

The ecology of juvenile Rhabdosargus holubi (Steindachner)
(Teleostei:Sparidae)

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INTRODUCTION

Estuaries have received considerable attention from biologists in southern Africa in the past thirty years. Professor J.H. Day and the Zoology Department of the University of Cape Town have undertaken ecological surveys of a large number of estuaries of a variety of types, laying the important groundwork for more detailed studies of single species or problems. Their studies showed that the fish populations of southern African estuaries consist mainly of marine species. Why and when these species enter or leave the estuaries and whether they grow or breed in them were not established. The only relatively detailed study of a fish in southern African estuaries was that of Talbot (1955) who investigated aspects of the growth, feeding and reproduction of Rhabdosargus globiceps (Cuvier). No quantitative information has been published on any of the fish species, perhaps due to the difficulty of obtaining such data; results such as population estimates, mortality rates and growth rates are particularly unreliable when the area being investigated is in direct contact with the sea. Studies relating to even a single species of fish are hampered by immigration and emigration between estuary and sea, which make the population continuously variable. The larger estuaries are also difficult to sample adequately, especially with regard to netting, except perhaps on a very large scale using expensive commercial equipment.

Along the southern African coast many of the smaller estuaries with a limited catchment area are cut off from the sea for most of the year due to erratic rainfall and longshore drift of sand. These closed or 'blind' estuaries of south eastern Africa, of which there are at least thirty in the eastern Cape Province, provide excellent

areas for studying estuarine fish populations which enter when the estuary is open to the sea, but become isolated once it closes. These fish which become cut off in closed estuaries are subject to a new series of conditions. They are exposed to the wider temperature and salinity fluctuations of an estuary and they are forced to utilise the food resources of the estuary. Additionally they may be subjected to predation from piscivorous birds, and those predatory fish which are also cut off in the estuary. These factors will affect the size, mortality, and growth rates of a fish population. Since the fish are isolated no recruitment from the sea can take place, and any increase in numbers would have to come from breeding within the estuary.

It is inevitable that the most numerous species should receive attention first. Rhabdosargus holubi (Steindachner) (Synonymy : Sargus holubi Stndr, Austrosparus tricuspidens Smith, Rhabdosargus tricuspidens (Smith)) (Plate 1) is one of the most abundant fish in the estuaries of the eastern Cape Province. According to Smith (1965) it is endemic to southern Africa, occurring from the Cape to Zululand, being most common between Mossel Bay and East London. It is largely replaced by Rhabdosargus sarba (Forskäl) in the north of its range and by Rhabdosargus globiceps (Cuvier) in the south. Commonly called the 'flatty' or silver bream it is considered only as being of nuisance value by fishermen, seldom exceeding 30 cm in length.

In this study the ecology of R.holubi was examined in relation to the closed West Kleinemonnd estuary which is a small 'blind' estuary typical of those found along the eastern Cape coast. The growth, mortality and population size of R.holubi in this estuary were

investigated and linked where possible with predation and detailed studies into the tolerances, food, and feeding of the species. It was not known at the commencement of the study whether breeding could occur in estuaries, although Smith (1965) stated that R.holubi does breed in estuaries. During the course of the sampling programme in the West Kleinemonde estuary, measurements of the growth and population structure of two other species of fish were recorded for comparison with R.holubi : the sparid Lithognathus lithognathus (Cuvier) (White Steenbras) and the piscivorous carangid Hypacanthas amia (L.) (Leervis or garrrick).

Laboratory studies on R.holubi were possible due to the fact that large numbers of this species could be caught in eastern Cape estuaries and transported to Grahamstown, where they could be maintained in captivity for up to four months in recirculating seawater aquaria. During the course of the study over 2000 fish were kept in captivity at different times for a variety of experiments. Another 2500 were preserved for gut analyses and lipid determinations, while over 5000 were captured, examined and released in the field.

PART I

POPULATION STRUCTURE AND GROWTH

INTRODUCTION

In common with many of the smaller estuaries of south east Africa, that of the West Kleinmond may be closed for long periods by a sandbar which is built up at the mouth by longshore drift and south westerly winds. It is usually only open to the sea for short periods after heavy rain in the catchment area has caused a rapid rise in water level. During the times of opening marine fish populations can enter the estuary. The populations which enter at these times become isolated when the mouth closes. The species of fish which most commonly become isolated in the West Kleinmond estuary are Rhabdosargus holubi, Lithognathus lithognathus and Hypacanthus amia, together with several species of Mugil. During the period from 1971 to 1973 a large number of R.holubi became isolated in the West Kleinmond estuary thus affording an opportunity to examine certain aspects of their biology. During this period the estuary opened on two occasions, once at the commencement of the survey and again after ten months. Thus the study period spanned a complete opening and closing cycle and provided an opportunity to follow any possible changes in population structure. This first part of the study is devoted to the population structure and growth rate of R.holubi.

MATERIALS AND METHODS

1. Sampling area

The West Kleinmond river is situated 16 km north of Port Alfred (Fig. 1). It is 18 km long and has a catchment area of 52 km². Freshwater from the river flows into the estuary over an artificial

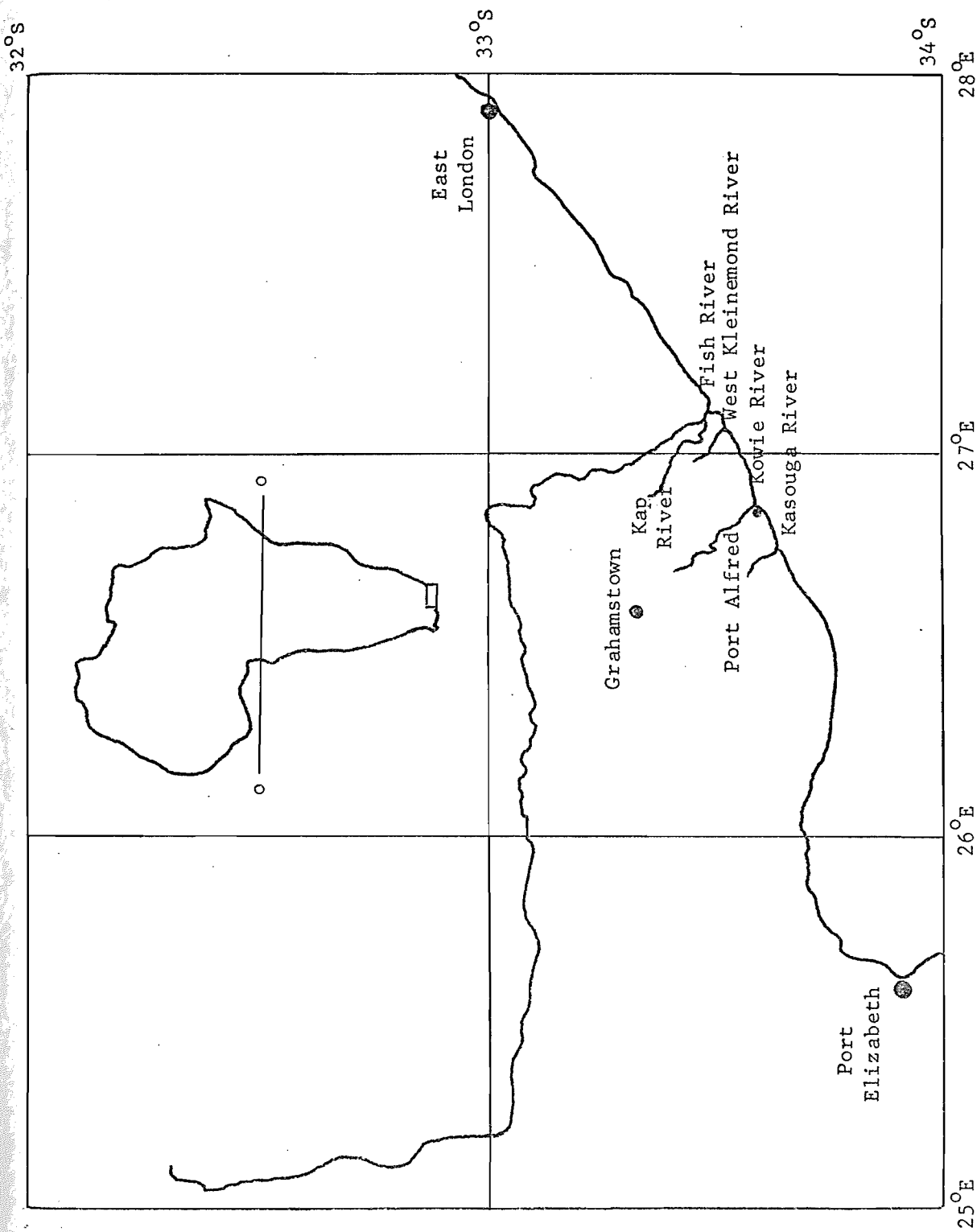


Figure 1: South east Africa, showing positions of estuaries investigated. Insert: Africa, showing position of map area.

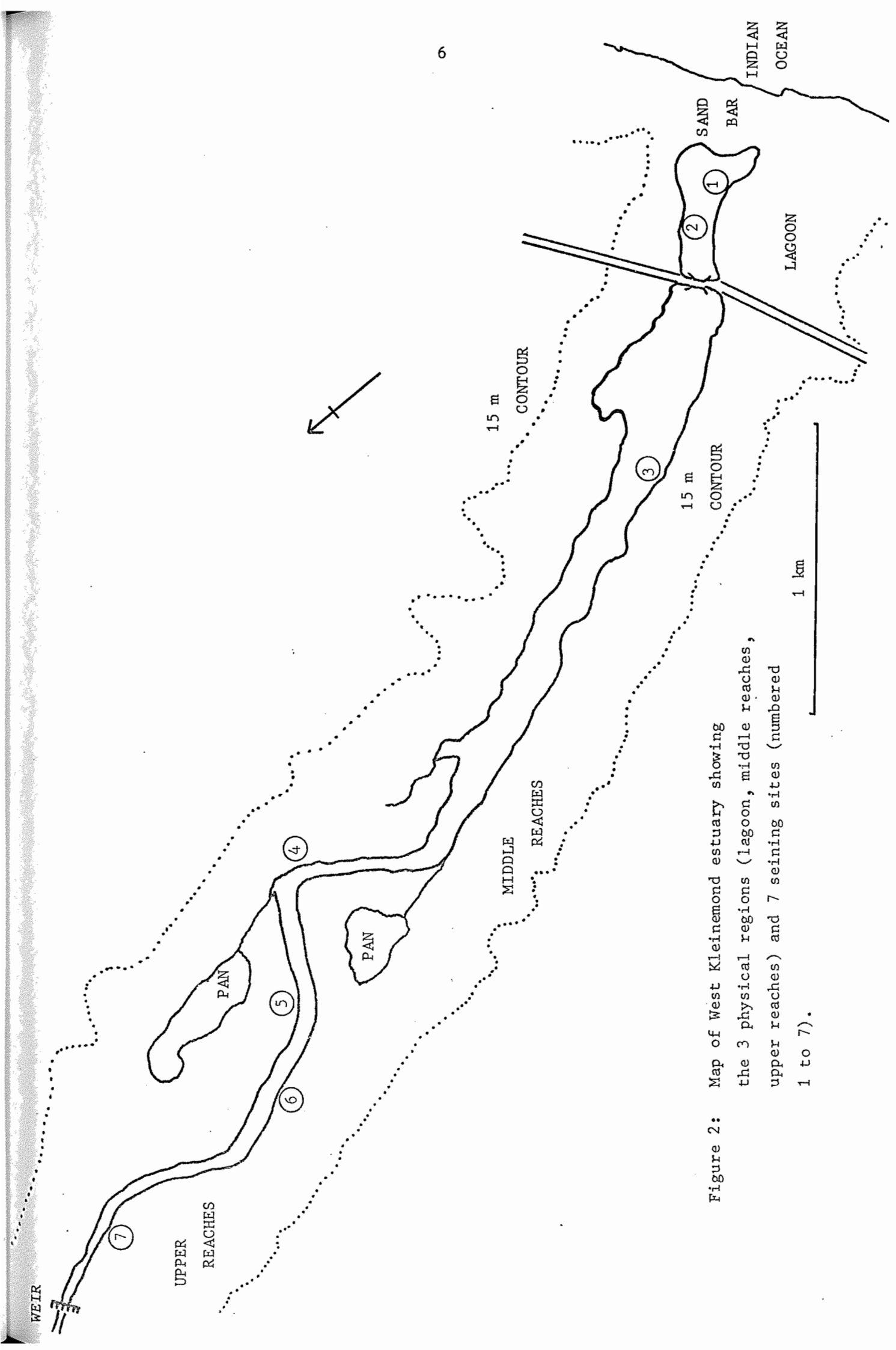


Figure 2: Map of West Kleinmond estuary showing the 3 physical regions (lagoon, middle reaches, upper reaches) and 7 seining sites (numbered 1 to 7).

weir which divides estuarine water from riverine water 5 km from the sea. The estuary is divisible into three regions (Fig. 2). At the seaward end is a 200 m wide lagoon not more than 3 m deep. This area has a sandy substrate and extensive shallow margins on which the submerged macrophyte Ruppia spiralis L. grows. The lagoon is separated from the middle reaches by an embankment and bridge. The middle reaches narrow from 200 m in width to about 50 m 2 km above the bridge and do not exceed 2 m in depth. They are similarly sandy, with R. spiralis commonly present over the whole width. The 2.5 km long upper reaches (Plate 2) are only 20 to 30 m wide and are characterised by steep muddy banks and deeper water of up to 5 m.

Rainfall in the catchment area is erratic and seasonal, usually occurring in spring and autumn and averaging about 525 mm per annum. During the period of study the heaviest rains which caused the estuary to open came in spring. Thus the estuary may remain closed for long periods. West Kleinmond estuary opened to the sea in September 1970 and closed in December 1970. It reopened in August 1971 and remained open until December 1971. Thus it was cut off from the sea between January and August 1971 and from February 1972 onwards. This study of population structure and growth was carried out from January 1970 until March 1973.

2. Sampling of fish

Fish were sampled using a 33 m long seine net, 4 m deep with a 1.3 cm bar mesh size and without a bag. This net was laid using a boat powered by an outboard motor. It was towed out in a semi-circle from the bank (Plate 2) and then pulled in by hand. The soft nature of the substrate and the steep banks in the upper reaches precluded pulling the seine out from the bank by hand. The mesh size of the



Plate 1. Rhabdosargus holubi (Steindachner)
(Courtesy J.L.B. Smith Institute of Ichthyology)

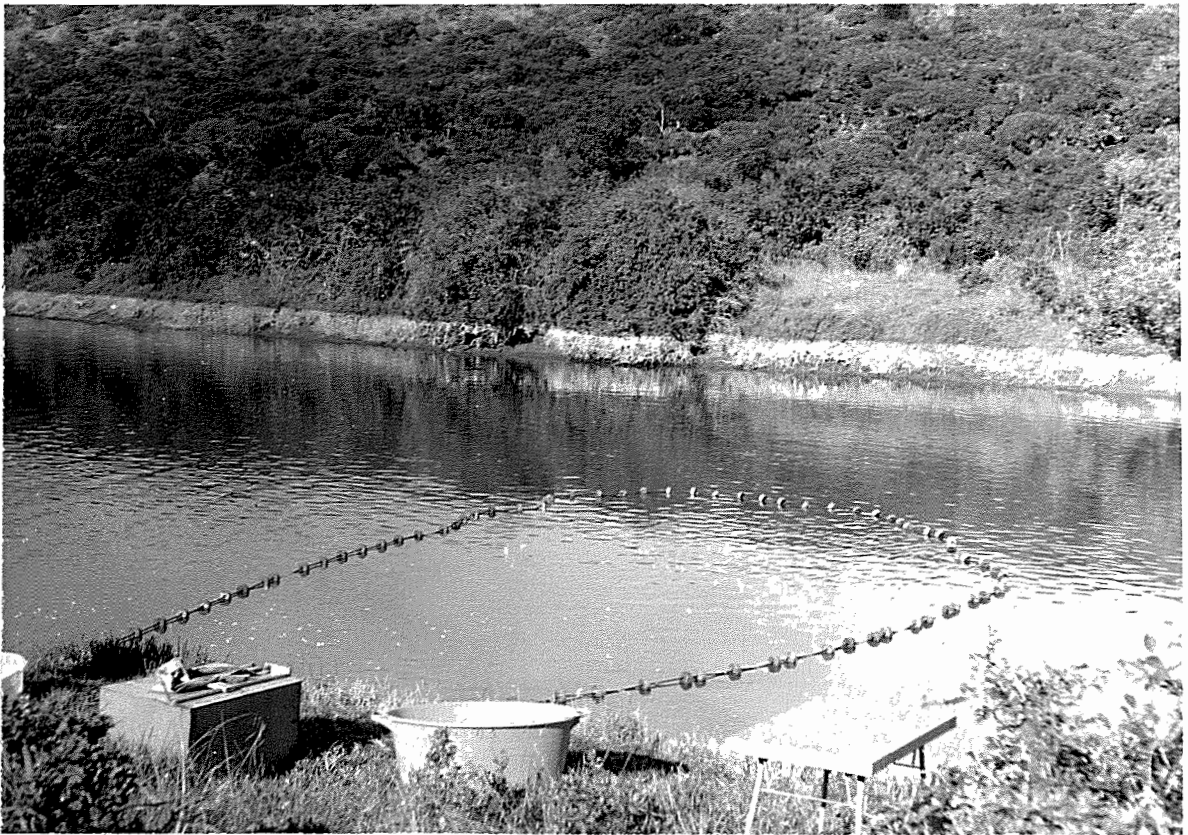


Plate 2. Seine net ready for pulling in at Station 6 in the upper reaches of the West Kleinmond estuary. Note flood level mark on west bank.

net was sufficiently small to catch all R.holubi above 4 cm standard length (S.L.). Seining was carried out once a month at seven sites along the estuary (Fig. 2). Seven seining operations were performed at each site and the duration of each was approximately five minutes. Usually it took one week to work all seven stations. Length and weight measurements were taken in the field and all fish were returned to the estuary. The fish were weighed to the nearest gram on an Ohaus Scale Corporation "Dial-O-Gram" balance housed in a windproof shelter. R.holubi over 8 cm could not satisfactorily be marked with these tags because of the size of the tags in relation to the size of the fish.

The tag was inserted through the epaxial musculature below the dorsal fin at the junction of the hard and soft dorsals, thus the anchor of the tag lay on one side of the body and the vinyl tube bearing the number trailed on the other (Plate 3). The antibiotic "Terramycin" (in topical ointment form) was administered to the tagging wound to prevent infection. Chilvers and Gee (1969) have reported adverse effects of Floy FD67 anchor tags on freshwater fish in Lake Victoria. In order to determine the possible effects of tagging and marking on R.holubi a field trial was undertaken in the West Kleinmond estuary. 16 fish were kept for nine months in a 2 x 1.5 x 1.5 m plastic mesh cage (mesh size : 1.3 cm bar) (Plate 4). Seven R.holubi were marked with Floy FD67 anchor tags using the method described above, while the remaining nine had one or other of the pelvic, pectoral, anal, dorsal or caudal (upper and lower lobes) fins clipped. The cage was put into the upper reaches of the estuary so that the top was 1.5 m below the surface of the water. Although the fish could feed on the substrate through the mesh no aquatic macrophytes were present near the cage. The cage was lifted at monthly intervals and the fish measured and weighed. The experiment was terminated after nine months.



Plate 3. Rhabdosargus holubi 7 months after marking with Floy FD 67 anchor tag.

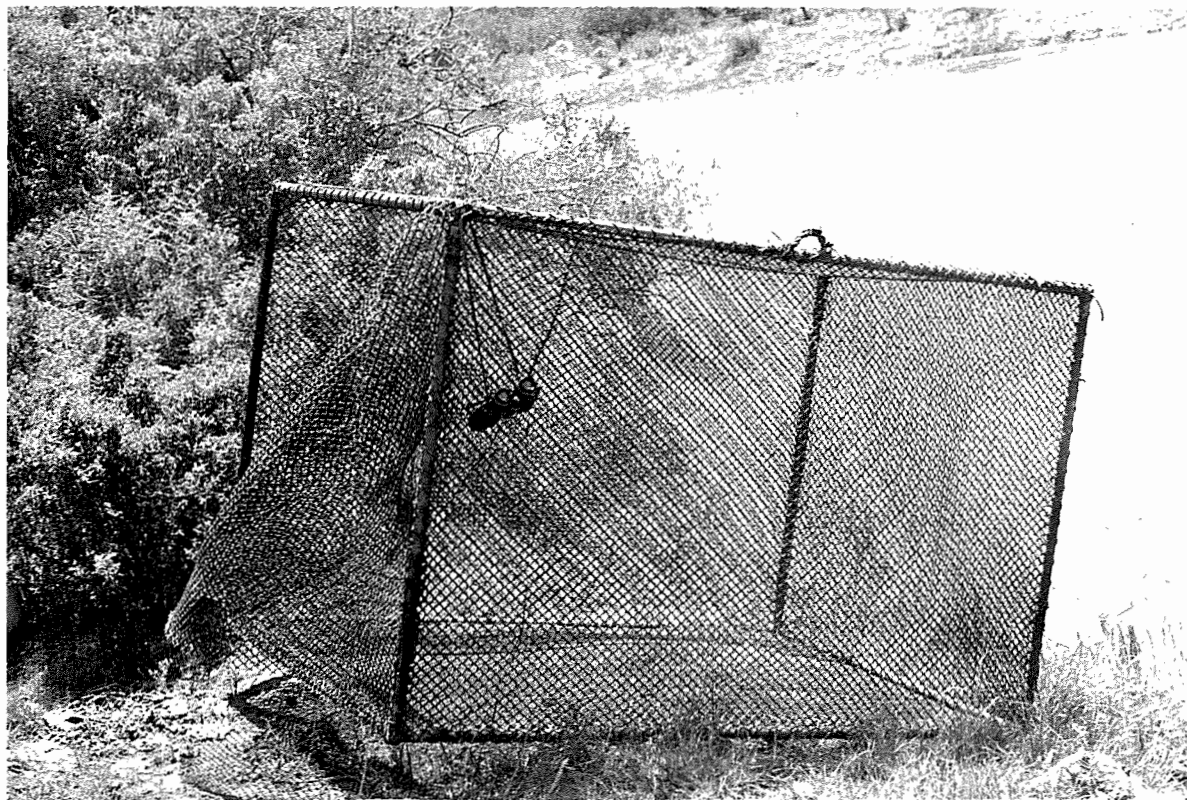


Plate 4. Cage used in 9 month experimental study of the effects of tagging on Rhabdosargus holubi, at Station 6 in the West Kleinmond estuary.

The length - weight relationships of R.holubi and L.lithognathus were computed using a Hewlett-Packard 9810A calculator. The growth rates of R.holubi were calculated from length frequency histograms and tag returns. The growth rates of L.lithognathus and H.amia during 1971 were calculated from length frequency histograms. Scales and otoliths of R.holubi were examined with a view to age determination and growth rate. Scales were collected from captured R.holubi and were removed from the anterior region just above the lateral line.

RESULTS

Investigation of scales from 410 R.holubi revealed that they were of no value in determining age or growth rate in the population studied although it is possible that in adults the rings might be useful. Scale rings were laid down but were irregular and did not correspond to season or any observed external phenomena. The scales from tagged recoveries showed no consistent increment of circuli although in some instances a ring was laid down that might have corresponded with the time of tagging. Talbot (1955) found that scale readings did not give consistent values in Rhabdosargus globiceps but otoliths did give some useful information on age when marine and estuarine individuals were examined. An opaque band and a transparent band were apparently laid down each year but estuarine individuals laid down only a transparent band for most of the year. The R.holubi in this study were all estuarine and were mostly 0 group fish and thus the otoliths provided no information on age or growth.

In the experimental cage in the West Kleinmond estuary (Plate 4) although one of the tagged R.holubi disappeared two weeks after marking, no mortality occurred in the subsequent nine months. None of the remaining fish grew in length and over the nine months there was a mean

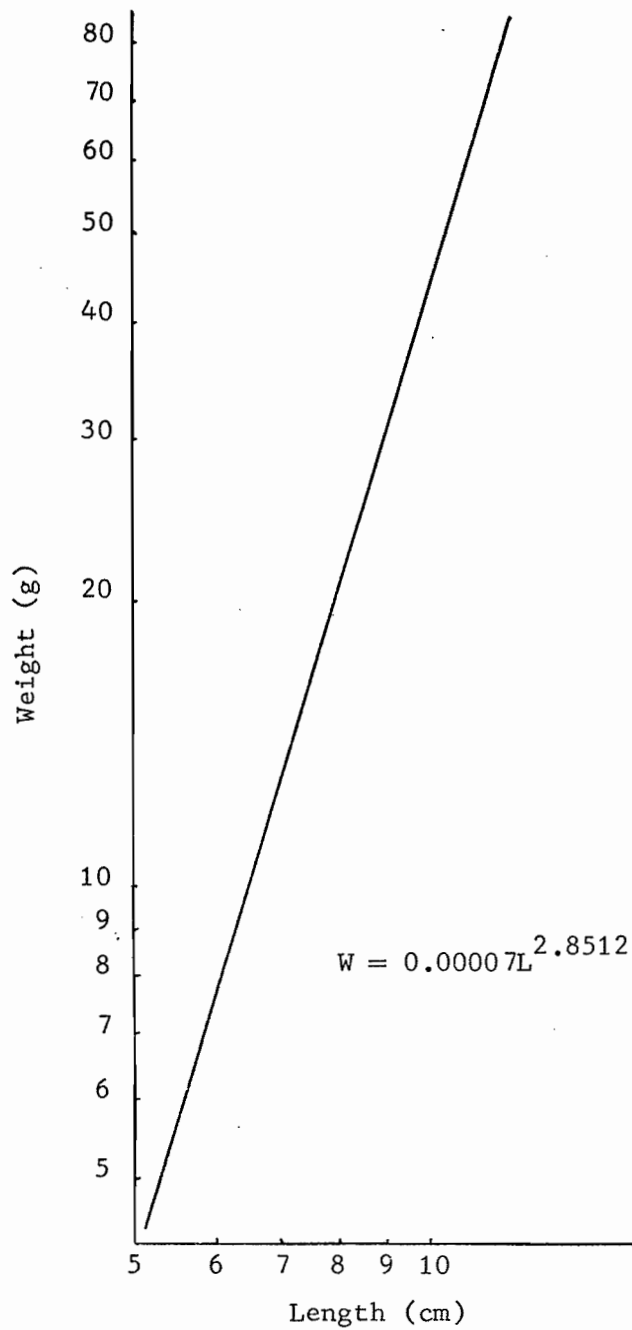


Figure 3. The length-weight relationship of juvenile *Rhabdosargus holubi* plotted on logarithmic axes, line based upon 1416 fish, correlation coefficient = 0.999.

weight loss of 19%. The lack of growth can probably be ascribed to the near starvation conditions under which the fish were kept; the substrate beneath the cage had no plants growing in it and the biomass of benthic invertebrates was only about 1 g dry weight per m^2 . This latter is in contrast to the value of 5.9 g/m^2 which was the mean biomass for the entire estuary (Batchelor, 1972). The lack of growth of tagged fish in the cage was in marked contrast to that of tagged fish released into the estuary which did show growth. In view of the low mortality under the severe conditions of the cage experiment it appears reasonable to assume that tagging of fish did not cause undue mortality.

The standard length - wet weight relationship of juvenile R.holubi was obtained from measurements on 1416 fish and is shown in Figure 3. The relationship between weight and length can be expressed using the formula $w = aL^b$, where a is a constant and b an exponent. The regression of weight upon length for the size group of juvenile R.holubi present in the West Kleinmond estuary is:

$$w = 0.00007L^{2.8512}$$

Similar regressions for the L.lithognathus present are:

- a) First year fish, 60 - 120 mm S.L.: $w = 0.00001L^{3.1222}$
- b) Second year fish, 120-200 mm S.L. : $w = 0.00003L^{2.9453}$

Length frequency histograms for R.holubi collected in 1971 - 1973 are shown in Figure 4. The sample size for each month is also indicated in the figure. Histograms are not shown for months where no shift occurred in the modal size such as between January and July 1971. The histograms show that in January 1971 the modal size was

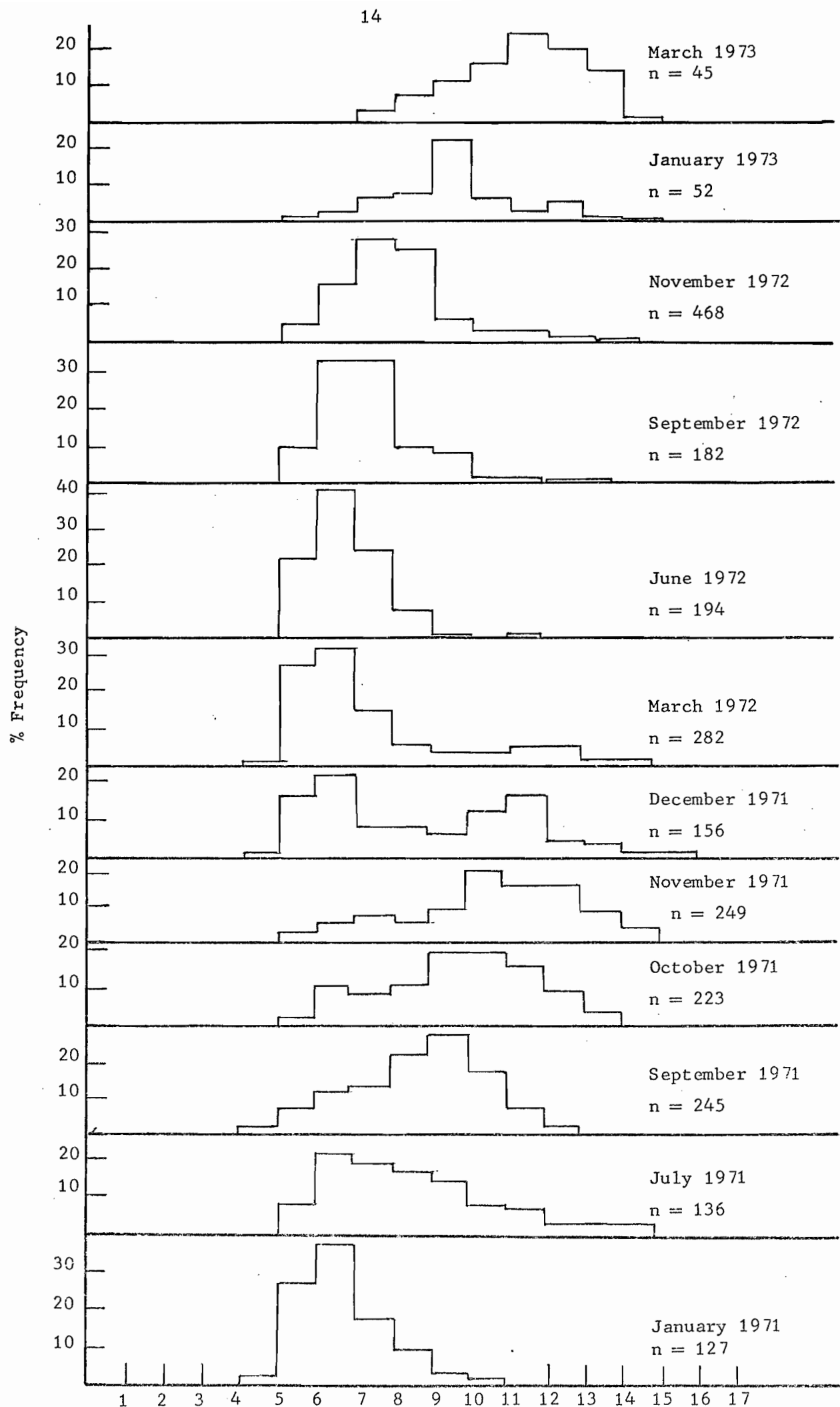


Figure 4: Population structure of *Rhabdosargus holubi* in the West Kleinmond estuary 1971-1973. Months in which no growth occurred have been omitted. n is the number of fish in sample.

6 cm and the population unimodal. In December 1971 the population had become distinctly bimodal. This was due to the appearance of fish in the 5 - 7 cm class. This new group then became the dominant element in the population, and the earlier population from 1971 had mostly disappeared by March 1972.

In August 1971 the estuary had opened to the sea. At this time a new population consisting mainly of juveniles of 1 - 2 cm S.L. invaded the estuary. These fish were however too small to be sampled by the seine net until they had reached a length of about 5 cm S.L. in December 1971. Thus only in December 1971 did the catch become bimodal. By the end of January 1972 the mouth of the estuary had completely closed. Before this occurred most of the first 1971 population had apparently emigrated, leaving a new unimodal population of juveniles in the estuary.

The length frequency histograms (Fig. 4) show that in both years a growth of 60 mm took place in summer between the months of September and March. The summer growth rate was thus about 10 mm per month. Only 13 tagged recoveries were made in 1971 but they all showed growth. The overall mean monthly growth rate from tag returns was 5 mm (standard deviation 3.37) but this growth was clearly different between summer and winter. The mean growth rate of seven fish tagged and captured between September and March (summer) was 10.3 mm while the mean growth rate of six fish tagged and captured between April and August (winter) was only 3.5 mm. The increase in the modal size of L.lithognathus from January to December 1971 is shown in Figure 5. It is evident that L.lithognathus grew 60 mm in their first year, thus showing the same growth rate as 0 group R.holubi. One year old L.lithognathus were also present in the estuary at the beginning of

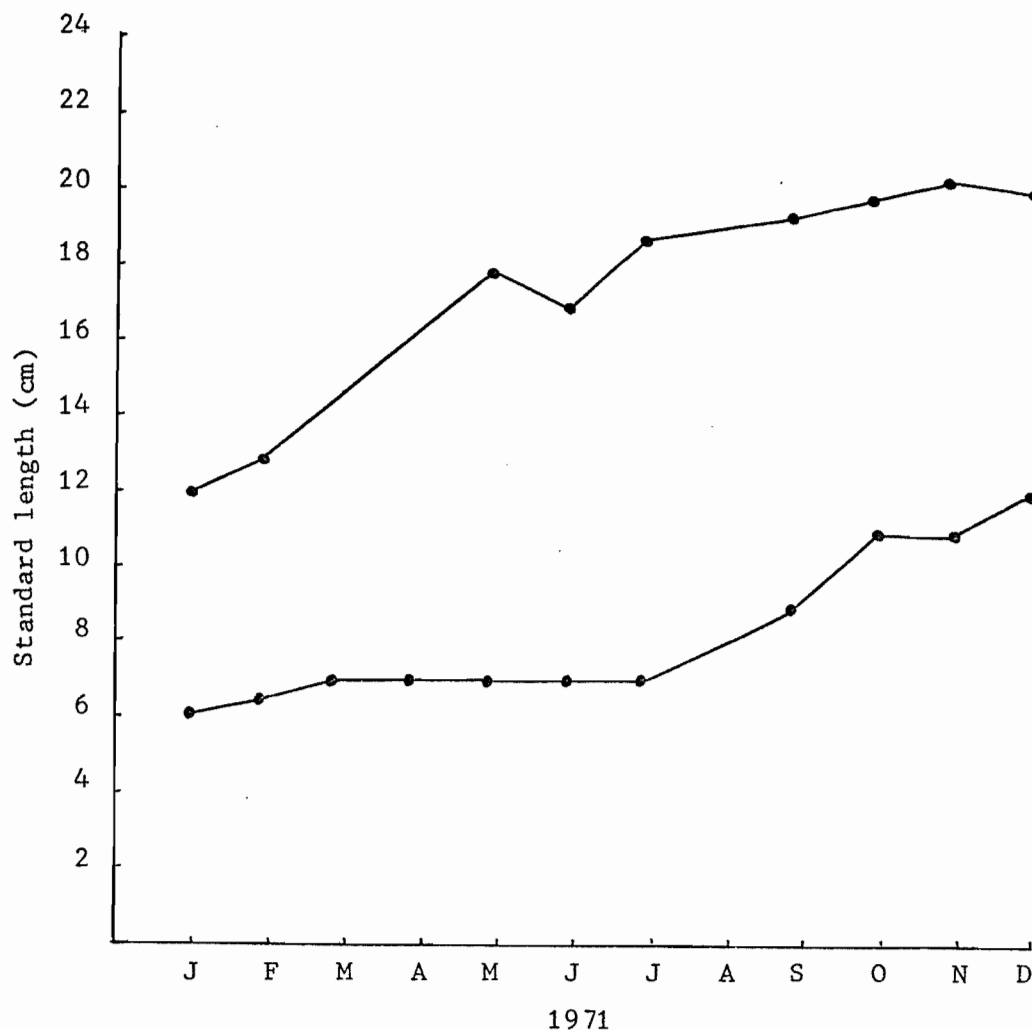


Figure 5: The increase in modal length of 12 size groups of Lithognathus lithognathus in the West Kleinmond estuary during 1971.

1971 and grew 80 mm per annum. Although only 50 H.amia were captured in 1971 the increase in their modal size from January to July 1971 was 110 mm (from 90 mm S.L. to 200 mm S.L.).

The University of Cape Town Ecological Survey records provide data concerning the reproductive maturation of R.holubi. In fish from Durban Bay it is evident that male R.holubi mature at about 14 cm but the smallest size in which ripe gonads were found was 26 cm. Female fish mature at about 13 cm and ripe gonads were found in fish of 14 cm.

It is very likely that R.holubi exhibits hermaphroditism since many of the sparidae are hermaphroditic, including Rhabdosargus sarba (Kinoshita, 1939). Few fish of 13 cm or over were captured in the West Kleinmond estuary and none of those examined had mature gonads. The gonads of all fish dissected, when visible at all, were small, thin and threadlike. Thus no mature fish would appear to occur in the West Kleinmond estuary even if they have reached a size at which maturity can occur.

DISCUSSION

The length-weight relationship of juvenile R.holubi shows that growth is allometric, that is that length and weight do not increase proportionally. The b value from the equation $w = aL^b$ is 2.8512 and is significantly different from 3 ($P > 0.002$, $t = 3.72$, d.f. = 18) which is the b value obtained when growth is perfectly isometric. The length-weight relationship calculated here is for juveniles only and should not be extrapolated since it is likely that the relationship changes with maturity. The length-weight relationship of L.lithognathus changes markedly after the first year; the b value changing from 3.1222 to 2.9453. Little data are available on the length-weight relationships among other Sparidae, although Suau (1970) showed that the b value of Lithognathus mormyrus (L.) was 2.956 for fish between 100 and 200 mm in length, a value very comparable to that of 2.9453 obtained here for L.lithognathus of similar length.

The growth results from length frequency data and tag returns are similar, both indicating a total summer increment of about 60 mm for juvenile R.holubi. Length frequency histograms do not show growth over winter although the tag returns suggest that growth does not entirely cease at this time. According to the 1971 histograms

growth began in September, immediately after the estuary had opened to the sea, but since growth also began in September 1972 when the mouth remained closed it seems unlikely that the opening had much influence on growth in 1971. The fact that the tagged fish, released into the estuary grew at the same rate as the overall population indicates that tagging does not significantly affect growth.

0 group L.lithognathus in the West Kleinmond estuary showed a similar growth pattern to R.holubi, having a 60 mm annual increment. However first and second year L.lithognathus were present in the estuary, unlike the situation in R.holubi.

Talbot (1955) recorded that R.globiceps has an annual growth of 60 mm, the same as the results obtained here for R.holubi. On the New South Wales and Queensland coasts Kesteven and Serventy (1941) state that the subtropical sparid Roughleyia australis Gunther had first and second year increments of 110 mm and 120 mm respectively, which is far greater than R.holubi although it is interesting to note that it is similar to the growth obtained here for H.amia. Although R.holubi is a warm temperate species its annual growth rate is similar to that of juvenile benthic species from colder waters; Percy (1962) showed that juvenile Pseudopleuronectes americanus (Walbaum) grows 60 mm per year in Connecticut waters; Macer (1967) working in the Irish Sea demonstrated that Pleuronectes platessa L. grows 60 mm per year; similarly Jones (1973) reports a 60 mm annual increment for 0 group Scophthalmus maximus (L.). Caldwell (1955) working in the warm temperate waters of Florida showed that 0 group Diplodus holbrooki (Bean) grew 60 mm during summer and almost ceased growth in winter, exhibiting the same pattern as R.holubi. An annual increment of 60 mm thus occurs in many 0 group juvenile marine fish. The fact

that juvenile R.holubi and L.lithognathus also grow 60 mm per year in the closed West Kleinmond estuary is therefore extremely significant and suggests that the closed estuary did not unduly restrict growth.

Brown (1957) stated that food supply is the most potent factor affecting the growth of fishes since growth cannot take place unless sufficient food is available. Other factors especially physical ones, such as temperature and salinity variations, may also affect the growth rate of fish (Kinne, 1960) and these factors may be of considerable importance where estuarine fish are concerned, since both are liable to fluctuate. In view of the similar growth in 1971 and 1972 it seems reasonable to assume that the environment was similar in the two periods of closure and that the food supply in the West Kleinmond estuary was adequate for both maintenance and growth. The quality of the food as well as the quantity must be of importance and this will be significant in a closed estuary where the variety of different foods available may be restricted.

When the mouth of the estuary opened in August 1971, the then resident population of juvenile R.holubi emigrated and the estuary was recolonised by smaller juveniles. Obviously the extent of the re-population must depend upon the length of time the estuary remains open. In this survey this period was never less than one month, thus allowing time for a considerable exchange of fish to take place. The time of year at which the mouth opens is of importance with regard to the size of R.holubi which enter. Both openings of the West Kleinmond which occurred in the course of this study took place in August, and allowed populations of 1 to 2 cm S.L. fish to colonise the estuary. Wallace (1973) has suggested that the spawning times of several species in Natal coincide with the onset of spring rains

which cause many of the estuaries to open. The data here would seem to support this idea.

When closed estuaries are open to the sea they must be regarded as equivalent to permanently open estuaries, such as that of the Kowie River (Fig. 1). One hundred R.holubi were captured by seining in the Kowie estuary. All ranged in size from 5 to 15 cm S.L. and were thus probably 0 group fish. Only one fish of longer than 15 cm S.L. was captured in the Kowie estuary. All records of larger R.holubi from the University of Cape Town records are from the sea or the lower reaches of large open estuaries. It is clear from the size frequency data that only juvenile R.holubi utilise the closed West Kleinmond estuary, and the apparently low numbers in the inshore coastal waters makes it probable that estuaries in general form extremely important areas for growth in the first year of life.

PART II

POPULATION DYNAMICS

INTRODUCTION

Although Day (1967) discussed the biology and interrelationships of some of the fish species in Knysna he was not able to give any quantitative data due to the complete absence of work of this nature in southern African estuaries. The closed West Kleinmond estuary would appear to provide an excellent environment for a detailed study of the population dynamics of estuarine fish. As described in Part I the West Kleinmond estuary was subject to two separate invasions of juvenile Rhabdosargus holubi in 1971 and 1972. Although many marine fish do invade estuaries there has been no attempt to quantify the recruitment or to analyse the possible mortality which may occur before the fish return to the sea. The closure of the West Kleinmond estuary from the sea made it possible to investigate quantitatively the population sizes and mortality rates of R.holubi which became isolated from the sea from January to July 1971 and again from March to November 1972.

Estimates of the numbers of fish in a population are usually imprecise, mainly due to sampling difficulties, and this study is no exception. Provided that several independent techniques are used however, and the limitations of the methods realised, the resulting data are meaningful when comparing relative sizes of populations.

Once the fish are isolated they become subject to a new group of predators, namely piscivorous birds. The shallow waters of the estuary make them particularly vulnerable to bird predation when compared with

the deeper waters of the sea. It was decided that an attempt should be made to assess the relative importance of this bird predation on the fish population. The numbers of different piscivorous birds and their food were therefore investigated at the West Kleinmond and Kasouga estuaries during 1972.

MATERIALS AND METHODS

1. Sampling of fish

The capture of R.holubi was undertaken over one week in every month at seven sites along the estuary (Fig. 2), as described in Part I. Fish over 8 cm S.L. were tagged with Floy FD67 anchor tags (Plate 3). Fish under 8 cm S.L. were fin-clipped using the right or left pelvic fin on alternate months in order that the time of marking could be traced upon recapture. The time of marking could then be determined according to the amount of regeneration of the clipped fin. For estimation purposes, it was only necessary to be able to distinguish those fish marked in the previous month from those marked earlier in the year.

The fish Hypacanthas amia (L.) was occasionally captured in the seine net in the West Kleinmond estuary. This species is a fish eater (Smith, 1965; Day, 1967) and a possible predator on R.holubi. The stomach contents of all H.amia captured in the West Kleinmond estuary in 1972 were therefore analysed.

2. Methods of statistical analysis of mark-recapture data

Estimates of the numbers of R.holubi present from January to August 1971 and from March to November 1972 were made from mark-recapture data. A great variety of mathematical treatments for

mark-recapture data are available and recent reviews include those of Pope (1961), Jones (1965), Cormack (1968) and Robson and Regier (1968). Capture-recapture experiments consist of marking and releasing animals on one occasion and subsequently resampling and recording the number of marked and unmarked animals in the recapture sample. The various methods of estimating population size all rely essentially upon the ratio of marked to unmarked animals in the recapture sample. Marking may either take place on a single occasion or on a regular series of occasions. In the first simple method the proportion of marked individuals remains constant whereas in the multiple method the number of marked individuals increases as the experiment proceeds. A considerable number of mathematical refinements have been devised to account for such factors as recruitment (Jolly, 1965) or differential mortality between marked and unmarked animals (Jones, 1965).

As shown in Part I there was no significant differential mortality between marked and unmarked animals in the West Kleinmond estuary; neither was there any immigration or emigration as the population was isolated from the sea. Since the population consisted of juveniles no recruitment could occur due to breeding. Therefore these factors did not have to be taken into account when choosing estimation methods. Confidence intervals in all treatments are related to the number of recaptures and the ideal situation may be impossible to attain under field conditions. Thus it was decided to use both the simple Petersen method and the more complex multiple mark-recapture method advocated by Robson and Regier (1968) in order to obtain two independent series of estimates for comparative purposes.

Both methods rely upon similar principles and require that the following conditions be present during the experiment:

1. marked fish become randomly distributed in the population
2. marked and unmarked fish undergo the same mortality
3. samples must be drawn from the population at random
4. marking must in no way influence subsequent capture
5. there must be no recruitment into the population

Buck and Thoits (1965) checked a number of Petersen estimates by draining censuses and by the use of rotenone and found that the errors of the estimates varied from 1% to 96%. They stated that the major source of error in the artificial ponds that they investigated was the lack of random distribution of marked fish which led to a disproportionally high number of marked individuals being recaptured. Perhaps, however, the lack of random distribution could be attributed to the fact that the fish were resampled after only 48 hours.

During the study in the West Kleinmond estuary four weeks elapsed between each sampling, allowing adequate time for marked fish to become distributed in the population. Most recoveries were on fin clipped fish and thus no information could be obtained about movement from these individuals. However tagged fish recoveries did show that the fish became randomly distributed through the population. In this respect the relatively small size of the West Kleinmond estuary was a definite asset. The use of Floy FD67 anchor tags and fin-clipping apparently had no significant differential effect on mortality and did not appear to increase the chance of recapture. Since the population was enclosed and juvenile, no immigration, emigration or recruitment through breeding could occur.

The Petersen method consists of marking animals on one occasion and sampling for recaptures on a single subsequent occasion. In

such a two sample experiment the proportion of marked to unmarked fish in the recapture sample provides an estimate of the proportion of marked to unmarked fish in the population from which the sample was drawn. Thus in this method where samples were taken monthly, each pair of monthly samples was used to obtain an estimate of the population. A period as long as one month between samplings made no difference to Petersen estimates because no emigration or recruitment could occur. Natural mortality in between samplings does not violate any of the conditions for valid estimates as it occurs independently of marking. The formula for the Petersen estimate is : $N = \frac{mc}{r}$ with a standard error of $N = N \frac{(N - m)(N - c)}{mc(N - 1)}$ (Robson and Regier, 1968) where N is the estimate of the population, m is the number of fish marked, c is the number of fish in the recapture sample and r is the number of marked recaptures.

The multiple mark-recapture method advocated by Robson and Regier (1968) was used to obtain successive estimates of N using the accumulated data from previous months. The rationale underlying this technique is that the proportion of fish not caught at all in the experiment is an estimate of the probability of not being caught. The chief limitation of this method is the increasing complexity of the calculations involved, as the number of samplings increases, especially where relatively large numbers are being dealt with. The limitation was overcome by employing a computer programme devised by J.G. Greener (see appendix 1) based upon Luther (1963). Standard errors were calculated from the formula:

$$S.E. (N) = \frac{N - U}{\Delta N} \quad (\text{Robson and Regier, 1968})$$

where U is the cumulative unmarked catch. The quantity ΔN may be obtained from the formula:

$$N = \frac{U}{N} - (N - U) \left(\frac{a}{N(N - a)} + \frac{b}{N(N - b)} + \dots \text{etc.} \right)$$

where a , b , c , etc. are the numbers of fish in each successive catch.

4. Mortality

Mortality rates were calculated from the two series of population estimates as well as from catch per unit effort data using the method of Ricker (1958). The catch per unit effort is an expression of the mean number of fish caught per seining operation. The population estimates and the catch per unit effort results are completely independent of each other since the former depend upon the ratio of marked to unmarked fish captured, while the latter depends upon the actual number of fish caught. The instantaneous mortality rate, and the actual mortality rate were calculated from the formula given by Ricker (1958). The formulae are as follows:

$$a = 1 - e^{-i}$$

$$i = \log e \frac{N_t + 1}{N_t}$$

a = actual mortality
 i = instantaneous mortality
 N_t = population at time x
 $N_t + 1$ = population at time $x + 1$.

The instantaneous mortality rate indicates what fraction of the population is dying at any given time. All mortality in the West Kleinemond estuary was assumed to be natural mortality as there was little or no angling.

Regular counts were made of piscivorous birds at the West Kleinemond estuary during 1972 and at the Kasouga estuary (Fig. 1) in the latter part of 1972. During 1972 regurgitated pellets of Ardea cinerea L. (grey heron) were collected from the Kap River heronry (Fig. 1) 8 km north of the West Kleinemond estuary.

RESULTS

1. Population estimates

The monthly mean number of fish caught per seining operation during 1971 and 1972 expressed as catch per unit effort is plotted in Figures 6 and 7 respectively. The catch per unit effort from

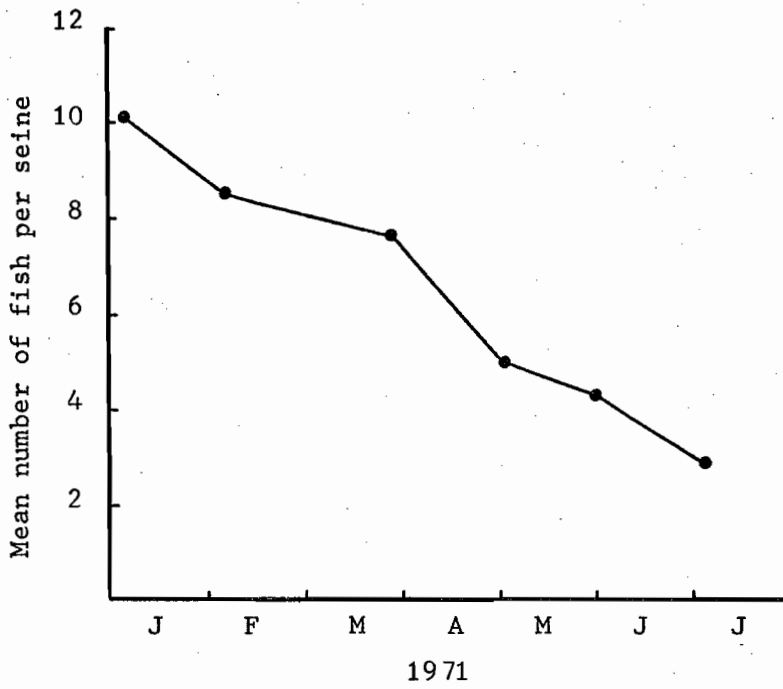


Figure 6: Decline in catch per unit effort in West Kleinemonde estuary during 1971.

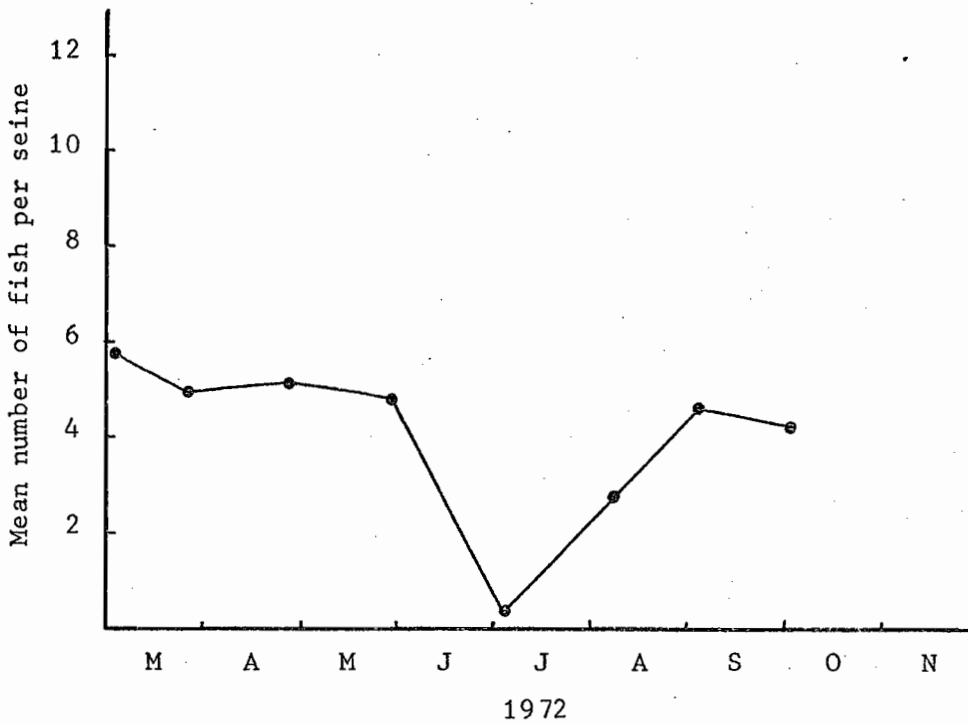


Figure 7: Catch per unit effort in West Kleinemonde estuary in 1972.

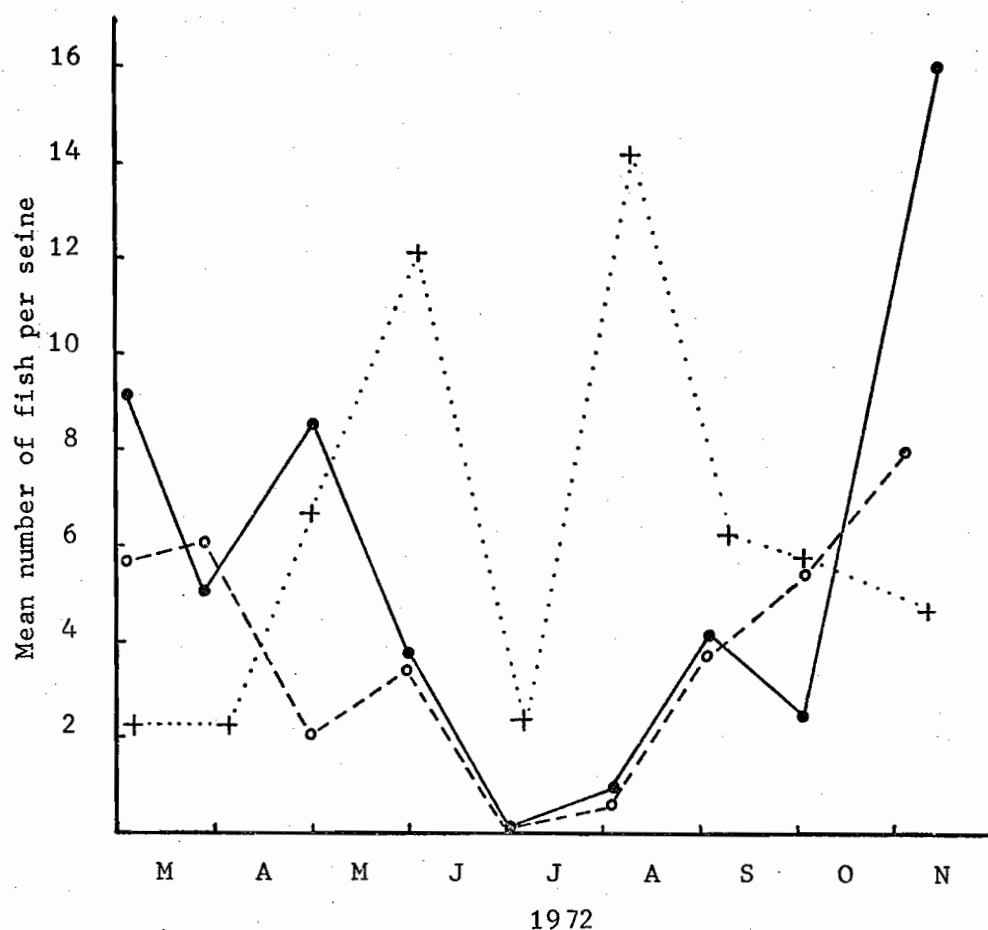


Figure 8: Catch per unit effort of Rhabdosargus holubi in the three major regions of the West Kleinmond estuary during 1972. Continuous line : lagoon; broken line : middle reaches; dotted line : upper reaches.

January to July 1971 showed a steady decline from a mean catch of about 10 fish per seine haul to a mean catch of 3 fish per haul. This decline in catch could either be due to a seasonal effect on the availability of R.holubi or to a decline in the population. During 1972 the overall catch per unit effort remained relatively stable at 4.5 fish per seine haul from March to May, declined to a minimum of 0.5 fish per seine haul in July and increased again in August, until by September the levels of March to May were attained. Thus except for the winter decline the catch per unit effort remained relatively stable in 1972. The abnormally high catch per unit effort in October 1972 was due to a single shoal of over 200 R.holubi being captured in the lagoon in one seine haul. Figure 8 shows the catch per unit effort

in the three major regions of the estuary in 1972. In March 1972 catches were moderate in the lagoon and middle reaches but low in the upper reaches of the estuary. With the approach of winter (May, June) the fish appeared to move up the estuary; the catch per unit effort in the lagoon and middle reaches declined whereas in the upper reaches it increased. In the middle of winter (July) when the water temperature fell as low as 11°C it was extremely difficult to catch any fish and during July 1972 only 15 fish were captured due to this winter effect. It is possible that in July 1971 the catch per unit effort was influenced by the winter effect, although none was observed.

Population estimates for 1971 and 1972 using Petersen and multiple mark-recapture methods are shown in Table 1. Due to adverse weather conditions affecting the seining programme it was necessary to combine the data for March and April 1971. Both estimation methods, which are independent of one another since they rely upon a different number of recaptures, show very similar results. The population in 1971 was initially about 55000 but by the end of seven months had declined to only about 11000 individuals. In 1972 the population remained relatively constant at about 13000 individuals. Standard errors of the population estimates are directly related to the number of recaptures. Thus in this study the Petersen estimates have a much higher standard error than the multiple estimates since they rely only upon recaptures marked in the previous month. None of the fish marked in 1971 were recaptured in 1972, supporting the interpretation put forward in Part I, namely that the 1972 population probably contained no individuals from the 1971 population.

2. Mortality

Instantaneous and actual monthly mortality rates for January

TABLE 1

Population estimates (with standard errors) for Rhabdosargus holubi in the West Kleinemonde estuary during the periods of closure.

Month	Petersen		Robson & Regier	
	Estimate	S.E.	Estimate	S.E.
1971				
Feb	55360	55107	55360	23423
Mar/Apr	39600	19590	42678	10209
May	22027	10843	21933	3223
June	14418	7078	14061	1449
July	11016	5392	11953	1018
1972				
Apr	14674	7215	14674	6460
May	15966	9006	24131	1380
June	14679	8354	13405	2424
Sept	17654	12351	12925	1680
Oct	14833	10366	12221	1211
Nov	13773	5486	11588	771

TABLE 2

Instantaneous mortality rates (i) and actual mortality rates (a) of Rhabdosargus holubi in the West Kleinemonde estuary in 1971.

Method	Petersen		Robson & Regier		Catch/Unit effort	
	i	a	i	a	i	a
1971						
Jan					0.15	0.14
Feb	0.33	0.28	0.26	0.23		
Mar/Apr	0.58	0.44	0.67	0.49	0.49	0.39
May	0.42	0.34	0.44	0.36	0.09	0.09
June	0.27	0.24	0.16	0.15	0.05	0.39
July						

to July 1971 are shown in Table 2. The overall mortalities from the beginning of January until the end of July 1971 were: calculated from the Petersen estimates : 80%, from the multiple estimates : 78%, and from the catch per unit effort : 74%, thus the three methods are in agreement. The decline in the 1971 population according to the two estimation methods is not linear, therefore it is not possible to give a mean monthly mortality rate. The estimates and catch per unit effort data for 1972 showed that the population remained stable with mortality insufficient to be revealed by any of the estimates. Therefore no mortality rates have been calculated for 1972.

3. Predators

a) Fish: The only piscivorous fish apparently present in the West Kleinmond estuary during 1971 and 1972 was Hypacanthus amia. The H.amia present in the West Kleinmond estuary in 1971 were relatively small (Part I). At this time R.holubi were too large to have fallen prey to these small H.amia in 1971, especially in the first half of the year when most of the mortality took place. During 1972 the H.amia were larger but the numbers were low as is revealed by the fact that only 14 were caught. Of these 14, one contained a single R.holubi in the stomach while the rest had been feeding on Mugil spp. or Gilchristella aestuarius (Gilchrist and Thompson).

b) Birds: Regular counts of piscivorous birds were undertaken during 1972 on both the West Kleinmond and Kasouga estuaries. The latter is a closed estuary 12 km south of Port Alfred. It is 3 km long and not more than 5 m deep. It was cut off from the sea in 1972. At the times of the bird counts at Kasouga estuary seining was also carried out in order to obtain a relative estimate of the size of the population of R.holubi. The catch per unit effort of R.holubi at Kasouga estuary in 1972 varied between 10.0 and 14.0 fish per seine.

This is high when compared with the catch per unit effort at West Kleinemonde estuary in 1972 (Fig. 7). The mean number of piscivorous birds at the West Kleinemonde and Kasouga estuaries in 1972, together with the highest and lowest numbers recorded on a single day, are shown in Table 3.

TABLE 3

Number of fish-eating birds present on any one day on the West Kleinemonde and Kasouga estuaries in 1972. The mean numbers ($\bar{x}$) were obtained from 50 counts at West Kleinemonde estuary (March - November) and from 10 counts at Kasouga estuary (July - November). The highest and lowest numbers recorded on any single day are also shown.

Species	Common name	West Kleinemonde			Kasouga		
		$\bar{x}$	high	low	$\bar{x}$	high	low
<u>Ardea</u>							
<u>cinerea</u> L.	grey heron	2	4	1	7	8	5
<u>Casmerodius</u>	great white						
<u>Albus</u> (L.)	heron	1	1	1	3	3	3
<u>Egretta</u>							
<u>garzetta</u> (L.)	little egret	2	4	1	6	7	5
<u>Phalacrocorax</u>	reed						
<u>africanus</u> (Gmelin)	cormorant	11	30	2	69	118	20
<u>P.carbo</u> (L.)	white breasted cormorant	1	6	0	9	13	4
<u>P.capensis</u>	Cape						
(Sparman)	cormorant	1	3	0	3	4	1
<u>Anhinga rufa</u>							
(Lacépède et Daudin)	darter	3	6	1	4	8	0
<u>Geryle rudis</u> (L.)	pied kingfisher	4	4	4	4	4	4
<u>Megaceryle</u>	giant						
<u>maxima</u> (Pallas)	kingfisher	2	2	2	2	2	2
<u>Corythornis</u>	malachite						
<u>cristata</u> (Pallas)	kingfisher	2	2	2	2	2	2
<u>Haliaeetus</u>							
<u>vocifer</u> (Daudin)	fish eagle	2	2	2	2	2	2

Regurgitated pellets of Ardea cinerea from the Kap River heronry were analysed with regard to fish remains. Kap River heronry is only 8 km from West Kleinmond estuary while the next nearest heronry is 30 km away. Since herons from Kleinmond were observed to fly off in the direction of Kap River it is assumed that those which fished on the West Kleinmond estuary came from Kap River heronry. Hibbert-Ware (1940) showed that due to the action of the bird's digestive enzymes heron pellets give an underestimate of the predominance of fish in the diet of A.cinerea. All 41 pellets of A.cinerea examined from Kap River contained assemblages of fish scales and it is concluded that the scales are representative of the range of fish normally taken by the grey heron from the West Kleinmond estuary. All scales were indistinguishable from those of R.holubi and their shape was different from that of most of the scales from Mugil spp. and Lithognathus lithognathus (Cuvier), the other relatively common fish in the West Kleinmond estuary. No other hard parts of fish were found in the heron pellets.

c) Crabs: The crab Scylla serrata (Forskål) is known to include fish as part of its diet. About 2000 were present in the West Kleinmond estuary during both 1971 and 1972 (Hill, pers.comm.). The population in 1971 consisted solely of juveniles which only attained maturity in 1972. Their effects upon the population of R.holubi are at present unknown.

DISCUSSION

Chilvers and Gee (1969) found that FD67 anchor tags caused a high mortality rate in aquarium kept Tilapia melanopleura x T.zillii hybrids, Bagrus docmac Forskål and Labeo victorianus Boulenger. They also state that the caudal peduncle is a better position than the

shoulder from a consideration of both tag retention and the effect of the presence of the tag on growth of T.melanopleura x T.zillii hybrids. Kennedy (1970) using Floy FT2 tags showed that 100% mortality occurred in tagged, captive Anoplopoma fimbria (Pallas) within one year due to infection of the tagging wound. By contrast Carline and Brynildson (1972) showed in two successive trials that FD67 anchor tags did not affect survival of Salvelinus fontinalis (Mitchill). All the above cases involved freshwater fish and no antibiotics were used.

Evidence from tagged R.holubi kept in an enclosure in the West Kleinemond estuary (Part I) showed that Floy FD67 anchor tags caused little or no mortality and tag loss was negligible. Thus it seems reasonable to conclude that there was no differential mortality between tagged and untagged R.holubi in the West Kleinemond estuary, and therefore the conditions for valid estimates were not violated in this respect.

It is apparent from the results that the size and mortality rates of the 1971 and 1972 populations of R.holubi in the West Kleinemond estuary were very different. Initially the 1971 population was large (about 50000) but it declined until it reached 11000, at which time the estuary opened to the sea, permitting no further estimates. The second survey (1972) revealed that the new population was few in numbers, at a level in fact just above the low point reached in 1971. This small population remained stable for the rest of the survey period showing little or no mortality.

The standard errors for the population estimates are rather wide, especially for the Petersen method (Table 1) which relied upon fewer recaptures, but the trends from the different methods are very similar and are supported by the catch per unit effort data (Figs 6, 7 and 8).

The catch per unit effort in the West Kleinmond estuary measured the overall decline in the number of R.holubi in 1971, but as a measure of population size catch per unit effort must be treated with caution. Although catch per unit effort data are a useful measure of the availability of fish they cannot be used in isolation to provide any quantitative information unless a regular series of samplings is performed. For instance in July 1972 the catch per unit effort dropped very low (Fig. 8) due to the inaccessibility of the fish to the sampling gear, but the population had not declined. Thus catch per unit effort does not give any direct information on actual population size but is a good index of comparative abundance when a standardised sampling procedure is used. The relationship between population size and catch per unit effort is not direct however, and may be unreliable especially when the population is large. This is probably due to the efficiency of the sampling gear; for example when fish are numerous a further increase in numbers may not bring a corresponding increase in the catch per unit effort as the efficiency of the net may be near maximum. This was demonstrated by the results obtained in this study where in 1971 a reduction of over 50% in the population from February to May did not bring a similar reduction in the catch per unit effort which only fell by 40%.

The decline in the population of R.holubi over a seven month period in 1971 was the same when measured by three different methods, thus there is little doubt that the overall mortality was of the order of 80%. The monthly mortality varied between 49% and 9%, being mostly around 30%. High mortality rates amongst juvenile fish have been reported by other workers. Thomson (1959a) using Petersen and Schnabel estimates found that the mortality rate of Rhabdosargus sarba over 11 months in Lake Macquarie estuary Australia was 78%, but some

of this was thought to be due to emigration. Macer (1967) working in the Irish Sea on populations inhabiting shallow sandy bays found a mortality rate of 40% per month among 0 group Pleuronectes platessa, while Riley and Corlett (1965) found the mortality of this species to vary between 30% and 50% per month. In Connecticut, Percy (1962) found a mortality rate of 31% per month among juvenile Pseudopleuronectes americanus. Thus the mortality rate of juvenile R.holubi is of a similar magnitude to that of other benthic species which have been investigated. The causes of this high mortality have not been established previously with any certainty, but predation was the factor most favoured by Macer (1967) and Riley and Corlett (1965).

The chief predators in the West Kleinmond estuary were: the fish H.amia, the crab S. serrata and a number of species of birds. The numbers of H.amia present during 1971 were very insignificant and only one of the 14 examined in 1972 had been eating R.holubi. S.serrata was plentiful during the period in which the population of R.holubi declined but was also present in 1972 when the numbers of R.holubi remained constant. Thus it appears that the crabs probably did not contribute significantly to the population decline in 1971.

The counts of piscivorous birds at West Kleinmond estuary and Kasouga estuary reveal that their numbers may be related to the density of R.holubi. The population of R.holubi in West Kleinmond estuary during 1972 was relatively low as was the number of fish-eating birds, whereas the number of R.holubi in Kasouga estuary during 1972 was high with a correspondingly high density of birds. The catch per unit effort at Kasouga estuary in 1972 was similar to that for West Kleinmond estuary. Unfortunately no detailed counts were kept for this estuary during 1971. The shallow nature of these estuaries makes

them fishable over much of their area by Ardea cinerea. Phalacrocorax carbo (L.) is capable of fishing over the entire estuary since it has been recorded diving to 9.5 metres and regularly dives to 3 metres (Green, 1968).

Thus the fish populations are extremely vulnerable to bird predation. The birds which were present in largest numbers and which fluctuated most were A.cinerea, P.carbo and P.africanus (Gmelin), whereas the low number of resident kingfishers did not change. Owen (1955) showed that A.cinerea in England most frequently took fish of a length between 10 and 16 cm (presumable total length), this overlaps well with the size range of 6 to 16 cm (total length) for R.holubi in the West Kleinmond estuary. No evidence is available on which species of fish the two cormorants, P.carbo and P.africanus, took from West Kleinmond estuary but R.holubi may have formed a significant proportion of their diet since it was one of the most numerous species. Van Dobben (1952) showed that fish with a length of between 10 and 30 cm were most frequently taken by P.carbo. Similarly Bowmaker (1963) stated that P.africanus consumes fish of between 2 and 20 cm. Thus the size ranges of the fish normally eaten by the cormorants overlaps with the size range of R.holubi present in West Kleinmond estuary during 1971 and 1972 and in Kasouga estuary in 1972. Junor (1969) has shown that the daily food requirements of A.cinerea, P.carbo and P.africanus are 245 g, 304 g and 95 g respectively. Those of Ceryle rudis and Megaceryle maxima are only 13 g and 55 g respectively. Therefore the former three species could be expected to have had a much more marked effect on the fish population. For example 10 grey herons could be expected to consume at least 100 20 g fish per day while 10 pied kingfishers would only consume about 5 20 g fish per day. It would appear possible that the mortality rate among juvenile R.holubi

in the West Kleinemonde estuary was controlled in a density dependent fashion, large numbers of fish giving rise to a high level of predation by fish-eating birds. This resulted in a rapid decline in the numbers of fish and a consequent drop in the level of predation.

The interesting winter effect shown in the 1972 survey when R.holubi moved to the deeper water of the upper reaches of the estuary would perhaps make capture by piscivorous birds other than P.carbo and P.africanus more difficult. Without any direct evidence it is only possible to postulate that perhaps the winter effect would decrease the level of predation on a large population for two months of the year.

Therefore, although the mortality rate among juvenile R.holubi was found to be similar to that in other marine species, it was possible in the context of a closed estuarine environment to demonstrate the possible causes of this mortality. Much of this appears to be due to those species of piscivorous birds which are able to utilise a shallow water habitat for feeding when the opportunity arises, that is, when prey are relatively easily caught.

Although predation was a possible cause of the high mortality of R.holubi in 1971 other factors could not be excluded. The tolerance of R.holubi to different temperature and salinity combinations was unknown as was the diet and feeding habits in relation to the availability of food.

PART III

SALINITY AND TEMPERATURE TOLERANCE

INTRODUCTION

It is apparent from Parts I and II that many Rhabdosargus holubi spend the juvenile phase of their life history in closed estuaries such as that of the West Kleinmond River. Surveys of southern African estuaries have shown that they are exposed to extreme fluctuations in temperature and salinity. Flooding may flush out a previously hypersaline estuary with freshwater. Cool seawater entering at flood tide may cause a drop in water temperature of 10°C in a few hours. Clearly fish living in these estuaries must be able to cope with these conditions. No work has been done on the salinity and temperature tolerance of any of the southern African estuarine fish species. In view of the high mortality of R.holubi in the West Kleinmond estuary during 1971 and the lack of information about the physical tolerances of the species, it was decided to undertake an investigation into its ability to withstand variations in salinity and temperature. The salinity and temperature tolerance, and osmoregulatory ability of juvenile R.holubi were determined. The results are then related to the known distribution of the species using physical data collected from the West Kleinmond estuary and previously published data on other southern African estuaries.

MATERIALS AND METHODS

1. Temperature and salinity in the West Kleinmond estuary

Surface and bottom salinity and temperature measurements were made between 0900 and 1000 hours at monthly intervals in the West Kleinmond estuary from September 1970 until October 1971. Continuous temperature recordings were made in the West Kleinmond

estuary during the winter months of June and July 1972 and the summer months of February and March 1973. These recordings were made using an Oceanographic Engineering Corporation Model 401 temperature monitor and recorder. The thermistor probe from the monitor was placed one metre below the water surface 3.5 km from the mouth of the estuary. Salinities were calculated from chloride determinations made on an American Instruments Corporation Chloride titrator.

2. Temperature and salinity tolerance experiments

R.holubi were collected by seine netting from three similar closed estuaries, the East and West Kleinmond and Kasouga estuaries (Fig. 1). Fish were captured between July and November of 1971 and 1972 and all experiments were performed during these periods. A total of 1023 juvenile fish of between 4.5 cm and 12 cm standard length were used in the experiments. After initial trials at different acclimation temperatures and salinities it was decided to acclimate the fish at 20°C ($\pm 2^{\circ}\text{C}$) in 35‰ seawater for one week prior to use. The acclimation chambers were either 225 litre asbestos tanks lined with inert epoxy resin or a 400 litre fibreglass tank. All tanks were equipped with air-lift sand bed filtration systems. Fish were fed with prawns, either Upogebia africana (Ortmann) or Callinassa kraussi Stebbing (Crustacea, Anomura). Prawns were added to the tanks at least once every two days until the fish stopped feeding. Experiments were performed in 12 litre plastic bowls using 10 litres of water. A random selection of 10 fish were used in each experiment and were transferred directly from the acclimation tanks to the experimental containers. Initially, experiments were run for 100 hours and the water changed after 50 hours. Later however, it was found that no change in percentage survival occurred after 600 minutes, and subsequently all experiments

were run for 1000 minutes (16.6 hours) without any change of water. Water in the experimental bowls was brought to the required temperature before the introduction of the fish. Where water was changed it was preheated to the experimental temperature before substitution. Temperature control ($\pm 0.1^{\circ}\text{C}$) was achieved using either 0.75 kw Buhler heater stirrers or 1 kw Tecam heater stirrers. Temperatures below ambient were obtained by use of cooling coils from a refrigerator unit in conjunction with heater stirrers. During experiments the water was kept aerated.

Salinities of below $35^{\circ}/\text{oo}$ were obtained by diluting seawater with Grahamstown tap water (Cl^{-} below 1 milliequivalent per litre). Thus the freshwater used was of a similar chemical composition to that flowing into the Kleinmond and Kasouga estuaries. Salinities of above $35^{\circ}/\text{oo}$ were obtained by mixing Grahamstown tap water and seawater evaporated to $85^{\circ}/\text{oo}$ from a local saltworks.

Prior to death, fish swam upside down and when dead floated upside down. Death was marked by a cessation of opercular movement. The time of death during low temperature experiments was difficult to measure because respiratory movements were greatly reduced, especially below 10°C . In this case fish thought to be dead were transferred to water of the same salinity but at a temperature 5°C higher than the experimental temperature; if opercular movement was resumed within 30 minutes fish were returned to the experimental container.

Results were analysed by the fitting of a response surface using a method similar to that described by Costlow et al (1960). The equation which best fitted the observed data was a simple parabolic regression:

$$y = a + bx + cx^2$$

The terms upper and lower lethal

limits used here refer to those combinations which cause 100% mortality. It was felt that this is a more meaningful measure than 50% mortality since a relatively small number of fish were used in each experiment.

3. Osmoregulation

Juvenile R.holubi were collected from the West Kleinemond estuary and acclimated in the same way as those used in the tolerance experiments. Fish were fed twice daily on "Tetramin" brand tablet fish food. Experiments were performed in 30 litre plastic bowls containing 25 litres of water. A random selection of five fish was used in each experiment. They were transferred directly from the acclimation tanks to the experimental containers. All experiments were run at 20°C and the water was aerated continuously. Water of different salinities was obtained as previously described.

Fish were anaesthetised with Sandoz MS222 and blood was removed from the heart using 1 mm bore capillary tubes. Blood serum was separated out by immersing the blood in liquid paraffin and allowing it to clot. The osmotic pressure of the blood serum and external media was measured in milliosmoles per litre using a Hewlett-Packard 302B Vapor Pressure Osmometer. One hundred fish were used in the investigation.

RESULTS

1. Estuarine temperatures and salinities

Bottom temperatures in the upper and lower reaches of the West Kleinemond estuary from September 1970 until October 1971 are shown in Figure 9. Surface water was usually 1 - 2°C warmer than bottom water. There is a clear annual cycle with winter temperatures of about 12 - 17°C and summer temperatures of about 22 - 27°C. Figure 10

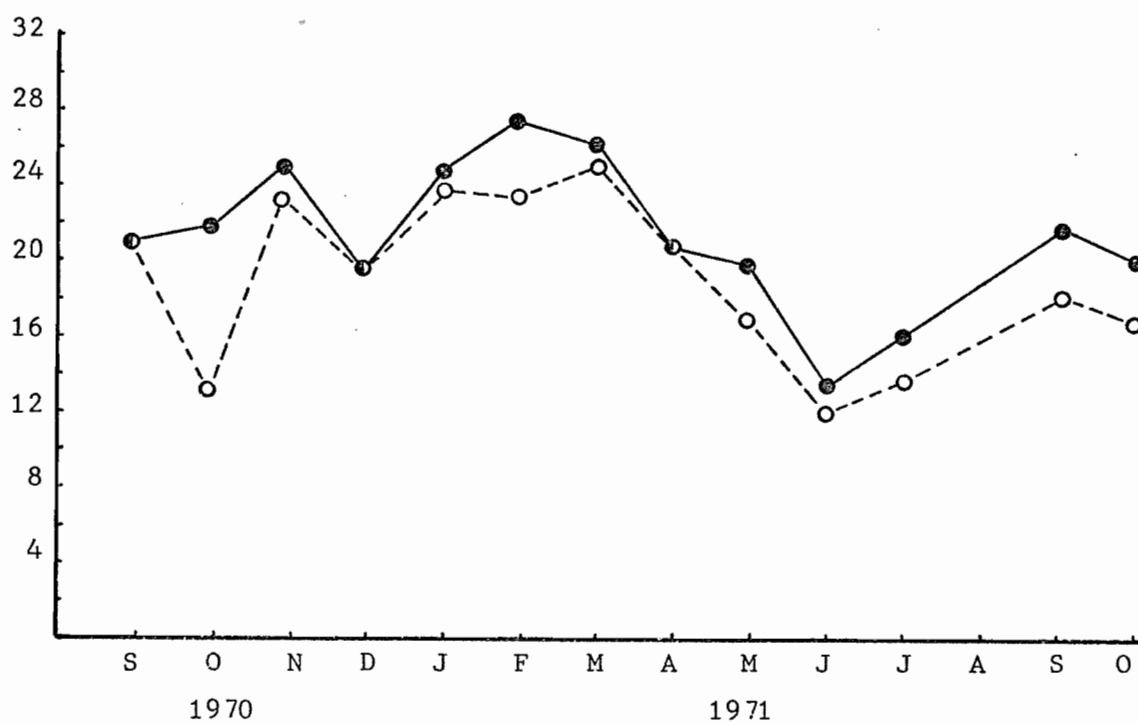


Figure 9: Bottom temperatures in the West Kleinemonde estuary from September 1970 until October 1971. (—) upper reaches of estuary; (-----) lower reaches of estuary.

shows the daily high and low temperatures for a period of 44 days during the winter months of June and July 1972 and the daily high and low temperatures for a period of 17 days during the summer months of February and March 1973. These continuous temperature records show that diurnal temperatures only fluctuated very slightly, usually about 2°C . The highest temperature recorded in the West Kleinemonde estuary during the survey was 28°C and the lowest 10.7°C . Hill and Allanson (1972) showed that the temperature in the open Kowie estuary, only 16 km south of the West Kleinemonde estuary, did not fall below 11.5°C or rise above 27°C , except in the shallow margins. These temperatures are very similar to those obtained for the West Kleinemonde estuary, but the daily fluctuations in the Kowie estuary caused by tidal movements were as much as 5° in winter and 10° in summer.

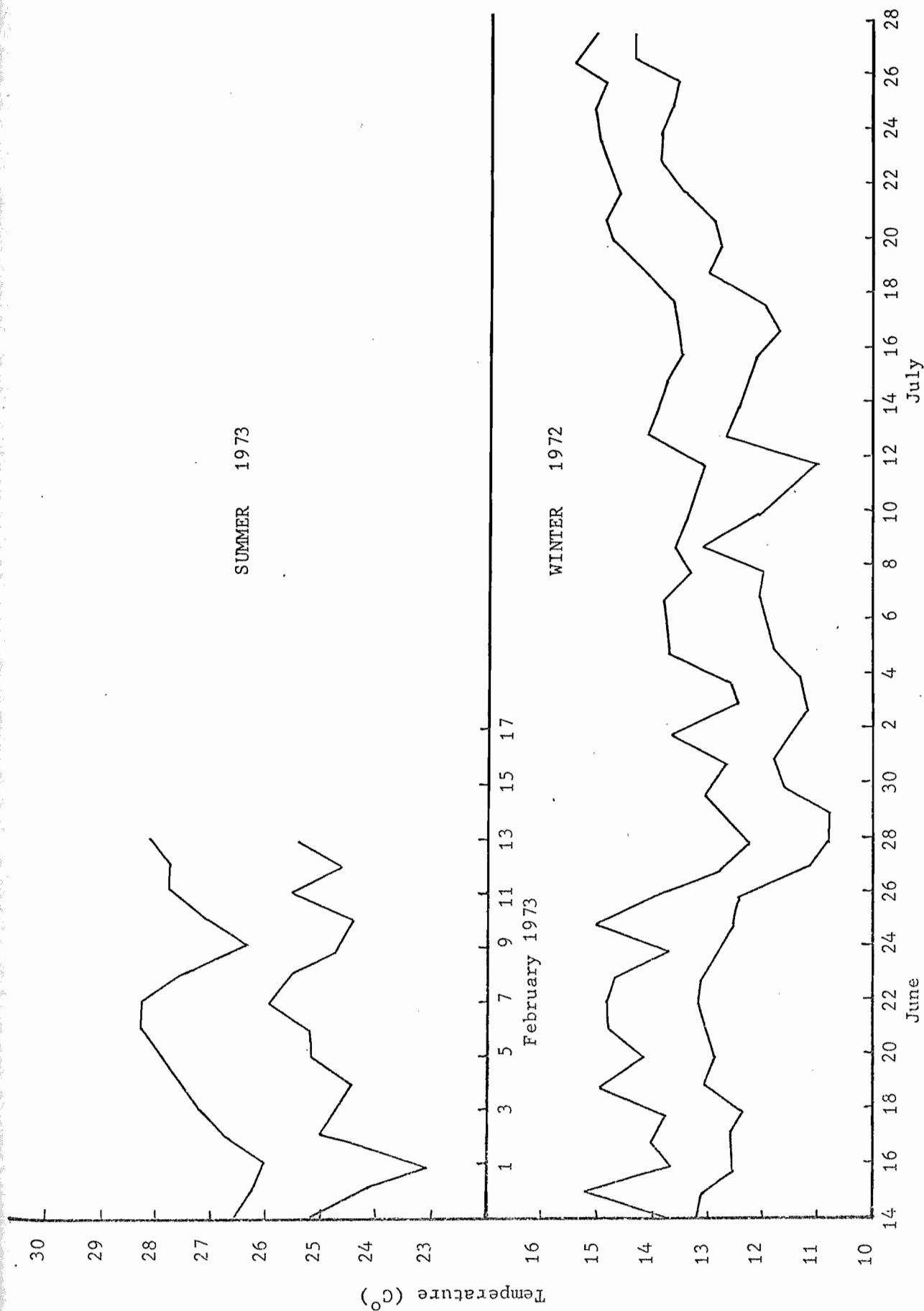


Figure 10: Daily high and low temperatures during summer and winter in the middle reaches of the West Kleinmond estuary.

The bottom salinity in the upper and lower reaches of the West Kleinemonde estuary did not drop below $10^{\circ}/\text{oo}$ while the estuary was closed (January-July 1971). There was a slight salinity layering in the upper reaches where the surface salinity was generally $5^{\circ}/\text{oo}$ lower than the bottom salinity but there was little layering at the seaward end of the estuary. When an estuary is closed variations in salinity are a result of the amount of freshwater flowing into the system and the amount of evaporation. Thus except in the case of floods, which are usually of less than 48 hours duration, salinity changes in a closed estuary are slow. This is in contrast to the situation in an open estuary where daily salinity fluctuations may occur as a result of tidal exchange. In August 1971 when the West Kleinemonde estuary flooded, a drop in salinity from $24^{\circ}/\text{oo}$ to $2^{\circ}/\text{oo}$ was recorded within 24 hours before the estuary opened and became tidal. While it remained open the salinity at the seaward end was never less than $33^{\circ}/\text{oo}$ and the salinity at the landward end was not less than $6.5^{\circ}/\text{oo}$ on the surface, or $19^{\circ}/\text{oo}$ on the bottom.

2. Tolerance experiments

In most experiments R.holubi showed either 100% mortality or 100% survival. Thus there appeared to be little variation between animals. The combinations of temperature and salinity which proved lethal are shown in Table 4. Freshwater was lethal at all temperatures. In all salinities other than $35^{\circ}/\text{oo}$, where the upper lethal temperature was 33°C , the upper lethal temperature was between 28°C and 31°C . The lower lethal temperature was between 5°C and 13°C . The results of experiments using animals which had been acclimated at other than 20°C and $35^{\circ}/\text{oo}$ are shown in Table 5. Reducing the acclimation salinity to $17.5^{\circ}/\text{oo}$ or $8.7^{\circ}/\text{oo}$ did not alter the upper or lower lethal temperature limits. Reduction

TABLE 4

Combinations of salinity and temperature which proved to be lethal to R.holubi on exposure for 1000 minutes. All fish were acclimated at 20°C in 35‰/oo seawater.

Lethal salinity ‰/oo	Upper lethal temperature	Lower lethal temperature
Freshwater	Lethal at all temperatures between 5 and 35°	
0.7	31	
1.75	31	
3.5	31	10
10	31	7
25	33	
30		5
35	33	
42.5	31	10
63	28	13
68	28	
70	20	

TABLE 5

Lethal temperature and salinity combinations of fish acclimated to a range of salinities and temperatures.

Acclimation conditions		Lethal combination	
Temp (°C)	Salinity (‰/oo)	Temp (°C)	Salinity (‰/oo)
20	17.5	31	17.5
20	17.5	7	17.5
20	8.7	31	8.7
20	8.7	7	8.7
15	35	15	freshwater
15	35	33	35
15	35	15	70

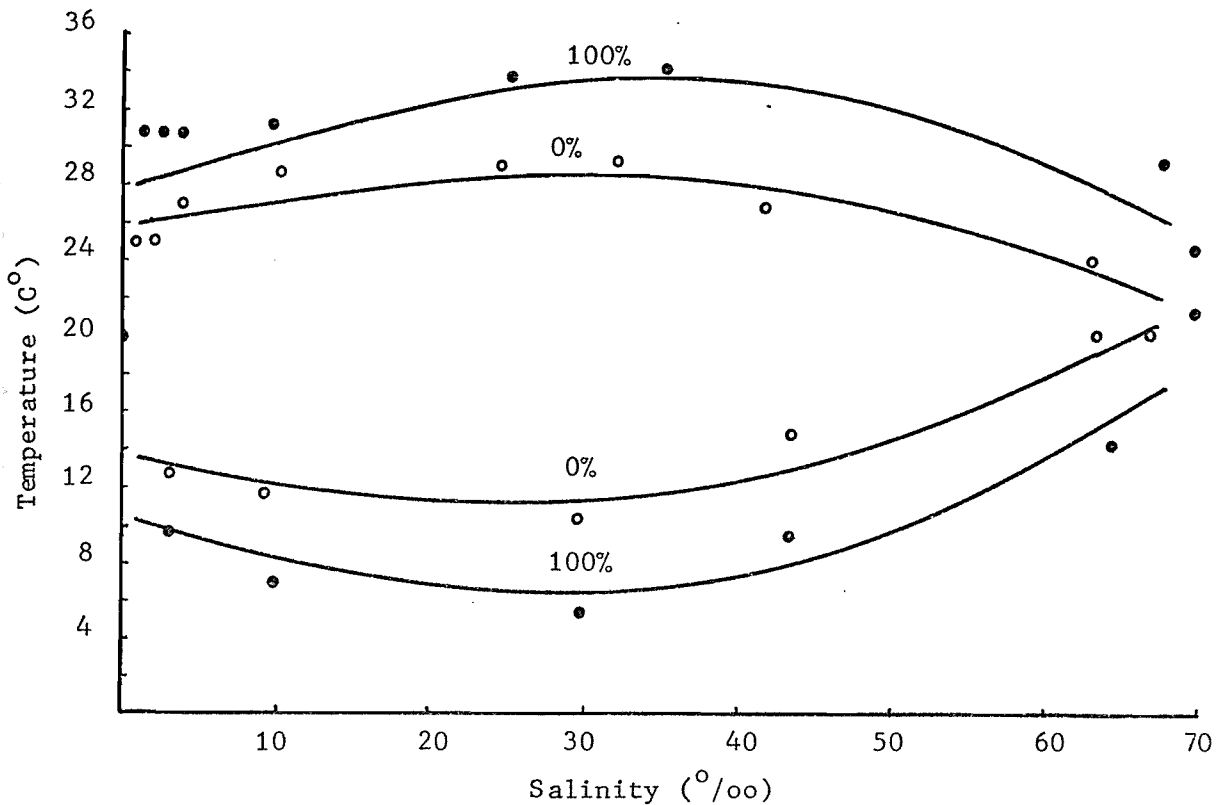


Figure 11: 0% and 100% mortality curves for juvenile Rhabdosargus holubi under 36 combinations of salinity and temperature.

of acclimation temperature to 15°C did not alter the lethal salinity limits. Curves calculated from the 0% and 100% mortality of juvenile R.holubi exposed to 36 combinations of temperature and salinity are shown in Figure 11. The curves were calculated using parabolic regressions and the correlation coefficients for the regressions were significant at a 0.02 level. The chief limitations of the curves in Figure 11 are that they do not emphasize that freshwater and 70‰ seawater are lethal at all temperatures. They do however show that interaction between temperature and salinity with regard to mortality is minimal, especially at 12 - 26°C and 1 - 40‰.

3. Osmoregulation

Figure 12 shows the time taken by R.holubi for osmotic adjustment

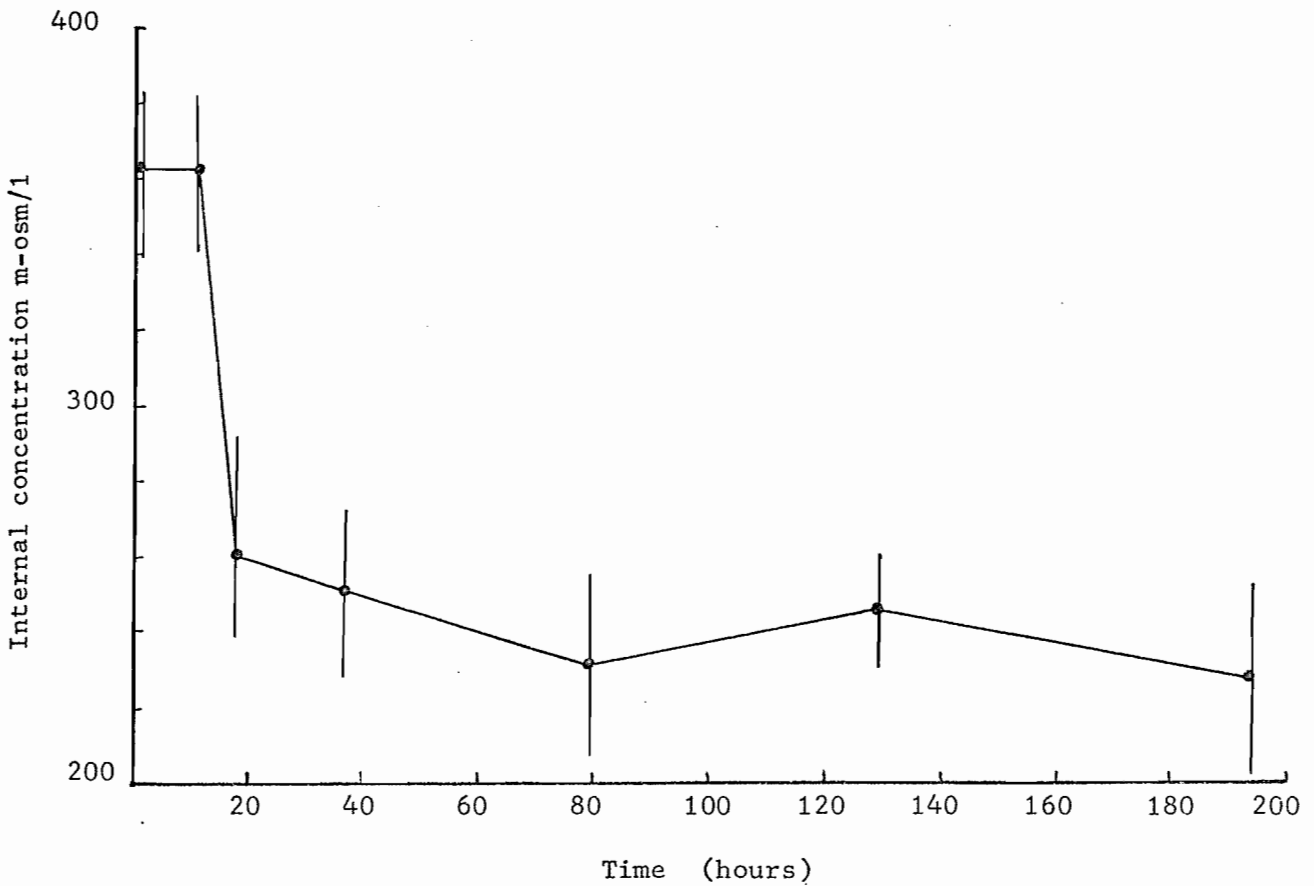


Figure 12: Mean time taken for Rhabdosargus holubi to adjust its internal osmotic pressure in water of 140 m-osm/l. (Approx. 3.5‰). Actual range of individual variation shown for each point (mean of 5 fish).

to occur in water of 140 m-osm/l (approx. 3.5‰). There is no change in the internal concentration until after 10 hours and adjustment is complete by 30 hours. As a result of this experiment all subsequent measurements of the osmotic concentration of the blood were made after 30 hours exposure to a new salinity. Figure 13 shows the osmotic concentration of the blood of R.holubi after 30 hours in a range of salinities, from nearly freshwater to twice the concentration of seawater. The mean osmotic concentration of the blood in seawater was 370 m-osm/l. At a salinity of 1‰ the internal concentration dropped to 216 m-osm/l. In 65‰ the internal concentration rose to a mean of 381 m-osm/l. The actual

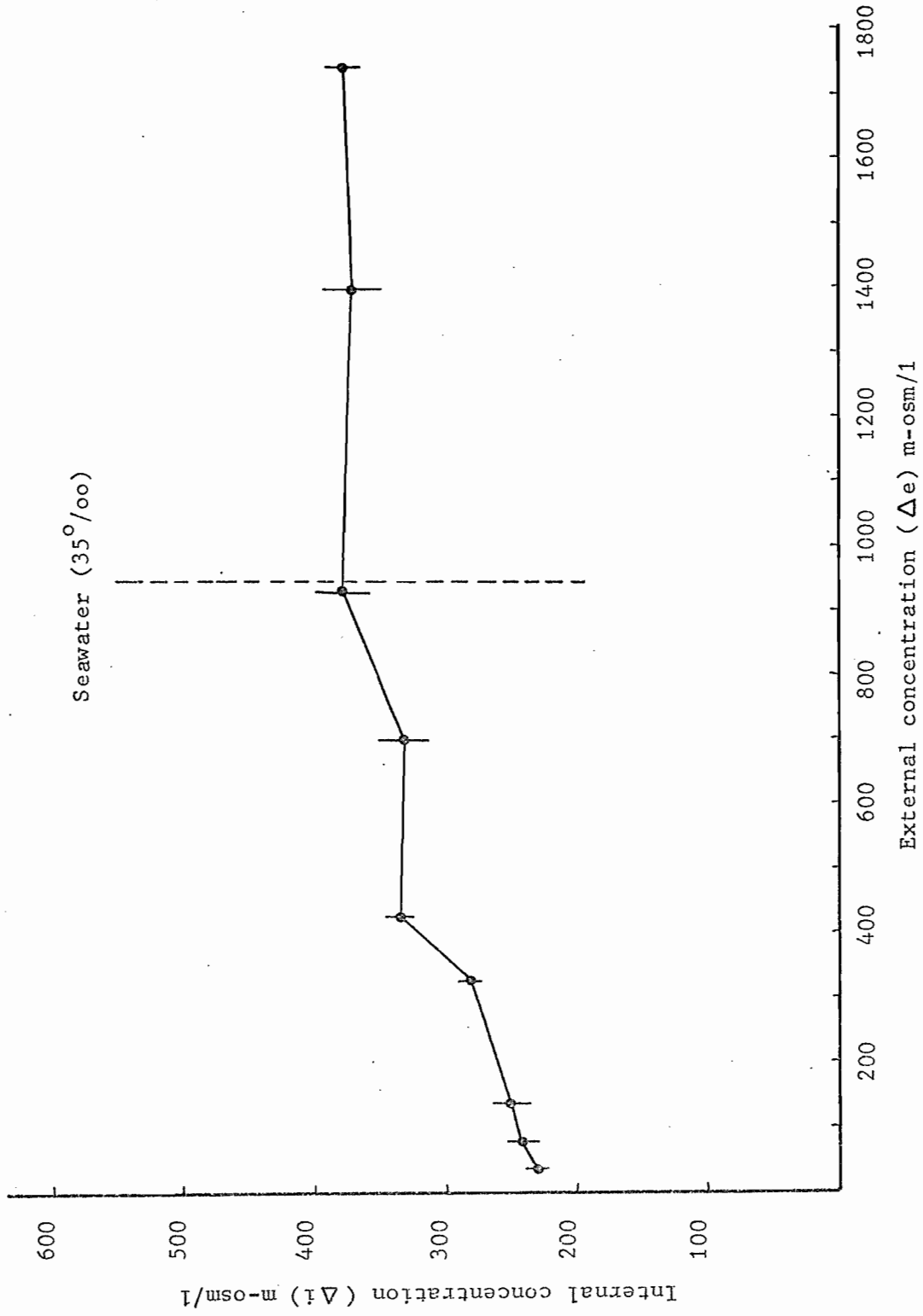


Figure 13: Osmotic concentration of the blood of R. holubi after 30 hours in a range of salinities from 1‰ to 65‰. Actual range of individual variation shown for each point (mean of 5 fish).

range of individual variation is shown in Figure 13. Clearly R.holubi is a strong regulator, being hyposmotic in high salinities and hyperosmotic in low salinities, with very little change in internal osmotic pressure over an extremely wide salinity range.

DISCUSSION

There is little interaction between salinity and temperature with regard to the tolerances of R.holubi at salinities below 35⁰/oo. The data indicate that R.holubi is extremely tolerant of changes in salinity, and can survive within the salinity range of 1⁰/oo to 70⁰/oo. It cannot however penetrate freshwater or water with a salinity of above 70⁰/oo. There are no records of R.holubi from either of these two extremes although in the St.Lucia estuary in Zululand R.holubi was recorded by Millard and Broekhuysen (1970) at salinities between 7.2⁰/oo and 37.6⁰/oo. Green (1968) states that most marine teleosts in seawater of 35⁰/oo keep the osmotic pressure of their blood between 350 and 500 m-osm/l. In 35⁰/oo seawater R.holubi keeps its internal osmotic pressure at 370 m-osm/l but can regulate extremely well and maintains a relatively constant internal osmotic pressure over a range of salinities from 1⁰/oo to 65⁰/oo. When exposed to a medium as low as 40 m-osm/l the fish can maintain an internal concentration of 216 m-osm/l. At the high external concentration of 1740 m-osm/l the blood concentration is maintained at about 380 m-osm/l which is only slightly higher than that in 35⁰/oo. A similar phenomenon occurs in the flounder (Platichthys flesus (L.)) which like R.holubi spends its juvenile phase in estuaries that may have very reduced salinities. In full seawater the flounder maintains its blood at 518 m-osm/l but in water of 165 m-osm/l it drops its internal concentration to 286 m-osm/l (Henschell, 1936), apparently with no adverse effects. As is shown in Figure 12 the internal



concentration of R.holubi in reduced salinities does not drop until after 10 hours, this could be of importance since it may be subject to short term changes in salinity, such as high and low water in tidal estuaries, or severe flooding in the case of closed estuaries.

The salinities in the estuaries between Mossel Bay and St. Lucia, where R.holubi is most common, do not usually approach the lethal limits. According to Macnae (1957) the salinity of the open Zwartkops estuary ($25^{\circ}40$ E, $33^{\circ}50$ S) is always only slightly less than seawater. Hill (1966) working on the Umlalazi estuary ($31^{\circ}50$ E, $28^{\circ}55$ S) in Zululand, which is open to the sea, showed that the salinity at the seaward end of the estuary varied between $20^{\circ}/\text{oo}$ and $35^{\circ}/\text{oo}$ while the upper reaches varied between $4^{\circ}/\text{oo}$ and $19^{\circ}/\text{oo}$, according to tidal influence. These values are all well within the salinity tolerance of juvenile R.holubi.

It is evident from the experiments that the upper and lower temperature limits of R.holubi are about 30°C and about 10°C respectively (Fig. 11). South eastern Africa has a warm temperate climate and the temperatures in the West Kleinmond and Kowie estuaries range between 11°C and 28°C . Further north temperatures become sub-tropical; the winter temperatures reported by Broekhuysen and Taylor (1959) in the tidal basin of the Kosi estuary ($32^{\circ}50$ E, $27^{\circ}00$ S) were about 20°C .

The southern limit of R.holubi appears to be Milnerton ($18^{\circ}30$ E, $33^{\circ}50$ S) near the Cape of Good Hope (Millard and Scott, 1954). It has not been recorded elsewhere from the Atlantic coast.

The northern limit probably lies between St. Lucia estuary ($32^{\circ}30' \text{ E}$, $28^{\circ}00' \text{ S}$), where R.holubi is common (Millard and Broekhuysen, 1970) and Kosi estuary in Zululand where it has not been recorded.

Experimental evidence suggests that the distribution of R.holubi may be related to temperature. The temperatures of the west coast estuaries may drop below the lower lethal limit for R.holubi. Day (1958) reports winter temperatures of below 10°C at Langebaan ($18^{\circ}00' \text{ E}$, $33^{\circ}00' \text{ S}$) on the Atlantic coast. The sub-tropical temperatures of the tidal basin of the Kosi estuary in the north east can exceed the lethal limits. Summer temperatures measured in this estuary in 1967 were 31°C with temperatures as high as 39°C in the shallows (Hill, pers.comm.). Thus R.holubi appears to be restricted by temperature to south eastern Africa, in the area between the Cape of Good Hope and Zululand.

It is apparent that temperatures and salinities in the closed West Kleinmond estuary seldom approach the lethal limits of juvenile R.holubi. Therefore these physical factors are unlikely to have contributed to the high mortality in the West Kleinmond estuary in 1971.

PART IV

FOOD AND FEEDING

INTRODUCTION

Rhabdosargus holubi spend at least the first year of their life in estuaries. Juvenile R.holubi are certainly not common in the inshore coastal waters, and it seems that they prefer to inhabit estuaries even although these are physically unstable and the mortality rate from predation may be relatively high. Having entered an estuary at a very young age they are able to grow to near maturity before migrating back to the sea. Since growth takes place in closed estuaries food must be available, but the diet and feeding habits of R.holubi have not previously been studied. Thus it was not known whether such factors as the food preferences of juvenile R.holubi, in relation to the food available in the estuary as opposed to the sea, were a possible reason for their invading estuaries.

In view of the lack of information on the diet of this species, the possible significance of food with regard to the role of estuaries in the biology of R.holubi, and the high mortality rate in the West Kleinmond estuary in 1971, an investigation into the diet, food consumption rate and feeding habits of R.holubi was undertaken. Since R.holubi occurs in open and closed estuaries this study included fish from both areas. This study involved mainly juvenile R.holubi since only this stage is found in closed estuaries. Fortunately the Ecological Survey Records of the University of Cape Town contain data relating to the diet of adult R.holubi from coastal waters. These records also show that adult R.holubi are occasionally found in small numbers in the lower reaches of some of the larger open

estuaries. One such adult R.holubi was captured in this survey in the lower reaches of the open Kowie estuary.

MATERIALS AND METHODS

1. Study areas

Samples of not less than 20 R.holubi were collected in summer 1971 and during the spring, summer, autumn and winter of 1972/73 from the West Kleinemonde estuary. Fish from another closed estuary, that of the Kasouga River, were collected in 1972 for comparison with those from the West Kleinemonde estuary. Samples for comparative purposes were also obtained from the Kowie and Msikaba estuaries (Figs 1 and 17) which are permanently open to the sea. Fish were captured by seining as described in Part I and were immediately preserved in 10% formalin for stomach analyses in the laboratory.

2. Stomach analyses

Over 2000 individual stomachs were analysed in the course of the investigation, which was both quantitative and qualitative. Stomach contents were sorted into taxonomic groups, counted and dried to constant weight at 70°C. The food items were analysed numerically by frequency of occurrence and gravimetrically by dry weight. Data relating to the stomach contents of adult R.holubi from University of Cape Town records were analysed according to frequency of occurrence only since no weights were recorded. The frequency of occurrence method shows what organisms are being eaten but gives no quantitative information, whereas the gravimetric method emphasizes the importance of the major food items but masks the smaller items.

Large amounts of plant material were found in the stomachs of R.holubi. In view of the apparent absence of cellulase in teleosts

(Lagler et al, 1962) the plant material was studied in more detail. The aquatic macrophyte Ruppia spiralis L. from the stomachs of R.holubi from the West Kleinmond estuary was therefore investigated microscopically using a Jeol JSMU3 scanning electron microscope. The total organic nitrogen content of R.spiralis from the West Kleinmond estuary, and from the stomach and hindgut of R.holubi was determined by the micro-Kjeldahl procedure (Bradstreet, 1965; Webb, pers.comm.).

3. Analyses of epiphytic diatoms

Diatoms were removed from R.spiralis and Hypnea spicifera (Suhr) Harvey by ultrasonication of the material for five minutes at 20 Khz using a Bandelin Sonorex. The diatomaceous material was dried to constant weight at 70°C. Organic material was then digested using 5% KOH. Inorganic material was recovered by centrifuging for 30 minutes at 8000 rpm in an MSE Ultracentrifuge. The inorganic material was redried at 80°C and the organic content determined by difference.

4. Food consumption and feeding periodicity

These parameters were investigated by sampling R.holubi from the West Kleinmond estuary over 24 hour periods in March 1972 and April 1973. The method used was an adaptation of that described by Bajkov (1935). The rationale behind this method is that the daily food consumption of fish under natural conditions can be measured by comparing the quantity of food in the stomachs of newly caught fish and the rate of food passage from the stomachs of newly caught fish held in captivity for several hours. The method has a serious limitation in that it presupposes that capture and handling do not affect rates of digestion. In spite of this obvious source of error this

technique was used in this study since it was the only relatively simple procedure which so far had been devised for estimating the food consumption of fish under field conditions. Whatever method is used however, it would have to involve handling.

In the study 20 fish were captured by seining every three hours (1972) or every four hours (1973). Ten of the fish were sacrificed and preserved immediately while the remaining ten were kept alive for three hours (1972) or four hours (1973) in a tank of aerated water, before being killed and preserved. The quantity of food in the stomachs was analysed in the laboratory, and estimates of the daily food consumption were obtained from the sum of the differences between the mean weight of the stomach contents of fish (a) kept alive for three or four hours and (b) those from a subsequent sample which were killed immediately.

The regular sampling every three or four hours also provided data on feeding periodicity. In order to watch the feeding behaviour or R.holubi visual observations were made during the day and night using SCUBA.

RESULTS

1. Diet

The stomach contents of R.holubi from the estuaries sampled are shown in Tables 6 and 7 analysed according to frequency of occurrence and dry weight respectively. The number of fish in each sample is shown. Aquatic plants formed the bulk of the stomach contents in almost all cases. The species of plant eaten varied considerably between the different estuaries and the following species were recorded in the stomachs of R.holubi:

Percentage frequency of occurrence of food items in the stomach of Rhabdosargus holubi.

ESTUARY	WEST KLEINEMOND				KASOUCA		KOWIE		MSIKABA
SEASON	Summer 1971	Autumn 1972	Spring 1972	Summer 1973	Winter 1973	Autumn 1972	Winter 1972	Autumn 1972	Summer 1973
Number of fish in sample	21	175	58	50	49	100	28	30	16
Food item									
1. Aquatic macrophytes and green algae	52	79	74	86	84	89	78	23	93
2. Red algae								57	
3. Amphipoda	76	10	35	4	4	22	72		4
4. Isopoda	10	10	24	6	20	4	6		
5. Bivalvia								30	43
6. <u>Assimineae</u>		6	9	8		3	6		7
7. Ostracoda		2		4				13	13
8. <u>Callianassa</u>	10	2				2			
9. <u>Upogebia</u>								23	
10. Polychaeta		1	17		2			7	
11. Oligochaeta			5						
12. Crabs		1					6		
13. <u>Palaemon</u>		1	2				6	7	17
14. Fish (Unid.)		1	1		2			2	2

TABLE 7

Percentage occurrence of food items according to dry weight in the stomach of Rhabdosargus holubi.

ESTUARY SEASON	WEST KLEINEMOND				KASOUGA		KOWIE		MSIKABA
	Summer 1971	Autumn 1972	Spring 1972	Summer 1973	Winter 1973	Autumn 1972	Winter 1972	Autumn 1972	Summer 1973
Number of fish in sample	21	175	58	50	49	100	28	30	16
								**	
Food item									
1. Aquatic macrophytes and green algae	51	88	79	92	83	92	71	80	90
2. Amphipoda	38	1	3			2	19	1	
3. Isopoda	1	3	13	2	15	1	1		
4. Bivalvia								4	
5. <u>Assimineae</u>		4	2	5		4	1		6
6. Ostracoda								1	
7. <u>Callianassa</u>	10	3				2			
8. Polychaeta		1	1						
9. Oligochaeta			1						
10. Crabs		1					2		2
11. <u>Palaemon</u>		1	1				8	11	
12. Fish (Unid.)		1	1		3			5	2

** Samples lost prior to weighing.



Plate 5. Scanning electromicrograph of Ruppia spiralis from the West Kleinmond estuary, showing layer of epiphytic diatoms.

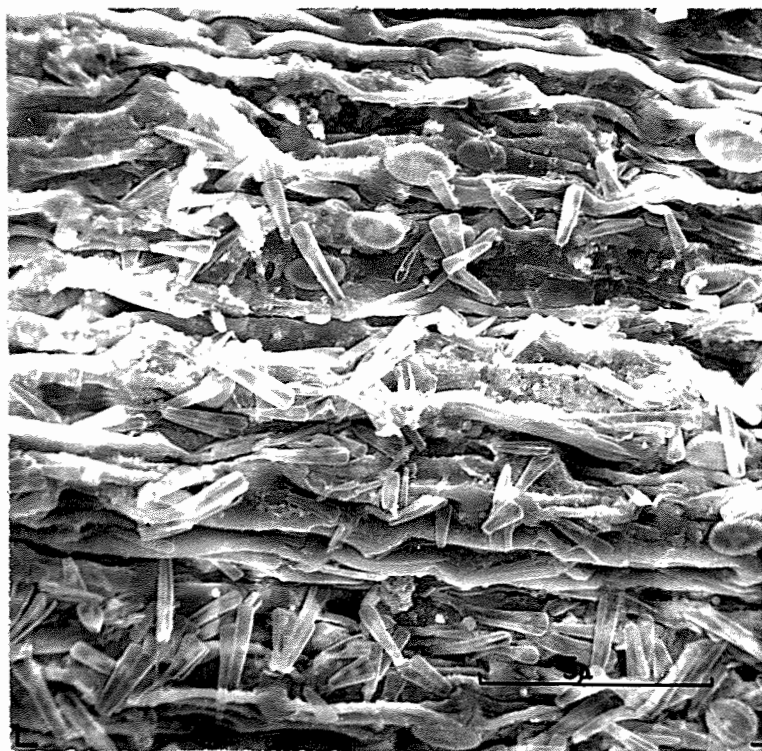


Plate 6. Scanning electromicrograph of Ruppia spiralis from the stomach of Rhabdosargus holubi, showing layer of epiphytic diatoms.

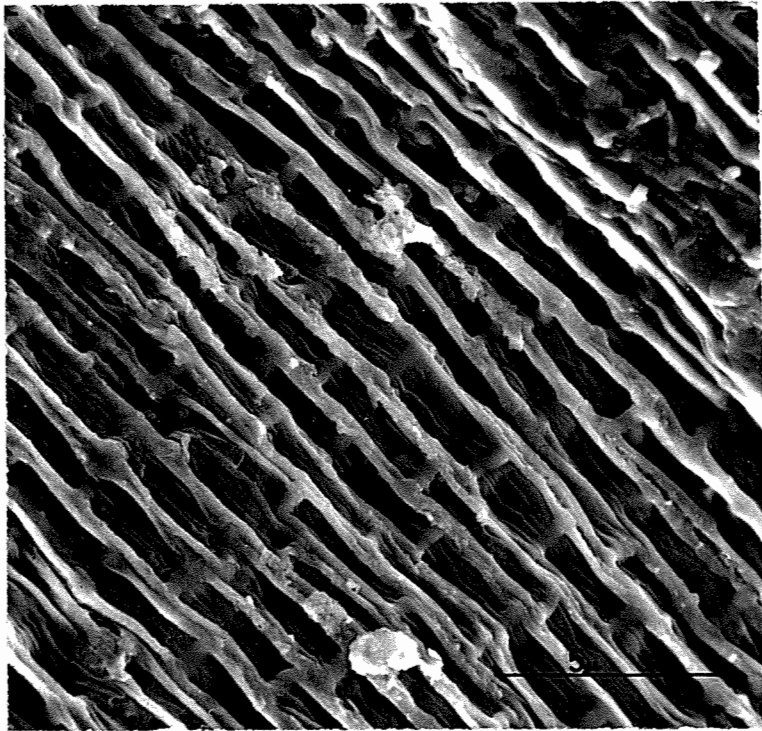


Plate 7. Scanning electronmicrograph of Ruppia spiralis from the hindgut of Rhabdosargus holubi. Note absence of epiphytic diatoms. The R.spiralis in Plates 6 and 7 was from the same individual.

The scanning electron microscope revealed that R.spiralis from the West Kleinmond estuary and from the stomachs of R.holubi from the West Kleinmond estuary was covered with a layer of sessile diatoms (Plates 5 and 6). The chief components of the diatom flora were:

Licmophora ehrenbergii (Kützing)

Cocconeis scutellum Ehrenberg

Cocconeis placentula Ehrenberg

Cocconeis dirupta Gregory

Synedra crystallina Kützing

Achnanthes minutissima Kützing

R.spiralis from the intestine immediately following the stomach and from the hindgut showed very few or no diatoms (Plate 7). In addition to R.spiralis the hindgut contained large amounts of empty diatom frustules. Total nitrogen determinations on naturally diatom-free R.spiralis from the West Kleinmond estuary, and from the stomach and hindgut of R.holubi are shown in Table 8. There is little difference in total nitrogen content between diatom free R.spiralis collected from the West Kleinmond estuary and R.spiralis from the hindgut of R.holubi, again indicating that R.spiralis is not subject to digestion in the gut of R.holubi. The higher nitrogen values from the stomach material can be ascribed to the presence of diatoms.

TABLE 8

Percentage total nitrogen content of Ruppia spiralis samples from 8 Rhabdosargus holubi and from West Kleinmond estuary.

Diatom free <u>R.spiralis</u> from West Kleinmond	S.Error	<u>R.spiralis</u> from hindgut of <u>R.holubi</u>	S.Error	<u>R.spiralis</u> from stomach of <u>R.holubi</u> with diatoms	S.Error
3.9	0.14	4.07	0.24	5.17	0.02

Table 9 shows the amount of diatomaceous material (expressed as a percentage of total plant dry weight) on R.spiralis from the West Kleinmond estuary and from the stomachs of eight R.holubi. It is evident that about 50% of the dry weight of the macrophyte was contributed by diatoms. Table 9 also shows that about 27% of the dry weight of diatoms was composed of tissue. The higher amount of diatom material on R.spiralis from the stomach suggests that the fish may be able to select weed which has a high density of diatoms. The lower amount of tissue in the stomach diatoms was probably due to some digestion having occurred prior to the death of the fish. An investigation into the red algae consumed by R.holubi in the Kowie estuary showed that 26% of the dry weight of the plant was made up of diatoms and 20% of Bryozoa (Ectoprocta), thus making a total of 46%. This latter is very similar to the proportion of the dry weight of R.spiralis made up by diatoms in the West Kleinmond estuary. Thus it is clear that a very significant proportion of the material classified as plant consumed by R.holubi is made up of either diatoms or Bryozoa.

TABLE 9

Dry weight of diatomaceous material on Ruppia spiralis from R.holubi and from West Kleinmond estuary (expressed as a percentage of total dry weight of plant) and percentage organic tissue in the diatoms.

Stomach of <u>R.holubi</u>		West Kleinmond estuary	
% diatom on plant	% organic tissue in diatom	% diatom on plant	% organic tissue in diatom
55.59	9.6	41.18	27.21

Although both frequency of occurrence and dry weight analyses emphasise the amount of plant material consumed, significant quantities of such benthic invertebrates as amphipods, isopods, bivalves, gastropods,

and polychaetes were eaten. As was the case with the plants, the species of animals that were eaten varied according to their availability in the estuary. Bivalves and the burrowing prawn Upogebia africana (Ortmann) were found in animals from the Kowie estuary but not from the West Kleinmond estuary where they do not occur. Similarly the burrowing prawn Callianassa kraussi Stebbing which is common in closed estuaries but absent from open estuaries was consumed only in the West Kleinmond and Kasouga estuaries and not in the Kowie or Msikaba estuaries. Amphipods and isopods were commonly eaten in the closed estuaries but not in the open ones, although they are present in both. A possible reason for the absence of these two groups of animals in the stomach contents of open estuary fish is that the water of open estuaries is usually less transparent than that of closed estuaries, thus making it more difficult for the fish to locate small, relatively fast moving, animals. Small numbers of polychaetes and the gastropod Assimineia sp. were found in fish from both open and closed estuaries. These animals remain on the substrate and are slower moving than isopods or amphipods and would be easier to catch. Although the amount of animal material varied between 8 and 49% of the gut contents by dry weight there appeared to be few seasonal trends. In the West Kleinmond estuary the prawn C.kraussi formed 10% of the stomach contents in summer but at other times of the year little or no C.kraussi were eaten. There is no apparent reason why this species should only be consumed in the warmer months, unless higher temperatures perhaps induce these prawns to leave their burrows more often, for it is only when they are out of their burrows that R.holubi can capture them.

As has been described in Parts I and II R.holubi migrate to the sea before reaching maturity. At about this time the teeth undergo a

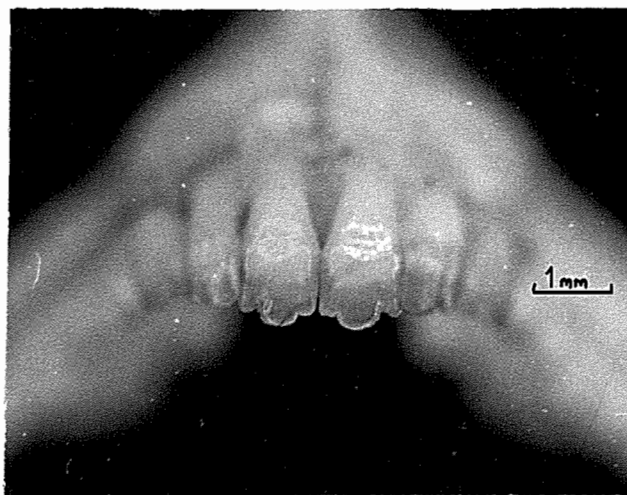


Plate 8. Upper incisors of juvenile Rhabdosargus holubi (8 cm S.L.) showing tricuspid form.

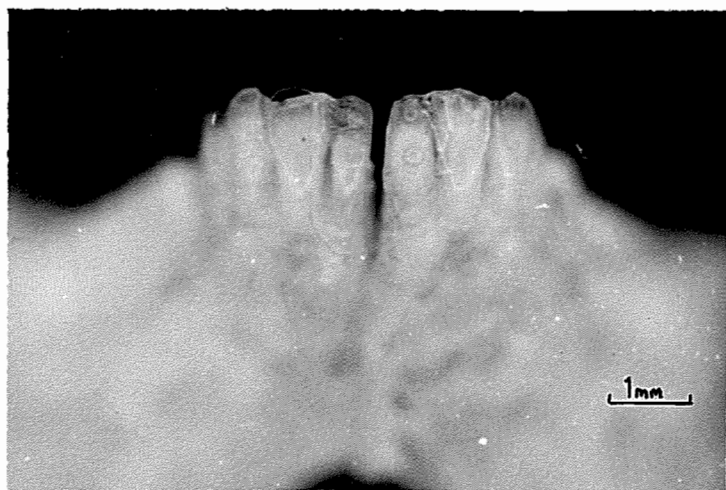


Plate 9. Lower incisors of juvenile Rhabdosargus holubi (8 cm S.L.)

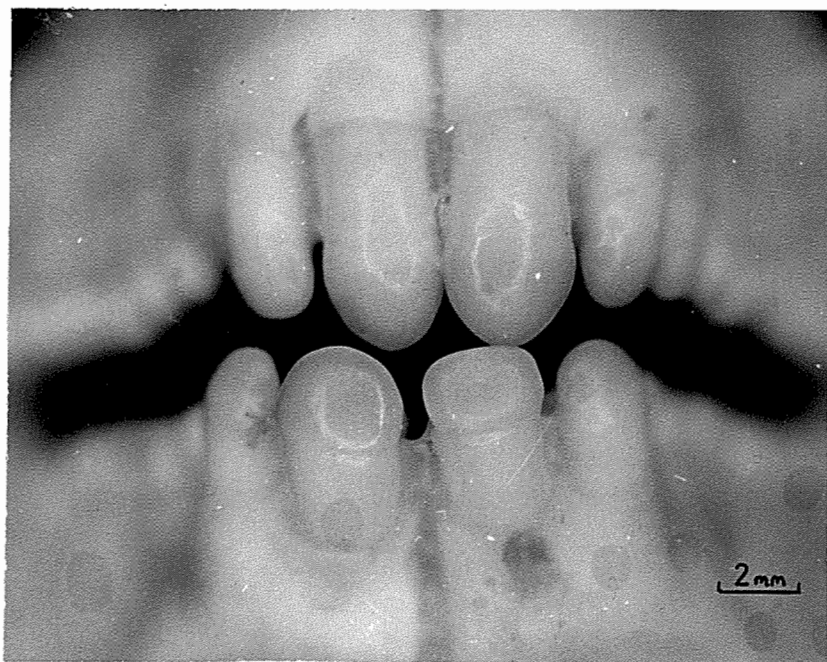


Plate 10. Upper and lower incisors of adult Rhabdosargus holubi (26 cm S.L.).

change. The flattened tricuspid teeth of the juveniles (Plates 8 and 9), ideal for biting off lengths of aquatic plants, are replaced by rounder and heavier front teeth in the adults (Plate 10). This change in dentition is accompanied by a change of diet. The diet of adult R.holubi (over 18 cm S.L.) from the sea and lower reaches of large open estuaries analysed from University of Cape Town records is shown in Table 10 and shows the predominance of animal material in the diet, especially bivalve molluscs (of the genera Loripes, Tivela and Donax). A single male R.holubi of 26 cm S.L. was captured in the lower reaches of the Kowie estuary in 1973 and its stomach was packed with the gastropod Nassa kraussiana (Dnkr) and the remains of one bivalve. It is significant that none of the juveniles in the Kowie estuary had been feeding on N.kraussiana which is extremely abundant there. The teeth of juvenile R.holubi may not be sufficiently robust to deal with this gastropod.

TABLE 10

Percentage frequency of occurrence of food items in the stomachs of Rhabdosargus holubi above 18 cm S.L. (Data courtesy U.C.T.).

Food item	Durban Bay	Bushmans estuary	Breede estuary
Plant material	4	15	33
Bivalvia	54	38	44
Polychaeta	9		11
Amphipoda	6		
Penaeids	7		
<u>Haminea</u>	2		11
Sipunculids	3		
<u>Upogebia</u>		8	
<u>Callianassa</u>		8	
<u>Nassarius</u>		8	
<u>Palaemon</u>		8	
Crabs	5	15	
<u>Echinodiscus</u>	7		
Number in sample	29	11	5

2. Food consumption and feeding periodicity

Since smaller fish eat relatively more food, expressed as a percentage of body weight, than larger fish (Jones, 1973), it was necessary to determine the relationship between food intake and size in order that valid consumption estimates could be made. Comparisons between samples of different mean weights would be misleading. Using the methods of Jones (1973) the relation between the weight of food in a full stomach and the fish weight was used as an index of feeding rate. This relationship, in the case of R.holubi, was linear and the equation for the regression is:

$$y = 5.3x + 10.2 \quad (r = 0.7243, P < 0.001)$$

The percentage fullness of the stomach of each fish captured in the 24 hour samplings could be calculated using the above linear relationship between the weight of fish and the weight of food in a full stomach, and the absolute weight of food in the stomach corrected to that of a fish of 5 g dry weight - the mean weight of fish captured.

The results of the food consumption experiments are shown in Tables 11 and 12. Both the mean actual dry weight and corrected dry weight of food in the stomachs is shown, together with the percentage fullness of the stomach calculated from the regression above, from which the amount of food present in the stomach of a 5 g fish could be estimated. The mean dry weight of food consumed every three or four hours by a fish of 5 g was calculated by subtracting the dry weight of food present in the stomach after three or four hours in captivity, from the dry weight of food present in the stomach of fish newly caught from the estuary at the time the captive fish were killed. The total food consumption over 24 hours was then estimated by summing the estimates of the amount of food eaten every three or four hours. Since all the stomachs contained some food

TABLE 11

Results of experiment in March 1972 at West Kleinmond estuary to calculate daily food intake of juvenile Rhabdosargus holubi (Corrected for 5 g fish). All weights in grams, (a = killed after 3 hours).

sample time	$\bar{x}$ weight of food in stomach	$\bar{x}$ dry weight of fish	% fullness	$\bar{x}$ dry wt cor. 5 g	$\bar{x}$ dry wt consumed by 5 g fish
1045	0.0213	5.2	56.4	0.0207	
1045a	0.0189	7.95	36.1	0.0132	0.0067
1345	0.0274	7.59	54.3	0.0199	
1345a	0.0243	10.04	38.3	0.0141	0.0090
1645	0.0292	6.82	63.0	0.0231	
1645a	0.0104	5.84	25.3	0.0093	0.0215
1945	0.0190	2.35	83.9	0.0308	
1945a	0.0171	2.05	81.2	0.0298	
2245	0.0033	1.88	16.4	0.0060	
2245a	0.0018	1.33	10.4	0.0038	
0145	0.0014	2.67	5.7	0.0021	
0145a	0.0006	2.32	2.7	0.0010	
0445	0.0002	3.03	0.8	0.0003	
0445a	0	2.81	0	0	0.0080
0745	0.0116	8.1	21.8	0.0080	
0745a	0.0188	12.0	25.5	0.0094	0.0113
Total — —					0.0565 g/day
					= 1.1% of body weight

TABLE 12

Results of experiment in April 1973 at West Kleinmond estuary to calculate daily food intake of juvenile R.holubi (corrected for fish of 5 g dry weight). All weights in grams. (a = killed after four hours).

Sample time	$\bar{x}$ weight of food in stomach	$\bar{x}$ dry weight of fish	% fullness*	$\bar{x}$ dry wt cor. 5 g	$\bar{x}$ dry wt consumed
1000	0.0273	4.88	78	0.0286	0.0286
1000a	0.0030	4.4	9.2	0.0034	0.0277
1400	0.0307	4.9	84.8	0.0311	
1400a	0.0140	5.64	34.9	0.0128	0.0366
1800	0.0707	7.97	134.7	0.0494	
1800a	0.0369	6.53	82.4	0.0302	0.0002
2200	0.0007	9.52	1.22	0.0005	
2200a	0.0025	6.31	5.68	0.0021	0.0002
0200	0.0003	5.53	0.73	0.0003	
0200a	0.	5.16	0	0	0.0002
0600	0.0002	4.91	0.47	0.0002	
0600a	0	4.81	0	0	
Total — — — — —					0.0931 g/day
					= 1.9% of body weight

* Derived using equation on page 67. Values over 100% indicate probable distension of stomach.

at the end of the captivity periods it was evident that complete digestion of the stomach contents did not occur in either the three hour or the four hour experiment. The daily food consumption of 0.0565 g dry weight per day (1.1% of body weight) measured by the three hour interval method in 1972 was less than that of 0.0931 g dry weight per day (1.9% of body weight) measured by the four hour

interval method in 1973. The reasons for this difference may be due to differential rates of digestion due to stress from capture and handling, therefore these results should be treated with caution.

Table 13 shows the amount of animal and plant material (expressed as a percentage of body weight) consumed per day in 1972 and 1973. These data were obtained from analyses of the stomach contents of fish captured in the 24 hour samplings and represent the proportions of the overall food consumption figures (Tables 11 and 12) made up by animal and plant material. Whereas the percentage of animal material consumed in 1973 is only slightly more than in 1972 the amount of plant material in 1973 was twice that in 1972.

TABLE 13

Amount of animal and plant material consumed per day by R.holubi in the West Kleinmond estuary (Expressed as percentages of body weight).

Food	March 1972	April 1973
Animal	0.45	0.55
Plant	0.67	1.35
Total	1.1	1.9

It can be seen from Tables 11 and 12 that virtually no food was consumed between about 1900 hours and 0600 hours. In both 1972 and 1973 R.holubi fed from dawn to dusk (Fig. 14). It was evident that the consumption of plant material went on throughout the daylight hours, reaching a peak in the late afternoon. Animals however, were

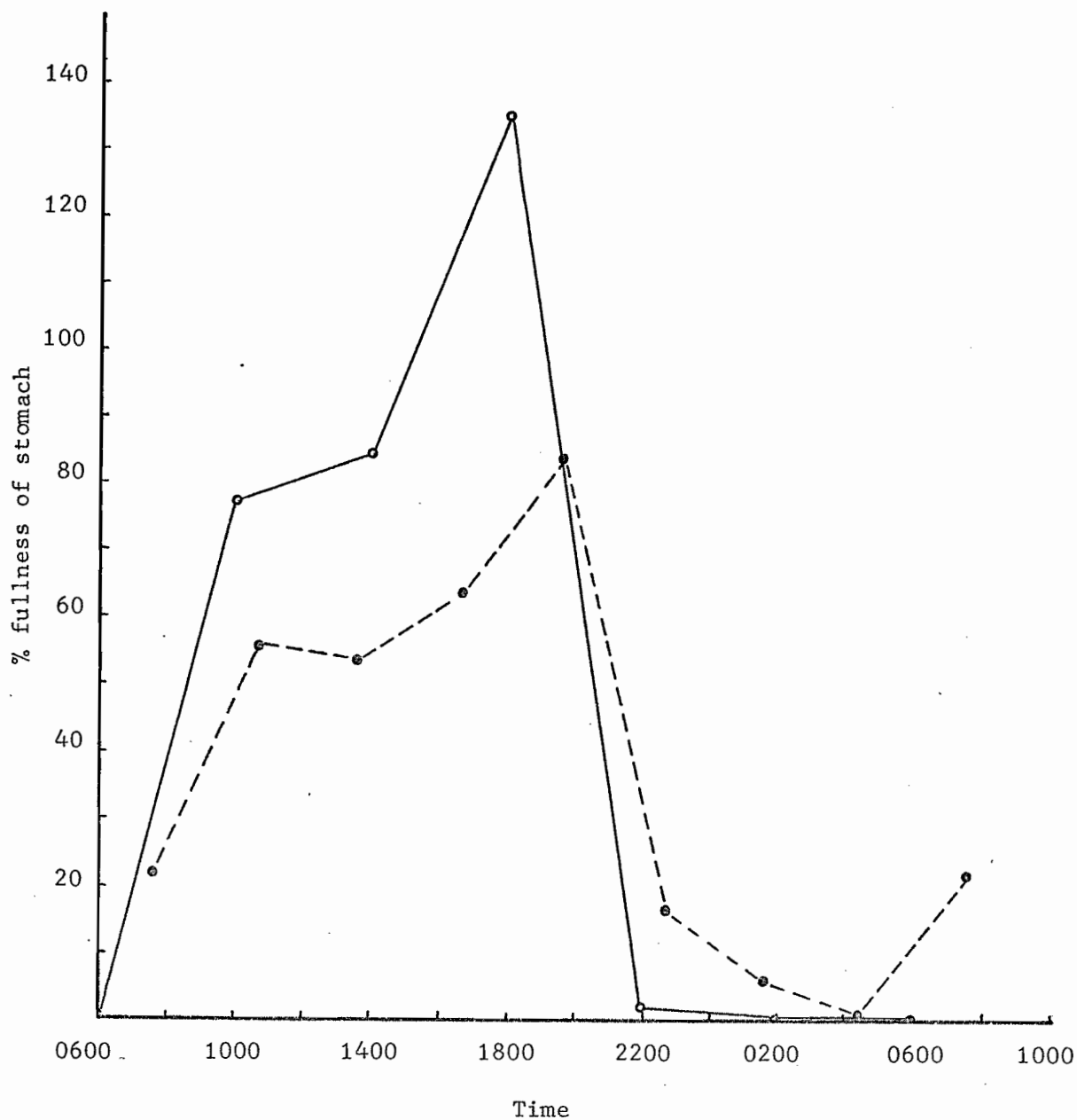


Figure 14. Feeding periodicity of R.holubi in the West Kleinmond estuary. (—) April 1973 4 hour sampling, (-----) March 1972 3 hour sampling. Based on Tables 11 and 12. (Fullness over 100% : See Table 12, page 67).

eaten only in the morning. There is no apparent reason why animals should only be consumed in the morning since both amphipods and isopods are just as available to the fish in the afternoon as the morning (Bolt, pers.comm.).

SCUBA observations during daylight hours showed that R.holubi were to be found mainly in shoals of between 10 and 400 individuals, the commonest number being about 50. The shoals occurred mainly along the edges of the beds of R.spiralis. It is likely that these beds were too thick and matted to allow fish an easy passage through them. R.holubi were observed to graze along the vertical walls of edges of the R.spiralis beds, biting off pieces of plant material. Night diving with underwater torches showed that by 2100 hours R.holubi were inactive and resting on the bottom, apparently asleep since they could be approached and touched before swimming away. At night the fish did not shoal and were noticeably evenly spaced. The vulnerability of R.holubi to predation by the crab Scylla serrata, observed to be active at night, could thus be considerable.

DISCUSSION

Both the analysis by frequency of occurrence and by dry weight indicate the predominance of plant material in the stomach contents of juvenile R.holubi. The results show that R.spiralis eaten by R.holubi from the West Kleinmond estuary passed through the alimentary canal apparently unaltered, suggesting that the macrophytes are not utilised as a food source. Nevertheless the epiphytic diatoms on R.spiralis were subject to digestion and may form up to 50% of the dry weight of the plant taken in. Thus the macrophyte is eaten because of its coating of diatoms. R.holubi apparently cannot separate diatoms mechanically from R.spiralis, separation can only be carried out by chemical digestion in the stomach. Diatoms are vulnerable to digestive enzymes because of the raphe and the numerous pores in the frustule and because of the lack of a cellulose wall. The cellulose wall of the R.spiralis however protects the cell contents of the macrophyte from digestion. The

presence of a cellulase enzyme in fish has not been established, so unless the cell walls of the R.spiralis could be damaged mechanically in the mouth, pharynx or gut, no digestion of the cell contents is possible. There was no evidence of any physical damage to R.spiralis in the gut of R.holubi. Thus a large part of the plant material consumed by R.holubi is not available to it as food. A related phenomenon was reported by Fish (1951) with regard to Tilapia esculenta Graham from Lake Victoria. This species feeds on phytoplankton from which only the diatoms are digested, the blue green and green algae being passed out unaltered. Animals are seldom found among the plant material in the stomachs of juvenile R.holubi, making it unlikely that the plants are consumed for the animals that might be sheltering in them. Feeding only occurs during daylight hours which suggests that visual stimuli may be important in the location and selection of food. Extensive examination of R.spiralis from the West Kleinmond estuary showed that many plants had only a sparse cover of diatoms or none at all, therefore the fish must exhibit choice in only taking parts of the plant with an adequate covering of diatoms.

Juvenile R.holubi appear to show a definite preference for plant material because in all the estuaries investigated they showed a predominance of plant material in the stomach. In the Kowie estuary they even consumed red algae (Table 6): known to be a rich source of diatoms (Giffen, 1970). The results from the present study indicate that diatoms and Bryozoa make up 46% of the dry weight of the red algae. The fact that R.holubi consumes plant material in order to gain access to the sessile epiphytic diatoms or Bryozoa is very significant since aquatic plants are recorded as being a major food item in other sparid fishes. Talbot (1955) states that filamentous algae were the commonest food

item occurring in estuarine Rhabdosargus globiceps and that the stomachs were often packed with this material, no animal remains being present. Talbot (1955) also reports that three other sparid fish, Sarpa salpa (L.), Diplodus sargus L. and D.trifasciatus (Raf.), consume considerable quantities of plants in the Klein River estuary at Hermanus near Cape Town. Darnell (1961) working on Lake Pontchartrain, Louisiana, stated that one of the most frequently consumed vascular plants was Ruppia maritima L. and that in a few of the fish species, notably the sparids, vegetation made up a large portion of the diet, as in Lagodon rhomboides (L.) (6 - 48%) and Archosargus probatocephalus (Walbaum) (54%). Thomson (1959b) showed that algae are a major food item of R.sarpa (Forskäl) in Lake Macquarie, Australia. He stated that all the omnivores fed in the weed zone of Lake Macquarie and suggested that the consumption of Zostera sp., by a number of omnivorous species, may be solely for the sake of the attached Bryozoa.

During 1971 there was an almost complete absence of R.spiralis in the West Kleinmond estuary, at which time animals predominated in the diet of R.holubi. The reasons for the absence of R.spiralis are not known but other macrophytes such as Potamogeton sp. were present, and did form part of the diet of R.holubi (Tables 6 and 7). It is interesting that the animals most commonly consumed in 1971 were amphipods, this fact is presumably related to their abundance and availability. If in fact amphipods were more abundant in the estuary in 1971 perhaps this was related to the absence of large areas of macrophytic vegetation. In subsequent seasons, when there were large quantities of R.spiralis in the West Kleinmond estuary, the number and weight of isopods eaten exceeded that of amphipods. There is however no evidence of a correlation between the numbers of these crustaceans and the amount of plant material present.

In 1972 with the advent of large quantities of R.spiralis in the West Kleinemonde estuary R.holubi turned to the consumption of large amounts of this macrophyte in order to gain access to the sessile diatom flora. It is significant in this respect that the growth rates of R.holubi in 1971 and 1972 were similar. This suggests that the greater proportion of animal food and smaller quantities of plant material eaten in 1971 were as nutritionally valuable as the very great amounts of plant material eaten in 1972. It is important to note that only about 27% of diatoms is made up of digestible organic tissue, the remainder being siliceous frustule, so in terms of tissue weight consumed per day it is possible that in 1972, animal food, which was at about the same level as in 1971 (Table 13), was as important as plant food, despite the very large quantities of plant material eaten in 1972.

The daily food consumption figures of 1.1% of body weight (1972) and 1.9% of body weight (1973) must be considered as underestimates since in some cases individual fish had more than 1.9% of their body weight in their stomachs at a given time. In neither year would it appear that the periods between sampling were long enough to allow sufficient digestion to occur. The four hour interval in 1973 gave a higher consumption figure than the three hour interval in 1972 suggesting that it was time that was responsible for the underestimates. It is very likely that the rate of digestion was affected by stress due to capture and handling. Jones (1973) working on the turbot (Scophthalmus maximus (L.)) in a similar series of experiments, obtained a food consumption figure of 2.5% of body weight per day, which was considered too low, and stress was suggested as a possible reason. It is difficult to envisage any method of capturing fish which would not subject them to stress and so affect the rate of

digestion. Laboratory studies on rates of digestion have been reviewed by Windell (1966). Due to the many different variables however, such as food type, ration size, and activity, which affect digestive rates, the extrapolation of laboratory results to fish feeding under natural conditions in the field is likely to lead to considerable inaccuracy. Food consumption in fish is affected by temperature (Kinne, 1960; Warren and Davis, 1966; Jones, 1970; Peters and Boyd, 1972) and thus the figure of 1.9% of body weight obtained in April when the water temperature is about 18°C (Part III) would be less than could be expected under similar experimental conditions in the middle of summer (December - January).

It would appear that during the juvenile phase of its life history, which is spent in estuaries, R.holubi is an omnivore with a marked preference for plant material covered with diatoms. The reasons for juvenile R.holubi consuming diatoms in preference to animal material are not clear since the amount of digestible tissue gained from the diatoms may be less than from an equivalent amount of animal food. Possibly plant food is more accessible and requires less energy to find than animal food. Animal food varies considerably from one estuary to another according to its abundance and availability but amphipods, isopods, small gastropods and polychaetes were the main animal food in the estuaries investigated. The migration to the sea at maturity is accompanied by a change in the form of the teeth and a reduction in the amount of plant material in the diet. At this stage the fish switch to a diet of predominantly bivalve molluscs. The life cycle of R.holubi in which the juveniles are found in estuaries and the adults mainly in the sea may be related to the food preferences of the different age groups. Bivalve molluscs which make up a considerable portion of the diet of adult R.holubi are scarce in

southern African estuaries (Day, 1951) but are relatively common in the sea. Conversely the food eaten by juvenile R.holubi may not be present in any quantities in the sea. Most of the plants and their diatom flora, which form a prominent part of the diet of juveniles in estuaries, are absent from the sea. A notable exception is the red algae which are washed into open estuaries, such as that of the Kowie River, and are readily eaten by juvenile R.holubi. However on the southern African coasts most of these red algae are found on the lower intertidal zones of rocky shores where they may be inaccessible to R.holubi due to such factors as wave action and currents.

The teeth of juvenile R.holubi are specialised for feeding on plant material and although these teeth are used for eating animals they change with maturity. This change in dentition does not take place in R.globiceps or R.sarba which have teeth similar to adult R.holubi. Green filamentous algae are a major food item of both juvenile R.globiceps (Talbot, 1955) and R.sarba (Thomson, 1959b), but macrophytes would appear to be less important. The reverse is the case in juvenile R.holubi which seem to prefer macrophytes that would require more specialised grazing teeth since the vascular tissue of these plants makes them very much tougher than algae. The red algae consumed by R.holubi in the Kowie estuary are likewise very much tougher than green filamentous algae.

This suggests that perhaps the tricuspid teeth of the juveniles of R.holubi are a specialisation which developed to enable them to utilise more effectively the large plant food resources of south east African estuaries. Therefore it is likely that the food requirements of both the juveniles and the adults determine to a considerable extent at what stage R.holubi enters and leaves estuaries.

PART V

CONDITION AND FEEDING

INTRODUCTION

Juvenile Rhabdosargus holubi which become trapped in closed estuaries such as that of the West Kleinmond are dependent upon the quality and quantity of the food resources of the estuary. Feeding and growth took place in the closed West Kleinmond estuary from 1971 until 1973 but nothing was known about the level at which R.holubi were able to feed relative to similar fish in open estuaries, nor was it known whether the amount of food eaten varied seasonally.

Since weight but not length of fish usually varies according to the feeding level or reproductive state, assessments of condition, 'well-being', or 'fatness' of fish have generally employed measurements of the length - weight ratio. These condition factor analyses have long been used in fisheries biology as a relatively rapid means of assessing factors such as the feeding level in one system as opposed to another. More recently Brett et al (1969) showed experimentally, using Oncorhynchus nerka Walbaum, that the fat fraction of the body can be used as a measure of the feeding level of a fish without particular reference to temperatures. He went on to state that fat assessment should be of value to the ecologist as a means of assessing the general feeding level of any particular population, providing that metamorphic changes are taken into account. The main function of lipids is as an energy reserve and, according to Tashima and Cahill (1965) lipids in fish are stored at or near the sites of utilisation since there is no specialised adipose tissue as in birds and mammals. The oxidation of fatty acids provides for sustained muscular contractions such as are used in normal swimming activity, while glycogen is the major source for violent and sudden muscular activity.

Since lipid levels are not affected by short-term stress (Love, 1970), as are for example carbohydrates such as glycogen, it was decided to employ determinations of the total lipid content of the body together with condition factor analyses in order to evaluate the feeding level of juvenile R.holubi in the closed West Kleinemonde estuary in relation to that of similar fish captured in the open Kowie estuary. As only juvenile fish were involved in the investigation, the results of lipid analyses and condition factor analyses were not complicated by cyclical changes in lipid level and weight, associated in adults with the formation of gametes.

Experiments to determine the effect of feeding level on the total lipid content and condition of juvenile R.holubi were undertaken in the laboratory in order to provide some basis on which to judge results obtained from animals collected from estuaries.

MATERIALS AND METHODS

1. Experimental studies

All experiments were conducted at a salinity of 35⁰/oo in the 225 l and 400 l tanks described in Part III. Fish between 6 and 14 cm S.L., captured by seining in the West Kleinemonde estuary were transported to the laboratory in plastic bags supplied with aeration and transferred directly to the experimental tanks. 'Tetramin' brand tablet fish food was supplied and most newly captured fish began feeding within 24 hours. Experiments were commenced one week after capture. Ten fish, all of the same batch, were used in each experiment. The experiments were performed between March and June 1973.

a) Starvation experiments : groups of fish were starved for 2, 4, and 8 weeks at 22⁰C before being killed and analysed.

b) Feeding experiments : two groups of fish held at 22°C were fed to excess twice daily for 7 weeks, one group on 'Tetramin' brand tablet fish food and the other on the small carid shrimp Palaemon pacificus Stimps, collected from the east lagoon of the Kowie estuary. Excess uneaten food was removed after one hour. One further group of fish was held at 15°C and fed to excess twice daily for 7 weeks on 'Tetramin' brand tablet fish food.

2. Sampling of fish from West Kleinmond and Kowie estuaries

Ten R.holubi were collected from each estuary once a month from December 1972 to October 1973 by seining in the manner described in Part I. Fish were immediately placed in crushed ice and subsequently deep frozen to - 25°C within about 8 hours of capture. All fish captured were between 6 and 14 cm S.L. Attempts made to collect juvenile R.holubi from coastal rock pools at low tide using rotenone were unsuccessful.

3. Determination of total lipid content

Total lipids were determined for each fish by the Groves procedure for dry extraction of fat (Brett et al, 1969). An outline of the method is as follows:

Frozen fish were sliced longitudinally and their abdominal cavities cut open, the fish were then placed on preweighed aluminium foil trays and dried to constant weight at 70°C in a vacuum oven. The dried fish were then crushed and transferred, together with the aluminium foil, to a 500 ml erlenmeyer flask fitted with an aluminium foil wrapped stopper. The material in the flask was extracted with four successive 200 ml portions of n- hexane. Each extraction proceeded, with occasional shaking, for 24 h. After the final extraction the hexane was poured off through preweighed filter paper to prevent loss of suspended

particulate matter. Final traces of hexane were removed from the fish in a vacuum oven at 40°C.

The fat content of the food items of R.holubi was determined in a Soxhlet apparatus using anhydrous diethyl ether extraction. n-Hexane was not used in these determinations as it was found to contain traces of an unknown impurity. This impurity did not affect the results of the Groves method which relied upon the difference in weight between dried fish and fat-free dried fish, but could have influenced the results from the Soxhlet method where the fat itself was collected and weighed.

4. Condition factor analyses

The relationship between length and weight provides the basis on which condition factors may be calculated. The formula used for calculating condition factor was:

$$C.F. = \frac{W \times 100}{L^b}$$

The exponent b was obtained from the relationship between length and weight and in the case of juvenile R.holubi has the value 2.8512 (Part I). There was no change in the length - weight relationship of R.holubi between 5 and 15 cm S.L. (Fig. 3), thus the value of 2.8512 could be used to calculate the condition factor of fish from both the West Kleinmond and Kowie estuaries in addition to the fish kept under experimental conditions in the laboratory.

RESULTS

LIPID ANALYSES

1. Experimental studies

a) Starvation experiments : The total lipid and water content of

juvenile R.holubi held for 2, 4 and 8 weeks without food at a salinity of 35⁰/oo and a temperature of 22⁰C are shown in Table 14. Although each batch consisted of individuals collected on the same occasion, different batches were used for each of the experiments and as shown in Table 14 their initial fat and water values were different, therefore the percentage change in fat and water content are shown in Table 14. There was a significant inverse relationship between the fat and water content of the body, ($r = 0.599$, d.f. = 32, $P < 0.001$), the sum of the two constituents usually representing about 80% of the total body weight. A relationship between the fat content and the size of the body has been reported in Anguilla anguilla L. and Trachurus trachurus L. (Lovern, 1938; Arevalo, 1948), but this relationship did not exist in the size group of R.holubi used throughout this investigation. It is possible however that it may be present in a wider size range of R.holubi than were used in this study.

TABLE 14

The total lipid and water content of 3 groups of R.holubi starved for 3 different periods of time. (g/g = gram per gram body weight).

Period of starvation	LIPID				WATER			
	$\bar{x}$ initial lipid g/g	$\bar{x}$ lipid after starvation	S.E.	% change in lipid	$\bar{x}$ initial water g/g	$\bar{x}$ water after starvation	S.E.	% change in water
2 weeks	0.037	0.026	0.003	-31	0.79	0.77	0.004	+2.0
4 weeks	0.050	0.029	0.005	-43	0.80	0.76	0.004	+5.3
8 weeks	0.065	0.043	0.006	-34	0.83	0.77	0.008	+7.2

b) Feeding experiments : The lipid and water content of fish fed to excess are shown in Table 15. There was no significant difference in total lipid content between 'Tetramin' fed fish kept at 15⁰C and

those at 22°C. ($t = 1.98$, d.f. = 14, $P > 0.05$). There was however a significant difference between the two groups of R.holubi kept at 22°C. Those fed on 'Tetramin' tablets had a significantly higher fat content than those fed on P.pacificus ($t = 2.92$, d.f. = 17, $P < 0.01$). The total lipid content of the food items was therefore determined and is also shown in Table 15. The lipid content of 'Tetramin' tablets was found to be 17% of dry weight while the lipid content of P.pacificus was only 3% of dry weight. The latter determinations were made on winter animals but the fish were fed on summer individuals whose fat content may have been somewhat higher.

TABLE 15

The total lipid and water content of three groups of fish after feeding to excess twice daily for 7 weeks. (g/g = gram per gram body weight; d.w. = dry weight).

Temp C°	food	$\bar{x}$ ration g/g d.w.	$\bar{x}$ lipid content of food.g/g d.w.	$\bar{x}$ lipid content of fish	S.E.	$\bar{x}$ water content g/g	S.E.
15	Tetramin	0.05	0.17	0.069	0.008	0.71	0.009
22	Tetramin	0.13	0.17	0.084	0.004	0.69	0.004
22	<u>Palaemon</u>	0.10	(0.03)*	0.065	0.005	0.70	0.004

* determined from winter captured P.pacificus - fish were fed summer individuals.

Both groups of R.holubi which were given 'Tetramin' tablets were fed to excess and it is important to note that although those kept at 15°C consumed less than half the amount of food of those kept at 22°C (Table 15), their lipid content after 7 weeks was similar. This would appear to support the statement of Brett et al (1969) that the fat fraction reveals the feeding level of the fish irrespective of temperature.

2. Results from the West Kleinmond and Kowie estuaries

Figure 15 shows the total lipid and water content of juvenile

R.holubi from the two estuaries from December 1972 until October 1973. In the West Kleinmond estuary the lipid levels of R.holubi were at a maximum in summer (6.4%) and a minimum in winter (0.75%). Fish from the Kowie estuary showed little seasonal variation, their lipid level fluctuating between 1% and 2%. The results from the West Kleinmond estuary are similar to those obtained by Arevalo (1948) working on Trachurus trachurus L. in which there was a maximum lipid level (9%) reached at the end of summer and a minimum lipid level (1.5%) at the end of winter. The inverse relationship between fat and water which was present in the laboratory fish also occurred in the R.holubi from the two estuaries. Lipid analyses of epiphytic diatoms taken from Ruppia spiralis from the West Kleinmond estuary showed they had a mean lipid content of 9% dry weight. Epiphytic diatoms from red algae from the Kowie estuary had a mean lipid content of only 1.36% dry weight.

CONDITION FACTOR ANALYSES

1. Experimental studies

Condition factor reflects the feeding level of the various groups (Table 16). Starved fish held at 22°C had a very reduced condition factor when compared with fish fed to excess at 22°C. The group which were fed to excess at 15°C showed a lower condition factor than those fed to excess at 22°C, reflecting perhaps the lower food intake of the 15°C fish, although as previously stated the two groups had similar lipid levels.

2. Results from the West Kleinmond and Kowie estuaries

The mean condition factors of R.holubi captured in the West Kleinmond estuary from 1971 until 1973, and in the Kowie estuary in 1973 are shown in Figure 16. There was a seasonal fluctuation in the condition factor of West Kleinmond fish : their condition factor

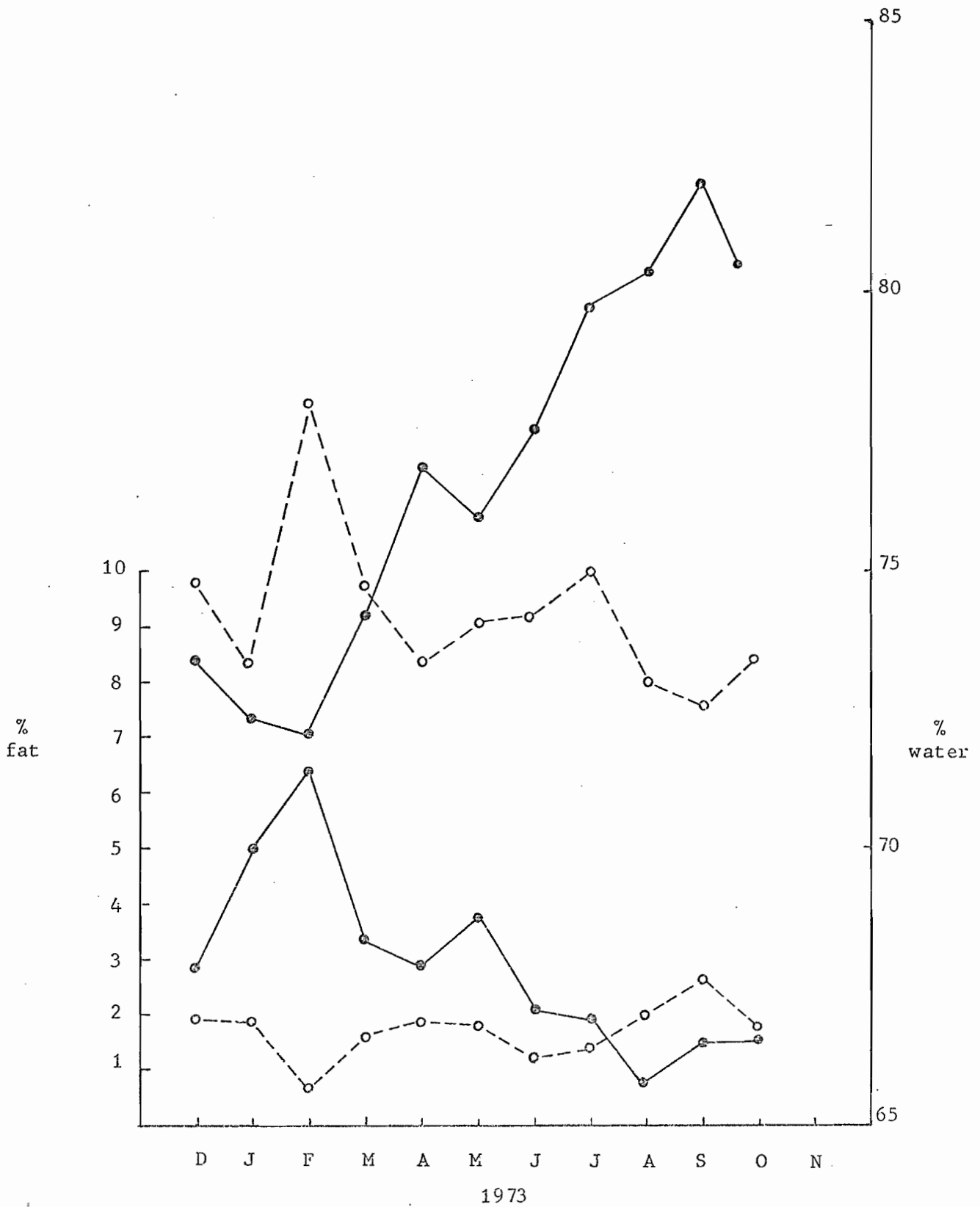


Figure 15: Mean monthly lipid and water content of *Rhabdosargus holubi* during 1973 from the West Kleinemonde (—) and Kowie estuaries (-----).

fell off in winter to a minimum value in August and was at a maximum in summer (December - January). R.holubi from the open Kowie estuary showed little seasonal change in their condition factor which remained at a similar level to that of summer fish from the West Kleinmond estuary.

TABLE 16

The condition factor of the three groups of starved fish and the three groups of fish fed to excess.

Treatment	Temp. °C	Duration of experiment	$\bar{x}$ Condition factor	Standard error
Starved	22	2 weeks	52	2.0
Starved	22	4 weeks	56	1.0
Starved	22	8 weeks	51	1.0
Fed 'Tetramin'	22	7 weeks	70	1.0
Fed 'Tetramin'	15	7 weeks	59	2.0
Fed <u>Palaemon</u>	22	7 weeks	64	1.0

DISCUSSION

The striking inverse relationship between the lipid and water fractions of the body of R.holubi has been demonstrated in other species by several workers (Brandes and Dietrich, 1956; Herrera and Munoz, 1957; Coppini, 1967). As the lipid level falls so the amount of water apparently increases and vice versa. Blaxter and Holliday (1963) have shown that the reason for this relationship is that most

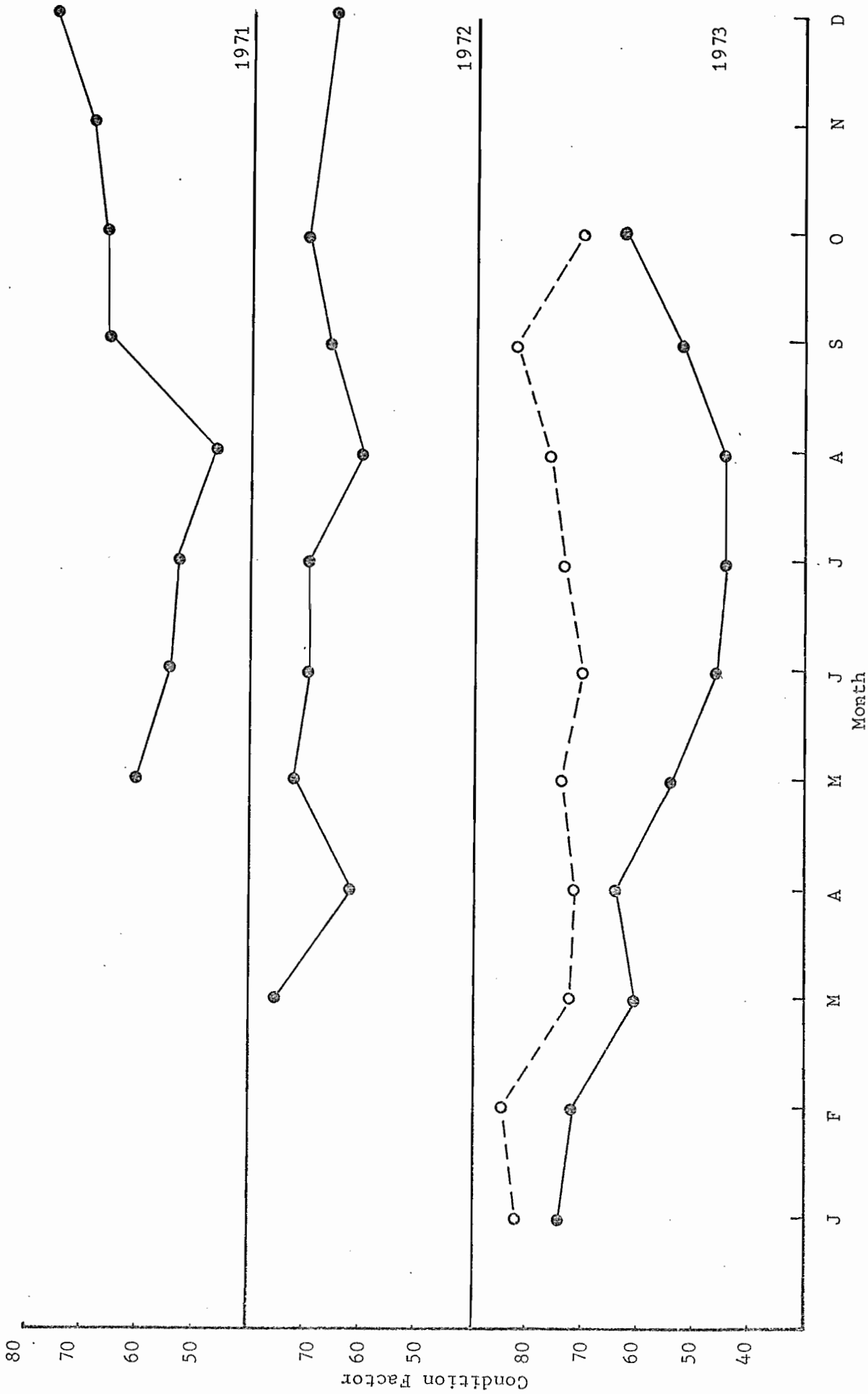


Figure 16: Condition factor of Rhabdosargus holubi in the West Kleinmond estuary (—) from 1971 to 1973 and in the Kowie estuary in 1973 (-----). Mean value for each month shown. (Standard error between 1.0 and 2.0 in all months).

measurements have been based on percentage changes, as was the case in this investigation. Where there is an absolute fall in the fat content the percentage of other substances, such as water, must rise relative to total body weight. In R.holubi the fat and water together make up about 80% of the body weight, a value similar to that reported by Iles and Wood (1965) for Clupea harengus L.. Although percentage changes in the water content of the body of fish may be misleading, the water content can be analysed relatively quickly, and provides a useful check on the accuracy of lipid analyses if the total of the two constituents is known.

The analyses presented here show that the lowest lipid levels were reached after 4 weeks of starvation. After 8 weeks the proportion of lipids present had risen. The percentage water content however reached a maximum at 8 weeks when it appeared that the lipid content had also risen. It is likely that the increase in the proportion of lipids at 8 weeks was due to the decrease in some other constituent such as protein, this suggestion is supported by the fact that the amount of water continued to increase. Starvation thus seems to reduce the total lipid content of the body, but only up to a certain point, beyond which other body components, possibly protein, may be utilised. Wilkins (1967) found that when C.harengus were starved, all the neutral triglyceride fat was metabolised but a residual amount of fat was always present in the body, representing structural and functional elements essential for survival. It appears possible from the experimental study that a similar situation may have existed in R.holubi kept under starvation conditions.

R.holubi which were fed to excess in the laboratory showed a markedly higher lipid content than the individuals which were starved.

Edwards et al (1972) found that the relative proportions of lipid and protein did not change in relation to feeding level in Gadus morhua L. since energy is apparently not stored as lipid in the cod. It does seem however that in the experimental R.holubi the lipid content was dependant upon feeding level, but not upon temperature. Although the amount of food consumed decreased with temperature, the lipid content remained the same (Table 15).

The seasonal trend in lipid content in R.holubi from the West Kleinmond estuary appears to be absent from Kowie fish. The fat content of fish from both estuaries fell below that of laboratory fish which had been starved. Evidently the Kowie fish which had a consistently low lipid content were not starving throughout the year since their condition factor was always relatively high, at a similar level in fact to fish which had been fed to excess in the laboratory at 22°C (Table 16). The reasons for the all year round low lipid levels in the Kowie estuary may be connected with the food supply, which although not deficient in quantity may have been low in lipid content. It was shown in Part IV that diatoms were important food items of R.holubi: the diatoms from the West Kleinmond estuary contained 9% fat while those from the Kowie estuary contained only 1.36% fat. As shown in Table 15, the lipid content of R.holubi is directly related to the lipid content of the diet. If Kowie fish were feeding predominantly on food containing only 1.36% fat while the West Kleinmond fish were consuming diatoms with a relatively high fat content of 9% it would be expected that there would be a difference in lipid content of the fish. The reasons for the lipid content of the diatoms in the two estuaries being different are not easily explained. It has been suggested however that the amount of oil present in diatoms may reflect their nutritional state (Morris, 1967). It thus appears possible that there was a relationship

between the total lipid content of the food and the total lipid content of the body of R.holubi. This does not explain however, why the lipid levels of Kowie fish (throughout the year), and West Kleinemonnd fish (in winter) should be lower than those of fish which had been starved for up to two months in the laboratory. Love (1970) states that the greatest care must be used in interpreting values obtained from fish kept in the laboratory, a number of changes in the various constituents of the body may take place after capture, for example the proportions of proteins may change in newly caught fish. The condition factor analyses show a more uniform and constant picture than the lipid analyses. As with the lipid content the condition factor of Kowie fish remained relatively stable throughout the year, at a level similar to that of fish fed to excess at 22°C. During summer the condition factor of West Kleinemonnd fish was also at a similar high level, but during winter declined to the level reached by fish fed to excess at 15°C. Evidently the actual amount of food eaten affects the condition factor, and since the temperature also affects the amount of food eaten then it too will influence the condition factor. These results therefore indicate that West Kleinemonnd fish carried on feeding throughout the winter but at a considerably reduced level, while the R.holubi from the open Kowie estuary fed at a similar level all year round.

The condition factor shows little correlation with the lipid content of the body which may perhaps be controlled far more by the lipid content of the food than the amount of food. For example the fish from the Kowie estuary were feeding on a diet relatively low in lipids compared to the West Kleinemonnd fish, and during summer had a correspondingly lower lipid level in the body.

Thus lipid content may perhaps be used as an index of the amount of lipid in the food while the condition factor may reflect the quantity of food eaten. The fall in condition and lipid level in the fish from the West Kleinmond estuary in winter suggests both a reduction in the quantity of food eaten and a reduction in the lipid content of the food. The former is consistent with the laboratory findings that a decline in temperature causes a decline in food consumption and a decline in condition. The latter suggests that the oil content of the diatoms may be lower in winter.

The winter temperatures in the West Kleinmond estuary remain consistently as low as $11 - 12^{\circ}\text{C}$ for at least one month, with a daily fluctuation of only $1 - 2^{\circ}\text{C}$ (Part III). It is possible that these low temperatures in the West Kleinmond estuary, which are only a few degrees above the lethal limit of juvenile R.holubi may perhaps reduce feeding to a level low enough to cause a drop in the condition factor as well as total lipid level. R.holubi in the open Kowie estuary do not lose condition or suffer a drop in their total lipid content during winter. Daily temperatures in the Kowie estuary in winter range between 11 and 16°C , the high tide causing a rise in temperature to at least 15°C (Hill and Allanson, 1971). Since at least one temperature peak always occurs in daylight hours, the fish may be able to feed at an increased level during the time the water is at a relatively high temperature. This would create the situation where feeding would occur at least once every 24 hours, but for much of the remainder of the time, when the temperature falls to around 11°C , metabolic activity would be very low. Thus the amount of food consumed during the warmer part of the tidal cycle may be sufficient to maintain the condition factor and lipid level, taking into account the fact that metabolic activity would fall very low for half the day.

Thus, although it is possible to relate lipid changes to changes in the quantity of food eaten under laboratory conditions, it is evident that the lipid content of R.holubi under natural conditions cannot be used to assess the level of feeding without prior knowledge of such factors as the lipid content of the food and environmental temperatures. In this study it was not possible to relate the amount of lipid in the body with the feeding level of fish from the two estuaries. In fact, condition factor analyses provided more information on the probable level of feeding. The use of total lipid content alone to compare the feeding level of the fish from the two estuaries would have indicated for example that the Kowie fish were continually starving. Although lipid analyses proved useful in showing up the possible differences in the foods from the two estuaries, condition factor analyses showed at what level feeding was taking place.

Lipid analyses may provide useful information on feeding level in fish kept under controlled conditions in the laboratory, where all environmental parameters are known, but their value in field studies is extremely limited. It is not practical to monitor all the continuously variable factors which are likely to affect the lipid content of fish in a natural system, especially a marine or estuarine one. The complexity of lipid analyses compared with condition factor analyses, together with the difficulty of interpreting lipid content, indicate that lipid analyses are not a practical means of assessing the feeding level of fish populations.

In conclusion, R.holubi in the open Kowie estuary feed at a similar level throughout the year, maintaining a relatively constant condition; conversely the West Kleinmond fish consume more food during the summer months than the winter months, reaching a peak of condition in late

summer but declining in condition as the temperature falls over winter. This finding is consistent with the fact that R.holubi showed little or no growth during winter.

DISCUSSION

Rhabdosargus holubi is one of the most abundant fishes of the south eastern coast of southern Africa. It forms part of the migratory component of the estuarine fauna, since only the juveniles commonly occur in estuaries, the adults being almost exclusively found in the sea. Day (1951) defined the migratory component of the estuarine fauna as those animals which only spend part of their lives in estuaries. The juveniles occur in both open and closed estuaries and may become isolated in closed estuaries such as that of the West Kleinmond, by entering during the short periods in which they are open to the sea.

R.holubi has a similar geographical range to the endemic warm temperate intertidal group of animals designated by Stephenson (1947) as component C (south eastern component). This group is centred on the southern Cape coast between Port Elizabeth and the Cape of Good Hope with extensions as far north as Inhaca Island in Mocambique. R.holubi is endemic to south east Africa but its epicentre of distribution is around Port Elizabeth, therefore it represents a slight northward shift of component C, although it does not extend quite as far north as Inhaca Island (Fig. 17). Stephenson's (1947) component C includes numerous endemic species of warm temperate distribution whose actual temperature tolerances are unknown. It has been assumed that temperature controls the distribution of these species, but few have been investigated experimentally. The temperature tolerance of the common estuarine prawn Upogebia africana (Ortmann) has been established (Hill and Allanson, 1971), and coincides closely with that of R.holubi - this crustacean has a similar distribution to R.holubi. The existence of a discrete warm temperate fauna of south east Africa, whose geographical range is limited by temperature tolerance, appears possible

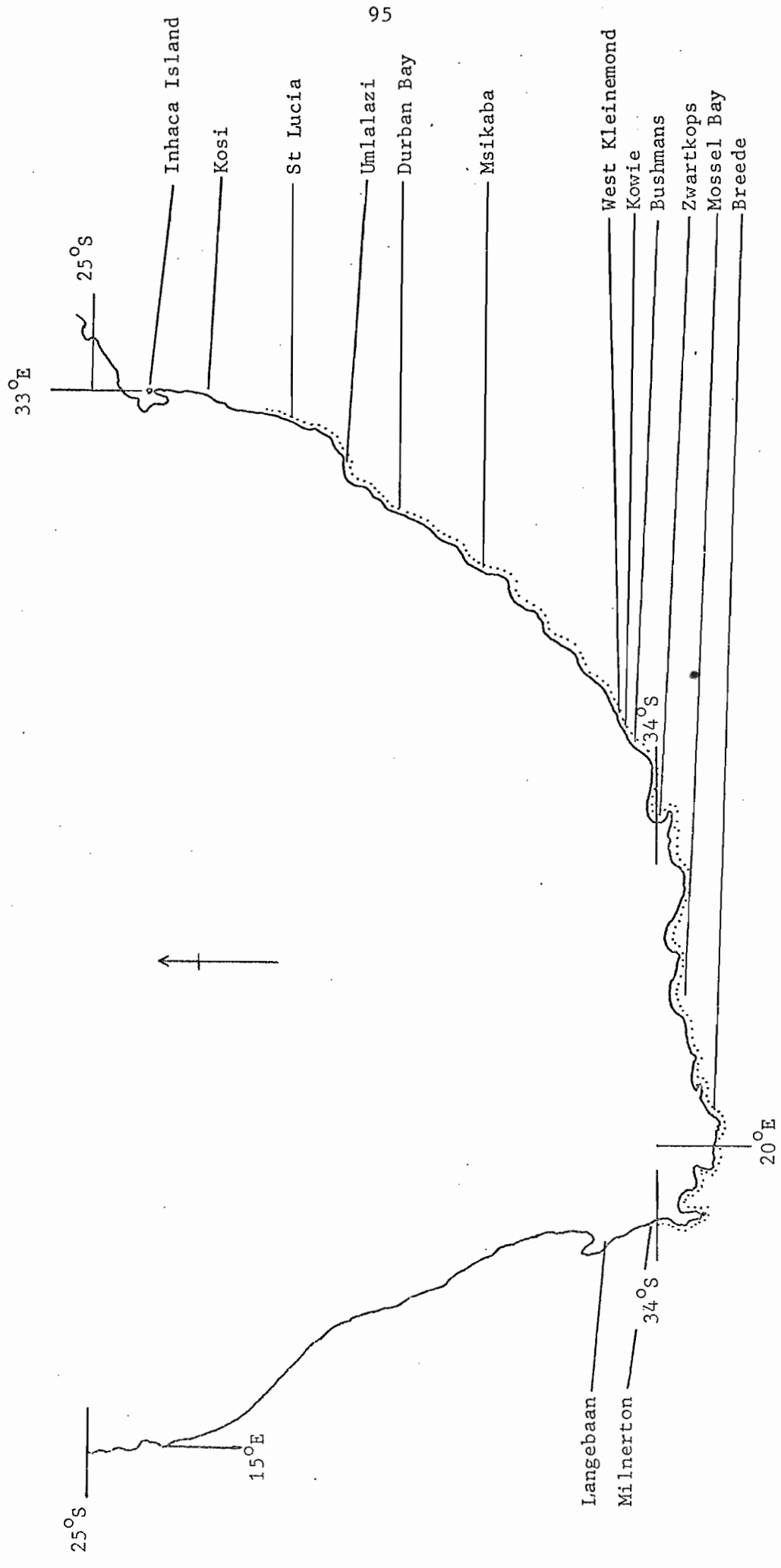


Figure 17: Southern Africa showing range of *Rhabdosargus holubi* (.....) and estuaries mentioned in text.

but further work on the tolerances of other species in the region would be valuable in this respect. The sparid fish Lithognathus lithognathus, which like R.holubi is common in south east African estuaries, obviously does not have the same temperature tolerances because it is able to live in the relatively cool waters of the Atlantic coast of south west Africa, and does not extend much further north than Port Elizabeth on the east coast (Smith, 1965).

This study has raised a number of points with regard to the ecology of R.holubi in the West Kleinmond estuary. In the light of the possibility that it may be beneficial for juvenile R.holubi to enter closed estuaries and the high mortality in the West Kleinmond estuary during 1971, the possible causes of the high mortality are significant and deserve further discussion.

The population of R.holubi in the West Kleinmond estuary at the beginning of 1971 was high relative to that at the beginning of 1972 when there was little subsequent mortality. A high density of fish could perhaps lead to starvation and thus mortality but there is no evidence to support this idea. Firstly, growth took place indicating that sufficient food was present, and secondly the cage experiment (Part I) showed that R.holubi could live for nine months under near starvation conditions without dying. Additionally R.holubi were starved for two months in the laboratory (Part V) with no mortality. There is a wealth of literature showing that most fish species can live for long periods without food. Clupea harengus for example can survive for 5 months without food (Wilkins, 1967), while Love (1970) quoting Boëtius (per.comm. 1968) states that the record for starvation is held by a specimen of Anguilla anguilla L. which lived for 1515 days (over 4 years) at 15°C without food. During this time it lost 76% of

its weight. Wilkins (1967) observed that when C.harengus were starving they became less timid and would perhaps be more liable to be caught by predators. The condition factor of R.holubi in 1971 was not abnormally low (Part V) and no large number of dead fish were found, which would have been the case if there had been large scale mortality due to starvation. The only dead fish ever found in the West Kleinmond estuary were juvenile Caranx ignobilis (Forskäl) which were thought to have been killed by low winter temperatures. Comparatively small numbers of these predatory fish, which are residents of subtropical and tropical waters, are able to penetrate as juveniles as far south as Algoa Bay (Smith, 1965) in summer, but probably do not survive through the winter.

If the estimate of 5.9g/m^2 of benthos in the West Kleinmond estuary (Batchelor, 1972) is approximately correct then at the highest population estimate, 2.5% of this would have been cropped daily by R.holubi (area of estuary : 67 hectares). It is important to note that this biomass figure does not include the prawn Callinassa kraussi, or any molluscs, the former are abundant and are eaten by R.holubi (Part IV). It is very difficult to arrive at a conclusion with regard to the amount of animal food consumed by R.holubi in 1971 since the food consumption rates are obviously variable. Additionally the biomass of benthos available to the fish will depend upon the production rates of the benthos for which there is no information. Evidently the feeding level drops off during winter, but this is more likely to be due to the fall in temperature than to a reduction in the availability of food.

Disease or parasitism must be considered in relation to the mortality. Ligula intestinalis (L.) was thought to have been responsible for large

mortalities among Rutilus rutilus L. in Chew Valley Lake near Bristol (Wilson, 1971) but there are few other recorded cases of parasites causing large scale mortality in fish. No obviously diseased juvenile R.holubi were ever seen. The only parasites discovered in or on R.holubi were occasional nematodes in the stomach and the ectoparasitic isopod Anilocra capensis Leach. The latter clings to the external surface, usually in the dorsal region of the head; none were found on West Kleinmond fish although approximately 1% of Kowie fish were parasitised with one individual. Thus parasitism can probably be ruled out as a cause of large scale mortality in the West Kleinmond estuary.

R.holubi seems to be restricted to south east Africa by its temperature tolerance, which, although ranging from about 10°C to about 28°C, will not permit it to enter tropical waters beyond Zululand or cool temperate waters of south west Africa. Within its range from the Cape of Good Hope to Zululand (Fig. 17) the distribution of the juveniles does not appear to be controlled by salinity or temperature. The distribution within an estuary such as the West Kleinmond is not related to salinity, even when a salinity gradient exists R.holubi do not show any preference for any particular salinity level. The experiments and estuarine conditions described in Part III have shown that they are able to withstand almost any of the temperatures and salinities which are likely to be encountered in the estuaries of south east Africa. As intolerance to physical conditions can be ruled out as being responsible for the mortality in 1971, predation is left as the most likely cause.

Although the evidence is by no means conclusive, there are indications that bird predation played an extremely important role in

the 1971 mortality. Other workers on juvenile marine fish almost all favour predation as being the major cause of mortality in the first year of life. Pearcy (1962) believed that cormorant predation on Pseudopleuronectes americanus was important but there was no direct evidence. In shallow closed estuaries containing juvenile fish populations which are accessible to fish eating birds, the activity of these birds assumes considerable importance. Herons and cormorants may have been largely responsible for an overall mortality of about 80% of the population of R.holubi in the West Kleinmond estuary in 1971 (Part II). In Part II the crab Scylla serrata was mentioned as a possible predator on juvenile R.holubi. In Part IV the significance of the activity of the crabs at night in relation to 'sleeping' R.holubi was stated. During 1971 the population of crabs in the West Kleinmond estuary consisted of individuals with a carapace width of 8 cm or less, and S.serrata of this size apparently consume little fish (Hill, pers.comm.), thus they probably did not cause the population decline in 1971. In 1972 however, the crabs had attained a size where fish form a greater part of the diet, but there was little or no mortality among the population of R.holubi that year. Thus even S.serrata of over 8 cm carapace width probably do not consume significant quantities of R.holubi in spite of the vulnerability of the fish at night.

Juvenile R.holubi enter estuaries from the sea at a length of between 1 and 2.5 cm. This fact together with the absence of adults in estuaries strongly suggests that the species spawns in the sea. Juvenile L.lithognathus, Mugil spp. and Hypacanthas amia have also been observed entering the West Kleinmond estuary in considerable numbers, and Wallace (1973) working in Natal has shown that several other species migrate into estuaries as fry, thus the phenomenon is by

no means restricted to R.holubi. This movement into estuaries may not be haphazard, for as Wallace (1973) has shown, the time of maximum recruitment of R.holubi and R.sarba in Natal coincides with the onset of the spring rains and the opening of the closed estuaries. Thus the fry are able to penetrate closed estuaries during the short period for which they are open. As to why there should be this movement between sea and estuary, there is no one obvious answer. A number of factors may possibly preclude adult R.holubi from staying in estuaries and conversely cause the juveniles to actively seek the estuarine environment. It may be necessary for the adults to spawn in the sea if the eggs are not tolerant of estuarine conditions. The work of Alderdice and Forrester (1971a, 1971b) and Alderdice and Velsen (1971) on the effects of salinity and temperature on early development of Clupea pallasii Valenciennes, Eopsetta jordani (Lockington) and Gadus macrocephalus Tilesius demonstrated that salinity or temperature, or a combination of both, may affect development of the eggs, and distinct optima for development exist. Thus it may be that adult R.holubi have to migrate back to the sea because the conditions in the estuary may be unsuitable for spawning and egg development. Nothing is known of the eggs of R.holubi but it is possible that the eggs may have requirements for development or fertilisation which can only be met under conditions found in the sea. Other workers have shown that the buoyancy of the eggs is important in relation to suitable spawning areas. Kuo et al (1973) showed that the eggs of Mugil cephalus L. did not become fertilised or remained undeveloped when allowed to rest on the bottom, but, if prevented from sinking development would ensue. Thus eggs spawned in an estuary may fail to develop because of inadequate water movement. This may explain why M.cephalus spawns in the sea where there is sufficient water movement to keep the eggs afloat. Gilchrist (1904) stated that the eggs of Rhabdosargus globiceps float

in seawater but if there is any slight movement of the water they 'float' away from the surface. It is not known whether the eggs of R.holubi float in seawater, but R.globiceps spawns only at sea where the eggs of this species would be expected to sink. Unfortunately due to the lack of availability of adult R.holubi no work on the reproduction of the species was possible.

Bertin and Arambourg (1958) state that many sparid fish undertake seasonal migrations between the estuary and the sea. Heldt (1943, quoted by Bertin and Arambourg, 1958) established that Aurata aurata L. enters coastal lagoons and estuaries of the Mediterranean in spring, in order to take advantage of the abundant animal food, but migrates back to the sea in autumn to spawn. It is significant in this respect that adult R.holubi do not return to the estuary once they have left, possibly as the fish age their salinity and temperature tolerance decreases, but a more likely reason for their not returning to the estuary is that enough suitable food is only present in the sea.

The diet of adult R.holubi is different from that of the juveniles. The teeth change with maturity and become suitable for catching and crushing animals such as bivalves, which are more abundant in the sea than in estuaries. The adult teeth are not well suited to cropping plants like those of the juveniles. Thus it may be that the food resources of the coastal waters of the sea better serve the food requirements of adult R.holubi than do the estuaries. The opposite situation exists among the juveniles which show a marked preference for consuming the epiflora and epifauna of aquatic macrophytes that are more abundant in estuaries than in the sea, except for large quantities of algae present in the intertidal and subtidal zones. Although the plant eating habit of R.holubi has been discussed in

some detail in Part IV, further mention of its significance is necessary. The discovery that only the diatoms on for example the macrophyte Ruppia spiralis are utilised, was due, initially, to the observation that the macrophyte in the faeces was not digested at all. Most analyses of the diet of fish rely upon an examination of the stomach contents, and this has probably led to the possibility being overlooked, that not all the plant material eaten may be directly of any value as food. There are of course fish which can make use of plant material, a good example is the freshwater cichlid Tilapia rendalli (Boulenger) which lives almost exclusively on aquatic macrophytes. Digestion in this species is preceded by the crushing of the plant by means of the pharyngeal teeth, thus the cell contents become accessible to the digestive enzymes. There are relatively few herbivorous marine fish, although many of the parrot fish (Scaridae) are herbivores, well adapted for eating algae. In addition to their anterior cutting teeth, they also possess pharyngeal teeth for grinding up plant cells.

The consumption of macrophytes by juvenile R.holubi for their attached flora is interesting from an evolutionary viewpoint. Firstly, as already mentioned it might have led to the development of the specialised teeth of juvenile R.holubi. It is possibly significant that the closely related R.globiceps and R.sarba do not possess specialised teeth in the juvenile phase of their life. Although they may consume a certain amount of filamentous algae they do not eat large quantities of very much tougher macrophytes such as Ruppia spiralis. Secondly, the development of the ability to eat macrophytes may be a step on the pathway to becoming totally herbivorous. The latter would however require the development of a method for breaking up the plant tissue, such as pharyngeal teeth, or the development of an enzyme capable of breaking down cellulose. Evolution of the herbivorous

habit would appear to be a retrograde step since animal food is usually more nutritious. In the context of an environment such as that of a closed estuary, however, being able to utilise fully the plant component might be advantageous and could help reduce interspecific competition. The latter exists at present to a certain extent because R.holubi still consumes some animal food such as crustaceans which are also eaten by juvenile L.lithognathus and doubtless other species.

Juvenile R.holubi in the West Kleinemonde estuary consume large quantities of plant material which is passed out in an undigested state. Relatively large amounts of plant material are introduced into the estuary in this way and must become part of the detritus chain, possibly forming an important substrate for micro-organisms and perhaps food for detritus feeding animals.

The number of juvenile R.holubi in a closed estuary may be to some extent fortuitous, relying as it does upon the length of time the estuary is open. The situation in a permanently open estuary is different in that immigration and emigration can take place at any time. Indications from catch per unit effort suggest however that the population density of R.holubi in the open Kowie estuary may be less than in the closed estuaries which have been investigated. This indication cannot be quantified at present, due to the impracticability of adequately sampling the Kowie estuary with the gear available. Large numbers of juvenile R.holubi may enter open estuaries, perhaps in search of food. In an estuary such as the Kowie there is little aquatic vegetation and thus a large proportion of R.holubi might be expected to leave the estuary. The generally quieter lagoons of closed estuaries with their often abundant aquatic vegetation are probably more attractive to juvenile R.holubi. Thus the species may congregate

in higher numbers in a closed estuary, such as the West Kleinmond, with its abundant vegetation.

Estuaries are generally quieter than the sea with respect to currents and wave action. Day (1964) found that the distribution of many animals in estuaries was determined by the strength of wave action and many of the estuarine animals are 'quiet water species'. The tremendous wave action and surge along the Indian Ocean shores of south east Africa (Stephenson and Stephenson, 1972; See Fig. 8.2) may be of significance to juvenile fish seeking food and would make it extremely difficult for juvenile R.holubi to take advantage of the intertidal and subtidal algae growing on the rocks. As was shown in Part II there is heavy predation upon juvenile R.holubi in estuaries, it is therefore unlikely that they enter estuaries in order to escape from predators. Various workers in North America and Europe such as Pearcy (1962), Riley and Corlett (1965) and Macer (1967) have shown that 0 group juveniles of several benthic species spend the first year of their life in shallow, relatively quiet and sandy bays where mortality from predation may be severe. On the south east African coast there are no quiet shallow bays of the type found in Europe, but there are a large number of quiet, shallow and usually sandy estuaries. These may provide the conditions necessary for juvenile benthic fish. Escape from predation may be secondary upon a more urgent requirement such as food which will enable the juveniles to grow rapidly.

The estuaries of south east Africa thus appear to play a vital role in the life cycle of R.holubi, and probably of other fish species which occur in them. The absence of suitable conditions for feeding, food supply, and growth in the rough inshore waters of the Indian Ocean make the less disturbed estuaries, with their often abundant aquatic vegetation, attractive to juvenile fish.

SUMMARY

1. Growth rates of a population of R.holubi in an estuary closed off from the sea were investigated. Juvenile R.holubi invade the estuaries at a length of 1 - 2 cm but migrate back to the sea at a length of about 14 cm without attaining maturity in the estuary. Adult R.holubi are rarely encountered in estuaries. The annual growth increment of 0 group R.holubi was 6 cm, most of which occurs in summer. The growth of Lithognathus lithognathus was also measured in the same closed estuary, 0 group fish of this species grew 6 cm per annum and 1+ group fish grew 8 cm per annum. The growth rates of R.holubi in a closed estuary are similar to those recorded for juvenile marine fish in the sea in the northern hemisphere.
2. Monthly estimates were made of populations of juvenile R.holubi in the closed West Kleinemonde estuary during 1971 and 1972 using mark - recapture methods. The Petersen method and multiple mark - recapture method gave comparable estimates, which were supported by catch per unit effort data. Mortality rates were calculated from the population estimates and catch per unit effort data. In February 1971, the population was 55000 but, had declined by 80% to 11000 by July 1971. In 1972 a population of different individuals, numbering about 12000, showed little or no mortality. The monthly mortality rate is similar to that of other juvenile benthic species which have been investigated. The high mortality rate in 1971 was density dependent and possibly mainly due to predation by piscivorous birds. The numbers of piscivorous birds could be related to the numbers of R.holubi.
3. Temperature and salinity tolerances of juvenile R.holubi were determined experimentally. Results indicated that they are

tolerant over a wide range of salinities ($0.7 - 70^{\circ}/\text{oo}$) and temperatures ($10 - 30^{\circ}\text{C}$) and that little interaction between temperature and salinity occurs. The species is also a strong osmoregulator, in $35^{\circ}/\text{oo}$ the internal osmotic concentration is held at 370 m-osm/l. At a salinity of $1^{\circ}/\text{oo}$ the internal osmotic concentration falls to 216 m-osm/l and at a salinity of $65^{\circ}/\text{oo}$ rises to 381 m-osm/l. When exposed to a new salinity the internal osmotic concentration does not change until after 10 hours. Results from tolerance experiments and salinity and temperature data from southern African estuaries suggested that the distribution of R.holubi may be controlled by temperature.

4. Stomach contents of over 2000 juvenile R.holubi were examined from fish captured in open and closed estuaries. Feeding only occurs during daylight hours. Juvenile R.holubi feed mainly on aquatic vegetation. This is not digested, and is passed out in an undigested state. Epiphytic diatoms which may form up to 50% of the dry weight of the plants together with sessile ectoprocts are digested in the stomach. A variety of benthic animals were also eaten. Maximum total food consumption was estimated at 1.9% of body weight per day. The teeth of juvenile R.holubi are specialised for grazing aquatic plants and differ from those of adult R.holubi which consume mainly bivalves and large crustaceans. It is suggested that the different food requirements of the adults and juveniles determine at what stage this species enters and leaves estuaries.
5. The condition factor and total lipid level of R.holubi from the closed West Kleinmond estuary and open Kowie estuary were measured monthly in 1972/3. In the laboratory, total lipid level could be related to feeding level, and was not affected by

temperature although food intake at 15°C was half of that at 22°C. Condition factor was affected by temperature. R.holubi from the West Kleinmond estuary showed a seasonal variation in lipid content (6.4% in summer, 0.75% in winter) but those from the Kowie estuary showed little or no variation (1 - 2%). It is suggested that the difference in the fat content of the fish between the two estuaries in summer was related to the lipid content of the food, which was much less in the Kowie estuary. The condition factor of West Kleinmond fish declined in winter while that of Kowie fish remained stable. It is considered that condition factor is a more sensitive index of the actual feeding level of fish than the lipid content since the latter may be affected by the lipid content of the food. Feeding level during winter in the closed West Kleinmond estuary probably declined due to the fall in temperature. Feeding level may have remained relatively stable in the open Kowie estuary, perhaps due to the influence of warmer sea temperatures.

6. R.holubi forms part of the migratory component of the estuarine fauna since only the juveniles are commonly found in estuaries. They may leave estuaries before reaching maturity in search of suitable food, such as bivalves, or because conditions in the estuary are not suitable for spawning. The rough nature of the coastal waters may make it difficult for juvenile R.holubi to feed on the algae growing on the rocks. Thus, in spite of a high level of predation, the quieter estuaries which abound in aquatic vegetation are a more suitable environment for the juveniles than the sea.

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APPENDIX 1

Computer programme for solution of multiple mark-recapture data.

The problem is essentially the solution of a large order polynomial. Generally the root required has a large numerical value. The method used in preparing the data is laid out by Robson and Regier (1968). There was one simplification added to the system: it was assumed that the number of specimens dead or injured on capture was zero. This simplification was justified in view of the large catches and insignificant mortality on capture.

Programme

Name: Fish

Language: Extended mercury autocode (EMA)

System: Autoload

Computer used: ICL 1901A 16K

Peripherals used: Card reader and line printer

The programme is in 2 chapters with 164 statements

The algorithm for solution of the polynomial was as outlined by Luther (1963).

Each root is subject to a test by substituting it into the polynomial and solving. Due to inherent errors the solution is rarely zero.

A check to see if the correct roots have been found is made by substituting both the root + 1% of itself and the root -1% of itself.

A correct root yields large positive and negative values for these two operations.

Data:

1. Order of polynomial forming numerator of equation
2. n negative roots of above polynomial
3. negative value of cumulative catch total
4. convergence limit for polynomial root routine
5. blank card

It is suggested that items 1, 2 and 3 be punched on one card (more if required) with at least two spaces between each number. Item 4 is usually of the order of 10^{-5} but may have to be varied if roots are not being found correctly.

Operating:

The programme is at present being run under an autoloader system and being dumped on PROGRAM ◁ DUMP in object form.

Enter programme at 20.

Stops: 1. HALTED 28
2. HALTED 01

1. All roots have been found. GØ to enter programme again and read new set of data.
2. All roots have not been found. GØ to read in new convergence parameter. (One card + blank card). GØ 20 to re-enter programme for new set of data.

The printout of the programme is shown in the following pages.

```

9-   CHAPTER 1
10-  A->49
11-  X->49
12-  B->20
13-  C->20
14-  D->20
15-  9)I=I
16-  N=N-1
17-  M=N
18-  SPACE25
19-  PRINT('THIS PROGRAM FINDS THE ROOTS OF A POLYNOMIAL')
20-  NEWLINE2
21-  SPACE35
22-  PRINT('OF DEGREE')
23-  PRINT(N)2,0
24-  NEWLINE2
25-  SPACE25
26-  PRINT('COEFFICIENTS ARE')
27-  NEWLINE
28-  I=0(1)N
29-  SPACE 42
30-  AI=A(1+1)
31-  DI=AI
32-  AI=AI/D0
33-  PRINT(DI)0,10
34-  NEWLINE
35-  REPEAT
36-  99)NEWLINE2
37-  READ(E)                !ACCURACY PARAMETER
38-  SPACE80
39-  PRINT('CONVERGENCE LIMIT IS')
40-  PRINT(E)0,8
41-  NEWLINE2
42-  G=A1
43-  G'=A1+100
44-  L=0
45-  B0=A0
46-  C0=-B0
47-  1)J=1(1)N
48-  BJ=AJ-GB(J-1)
49-  CJ=-BJ-GC(J-1)
50-  REPEAT
51-  G=(-2BN-CN)/(C(N-1))
52-  JUMP20,%MOD(G-G')<=E
53-  G'=G
54-  L=L+1
55-  JUMP1,L<850
56-  SPACE 40
57-  PRINT(' BOMB OUT ')
58-  NEWLINE2
59-  HALT
60-  JUMP99
61-  20)PRINT(-G)0,8
62-  27)I=I
63-  PRINT('IS A ROOT')
64-  PRINT(' SUBSTITUTION YIELDS')
65-  JUMPDOWN40
66-  F=G
67-  G=F-F/100
68-  PRINT('          1% DOWN ')

```

```

69-   JUMPDOWN 40
70-   G=F+F/100
71-   PRINT('          1% UP')
72-   SPACE2
73-   JUMPDOWN 40
74-   G=F
75-   JUMP28,N=1
76-   N=N-1
77-   JUMP25,N=1
78-   I=0(1)N
79-   AI=BI/BO ?
80-   REPEAT
81-   NEWLINE
82-   JUMP1
83-   25)PRINT(-B1)0,8
84-   G=B1
85-   JUMP27
86-   28)I=I
87-   SPACE40
88-   PRINT('ALL ROOTS HAVE BEEN FOUND')
89-   NEWLINE10
90-   HALT
91-   ACROSS 1/0
92-   40)A=A
93-   H=0
94-   G=-G
95-   I=0(1)M
96-   U=%EXP((M-I)%LOG(%MOD(G)))
97-   H=H+DI(%SIGN(%SIGN(G)+%PARITY(M-I)))U
98-   REPEAT
99-   PRINT(H)0,8
100-  G=-G
101-  NEWLINE2
102-  RETURN
103-  END
104-  CLOSE

```

```

105- CHAPTER0
106- VARIABLES 1
107- 1)READ(N)
108- SPACE40
109- PRINT('A POLYNOMIAL WITH ROOTS AS FOLLOWS ')
110- NEWLINE
111- I=1(1)N
112- SPACE20
113- READ(XI)
114- PRINT(-XI)0,10
115- X(I+N)=XI
116- AI=0
117- NEWLINE
118- REPEAT
119- NEWLINE
120- I=1(1)N
121- J=1(1)N
122- L=I+J-1
123- A=1
124- K=I(1)L
125- A=AXK
126- REPEAT
127- AJ=AJ+A
128- REPEAT
129- REPEAT
130- AN=AN/N
131- SPACE40
132- PRINT('HAS THESE NORMALISED COEFFICIENTS:')
133- NEWLINE
134- A0=1
135- I=0(1)N
136- SPACE20
137- PRINT(AI)0,10
138- PRINT('N**')
139- PRINT(N-I)1,0
140- NEWLINE
141- REPEAT
142- NEWLINE2
143- READ(F)
144- A1=A1-F
145- SPACE40
146- PRINT(-F)0,8
147- PRINT('IS SUBTRACTED FROM SECOND HIGHEST POWER')
148- NEWLINE2
149- SPACE 40
150- PRINT('AND HIGHEST POWER IS CANCELLED')
151- NEWLINE2
152- SPACE40
153- PRINT('COEFFTS. ARE NOW')
154- NEWLINE
155- I=1(1)N
156- SPACE20
157- PRINT(AI)0,10
158- PRINT('N**')
159- PRINT(N-I)1,0
160- NEWLINE
161- REPEAT
162- NEWLINE3
163- ACROSS 9/1
164- END

```