1 ‰ per day.

Average temperature: 20.3°C.

Concentrations at which deaths occurred (‰ NaCl).	Number of survivors.	Percentage survival.
7	3	60
10	0	0

TLm estimate: no estimate possible (will be in range 7 to 10 ‰).

Sodium sulphate tolerance: In the determination of this characteristic only a preliminary experiment could be performed and only five fish were used. As in all the preliminary experiments, the median limit of tolerance is estimated in the present experiment for an exposure period of 24 hours, since this is the interval between successive concentration increases. This estimate of the median tolerance limit and other survival data are presented in Table 15.

TABLE 15.

PRELIMINARY PROCEDURE: THE SURVIVAL OF Barbus trevelyani IN GRADUALLY INCREASING SODIUM SULPHATE CONCENTRATIONS.

Number of fish used: 5.
Date: June, 1964.
Rate of concentration increase: 0.44 ‰ per day.
Average temperature: 18.3°C.

Concentrations at which deaths accurred. (‰ Na_2SO_4).	Number of surviving fish.	Percentage survival.	Survival probit.
1.77	4	80	5.84
3.11	2	40	4.75
6.66	1	20	4.16
8.00	-	0	0

TLm estimate = 3.1 ‰.

Barbus asper Boulenger, 1911.

Sodium chloride tolerance: This is the only tolerance value which was estimated for this species, due to the small numbers of fish which could be obtained. The use of the initial procedure produced the data summarised in Table 16 and the preliminary results are given in Table 17. From these tables it is apparent that this species is far more tolerant of sodium chloride than the previous two, and the difference in the rate of salinity increase between the initial and preliminary experiments does not affect the survival of the fish to the same degree as other species.

TABLE 16.

INITIAL PROCEDURE: THE SURVIVAL OF *Barbus asper* IN RAPIDLY INCREASING CONCENTRATIONS OF SODIUM CHLORIDE.

Number of fish used: 5. Rate of concentration increase: 3 ‰ per hour.

Date: March, 1964. Average temperature: 20.5°C.

Concentrations at which deaths occurred. (‰ NaCl)	Number of surviving fish.	Percentage survival.
18	3	60
21	0	0

TABLE 17.

PRELIMINARY PROCEDURE: THE SURVIVAL OF *Barbus asper* IN GRADUALLY INCREASING SODIUM CHLORIDE CONCENTRATIONS.

Number of fish used: 10. Rate of concentration increase: 1 ‰ per day.

Date: April, 1964. Average temperature: 17.8°C.

Concentrations at which deaths occurred. (‰ NaCl).	Number of surviving fish.	Percentage survival.	Survival probit.
17	5	50	5.00
18	2	20	4.16
19	0	0	-

TLm estimate = 17.0 ‰ NaCl.

Barbus afer Peters, 1864.

Sodium chloride tolerance: This was the only solution in which *B. afer* could be tested, since very few fish were available. The results of the initial experiment and the preliminary experiment are given in Tables

18 and 19 respectively. While this species tolerated quite high concentration under conditions of rapid increase in salinity level, the tolerance of B. afer was significantly lower in the preliminary experiment with its slower increase in concentration. It can therefore be concluded that this species has a lower tolerance than B. asper.

TABLE 18.

INITIAL PROCEDURE: THE SURVIVAL OF Barbus afer IN RAPIDLY INCREASING SODIUM CHLORIDE CONCENTRATIONS.

Number of fish used: 5. Rate of concentration increase: 3 ‰ per hour.

Date: March, 1964. Average temperature: 20.5°C.

Concentrations at which deaths occurred (‰ NaCl).	Number of surviving fish.	Percentage survival.
18	0	0

TABLE 19.

PRELIMINARY PROCEDURE: THE SURVIVAL OF Barbus afer IN GRADUALLY INCREASING CONCENTRATIONS OF SODIUM CHLORIDE.

Number of fish used: 10 Rate of concentration increase: 1 ‰ per day.

Date: May, 1964. Average temperature: 17.1°C.

Concentrations at which deaths occurred (‰ NaCl).	Number of surviving fish.	Percentage survival.
8	0	0

TLm: No estimate possible.

Barbus pallidus A. Smith, 1841.

This species was subjected to preliminary experiments using sodium chloride, sodium sulphate and sea water, but unfortunately no experiments using the final procedure could be performed on this species which also exhibits a high sodium chloride tolerance.

Sodium chloride tolerance: Both the initial and the preliminary procedures were utilised in the estimation of this tolerance value, and the results of these experiments are summarised in Tables 20 and 21 respectively. The results of both experiments are in close agreement and indicate that this species is not affected by differences in the rate of salinity change, and has a high tolerance of sodium chloride, which is very similar to that of Barbus asper.

TABLE 20.

INITIAL PROCEDURE: THE SURVIVAL OF Barbus pallidus IN INCREASING SODIUM CHLORIDE CONCENTRATIONS.

Number of fish used: 5. Rate of concentration increase: 3 ‰ per hour.

Date: March, 1964. Average temperature: 20.5°C.

Concentrations at which deaths occurred (‰ NaCl).	Number of surviving fish.	Percentage survival.
18	0	0

TABLE 21.

PRELIMINARY PROCEDURE: THE SURVIVAL OF Barbus pallidus IN GRADUALLY INCREASING CONCENTRATIONS OF SODIUM CHLORIDE.

Number of fish used: 10. Rate of concentration increase: 1 ‰ per day.

Date: April, 1964. Average temperature: 19.8°C.

Concentrations at which deaths occurred (‰ NaCl).	Number of surviving fish	Percentage survival.	Survival probit.
17	8	80	5.84
18	5	50	5.00
19	0	0	-

TLm estimate = 18.0 ‰ NaCl.

Sodium sulphate tolerance: Only four fish were used for the preliminary experiment with sodium sulphate, due to the difficulty experienced in obtaining large stocks of this species in the field. The results are summarised in Table 22, from which it is apparent that the sodium sulphate tolerance is far smaller than the sodium chloride tolerance.

TABLE 22.

PRELIMINARY PROCEDURE: THE SURVIVAL OF Barbus pallidus IN GRADUALLY INCREASING CONCENTRATIONS OF SODIUM SULPHATE.

Number of fish used: 4. Rate of concentration increase: 0.44 ‰ per day.

Date: August 1964. Average temperature: 18.4°C.

Concentration at which deaths occurred (‰ NaCl).	Number of survivors.	Percentage survival.
7.11	0	0

TLm estimate: not possible, but would be in vicinity of 7 ‰ Na_2SO_4.

Sea water tolerance: For the preliminary experiment with sea water only three fish could be used and no estimate of the median tolerance limit could be obtained. The results of this experiment are summarised in Table 23 and the sea water tolerance is approximately equal to the sodium chloride tolerance of this species, in which respect it differs from B. holubi where the sea water tolerance exceeds the sodium chloride tolerance.

TABLE 23.

PRELIMINARY PROCEDURE: THE SURVIVAL OF Barbus pallidus IN GRADUALLY INCREASING CONCENTRATIONS OF SEA WATER.

Number of fish used: 3. Rate of concentration increase: 1 ‰ per day.

Date: March, 1965.

Average temperature: 20.9°C.

Concentrations in which deaths occurred (‰).	Number of surviving fish.	Percentage survival.
16	1	33
17	0	0

TLm estimate: between 15 and 16 ‰ of diluted sea water, no accurate estimate possible.

Labeo umbratus A. Smith, 1841.

Sodium chloride tolerance: The results of the preliminary experiment are set out in Table 24. This species therefore tolerates a slightly higher concentration than Barbus holubi but is not as tolerant as B. asper and B. pallidus.

TABLE 24.

PRELIMINARY PROCEDURE: THE SURVIVAL OF Labeo umbratus IN GRADUALLY INCREASING SODIUM CHLORIDE CONCENTRATIONS.

Number of fish used: 9. Rate of concentration increase: 1 ‰ per day.

Date: January, February, 1965.

Average temeperature: 19.6°C.

Concentrations at which deaths occurred (‰ NaCl).	Number of surviving fish.	Percentage survival.
12	2	22
13	0	0

TLm estimate: not possible, but would be between 11 and 12 ‰.

Sodium sulphate tolerance: The gradually increasing concentrations produced by the preliminary procedure caused decreasing survival, as summarised in Table 25. Labeo umbratus is the species with the highest tolerance of sodium sulphate, which even exceeds its tolerance of sodium chloride.

Significantly, the batch of Labeo umbratus used in these tests were obtained from the "brack" Fish River with a high sulphate content, as indicated in Table 50. Unfortunately no specimens of this species could be obtained from non-brack rivers to determine whether adaptation to high sulphate concentration had occurred in those populations of this species living in "brack" rivers.

TABLE 25.

PRELIMINARY PROCEDURE: THE SURVIVAL OF Labeo umbratus IN CONCENTRATIONS OF SODIUM SULPHATE.

Number of fish used: 10. Rate of concentration increase: 1 ‰ per day.

Date: January, 1965. Average temperature: 20.2°C.

Concentrations at which deaths occurred (‰ Na_2SO_4).	Number of surviving fish.	Percentage survival.	Survival probit.
9	9	90	6.28
12	8	80	5.84
14	6	60	5.25
15	0	0	-

TLm estimate = 15.2 ‰.

Sea water tolerance: The preliminary procedure was also used to estimate the tolerance of this species to sea water. The data yielded by this experiment are supplied in Table 26. The tolerance values exceed the sodium chloride tolerance of this same species, and its sea water tolerance is approximately equal to that of Barbus holubi and to its own sulphate tolerance values.

TABLE 26.

PRELIMINARY PROCEDURE: THE SURVIVAL OF Labeo umbratus IN GRADUALLY INCREASING OF SEA WATER DILUTIONS.

Number of fish used: 9. Rate of concentration increase: 1 ‰ per day.

Date: March, 1965. Average temperature: 21.5°C.

Concentrations at which deaths occurred (‰).	Number of surviving fish.	Percentage survival.
15	7	78
16	0	0

TLm estimate: not possible, would lie between 15 and 16 ‰.

Tilapia mossambica Peters, 1852.

This species was subjected to solutions of sodium chloride and sodium sulphate and to dilutions of sea water and produced very interesting results which provide a valuable basis of comparison between the different species tested.

Sodium chloride tolerance: An initial experiment produced the results summarised in Table 27, from which it can be concluded that the sodium chloride tolerance of this species is quite high. This contention is borne out by the two preliminary experiments which are reported in Table 28. The first experiment (series A) was disrupted when the tank bottom cracked. The experiment was repeated and the results are reported as series B in the table. The difference, in the concentrations tolerated, between the two experiments cannot be explained satisfactorily on the basis of the available evidence. The degree of similarity between the tolerance as established by the initial experiment and that estimated from the second preliminary experiment indicate that this species is not greatly affected by differences in the rate of salinity increase.

TABLE 27.

INITIAL PROCEDURE: THE SURVIVAL OF *Tilapia mossambica* IN RAPIDLY INCREASING SODIUM CHLORIDE CONCENTRATIONS.

Number of fish used: 5. Rate of concentration increase: 3 ‰ per hour.

Date: March, 1964. Average temperature: 20.5°C.

Concentration in which deaths occurred (‰ NaCl).	Number of surviving fish.	Percentage survival.
24	0	0

TABLE 28.

PRELIMINARY PROCEDURE: THE SURVIVAL OF *Tilapia mossambica* IN GRADUALLY INCREASING SODIUM CHLORIDE CONCENTRATIONS.

Series A: Number of fish used: 10. Rate of concentration increase: 1 ‰ per day.

Date: May, 1964. Average temperature: 19.8°C.

Series B: Number of fish used: 10 Rate of concentration increase: 1 ‰ per day.

Date: June, 1964. Average temperature: 18.3°C.

Concentrations at which deaths occurred (‰ NaCl).	Number of surviving fish.	Percentage survival.	Survival probit.
Series A:			
20	9	90	
23	2	(All 7 deaths due to leakage)	
26	1	10	
27	0	0	
Series B:			
17	8	80	5.84
19	7	70	5.52
20	0	-	0

TLm estimate (Series B): = 21.2 ‰ NaCl.

<u>Sodium sulphate tolerance</u>: As indicated by the preliminary results, as summarised in Table 29, the sodium sulphate tolerance of <u>T. mossambica</u> is very much reduced in comparison with its sodium chloride and its sea water tolerance, and is also lower than the sodium sulphate tolerance of <u>Labeo umbratus</u>.

TABLE 29.

PRELIMINARY PROCEDURE: THE SURVIVAL OF Tilapia mossambica IN GRADUALLY INCREASING CONCENTRATIONS OF SODIUM SULPHATE.

Number of fish used: 10. Rate of concentration increase: 0.44 ‰ per day.

Date: August, 1964. Average temperature: 21.1°C.

Concentration at which deaths occurred (‰ Na_2SO_4).	Number of surviving fish.	Percentage survival.	Survival probit.
8.00	8	80	5.84
8.44	7	70	5.52
8.88	6	60	5.25
9.33	3	30	4.48
10.22	0	0	-

TLm estimate = 8.9 ‰.

<u>Sea water tolerance</u>: In order to allow a comparison between the sea water tolerances of a number of species, the final procedure was used in the case of <u>T. mossambica</u>. Three groups of ten fish each were exposed to arbitrarily chosen dilutions of sea water. This final experiment was preceded by gradual acclimation to 17 ‰ and the fish were then transferred directly to the various concentrations. Even in undiluted sea water no deaths occurred within the 5,000 minute period. This species tolerates sea water to a remarkable degree, since both of the batches which were used for this final experiment

were obtained from freshwater sources. The results are indicated in Table 30.

TABLE 30.

FINAL PROCEDURE: THE SURVIVAL WITH TIME OF Tilapia mossambica IN DIFFERENT DILUTIONS OF SEA WATER.

Date: April, 1965. Acclimation: to 17 ‰.

Salinity (‰)	Number of fish used.	Average temperature (°C).	Time since start of experiment (minutes).	Number of live fish.	Percentage survival.	Survival probit.
27	10	20.9	5,000	10	100	--
30	10	21.0	5,000	10	100	-
35	10	20.6	5,000	10	100	-

Tilapia sparrmanii A. Smith, 1840.

This species was tested quite exhaustively, since as a cichlid it is supposed to have a high tolerance of sea water, but is known to have lower tolerance of sea water than its congener, T. mossambica (Crass, 1964).

Sodium chloride tolerance: All three procedures were utilised in the estimation of this value. The results of the initial experiment, as given in Table 31, indicate a fairly high tolerance of sodium chloride. The data of the preliminary procedure experiment are in agreement essentially (Table 32), and the different rates of increasing the concentration have apparently little direct effect. The survival results of the final experiment are provided in Table 33 and the median times of survival are plotted in figure 7. This figure reveals that once again a hyperbolic relation between the median time of survival and concentration is apparent, if concentration is plotted on a logarithmic scale. Estimates

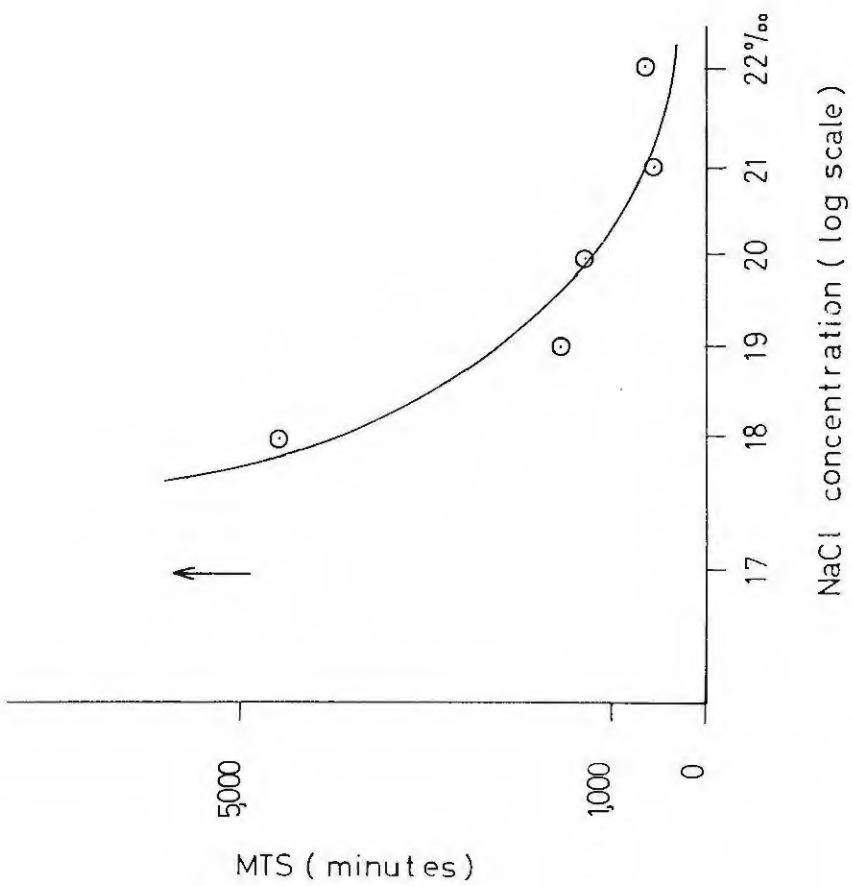


FIGURE 7: The relationship between median time of survival and sodium chloride concentration for *Tilapia sparrmanii*.

of the median tolerance limits for various exposure periods were also obtained from the results of the final experiment and are indicated in Table 34.

TABLE 31.

INITIAL PROCEDURE: THE SURVIVAL OF *Tilapia sparrmanii* IN RAPIDLY INCREASING CONCENTRATIONS OF SODIUM CHLORIDE.

Number of fish used: 5.
Date: March 1964.
Rate of concentration increase: 3 ‰ per hour.
Average temperature: 20.5°C.

Concentration at which deaths occurred (‰ NaCl).	Number of surviving fish.	Percentage survival.
24	0	0

TABLE 32.

PRELIMINARY PROCEDURE: THE SURVIVAL OF *Tilapia sparrmanii* IN GRADUALLY INCREASING CONCENTRATIONS OF SODIUM CHLORIDE.

Number of fish used: 10
Date: April, 1964.
Rate of concentration increase: 1 ‰ per day.
Average temperature: 19.1°C.

Concentrations at which deaths occurred (‰ NaCl).	Number of surviving fish.	Percentage survival.
19	9	90
20	0	0

TLm estimate: not possible, would be between 19 and 20 ‰.

TABLE 33.

FINAL PROCEDURE: THE SURVIVAL WITH TIME OF *Tilapia sparrmanii* IN DIFFERENT CONCENTRATIONS OF SODIUM CHLORIDE.

Date: July, 1964 Acclimation: up to 16 ‰.

Concentration (‰ NaCl)	Number of fish used.	Average temperature (°C).	Time since start of experiment (minutes).	Number of live fish.	Percentage survival.	Survival probit.
17	10	20.1	4,054	8	80	5.84
			5,000	8	80	
18	10	20.0	1,419	9	90	6.28
			2,244	7	70	5.52
			3,034	6	60	5.25
			5,194	5	50	5.00
19	10	19.8	176	9	90	6.28
			434	8	80	5.84
			694	7	70	5.52
			1,624	6	60	5.25
			3,154	5	50	5.00
			4,054	3	30	4.48
			4,474	2	20	4.16
			5,194	1	10	3.72
20	10	19.7	304	9	90	6.28
			634	8	80	5.84
			844	7	70	5.52
			944	6	60	5.25
			1,407	5	50	5.00
			1,624	4	40	4.75
			2,234	3	30	4.48
			2,839	2	20	4.16
			3,634	1	10	3.72
			4,054	0	0	-
21	10	19.4	239	9	90	6.28
			334	8	80	5.84
			363	7	70	5.52
			514	6	60	5.25
			574	5	50	5.00
			594	4	40	4.75
			724	3	30	4.48
			1,519	2	20	4.16
			2,234	0	0	-
22	10	19.9	424	9	90	6.28
			524	8	80	5.84
			534	7	70	5.52
			644	6	60	5.25
			784	5	50	5.00
			804	4	40	4.75
			844	3	30	4.48
			854	2	20	4.16
			874	1	10	3.72
			1,139	0	0	-

TABLE 34

FINAL PROCEDURE: THE ESTIMATED MEDIAN TOLERANCE LIMITS OF *Tilapia sparrmanii* TO SODIUM CHLORIDE FOR DIFFERENT LENGTHS OF EXPOSURE.

Length of exposure (minutes).	Median tolerance limit (%o NaCl).
5,000	17.9
1,440	19.9

Sodium sulphate tolerance: In sodium sulphate only a preliminary experiment was performed with *T. sparrmanii* and the results, as given in Table 35, indicate that the sulphate tolerance of this species is of the same order as that of *T. mossambica*. Similarly to the latter species, *T. sparrmanii* appear to be far more sensitive to sodium sulphate than to sodium chloride or sea water.

TABLE 35.

PRELIMINARY PROCEDURE: THE SURVIVAL OF *Tilapia sparrmanii* IN GRADUALLY INCREASING CONCENTRATIONS OF SODIUM SULPHATE.

Number of fish used: 10

Date: June, 1964.

Rate of concentration increase: 0.44 %o per day.

Average temperature: 18.3°C.

Concentrations at which deaths occured (%o Na_2SO_4)	Number of surviving fish	Percentage survival	Survival probit.
6.66	9	90	6.28
8.00	8	80	5.84
10.22	6	60	5.25
11.11	4	40	4.75
11.44	2	20	4.16
12.00	1	10	3.72
12.44	0	0	-

TLm estimate = 9.8 %o.

Sea water tolerance: Only the final procedure was used in the estimation of the tolerance of this species to sea water. Initially a concentration range of 17 to 22 ‰, as suggested by the sodium chloride experiments, was used, but only slight reductions in the number of survivors occurred. The group from 18 ‰ (which was unaffected) was transferred to 23 ‰, and two groups of ten fish each, picked randomly from the fish surviving in 17, 19 and 20 ‰, were transferred to 24 and 25 ‰ respectively. Due to the unavailability of more fish of this species, this procedure had to be adopted to obtain median times of survival significantly shorter than the 5,000 minute observation period in order to indicate those concentrations which are definitely lethal to this species. Tilapia sparrmanii does not tolerate sea water to the same extent that T. mossambica does, but is more tolerant of sea water than any of the other species tested in the present study. This conclusion is based on the survival results of Table 36; the estimated median times of survival are plotted in figure 8 which once again produces a hyperbolic relation between median time of survival and concentration on a semilogarithmic plot. Table 37 lists the estimates of the median tolerance limits for two different lengths of exposure, which are slightly higher than the Tlm's obtained in sodium chloride for the same exposure lengths.

TABLE 37.

FINAL PROCEDURE: THE MEDIAN LIMITS OF TOLERANCE OF Tilapia sparrmanii TO SEA WATER DILUTIONS FOR DIFFERENT LENGTHS OF EXPOSURE:

Length of exposure (minutes).	Median limit of tolerance (‰ salinity).
5,000	21.9
1,440	24.6

TABLE 36.

FINAL PROCEDURE: THE SURVIVAL OF *Tilapia sparrmanii* WITH TIME IN DIFFERENT DILUTIONS OF SEA WATER.

Date: April, 1964. Acclimation: up to 16 ‰.

Concentration (‰)	Number of fish used.	Average temperature (°c).	Time since start of experiment (minutes).	Number of live fish.	Percentage survival.	Survival probit.
17	10	20.5	4,320	9	90	6.28
			5,000	9	90	
18	10	20.5	5,000	10	100	
19	10	20.4	3,780	9	90	6.28
			5,000	9	90	
20	10	20.4	5,000	9	90	6.28
21	10	20.6	2,115	9	90	6.28
			3,555	7	70	5.52
			3,795	6	60	
			4,335	4	40	4.75
			5,000	4	40	
22	10	21.0	675	9	90	6.28
			2,115	7	70	5.52
			2,595	5	50	5.00
			3,795	4	40	4.75
			5,000	4	40	
23	10	21.2	1,705	9	90	6.28
			2,425	8	80	5.84
			3,145	7	70	5.52
			3,925	5	50	5.00
			5,000	5	50	
24	10	21.3	55	9	90	6.28
			85	8	80	5.84
			460	7	70	5.52
			1,025	5	50	5.00
			1,115	4	40	4.75
			2,425	2	20	4.16
			2,785	1	10	3.72
			4,285	0	0	–
25	10	21.5	415	8	80	5.84
			1,025	7	70	5.52
			1,115	6	60	5.25
			1,585	4	40	4.75
			1,705	3	30	4.48
			2,425	0	0	–

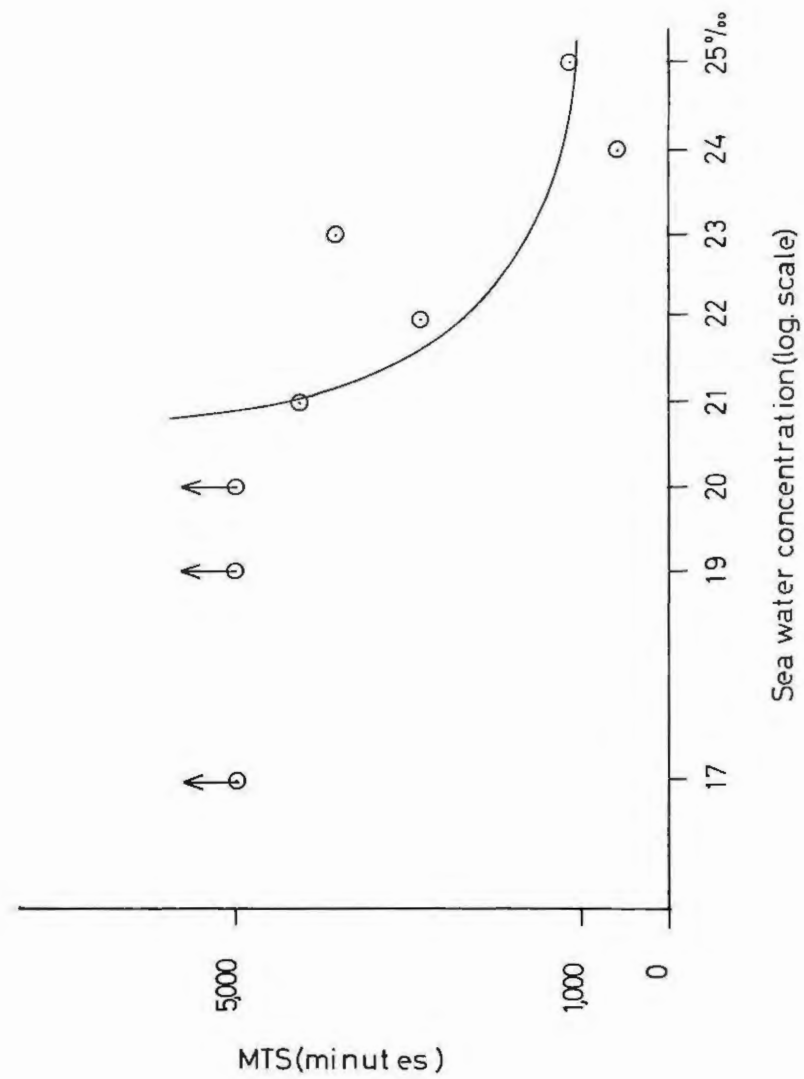


FIGURE 8: The relationship between median time of survival and sea water concentration for *Tilapia sparrmanii*.

Seven fish were transferred directly from 21 and 22 ‰, in which they had spent 5,000 minutes, to undiluted sea water. They survived for periods varying from 134 to 274 minutes. Tilapia sparrmanii can therefore withstand only a limited exposure to undiluted sea water.

Shock no doubt plays an important part in reducing the survival time to these values, but until the optimum rate of transfer to sea water can be determined, these values are the only ones available for use in any discussion on distribution.

Sandelia capensis (Cuvier and Valenciennes), 1831.

Sodium chloride tolerance: The results of Table 38 were obtained from an initial experiment while the data from the preliminary experiment are supplied in Table 39. Its tolerance in the latter experiment was appreciably lower than in the initial experiment and this species is therefore quite sensitive to large differences in the rate of concentration increase. The final procedure was also utilised and the survival figures are reported in Table 40. The estimates of the median times of survival, where possible, are given in Table 41, and the median tolerance limits are supplied in Table 42. From these results it is apparent that Sandelia capensis is one of the species that is fairly tolerant of sodium chloride.

Sodium sulphate tolerance: Only the preliminary procedure could be applied in the estimation of this tolerance and the results are reported in Table 43. In spite of the fairly high sodium chloride tolerance of this species its sodium sulphate tolerance is very low, indeed the lowest reported in the present study. Unfortunately no sea water experiments could be undertaken to provide information on the actual sea water tolerance of this species.

TABLE 38.

INITIAL PROCEDURE: THE SURVIVAL OF *Sandelia capensis* IN RAPIDLY INCREASING SODIUM CHLORIDE CONCENTRATIONS.

Number of fish used: 5.
Rate of concentration increase: 3 ‰ per hour.
Date: March, 1964.
Average temperature: 20.5°C.

Concentrations at which deaths occurred (‰ NaCl).	Number of surviving fish.	Percentage survival.
18	4	80
21	0	0

TABLE 39.

PRELIMINARY PROCEDURE: THE SURVIVAL OF *Sandelia capensis* IN GRADUALLY INCREASING CONCENTRATIONS OF SODIUM CHLORIDE.

Number of fish used: 10.
Rate of concentration increase: 1 ‰ per day.
Date: June, 1964.
Average temperature: 19.9°C.

Concentrations at which deaths occurred (‰ NaCl).	Number of surviving fish.	Percentage survival.	Survival probit.
10	9	90	6.28
12	8	80	5.75
13	5	50	5.00
15	3	30	4.48
16	1	10	3.72
17	0	0	-

TLm estimate = 13.2 ‰.

TABLE 40.

FINAL PROCEDURE: THE SURVIVAL WITH TIME OF Sandelia capensis IN DIFFERENT CONCENTRATIONS OF SODIUM CHLORIDE.

Date: February, 1965. Acclimation: up to 12 ‰.

Concentration (‰ NaCl).	Number of fish used.	Average temperature (°C).	Time since start of experiment (minutes).	Number of live fish.	Percentage survival.	Survival probit.
13	10	21.3	4,810	9	90	6.28
			10,000	9	90	-
14	10	21.2	485	9	90	6.28
			9,540	8	80	5.84
			10,000	8	80	-
15	10	20.7	4,810	9	90	6.28
			7,800	8	80	5.84
			10,000	7	70	5.52
16	10	21.0	1,930	9	90	6.28
			4,810	7	70	5.52
			5,830	6	60	5.25
			7,800	4	40	4.75
			10,000	4	40	-

TABLE 41.

FINAL PROCEDURE: THE ESTIMATED MEDIAN TIMES OF SURVIVAL OF Sandelia capensis IN DIFFERENT SODIUM CHLORIDE CONCENTRATIONS.

Sodium chloride concentrations (‰ NaCl).	Median times of survival (minutes).
13	no estimate possible
14	no estimate possible
15	17,780
16	6,683

TABLE 42.

FINAL PROCEDURE: THE ESTIMATED MEDIAN LIMITS OF TOLERANCE OF Sandelia capensis TO SODIUM CHLORIDE FOR DIFFERENT EXPOSURE LENGTHS.

Length of exposure (minutes).	Median tolerance limit (‰ NaCl.)
10,000	15.6
5,000	17.8
1,440	no estimate possible

TABLE 43.

PRELIMINARY PROCEDURE: THE SURVIVAL OF Sandelia capensis IN GRADUALLY INCREASING CONCENTRATIONS OF SODIUM SULPHATE.

Number of fish used: 5.

Date: August, 1964.

Rate of concentration increase: 0.44 ‰ per day.

Average temperature: 22.1°C.

Concentrations at which deaths occurred (Na_2SO_4).	Number of surviving fish.	Percentage survival.	Survival probit.
1.33	4	80	5.84
1.77	3	60	5.25
4.88	2	40	4.75
5.33	1	20	4.16
5.77	0	0	-

TLm estimate = 2.8 ‰.

THRESHOLD CONCENTRATIONS

It has been noted that the semilogarithmic relationship between median time of survival and Sodium chloride or sea water concentration can be described by a hyperbola. This is certainly true in figures 6 and 7, but less striking in figures 5 and 8 where a straight line could adequately define the relationship. The striking hyperbolic nature shown in figures 6 and 7 is adequately defined by the function:

$$C^nT = k$$

where C = concentration

T = median time of survival

n and k = constants

which has been found to define the action of numerous toxic agents on fish (Jones 1964). It is therefore not surprising that such a relationship should define other physiological stress conditions. From these curves it is possible to place some confidence upon threshold limits, estimates of which are taken from figures 5, 6, 7 and 8 by dropping a line from the asymptote to the salinity or sodium chloride axis. These estimates are summarised in Table 44.

TABLE 44.

THRESHOLD CONCENTRATION ESTIMATES.

Species	Solution	Threshold consentration
Barbus holubi	NaCl sea water	9% 14%
Tilapia sparrmanii	NaCl sea water	18% 21%

FREEZING-POINT DETERMINATIONS ON THE SERUM OF CYPRINIDS:

The two cyprinids used for these experiments, *Barbus holubi* and *Labeo umbratus*, were both subjected to freshwater and to dilutions of sea water to obtain serum samples for freezing-point depression measurements. While different dilutions of sea water were used for these two species (since their sea water tolerance differ as indicated previously and the dilutions were required to be definitely lethal) both species show a large increase in the serum freezing-point depression at the point of death in the sea water dilutions in comparison with the values obtained in freshwater. At these lethal salinity levels with the high values of external environment freezing-point depression (indicated as Δ_e in Tables 45 and 46), the freezing-point depression of the serum (indicated as Δ_i) may be nearly doubled, as is indicated from both these Tables and from figure 9.

TABLE 45.

THE FREEZING-POINT DEPRESSION OF THE SERUM OF *Barbus holubi* AT VARIOUS DILUTIONS OF SEA WATER.

Date: June, 1965.

Salinity of water.	Δe (°C)	Number of fish used.	State of fish at sampling.	Δi (°C).
0 ‰	+0.014±0.059	1	Alive	-0.543±0.019
13 ‰	-0.760±0.021	1	Point of death.	-0.847±0.033

TABLE 46.

THE FREEZING-POINT DEPRESSION OF THE SERUM OF *Labeo umbratus* AT VARIOUS SEA WATER DILUTIONS.

Date: June, 1965.

Salinity of water (‰).	Δe (°C).	Number of fish used.	State of fish at sampling.	Δi (°C).
0	+0.014±0.059	2	Normal	-0.519±0.027
			Normal	-0.498±0.027
18	-0.885±0.018	1	At point of death.	-0.982±0.036

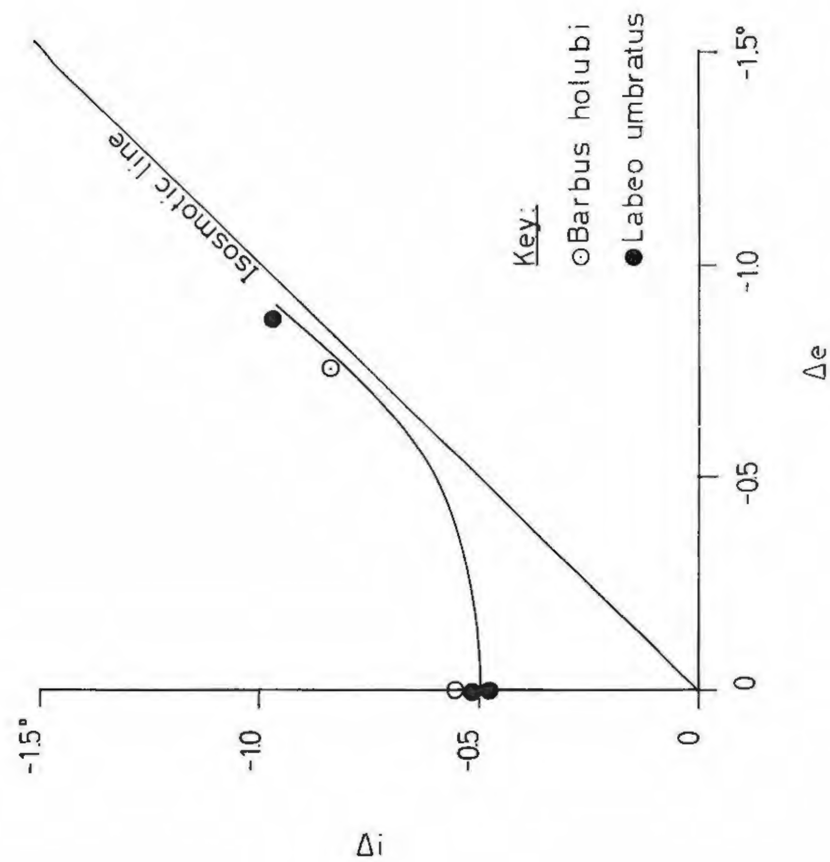


FIGURE 9: The freezing-point depression of the serum of Barbus holubi and Labeo umbratus from various dilutions of sea water.

Labeo umbratus was also subjected to solutions of sodium sulphate in order to obtain some information on the effect of this solute on serum freezing-point depression. The data are reported in Table 47 from which it is concluded that the serum freezing-point depression is not affected even by lethal concentrations of this salt, since at the latter concentrations the external environment is still hyposmotic to the blood of the fish. The cause of death in these concentrations cannot therefore be ascribed to osmotic failure, which occurred in both species tested in sea water, and must probably be due to some toxic action of the sodium sulphate. The relation between the serum and the external environment values is illustrated graphically in figure 10.

TABLE 47.

THE FREEZING-POINT DEPRESSIONS OF THE SERUM OF *Labeo umbratus* AT VARIOUS SODIUM SULPHATE CONCENTRATIONS.

Date: June, 1965.

Concentration of Na_2SO_4 (‰).	Δ_e (°C).	Number of fish used	State of fish at sampling.	Δ_i (°C).
0	+0.014±0.059	2	Normal	-0.519±0.027
			Normal	-0.498±0.027
10	-0.329±0.005	2	Normal	-0.499±0.017
			Normal	-1.035±0.15*
15	-0.457±0.016	4	Temporary loss of equilibrium	-0.567±0.016
			do	-0.581±0.015
			Permanent loss of equilibrium	-0.572±0.009
			do	-0.514±0.014

* This sample was very likely contaminated.

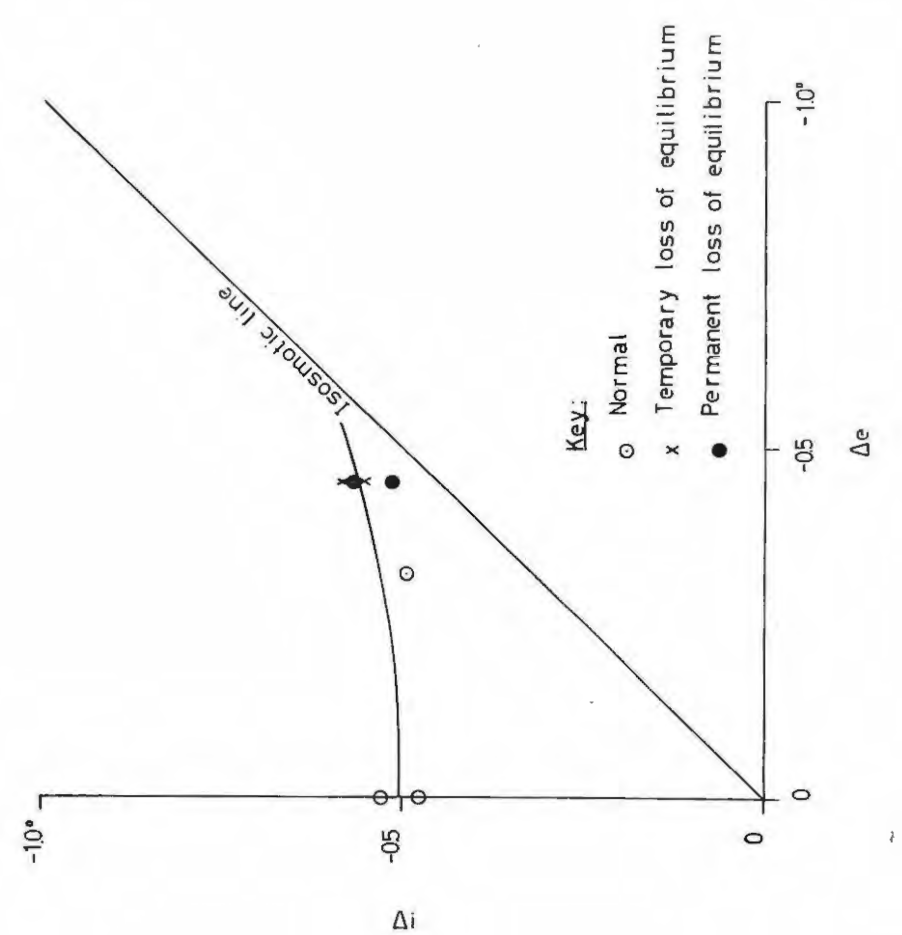


FIGURE 10: The freezing-point depression of the serum of Labeo umbratus from various concentrations of sodium sulphate.

GILL HISTOLOGY

The histological preparations indicate that no significant differences exist between the sections obtained from Barbus holubi exposed to sublethal concentrations of sodium chloride, and the sections obtained from B. holubi from freshwater. No evidence was found of any cells which approximate S-cells in structure in any of the gill sections examined. It can be concluded that S-cells are absent from Barbus holubi under these conditions.

5. DISCUSSION.

ZOOGEOGRAPHICAL ASPECTS.

Comparative tolerances to various solutions.

The estimated median limits of tolerance of the various species to sodium chloride, sodium sulphate and sea water are summarised in Tables 48, 49 and 50 respectively. The median time of survival estimates from the sodium chloride and sea water experiments are compared in figures 11 and 12. Where more than one 24 hour (1440 minutes) median tolerance limit estimate is available for a given species in a specified solution, the estimate from the final experiment was used, since it is statistically more reliable than the preliminary estimate. Where no estimates from the final experiment are available, the preliminary estimates are used. The estimates for the other exposure periods are obtained from the final experiments. For comparison between the various species the 24 hour median tolerance limits are used, since it is the only estimate which is available for all the species tested. While this exposure period has no special biological significance, it provides a useful indication of short term tolerance and can therefore be used for comparative purposes. For those species for which data are available the threshold concentration can also be used as a very useful means of comparison (Table 44).

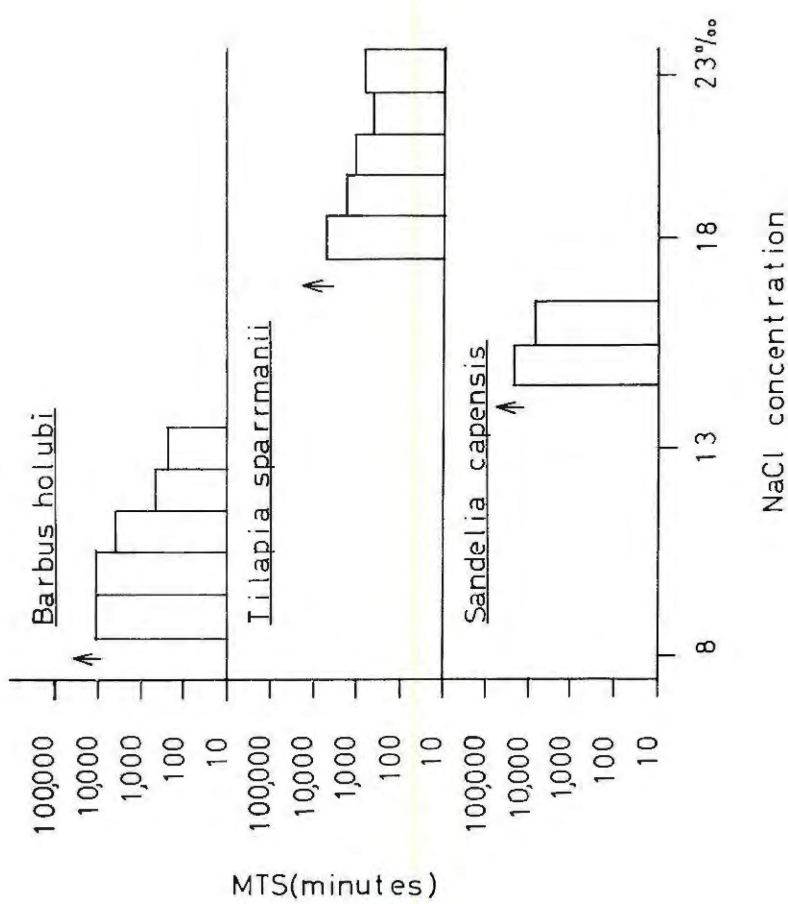


FIGURE 11: The median times of survival of *Barbus holubi*, *Tilapia sharrmanii* and *Sandelia capersis* in various sodium chloride concentrations.

TABLE 48.

THE MEDIAN TOLERANCE LIMITS OF VARIOUS SPECIES TO SODIUM CHLORIDE.

Species	Length of exposure (minutes)		
	1,440	5,000	10,000
Tilapia mossambica	21.2‰	-	-
T. sparrmanii	19.8‰	17.9‰	-
Barbus pallidus	18‰	-	-
Sandelia capensis	13.2‰	17.8‰	15.6‰
B. asper.	17‰	-	-
B. holubi.	11.8‰	11.4‰	10.1‰
Labeo umbratus.	11‰	-	-
B. afer.	8‰	-	-
B. trevelyani.	about 7‰	-	-

TABLE 49.

THE MEDIAN TOLERANCE LIMITS OF VARIOUS SPECIES TO SODIUM SULPHATE.

Species	Exposure lengths (minutes)		
	1,440	5,000	10,000
L. umbratus.	15.2‰	-	-
T. sparrmanii.	9.8‰	-	-
T. mossambica.	8.9‰	-	-
B. pallidus.	7.1‰	-	-
B. holubi.	6.8‰	5.3‰	-
B. trevelyani.	3.1‰	-	-
S. capensis.	2.8‰	-	-

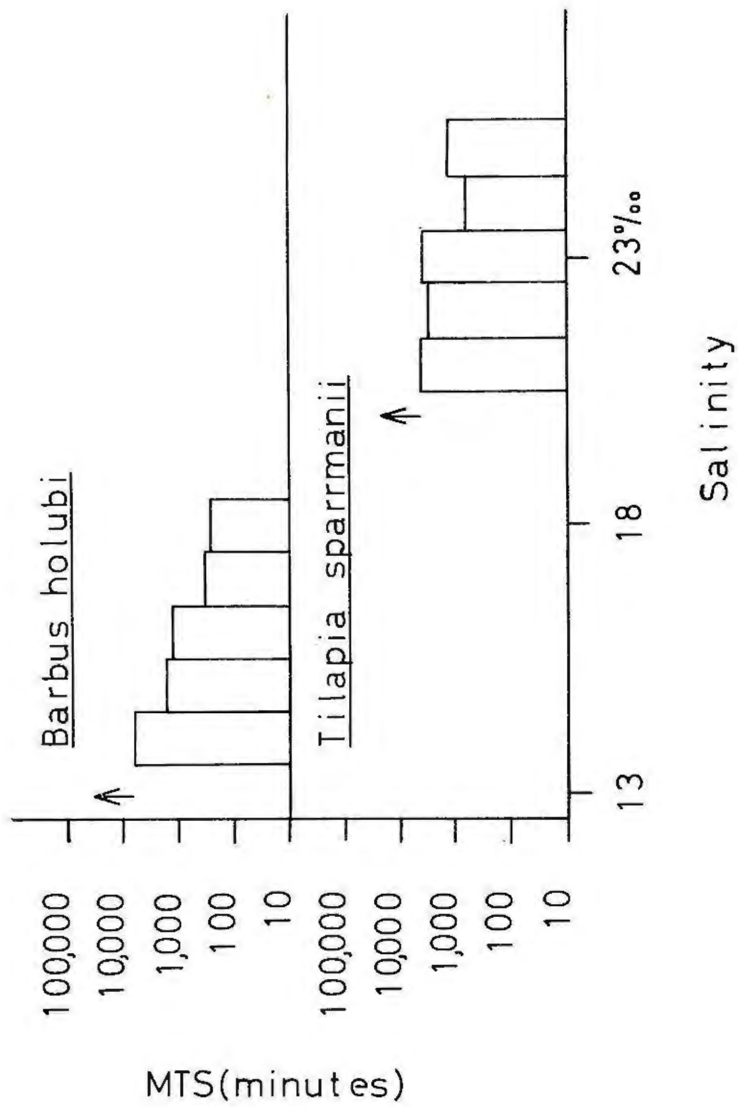


FIGURE 12: The median times of survival of *Barbus holubi* and *Tilapia sharrmanii* in sea water dilutions.

TABLE 50.

THE MEDIAN TOLERANCE LIMITS OF VARIOUS SPECIES TO SEA WATER.

Species	Exposure length (minutes)	
	1,440	5,000
T. mossambica.	35‰	35‰
T. sparrmanii.	24.6‰	21.9‰
B. holubi.	16.1‰	14.1‰
B. pallidus.	15‰	-
L. umbratus.	15‰	-

On the basis of 24 hour median tolerance limits the two cichlids, Tilapia mossambica and T. sparrmanii, have the highest tolerance to sodium chloride, but are not much more tolerant than some of the cyprinids, especially Barbus pallidus and B. asper. The cyprinids, however, vary widely in their tolerances. Sandelia capensis exceeds most cyprinids in sodium chloride tolerance. These interrelations are also borne out by the longer term median tolerance limits, where applicable.

Labeo umbratus is the species most tolerant of sodium sulphate, followed by the two cichlids and the Barbus species. Sandelia capensis has a very low sulphate tolerance. Tilapia mossambica exhibits the highest tolerance of sea water and lives in undiluted sea water for at least 5,000 minutes. Of the remaining species, T. sparrmanii is the most tolerant, but this species certainly cannot tolerate undiluted sea water for longer than a few hours. The three cyprinid species tested with sea water have approximately equal tolerances of sea water dilutions and, like Barbus holubi, B. pallidus and Labeo umbratus will probably survive only a few minutes in undiluted sea water. The estimated threshold concentrations support the above conclusions on differences between species.

SALINITY TOLERANCE AND SEA DISPERSAL

Sea dispersal under normal conditions: Tilapia mossambica with its ability to tolerate sea water for extended periods will no doubt be able to disperse through the sea from one river to another. That it in fact has done so is indicated by its present-day distribution in Southern Africa, since it occurs naturally in the Transkei rivers while its low cold tolerance, as established by Allanson, Ernst and Noble (1965), prevents it from utilising high altitude river capture which is the only other way in which it could have attained its present distribution range in the Transkei and the Eastern Province.

Tilapia sparrmanii has a lower salinity tolerance than T. mossambica and will not be able to disperse through the sea under normal conditions.

Barbus holubi is even less tolerant of sea water than the cichlid species. The absence of this species and other closely related species from the Eastern Province rivers must be due to the evolution of this species in the Orange system after the river captures, which have assisted in the dispersal of other species into the Eastern Province, had already taken place. External environmental conditions do not appear to have had a limiting effect on its distribution since this species has a high cold tolerance (Ernst, personal communication) and has thrived in small introduction experiments in the Eastern Province (Jubb, 1965; Gabie, 1965).

The sea water tolerance of Labeo umbratus is of the same order as that of B. holubi, and its Eastern Province distribution in the major rivers only also indicate that it has not made use of the sea as dispersal route, since it should occur in at least some of the other rivers as well if it had dispersed through the sea. L. umbratus is distributed over a far larger area of Southern Africa than B. holubi. Since both are large cyprinids, which would experience similar difficulties in the dispersal process, the only adequate explanation seems to be that L. umbratus has been present in the Orange River system far longer than B. holubi and could disperse through freshwater links, which were no longer available when B. holubi evolved in the Orange system.

Barbus pallidus also has a low tolerance of sea water which would prevent it from dispersing through the sea. Its distribution pattern is very similar to that of Tilapia sparrmanii (Jubb, 1963) and can therefore be considered as the result of distribution through freshwater links.

No data are available on the actual tolerance to sea water of the other species tested in the present study. From their 24 hour tolerances of sodium chloride, which are in all cases less than that of T. sparrmanii which cannot tolerate undiluted sea water, it appears that none of them will tolerate undiluted sea water. Supporting evidence is provided by the distribution ranges of these species. Barbus asper has closely related species in the Orange River system and in Natal (Farquharson, 1962) but none in the more isolated Transkei region where sea dispersal is the only possible method of entering the region. B. afer, which is very closely related to B. asper, has a very limited distribution and with its low sodium chloride tolerance, in comparison with that of B. holubi or L. umbratus, its sea water tolerance is expected to be very low. B. trevelyani is confined to the Buffalo River and its expected tolerance of sea water should be of the same order as that of B. afer. The presence of closely related species in the Orange River and the Natal systems indicate that the ancestral form of B. trevelyani must have reached the Buffalo River through freshwater links.

On the basis of its sodium chloride tolerance, which is slightly less than that of T. sparrmanii, it is expected that Sandelia capensis will not tolerate undiluted sea water.

Diluted sea water and dispersal: Low tolerance of sea water dilutions does not necessarily rule out all possibility of dispersal through the sea. Species that are unable to tolerate long term exposure to small dilutions of sea water, do however, live for varying short periods in undiluted sea water in direct-transfer experiments before showing signs of partial loss of equilibrium, from which no recovery appears possible. Under very favourable conditions this period may be long enough to allow dispersal through the sea. The average distance between river mouths in the Eastern Province area is approximately 4 miles and with a maximum recorded inshore current of about 4 knots (Harris, personal communication), the more tolerant species may just be able to survive the hour in sea water which is required for the current to transport the fish to the vicinity of a neighbouring river mouth. Serious difficulties, however, arise in the long drowned estuaries of the Eastern Province coast, since the fish will take an appreciable time to traverse such an estuary to reach sufficiently diluted water in which it can recover. Since even medium-sized rivers such as the Kowie and the Bushmans Rivers have estuaries of ten miles and longer, the fish will certainly not be able to swim very far before succumbing to high salinity. Even if the fish is swept up the estuary by tidal currents it is very unlikely that any species other than Tilapia mossambica would survive the extended exposure to nearly undiluted sea water. In the Kowie River, for instance, the fish may have to spend approximately four hours in water of high salinity before reaching a body of water which is fresh enough to allow recovery (Stocks, personal communication). Under conditions of exceptionally heavy runoff in the rivers, the fish may enter freshwater very near the mouth of the river, or even outside the mouth, but the strong outflowing currents of water will seriously hinder or prevent the fish from entering the river. At low tides after heavy rains, salinities as low as 6.04‰ have been reported from the Kowie River estuary, at a point about half a mile from the mouth (Hill, personal communication), which would certainly allow even the sensitive cyprinids to survive. No information is however available on the behaviour of sea currents under these conditions, and dispersal by this method must be considered as yet unproven, since it certainly has not allowed any of the cyprinid species to enter the Transkei rivers.

Another mechanism which could assist in the dispersal of intolerant species through the sea is the formation of isolated freshwater masses drifting on the denser sea water. There is little doubt that such freshwater "parcels" may be present under favourable conditions in currents (Mauchline and Templeton, 1964) and where mixing processes are limited in scope, such as in the longshore currents within the breaker zone.

The estimated threshold concentrations for B. holubi and T. sparrmanii indicate that where the stay in sea water is in any way being prolonged, the degree of dilution required to enable dispersal is considerably increased. Harris (personal communication) is of opinion that this mechanism does not operate off the very open coast of the Eastern Province and cannot therefore contribute to the dispersal of fish through the sea. As far as can be ascertained dispersal through the sea between Eastern Province rivers is only possible for those species tolerating undiluted sea water.

Available data indicate that during certain stages of the Pliocene and Pleistocene, when the distribution is suggested to have taken place, the rainfall over Southern Africa was larger than at present. Van Zinderen Bakker (1965) and Cooke (1965), however, agree that at its most the rainfall never exceeded 150% of the present average rainfall and it must therefore be concluded that these conditions could not have assisted materially in the dispersal of intolerant species beyond the possibilities available at present.

General conclusions: Data from the direct-transfer experiments, the 24-hour tolerance limits and the threshold concentrations all indicate that it is highly unlikely that any species, other than Tilapia mossambica, could have dispersed through the sea. From these figures it is evident that a considerable degree of dilution is necessary to enable even Tilapia sparrmanii to make use of this distribution route. Accordingly the probability of such a degree of dilution occurring under the conditions that prevailed during the Quarternary period is extremely small. The distribution patterns of the Eastern Province species further indicate that only in the case of Tilapia mossambica has sea dispersal played any role at all. All the other species are less tolerant of sea water and their dispersal can be explained in terms of freshwater links. The only secondary division species in the present study is therefore T. mossambica, while all the other species, including T. sparrmanii, are members of the primary division.

This conclusion contradicts the general scheme of Myers (1937) and of Darlington (1957), which consider all cichlids to be members of the secondary division. It must therefore be concluded that the family is an unsatisfactory unit for expressing salinity tolerance for zoogeographical purposes. The ideal unit for this purpose is obviously the species, although Heuts (1947) has demonstrated that even different subspecies may differ appreciably in their tolerance of sea water. For practical purposes some larger systematic unit could be used for expressing the salinity tolerance, but it will definitely not be the same unit in all families. In the light of the available evidence, the sub-family Cyprininae will be suitable for African cyprinids, since they all appear to have a low sea water tolerance. Among the cichlids, however, physiological groups of species are the only combinations with consistent tolerance of sea water. The genus Tilapia can be subdivided into two different physiological groups. Tilapia mossambica with its high tolerance belongs to a different subgroup of the genus than Tilapia sparrmanii and T. melanopleura Dumeril, 1859, which constitutes a low tolerance group.

Distribution ranges and tolerance of sodium chloride and sodium sulphate.

Comparison of the chemical composition of various local river waters (Table 51) and the sodium chloride and sodium sulphate tolerances (Tables 48 and 49) of the various species, indicate that the non-continuous distribution within the Eastern Province exhibited by most species (Barnard, 1943; Farquharson, 1962) is not due to the relatively high salt contents of some of the rivers since the 24 hour sodium chloride and sodium sulphate tolerances of even the most sensitive species, Barbus trevelyani, far exceeds the reported concentrations. While the concentrations tolerated under conditions of long-term exposure are expected to be lower than the short-term tolerances determined in the present study, observations during the acclimation phase of the experiments indicate that all the species tested should easily survive continuous exposure to the maximum concentrations reported. Other reasons for the discontinuous distribution patterns must therefore be found. One possible cause of such a pattern appears to be lack of cold tolerance, at least in Tilapia mossambica, as indicated by Allanson, Ernst and Noble (1965). Other important factors in this respect are ecological competition for food or other requirements, and the absence of suitable freshwater links at those times when a particular species is in a position to utilise them. All these factors would limit species to those rivers in which they can survive and have been able to reach previously. Further information on the ecology of the fish concerned and especially on river captures is essential to explain the present distribution patterns.

Labeo umbratus, which has the highest tolerance to sodium sulphate of all the species tested, is found in the brack rivers of the Eastern Province which have a higher sulphate content than the other rivers.

TABLE 51.

THE CHEMICAL COMPOSITION OF THE WATER OF SOME EASTERN PROVINCE RIVERS : (MAXIMUM CONCENTRATIONS REPORTED, IN TERMS OF SODIUM CHLORIDE AND SODIUM SULPHATE).

River	Maximum concentrations		Source of data
	NaCl (ppm)	Na_2SO_4 (ppm)	
Buffalo	435	93	1
Keiskama	23	3	1
Great Fish system	1,100	149	1
Kowie	1,005	64	1
Gamtoos system	4,971	not known	2
Kromme	126	15	3
Van Staden's	131	15	3

Explanation: 1 = Department of Water Affairs, Pretoria

2 = Resident Engineer, Gamtoos Canals, Patensie

3 = City Engineer, Port Elizabeth.

Its high tolerance could be of some value during very dry years, when the fish from the lower reaches will be confined to standing pools subjected to evaporation. On the other hand, the species with the lowest sodium sulphate tolerance, Sandelia capensis, is limited to the soft water of the shorter coastal rivers and to the fresh Kougha tributary of the Gamtoos system, but does not occur in the comparatively "brack" Groot tributary of the same river (Barnard, 1943). In the "brack" rivers high sulphate values caused by evaporation during occasional dry seasons, could act as a limiting factor on this species. Unfortunately no indication of the maximum sulphate concentration experienced under these conditions is available and the effects of the combined action of high chloride and sulphate concentrations on this and other species are as yet unknown, so that no definite conclusions can be reached.

It is interesting to note that the two species with the lowest tolerance of sodium chloride, Barbus trevelyani and B. afer, are also limited in distribution to one or only a few small coastal rivers respectively, which are all very low in total dissolved solids. It is however doubted whether the sodium chloride levels of the other local rivers could rise to such an extent as to be a limiting factor in the survival of these two species, since both of them can tolerate concentrations of at least 7‰ for 24 hours. Other additional factors must therefore also play a role in the limited distributions of these two species.

PHYSIOLOGICAL ASPECTS.

Specific tolerances to different ionic solutions.

When the tolerances of a particular species to the three types of solutions used are compared, a common tendency is exhibited by most of the species tested. In most cases the tolerance to sea water exceeds the sodium chloride tolerance which is much higher than the tolerance to sodium sulphate, all tolerances expressed in terms of concentration. This pattern is observed in Barbus holubi, Tilapia mossambica and T. sparrmanii and is partially evident in B. trevelyani and Sandelia capensis. The exceptions are B. pallidus in which the sodium chloride tolerance slightly exceeds its sea water tolerance, and Labeo umbratus which has a sodium sulphate tolerance of the same order as its sea water tolerance, both of which exceeds its sodium chloride tolerance. The general tendency applies to both short and long term exposures, where data for the latter are available.

From this general pattern the physiological effects of each type of solution can be deduced partially. As has been remarked earlier, concentration increase is used as a convenient measure of increasing osmotic pressures and of changing ionic proportions. The relative roles of increasing osmotic pressures and of changing ionic proportions can be determined when the lethal concentrations of the three solutions are set out with the osmotic pressures of each of these concentrations as is done in Table 52. It is immediately apparent that in some solutions the fish survive at osmotic pressures which are far higher than the osmotic pressures at which they die in other solutions. Indeed the solution with the highest osmotic pressure for a given concentration is the one the fish tolerate the best, that is sea water dilutions. The solution to which the fish are the most sensitive, sodium sulphate, has the lowest osmotic pressure of the three. Since osmotic pressure (per se) is not responsible for the difference in tolerances, the ions present in each type of solution must play an important part. In sodium sulphate, the osmotic pressure at which the fish die is so much lower than the osmotic pressures they can tolerate in sea water or sodium chloride, that it is certain that this solution has some toxic action. In a sodium sulphate concentration of 1‰ the number of millie-quivalents of sodium present is 9.28 as compared to 37.4 milli-equivalents of sodium present in a 1‰ solution of sodium chloride. While the sodium ion is known to be toxic to some degree (Doudoroff and Katz, 1953), the number of sodium ions in a sodium sulphate solution is therefore far less than the number of sodium ions in a sodium chloride solution of the same concentration (which the fish tolerates better), and sodium cannot therefore be responsible for the toxic effects of sodium sulphate. Thus the sulphate ion must have some toxic effect on the fish, since it is the only other constituent.

TABLE 52.

THE TOLERANCE OF THE VARIOUS SPECIES TO DIFFERENT IONIC SOLUTIONS IN TERMS OF THE OSMOTIC PRESSURES TOLERATED FOR 24 HOURS.

Species	24 hour median tolerance limits to					
	NaCl		Na_2SO_4		Sea water	
	‰	Osmotic pressure (atm.)	‰	Osmotic pressure (atm.)	‰	Osmotic pressure (atm.)
T. mossambica	21.2	17.4	8.9	4.5	35	30.3
T. sparrmanii	19.8	16.2	9.8	4.9	24.6	21.3
B. pallidus	18	14.7	7.1	3.6	15	12.9
S. capensis	17.8	14.6	2.8	1.4	-	-
B. asper	17	13.97	-	-	-	-
B. holubi	11.8	9.7	6.8	3.4	16.1	13.9
L. umbratus	11	9.0	15.2	7.7	15	12.9
B. afer	8	6.5	-	-	-	-
B. trevelyani	7	5.7	3.1	1.57	-	-

Osmotic pressures calculated from P = icRT with i = ionic constant, c = molar concentration, R = gas constant, T = absolute temperature (Moore, 1960).

The difference in tolerance to sea water dilutions and to sodium chloride solutions cannot be explained in terms of osmotic pressure either, since the most species tolerate higher osmotic pressures in sea water than in sodium chloride. The difference between the osmotic pressure tolerances in these two solutions is, however, smaller than the difference between the osmotic pressures tolerated in sodium sulphate and sea water respectively. The physiological differences between sea water and sodium chloride solutions are therefore smaller than between sea water and sodium sulphate. The slightly lower tolerance of sodium chloride can be explained partially by the slightly higher concentration of sodium ions in a sodium chloride solution than in a sea water dilution of the same weight per volume concentration, which would have an increased toxic effect on the fish. In addition the presence of other ions in the sea water makes sea water a socalled "balanced" solution (Jullien et al., 1959) with an optimal ionic composition, which gives rise to a higher tolerance of sea water by the fish. The toxicity of high concentrations of sodium ions has been well documented by Doudoroff and Katz (1953), while Garrey (1916) and Jullien et al. (1959) have found that the solution of mixed salts, which has the least toxic effect at high concentrations on fish, is one with a composition closely approximating that of sea water. Further experiments have proved that the important ions in this latter respect are the divalent magnesium and calcium ions. Heilbrunn (1956) and Brown and Danielli (1964) ascribe the action of these divalent ions to the stabilisation of cell membranes and the reduction in permeability which they produce.

The differences in tolerance to a certain solution, which are exhibited between the various species, cannot be adequately explained in terms of present physiological knowledge. In part it must be due to differences in osmo-regulatory and ionic-regulatory ability and in part to differences in the susceptibility of the body to toxic factors once regulation is reduced. The actual physiological mechanisms operating in this case would provide an interesting field for future investigation.

Further information on the physiological action of the different ions is provided by freezing-point depression data. In *Barbus holubi* held in a lethal concentration of sea water, death is correlated with a rapid increase in osmotic pressure of the blood, which indicates loss of osmo-regulatory ability, as is evident from figure 9. The same phenomenon operates in *Labeo umbratus* in lethal levels of sea water, but since the sea water concentration is higher than in the case of *B. holubi*, the final freezing-point depression of the *Labeo* serum is also higher. In both these species osmoregulation breaks down and the fish become isosmotic to the environment. These results do not allow any explanation of the slight difference in sea water tolerance between these species. The very small increase in osmotic pressure of the serum observed when *Labeo umbratus* is exposed to sublethal and lethal sodium sulphate concentrations confirms the postulated, toxic effects of the sulphate ion.

The symptom sequence in harmful concentrations.

The sequence of symptoms, as observed in a particular species, is the result of the progressive action of the lethal factors on the physiology of the fish. Many of these symptoms have also been described from fish exposed to toxic substances, for instance heavy metals, or to abnormal temperatures, so that they do not depend on the actual cause of the damage, but are the product of a general physiological breakdown process.

The white precipitate produced by some of these freshwater species has a mucous nature, and is probably due to the precipitation action of high salt concentrations on the mucous produced by exposed epithelial tissues, especially the gills. Why this precipitate has been observed in some species only, and also not in all the solutions to which the species in which it is found are exposed, is impossible to say.

The sensitivity of the fish to disturbance, their rapid movements and the eventual partial loss of equilibrium indicate definite interference with the normal functioning of the central nervous system. Similar symptoms have also been observed in low temperature work on fish, by Ernst (personal communication) and by the present author. At these low temperatures, the symptoms are accompanied by a slowdown of the impulse transmission rate in the nervous system, as demonstrated by Roots and Prosser (1962). Similar interference may result from inefficient osmotic and ionic regulation, since the operation of the nervous system depends to such a large degree on a constant internal environment and constant ionic concentrations of the extracellular fluid. The muscular tetany observed on death in some Tilapia sparrmanii can also be explained in terms of osmotic interference with the muscle cells, which are also dependent on the ionic content of the body for correct functioning. A different point of view is held by Jullien et al. (1959), who consider that the respiratory processes are the first to be affected by loss of regulatory abilities, since ingress of salts disrupt the acid-base balance of the blood, and therefore also the oxygen-carrying capacity of the blood. This would not only interfere with the functioning of the central nervous system, but soon leads to death. It can be added that *Sandelia capensis* never made use of its supra-branchial respiratory chamber by gulping air. The suggested interference with respiratory processes is not supported by this observation. The actual physiological process which causes eventual death is therefore still open to doubt.

Another symptom of damage by high salt concentrations, observed in some species, consists of a darkening of the body, often accompanied by an enhancement of other body colours in *Tilapia sparrmanii*. This change in colour is due to the dispersion of chromatophores within the skin, where they are normally present in the contracted state, and can also be explained in terms of the increased salt content of the body following the impairment of osmotic and/or ionic regulation by the high external salinities. Novales (1959) and Waring (1963) have both observed similar melanophore dispersion on the addition of sodium chloride "in vitro" to isolated frog skin. The absence of this symptom in some species is probably due to some peculiarity of their melanophore system, since it was absent from those species which did not show any signs of black pigmentation under normal conditions.

Length of survival and concentration.

The high survival times of the lower concentrations can be ascribed to the "resistance" which the fish offer to these concentrations. "Resistance" in this sense includes the regulation of absorbtion and excretion of harmful substances, and other physiological controls in the body. The sudden decrease in survival time experienced over the lethal range of concentrations has been ascribed by Brett (1956) to a replacement of "resistance" by "tolerance". "Tolerance" is the ability of the fish to live in sublethal and lethal concentrations for a limited time. This "tolerance" is not the result of any physiological regulation, but is the tolerance of the body cells themselves to a changed internal body environment and to harmful substances which have entered the body. The presence of a threshold, as established in all cases where sufficient data are available, indicates that at these threshold concentrations the fish pass from the zone of "resistance" into the zone of "tolerance" where death occurs. Understandably, the length of this tolerance period will be reduced very rapidly even by small increases in concentration, as is evident from the relation between median survival time and concentration over the tested concentration ranges. The "resistance-tolerance" concept has also been found to apply to the action of low temperatures (Brett, 1956) and to the effects of variations in external oxygen concentrations (Hughes, 1964).

Gill histology.

The results summarised previously indicate that in Barbus holubi no evidence of specialised acidophilic cells could be found. The conclusion is that these cells are absent from Barbus holubi, and that their presence is not even observable after an exposure to a sublethal sodium chloride concentration. Since this species does not osmoregulate in hypertonic solutions, according to the evidence of survival experiments and the determination of freezing-point depressions of its serum, there is no need for any structure on the gill to serve as excretory tissue. Even if the S-cells do have a chloride-excretory function, as maintained by most workers, they are not necessary in this species and are accordingly absent. The present investigation does not therefore throw any light on the actual function of these cells, because they are absent from the species studied. On the basis of this conclusion, the presence of these cells in other cyprinids (Datta Munshi, 1964), which also occur in freshwater only, cannot be explained and it is doubted if the cells seen by Datta Munshi can have an osmoregulatory function, in spite of their high chloride content.

6. SUMMARY AND CONCLUSIONS.

A number of experiments were undertaken to determine the tolerances of *Barbus holubi*, *B. pallidus*, *B. asper*, *B. trevelyani*, *B. afer*, *Labeo umbratus*, *Tilapia mossambica*, *T. sparrmanii* and *Sandelia capensis*, where possible, to solutions of sodium chloride, sodium sulphate, and sea water dilutions. These experiments consisted of two types, a simple survival experiment with rapidly or gradually increasing concentrations, and time-survival experiments in which the results were subjected to the probit transformation to provide estimates of median survival times and median tolerance limits.

Tilapia mossambica is the only species tested in the present study which can disperse through the sea. None of the other species are tolerant enough to allow dispersal through the sea, under normal conditions, and therefore have to disperse between river systems by means of freshwater links. The present distribution patterns provide additional evidence for the suggested dispersal methods. Very favourable conditions, including currents, heavy rains, or the socalled "freshwater" masses within the sea, could assist in the dispersal of intolerant fish through the sea between very closely situated river mouths, but conditions in the long estuaries and elsewhere appear to be limiting and prevent dispersal by this method.

Because of the inconsistent salinity tolerance of the cichlids used, it is concluded that the categories suggested by Myers can only be applied to the species, and not the family as done by Myers and Darlington. Only T. mossambica can be regarded as a member of the secondary division, and the other species are all members of the primary division.

The cichlids tolerate high sodium chloride concentrations better than any of the other species tested, and is followed by Sandelia capensis, and the various cyprinids. Two of the cyprinids with a very localised distribution have a comparatively low sodium chloride tolerance.

The species most tolerant of sodium sulphate is Labeo umbratus, which is closely followed by the other species. The species with the lowest tolerance, Sandelia capensis, also has a very limited distribution.

In all the species tested, with the exception of L. umbratus, the sea water tolerance exceeds the sodium chloride tolerance, which again exceeds the sodium sulphate tolerance the lowest (all concentrations expressed in parts per thousand). This relation is not due to differences in osmotic pressure of the solutions, and is the result of the toxic effects postulated for sodium sulphate on the one hand and the presence of beneficial divalent ions in the sea water on the other hand.

According to serum freezing-point depression determinations, the death of B. holubi and L. umbratus in sea water dilutions is correlated with the loss of osmotic regulation in high concentrations. In L. umbratus, death in sodium sulphate solutions is not due to osmotic interference with the body fluids. Sodium sulphate must therefore have some toxic effect on the fish.

The symptoms, which the various species exhibit on exposure to any of the test solutions, all adhere to the general pattern. Initially the fish show signs of increased nervous activity, which is soon followed by temporary loss of equilibrium. Permanent loss of equilibrium is the next step, and death follows after respiratory distress has become evident. In some species mucus secretion or melanophore expansion are also observed. All these symptoms can be explained by increased internal salt concentrations and interference with the central nervous system, after impairment of osmotic or ionic regulation by the high salt concentrations.

In all the time-survival experiments, the median length of survival and the various concentrations in which these values are obtained, satisfy the equation

$$C^{n}T = k.$$

The median tolerance limits of a species for a given solution also vary inversely with the length of time they were exposed to it. In the lower concentrations of each solution, the fish are in the "resistance zone", in which they can live for very long periods. The lethal concentration range is called the "tolerance zone" in which the fish can only survive for limited periods, till normal conditions can be restored or till the fish die.

No cells were found in the gills of Barbus holubi which agree in description with the so-called "chloride excreting cells" originally described by Keys and Willmer (1932). Whether the S-cells, the term preferred in the present study, are actually osmoregulatory in function cannot be answered from the present study, since they are completely absent from B. holubi with its limited osmoregulation abilities.

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