FEEDING ECOLOGY OF THE CICHLID FISH

SARCTHERODON MOSSAMBICUS IN

LAKE SIBAYA, KWAZULU

Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY of Rhodes University by STEPHEN H. BOWEN

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Résumé

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The feeding of the cichlid fish <u>Sarotherodon moesambicus</u> in Lake Sibaya, KwaZulu, was studied in relation to various biotic and abiotic features of the lake environment from January, 1973 to July, 1975. The primary goal of the study was to determine the cause of stunting and poor condition of adults in this population.

Juvenile and adult <u>S. mossambicus</u> feed on a mixture of detritus, bacteria and diatoms that occurs as a flocculent layer on sand substrates throughout the lake. Concentrated gastric acid, commonly at pH values of 1.5 and lower, lyses diatoms and bacteria which are subsequently digested in the intestine. This is the first report of digestion of bacteria by a fish, and the nutritional significance of the finding is discussed.

Juveniles feed predominantly on shallow sand terraces found along the margin of much of the lake, while adults usually feed in offshore waters at depths of 3 m and greater.

Benthic floc from feeding areas of juveniles and adults have similar concentrations of organic matter, total carbohydrate, soluble carbohydrate and calories, but differ markedly in respect to diatom and protein concentration. Diatom concentrations are generally high in terrace floc but are consistently low in floc from deep water. Protein concentration of benthic floc decreases with increasing depth from 0 - 5 m. Partial correlation analysis shows that protein and diatom concentrations have no correlation independent of their common relationship to depth, and the evidence implicates detrital bacteria as the primary source of protein in benthic floc. Probable causes of the observed distributions of diatoms and protein are discussed.

The protein content of benthic floc profoundly influences its nutritional value. A comparison with available data on animal nutrition shows that the ratio of digestible protein to digestible energy in floc in the shallows is high enough that this food resource would be expected to support good growth, but the ratio for deep water floc is so low that it would be expected to result in conspicuous malnutrition. Thus, inadequate dietary protein is identified as the principal cause of stunting and poor condition of adult <u>S. mossambicus</u> in Lake Sibaya. It is argued that the ratio of digestible protein to digestible calories can be expected to determine the food value of particulate organic matter that includes amorphous cetritus in other ecosystems.

Despite stunting and poor condition of adults, <u>S</u>. <u>mossambicus</u> are abundant in Lake Sibaya. The precocious breeding of this population is discussed as an adaptation that allows it to maximize reproductive output given the limited resources available to adults.

Juvenile <u>S</u>. <u>mossambicus</u> perform daily movements from deep water onto the terraces where they feed and then back into deep water. During periods of relatively low lake level when terrace waters were usually less than 1.5 m deep (1-73 to 1-74), juveniles were present on the terrace throughout daylight hours. At relatively high lake levels (7-74 to 8-75), juveniles were present on the terraces for only about five hours following sunset. Periods of heavy wave action interrupt feeding activity and result in reduced numbers of fish in terrace waters. The possible roles of predator avoidance and temperature in determination of daily feeding behavior are discussed.

Daily energy assimilation by juveniles, estimated by a field technique, is approximately 115 cal per g fish dry weight.

Introduction

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All activities of a consumer population are fueled and nourished by the food resources which it takes from its environment. In a given environment, the success of a consumer population depends to a large extent on its ability to develop a feeding strategy that adequately supplies its nutritional needs. A range of different possible feeding The range is defined in part by the population's strategies is available. ability to locate, capture and digest different food items, and in part by the opportunities offered by the environment. Some of the possible feeding strategies will be better than others. Each would bring the population into contact with a unique set of environmental factors, biotic and abiotic, that would influence the success of the population. Important biotic factors may include food quantity, food quality and exposure to predation, while exposure to severe weather conditions and temperature may be important abiotic factors. It is generally believed that a natural population selects a feeding strategy which brings it into contact with the set of environmental factors that maximizes population success. As conceived of here, a study of the feeding ecology of a consumer population is a study of how environmental factors associated with the population's feeding strategy affect the population's success.

This dissertation presents a study of the feeding ecology of the cichlid fish Sarotherodon mossambicus (Peters) in Lake Sibaya, KwaZulu. One special characteristic of this population has made it particularly interesting and important as a subject for such a study: while juveniles grow rapidly and are in good condition, the adults attain an abnormally small maximum size and are in extremely poor condition. Adult S. mossambicus in Lake Sibaya never develop the rounded, deep bodied shape that is characteristic of adults from other populations, but instead show extensive marasmus and generally look as if they suffer from severe . malnutrition. Because S. mossambicus is the most numerous fish in Lake Sibaya, the poor condition and small size of adults mean that this large lake has negligible fisheries potential (Pike, 1969). A desire to understand the factors responsible for the poor condition and small. maximum size of the adult fish has been the primary motivation of this study.

In addition, two other characteristics of the Lake Sibaya <u>S</u>. <u>mossambicus</u> population have recommended it for study. Firstly, the diet

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of these fish includes significant proportions of non-living particulate organic matter, commonly termed detritus, and heterotrophic microorganisms. Several authors have speculated on the ability of fish to digest these two materials and the food value they might have (Fish 1955, Odum 1970, Fryer and Iles 1972). The population of <u>S. mossambicus</u> in Lake Sibaya has provided an opportunity for a critical examination of these possibilities. Secondly, although several studies have reported observations on the feeding of <u>S. mossambicus</u> in exotic and artificial habitats, its feeding in a natural habitat such as Lake Sibaya has not been studied. In addition to its intrinsic scientific value, the study of a natural population may yield information valuable to the culture, management and conservation of <u>S. mossambicus</u> that could not be gained from study of populations in artificial or exotic habitats.

Finally, this is one of several studies of Lake Sibaya conducted by the Institute for Freshwater Studies at Rhodes University, Grahamstown, South Africa. As such, it makes an important contribution to the understanding of the Lake Sibaya ecosystem.

The working hypothesis of this study has been: The difference in the condition and growth of juvenile and adult S. mossambicus results from a difference in the quality of the food they utilize. Because slow growth and poor condition are apparent only as the fish become adult, it was thought that a comparison of juvenile and adult feeding would be most likely to reveal the factor or factors responsible for the condition of In order to concentrate on comparison of juvenile and adult adults. feeding, the feeding of fry has not been considered. The quality of food utilized was chosen as the most likely difference between juvenile and adult feeding since neither of the other two potentially important factors, the quantity of food available to juveniles and adults and the ability of juveniles and adults to utilize their food resources, appeared to be important in the Sibaya situation. The benthic floc on which these fish feed is abundant in all areas of the lake, and there is no reason to believe that adult and juvenile S. mossambicus differ in their abilities to collect or digest any given food item.

Thus, the study began with an investigation of the diets of juvenile and adult <u>S</u>. <u>mossambicus</u> in Lake Sibaya. When differences in the diets of juvenile and adult fish were found, an attempt was made to determine their nutritional significance, and how they were related to environmental

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factors in the lake such as the distribution of dietary components. As a general picture of the relationship between the population's feeding strategy and its environment emerged, I have attempted to account for the success of the <u>S</u>. <u>mossambicus</u> population in establishing itself in large numbers in Lake Sibaya, and the failure of <u>S</u>. <u>mossambicus</u> adults to attain the normal size and condition characteristic of adults of this species in other populations.



Chapter I. Lake Sibaya and its population of Sarotherodon mossambicus

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Lake Sibaya (32°40'E, 27°25'S) is the largest natural freshwater lake in Africa south of the rift valley. It lies at the seaward edge of a broad coastal plain of recently deposited tertiary sands which extends from the Lebombo Mountains east to the Indian Ocean (Fig. 2). A series of forested coastal dunes separate the lake from the sea. The lake surface, with an area of about 65 km², is approximately 20 m above mean sea level.



Figure 2. An ERTS photograph of the east coast of Africa in the vicinity of Lake Sibaya. Date August 2, 1972, altitude 900 km, code 1010-07131-301.

Lake Sibaya consists of a large main basin, two arms, one to the north and one to the west, and two smaller basins, one to the south and one to the southwest. The bathymetry of Lake Sibaya is shown in Figure 1. The maximum depth is 40 m and the mean depth is 13 m. Present basin profiles suggest that Lake Sibaya was originally an estuarine river valley that has been subsequently isolated from the sea by longshore dune formation (Hill 1969, 1975). It is believed that the steep sloped, deep channels of the western and northern arms reflect original river courses, but original



Figure 3. The terrace habitat along the southeastern shore of the main basin as viewed from the dune forest in January, 1973. Arrows indicate the deep edge of the terrace and the top of the steep slope. Lake level increases during 1973, 1974 and 1975 moved the water's edge up to the marginal trees by July, 1975. Photograph by M. Bruton.



Figure 4. The sheltered bay habitat (Fig. 11, site 4).

profiles in the main basin appear to be largely obscured by subsequent movement of the sand substrate.

A comment on the term detritus

Before I describe the different habitats in Lake Sibaya, it is necessary to digress briefly to comment on my use of the term detritus. Two definitions of this term are commonly used. Some authors use the term to mean dead organic matter of both animal and plant origin together with associated microorganisms, while others use the term to mean only the dead organic matter. The former definition was recently defended by Mann (1972a) who emphasized the way in which dead organic matter and living microbes function as a unit in trophic relationships. An animal that consumes dead organic matter inevitably also consumes any associated microorganisms. The opposite point of view was taken by Wetzel et al. (1972) who emphasized the independent natures of living and non-living organic matter. These authors pointed out the very different roles of microorganisms and non-living organic matter in the energy flux and material cycling of an ecosystem and thus argued in support of the second definition. My study of the feeding ecology of S. mossambicus has demonstrated that, also in trophic relationships, microorganisms and dead organic matter can play very different roles. To emphasize this important difference, I have used the term detritus to mean only the dead organic I have used the term benthic floc to describe the mixture of matter. detritus and microorganisms, including algae, which is found on benthic substrates throughout Lake Sibaya.

The structure of four important habitats in Lake Sibaya

Four habitats can be distinguished in Lake Sibaya with regard to bottom profiles, substrate type and depth. <u>Shallow terraces</u> are found along the shore in many parts of the lake (Fig. 3). Bottom profiles of this habitat slope gradually from shore to approximately 1.5 m depth and then slope very gently to the deep edge (Fig. 5). Terrace substrates at depths of less than 1.5 m are usually loose, rippled sand covered with a layer of floc a few millimeters in thickness. Deeper substrates may be loose or stabilized depending on the intensity of recent wave action.





The location of terraces in the main and south basins is shown in Fig. 6. This information has been compiled from aerial photographs, diving observations and observations made from a boat. Some small isolated terrace areas may not be illustrated here.

A second habitat is characterized by <u>steep slopes</u> which descend from the deep edge of most terraces to depths as great as 40 m (Fig. 5). The angle of these slopes, $25 - 30^{\circ}$ from horizontal, is approximately the critical angle of repose and thus the sand substrate is loose and often slides down the slope with the least disturbance from a diver. Most of the benthic floc on these steep slopes is interstitial. The location of steep slopes in Lake Sibaya is shown in Fig. 2.

The third habitat is characterized by its <u>gradual slope</u> in deep water (Fig. 5). This gradual slope begins at 2 - 4 m depth just a few meters from shore and may extend a kilometer or more to depths of 15 m. In the study reported here, a somewhat arbitrary depth limit of 15 m has been chosen for the gradual slope habitat because <u>S. mossambicus</u> is rarely found below that depth (Bruton 1973). The gradual slope substrate is stabilized sand covered with a layer of flocculent material a few millimeters thick. Small bits of macrophyte debris with a maximum dimension of 3 mm are present in variable amounts but are never dense enough to cover the substrate. The largest areas of gradual slope habitat known to me through diving observations combined with study of basin bathymetry are shown in Fig. 5. It is possible that many similar areas are present in the lake which have not been illustrated here.

A fourth habitat is found in <u>sheltered bay areas</u> (Fig. 4). This habitat is characterized by steep slopes close to shore and flat bottoms (Fig. 5). The substrate is mud covered by a layer of organic ooze.

As with most generalizations about ecosystems, this division of Lake Sibaya into four habitats is imperfect and a few areas less than 15 m in depth are not readily assigned to one of the four. In particular, it is difficult to determine whether some areas along the northern shore of the main basin should be described as deep terraces or shallow gradual slopes. Nonetheless, most areas are readily assigned to one of the habitat categories and I believe these generalizations accurately reflect important differences and similarities among various areas in the lake.





Physical and chemical characteristics

Physical and chemical characteristics of Lake Sibaya have been described by Allanson and Van Wyk (1969). Open waters of the lake are basically homothermal with complex and unstable thermal discontinuities over a range of 1 or 2 $^{\circ}$ C. Frequent periods of high velocity winds, predominantly from the south and north, maintain this homothermal condition and no persistent stratification has been observed. Open water temperatures measured at a fixed buoy in the main basin from April, 1970 to May, 1972 ranged seasonally from 18 to 28° C (Hart and Allanson 1975). Secchi disk values for the open water vary only slightly from 3.4 m. The waters are alkaline, pH 8.6, and have a high chloride ion concentration, 135 mg Cl⁻ 1⁻¹, but are otherwise fresh.

Lake level fluctuations in response to variable annual rainfall have been examined in a preliminary hydrological study of Lake Sibaya by Pitman and Hutchison (1975). Based on annual rainfall data and a number of estimated hydrological parameters for the lake basin and environs, these authors have simulated month-end lake levels from 1914 to 1969. Simulation results suggest that lake level varied over a 4 m range during those 55 years. During the course of the study reported here, lake levels have fluctuated over a 1.7 m range (Fig. 7). Lake level fluctuations are of particular interest because they influence markedly the biological nature of the terrace habitat. At high lake levels, large areas of flooded terrestrial vegetation provide both a substrate for aufwuchs development and shelter for invertebrates and small fish, especially fry. At low lake levels terraces are contracted and shallow, and a sand beach is present. Eulittoral pools occasionally develop between the shore and terrestrial vegetation. Although these pools are limited in extent and are exposed, they serve as nurserics for the fry of several species of fish (Bruton 1973).

Significant biological parameters

The standing crop of phytoplankton contains less than 5 mg chlorophyll a per liter, and has a maximum carbon fixation rate of 239 mg m⁻² d⁻¹ (Allanson and Hart 1975). Both values are very low for a warm subtropical lake. The desmid <u>Closterium</u> sp. and the diatom <u>Melosira</u> granulata are the most common phytoplankters. The most common zooplankter is <u>Pseudodiaptomus hessei</u> which occurs at a low mean density of less than six individuals per liter (Hart and Allanson 1975).

The most numerous fishes in Lake Sibaya are cichlids. <u>Tilapia</u> <u>sparrmanii</u> occurs mainly in gradual slope and sheltered bay habitats where its diet consists of diatoms, filamentous algae, submerged macrophytes, and insects (Minshull 1969). <u>T. rendalli swierstrae</u> adults appear to be restricted to sheltered bay habitats although juveniles are occasionally found on the terrace. Their main food items appear to be submerged macrophytes, diatoms and insects (Minshull 1969). <u>Pseudocrenilabrus</u> <u>philander</u> is abundant in all habitats and feeds primarily on benthic crustacea and aquatic insects (Allanson, Bruton and Hart 1974). In addition to the cichlids, <u>Clarias gariepinus</u> is common throughout the lake. This species is predatory and feeds primarily on fishes.

A three year study of the general biology of <u>S</u>. <u>mossambicus</u>^{*} in Lake Sibaya was reported by Bruton (1973). This is the only published study of a natural population of this species. Topics investigated included distribution, breeding and growth.

The vertical distribution of <u>S</u>. <u>mossambicus</u> in Lake Sibaya was determined with traps and gill nets set at different depths and with an echo-sounder. Gill nets laid to a depth of 13 m caught adult <u>S</u>. <u>mossambicus</u> only to 9 m. Echo traces showed targets, thought to be adult <u>S</u>. <u>mossambicus</u>, that were common to 12 m depth but did not occur below 18 m. In water less than 12 m deep, these targets were commonly found close to the lake bottom, but at greater depths they were generally limited to the upper 5 m of the water column. Juveniles were regularly caught in traps set on the bottom to 7 m, were uncommon from 7 to 12 m, and no juveniles were caught below 15 m.

Horizontal distribution varies with season. Most adults spend the cool season, May through July, in the open water. During the cool to warm transitional period, August through mid-September, adults migrate to shallower shoreward areas. Beginning in mid-September, adult males establish territories and build nests which they defend until January. Nests are most commonly found on the terrace in association with emergent vegetation. Females visit the nests, mate and return to deep water to incubate the eggs

* <u>Sarotherodon mossambicus</u> has recently been transferred from the genus Tilapia to the genus Sarotherodon (Trewavas 1973).







. Mean condition factor of adult <u>S. mossambicus</u> in Lake Sibaya. Each point represents the mean of values for over 30 specimens. Histograms indicate estimated time of scale ring formation. Arrows indicate time when adults migrate to deep waters for the cool season. Modified from Bruton (1973). in their mouths. After approximately twenty days incubation and brooding, the fry are released at the lake margin where they usually inhabit water less than 15 cm deep. Adults leave the terrace during the warm to cool transitional period, February through April, and move into sheltered bay and gradual slope habitats. At the beginning of the cool season, adults return to the open water.

A simpler pattern of seasonal distribution was found for juveniles. Juveniles are abundant on the terrace during the warm season and during both transitional periods, but during the cool season many move into deeper water where they are found close to the bottom.

Distribution and daily movements of juveniles were examined in detail. Bruton distinguished four habitats in littoral and sub-littoral zones of Lake Sibaya: shallow wave-washed terraces, barren slopes and sparsely vegetated slopes which lie below these terraces, and well vegetated sheltered bays. In seine net catches during daylight hours, juvenile <u>S. mossambicus</u> were most common in the terrace habitat where they represented over 98% of the catch, were less common in the slope habitat and were uncommon in the sheltered bay habitat. Thus, Bruton concluded that juveniles appear to prefer unvegetated areas in littoral and sublittoral habitats of Lake Sibaya.

Juveniles perform daily movements between steep slope and terrace habitats. They first enter terrace waters at about sunrise, the number of juveniles on the terrace reaches a peak at about midday, and most have left the terrace by sunset. At night, juvenile <u>S. mossambicus</u> lay motionless at 1 - 5 m depth on the steep slope or are dispersed in midwater.

Seasonal variation was found in the condition of adult <u>S</u>. <u>mossambicus</u>. As is common practice in fisheries biology, the term condition is used here to refer to a ratio of fish weight to fish length that reflects the relative fatness of a specimen. Bruton computed condition factors for <u>S</u>. <u>mossambicus</u> as

Fresh weight in gm x 100 $\overline{}$ Standard length in cm^{3.01}.

Adult condition factors were relatively high during the cool to warm transitional period but fell during the breeding season. After the breeding season, condition factors increased to approximately pre-breeding values (Fig. 8). Since the available methods failed to collect more than a few adult <u>S. mossambicus</u> during the cool season, no data are given for this time period, but Bruton did note that condition factors for the few

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specimens caught were low.

Analysis of scales collected throughout the year revealed that two rings of widely spaced circuli were formed annually, at times when condition factors were high or rapidly increasing (Fig. 8). Both were formed while adults inhabited shoreward areas; one during the warm to cool transitional period and one during the cool to warm transitional period. Based on the isometric relationship between scale radius and fish length, Bruton (1973) concluded that these scale rings were formed during periods of relatively rapid growth.

Scale rings were used to determine the age and growth of S. mossambicus The maximum age appears to be about 7 yrs for males and in Lake Sibaya. 6 yrs for females. Females first breed after one year when they have reached 8 cm SL (standard length, measured from the tip of the snout to the caudal peduncle) and males after one or two years when they have reached 10 cm SL. This is much earlier than in other populations of this species which breed for the first time after two or three years (Bruton and Boltt 1975). The growth of male and female S. mossambicus in Sibaya is the same during the first year and is equal to or greater than that reported for other populations of the same species. After the first year, growth is slower in females and both sexes grow more slowly and to a much smaller maximum size than in other While the maximum size in Sibaya is 26 cm TL (total length, populations. measured from the tip of the snout to the tip of the caudal fin), S. mossambicus in Inyamiti pan, 60 km northwest of Sibaya (Fig. 2) reach 43.2 cm TL (Coke, cited in Bruton and Allanson 1974).

Bruton (1973) believes that the precocious breeding and small maximum size of S. mossambicus in Lake Sibaya are part of an adaptive strategy that enables this population to overcome the effect of high mortality rates to which he suggests that fry and juveniles are subject, and also to take advantage of periodically renewed resources in the terrace habitat. He argues that by breeding precociously, generation time is shortened and a greater intrinsic rate of population increase results. Thus, high mortality rates can be tolerated when balanced by high recruitment rates. Precocious breeding would also be expected to change the age structure of the population such that young fish represent a larger proportion of the total population. Since only juveniles are known to feed in the terrace habitat, a larger proportion of the population would be able to take advantage of terrace food With heavy rains and the resultant rise in lake level, this part resources.

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of the lake receives inputs of ellochthonous material that provide a substrate for aufwuchs development, a possible food resource for <u>S</u>. <u>mossambicus</u>, and which may have food value itself. Thus, Bruton argued that the small maximum size of <u>S</u>. <u>mossambicus</u> adults results from an unusually early shunting of resources from growth to reproduction which allows this species to best utilize the unstable and periodically renewed terrace habitat.

Bruton's interesting hypothesis rests on two unexamined assumptions: firstly, the terrace food resources are in some way superior to other food resources in the lake and secondly, the small maximum size and poor condition of adults result from unusually early maturity. While the first assumption is at least plausible, the second is suspect. In a review of stunting or dwarfing in a variety of cichlids, Iles (1973) notes that adult fish in many populations which mature early remain in good condition, so early maturation alone is insufficient to account for the poor condition of adult <u>S. mossambicus</u> in Lake Sibaya. The validity of Bruton's assumptions are examined in the study presented here.



Figure 9. Jaw teeth from cichlids with different diets. (A) <u>Haplochromis macrops</u>, an omnivore, (B) <u>H</u>. <u>dentex</u>, a piscivore, (C) <u>H</u>. <u>obliquidens</u> and (D) <u>H</u>. <u>lividus</u>, both periphyton grazers, and (E) <u>S</u>. <u>mossambicus</u>. A through D redrawn from Greenwood (1974).



Figure 10. Lower pharyngeal bones and teeth from cichlids with different diets. (A) <u>Bathybates leo</u>, a piscivore, (B) <u>Haplochromis placodon</u>, a mollusc crusher, (C) <u>Tilapia esculenta</u>, a phytoplankton eater and (D) <u>S. mossambicus</u>. A through C photocopied from Fryer and Iles (1972).

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Chapter II. The diet of <u>Sarotherodon mossambicus</u> in Lake Sibaya as inferred from gut contents

As noted in the introduction, animals possess adaptations that facilitate utilization of a particular type of diet. Among the cichlids, the most important adaptations are found in jaw teeth, pharyngeal teeth and gut length (Fryer and Iles 1972).

The primary function of cichlid jaw teeth (Fig. 9) is to collect food items. Predators tend to have unicusped teeth that firmly grasp their prey while aufwuchs eaters tend to have broad bicusped and tricusped teeth which scrape food from various substrates. Plankton eaters and omnivores tend to have teeth with pcinted rather than broadened cusps. The jaw teeth of <u>S</u>. <u>mossambicus</u> are broadened and bicusped in the first row and tricusped in other rows (Fig. 9 E), and most closely resemble the teeth of periphyton grazers illustrated by Greenwood (1974).

Cichlid pharangeal teeth (Fig. 10) prepare food items for digestion. Many predatory cichlids have strong caniniform pharyngeal teeth which can rasp the flesh from vertebrate prey and rend invertebrate prey to allow digestive enzymes better access to digestible tissues. Many cichlids whose diet consists largely of snails have short stout molariform pharyngeal teeth that crush the shells and expose the soft tissues within to digestion. Cichlids which feed on algae have numerous fine pharyngeal teeth that break up algal aggregates and cut filaments into short lengths. The pharyngeal teeth of <u>S. mossambicus</u> (Fig. 10D) are numerous and fine, and closely resemble those of a plankton eating cichlid illustrated by Fryer and Iles (<u>op cit</u>).

Because plant cytoplasm is encased in digestion resistant cell walls, plant tissues are more difficult to digest than animal tissues. As a result, guts of herbivorous fish tend to be much longer relative to body size than guts of carnivorous fish. The relative gut lengths of omnivorous species fall between these two groups. This has been shown very elegantly by Fryer and Iles (<u>op cit</u>) who expressed the relative gut lengths of 106 cichlids in Lake Tanganyika and its affluents as the ratio of gut length to total fish length. From their data given in histogram form, I have calculated mean relative gut lengths of 0.67, 1.62 and 4.21 for carnivores, omnivores and herbivores, respectively. Relative gut lengths of 121 formalin preserved <u>S. mossambicus</u> from Lake Sibaya had a mean of 3.93. Because formalin destroys the elasticity of the gut, this value may be lower than one based on measurements from fresh specimens. In any case, the relative gut length as well as the form of the jaw teeth and the pharyngeal teeth implies that <u>S. mossambicus</u> is best suited to an herbivorous diet.

Consistent with this implication, most investigators report that the diet of <u>S</u>. <u>mossambicus</u> juveniles and adults is comprised primarily of benthic, epiphytic and planktonic algae, and to a lesser extent, macrophytes (Vaas and Hofstede 1952, Le Roux 1956, Munro 1967). In two investigations of <u>S</u>. <u>mossambicus</u> in culture ponds, zooplankton and benthic invertebrates constituted a large component of the diet (Le Mare cited in Hickling 1970, Le Roux 1956). Under natural conditions, <u>S</u>. <u>mossambicus</u> would protably be unable to compete for these food items with predatory fish better equipped for collection of such prey. <u>S</u>. <u>mossambicus</u> are also known to feed on terrestrial insects trapped on the water surface but these are probably important only during swarms when large numbers of insects become available. Thus, <u>S</u>. <u>mossambicus</u> can be described as an herbivore which depends on algae as a food resource but, like most fish species, can take advantage of a superabundance of other food items should they become available.

Two workers report an herbivorous diet for <u>S</u>. <u>mossambicus</u> in Lake Sibaya. Minshull (1969) examined 40 specimens and concluded that algae, primarily benthic diatoms, are the major food resource of this population although macrophytes, invertebrates and small fish were also ingested. Bruton (1973) examined 146 adults and an unspecified number of juveniles. He concluded that juveniles feed predominantly on a mixture of benthic diatoms and detritus while, in addition to this material, adults feed on terrestrial insects, macrophytes, periphyton, other fishes and possibly plankton. The relative importance of each of these components of the adult diet was not determined. These data disclose that, in its gross aspects, the diet of <u>S</u>. <u>mossambicus</u> in Lake Sibaya is not dissimilar to diets of <u>S</u>. <u>mossambicus</u> populations in other systems.

In this section I report the results of a more intensive examination of the diet of S. mossambicus in Lake Sibaya. The goals of this examination were firstly, to confirm the findings of Minshull (1969) and Bruton (1973), secondly, to describe in detail the mixture of benthic algae and detritus that these workers found to be the primary component of the diet of S. mossambicus in Lake Sibaya, and thirdly, to look for differences in

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the diets of juvenile and adult <u>S</u>. <u>mossambicus</u> that may have a bearing on differences in their condition and growth.

Methods

During all seasons, juvenile S. mossambicus were readily collected from the terrace with a 15 m long seine net. In a well vegetated sheltered bay and among emergent vegetation at the shallow edge of a gradual slope habitat, a throw net proved to be the most effective method for collection of juveniles. During the warm season and warm to cool transitional period, adults were readily caught with surface set gill nets in shoreward parts of the gradual slope habitat and to a lesser extent, in sheltered bay and terrace habitats. Seine catches on the terrace occasionally included adults. During the cool season, adults were collected with a frame trawl pulled along the bottom in a gradual slope habitat. The date and details of each collection are given in Table 1 and collection sites are shown in Fig.11. When more than 20 adult specimens were collected, approximately 20 were arbitrarily selected for examination. Some specimens were examined immediately after capture but most were preserved in 10% formalin pending examination. The coeloms of specimens to be preserved were opened to minimize post-mortem digestion.

In the laboratory, the microscopic composition of samples of gut content from the stomach and posterior intestine was determined by examination at 35, 100 and 400 times magnification with bright field illumination. A sample from the anterior intestine was examined if the stomach was empty. All specimens not collected from the terrace habitat were examined in this way. The uniform composition of gut contents of specimens collected from the terrace made microscopic examination of the gut contents of each specimen unnecessary. Thus, in large samples of fish from the terrace, gut contents of some specimens were examined microscopically while those of others were examined only macroscopically. Representative samples of adult and juvenile stomach content were examined by scanning electron microscopy. These samples were dried under vacuum and coated with gold-palladium.

The diatom species composition of a pooled sample of 10 juvenile gut contents was determined by Professor M.H. Giffen of Fort Hare University, South Africa. Frustules were cleaned with boiling acid and mounted permanently on microscope slides. The number of each diatom species in several transects across each slide was recorded.



Table 1. Details of collections of <u>S</u>. mossambicus for examination of gut contents. Site numbers refer to Fig.11.

Date	Site	Habitat	Method	No. Examined	Size
			~ .		
1 - 73	7	Terrace	Seine	32	Juvenile
1 - 73	1.	Terrace	Seine	6	Adult
4 - 73	1	Terrace	Seine	18	Juvenile
7 - 73	1	Terrace	Seine	97	Juvenile
12 - 73	1	Terrace	Seine	586	Juvenile
12 - 73	2	Sheltered bay	Throw net	10	Juvenile
12 - 73	3	Terrace	Throw net	10	Juvenile
12 - 73	1	Terrace	Seine	17	Adult
12 - 73	4	Sheltered bay	Gill net	2	Adult
12 - 73	5	Gradual slope	Gill net	24	Adult
12 - 73	6	Gradual slope	Gill net	12	Adult
12 - 73	7	Terrace	Gill net	9	Adult
12 - 73	8	Sheltered bay	Gill net	28	Adult
7 - 74	9	Terrace	Seine	20	Juvenile
2 - 75	10	Sheltered bay	Gill net	9	Adult
2 - 75	11	Terrace	Gill net	12	Adult
2 - 75	8	Sheltered bay	Gill net	22	Adult
3 - 75	1	Terrace	Seine	223	Small adult and juvenile
7 - 75	1	Terrace	Seine	80	Small adult and juvenile
7 - 75	12	Gradual slope	Trawl	45	Adult
			4	Real Anna State Street	•
•			Total =	1262	

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The amount of sand in the gut contents of 20 juvenile S. mossambicus was determined by weight and by volume. It was necessary to analyse a pooled sample rather than single samples because the volume of a single sample was too small to measure accurately with the available apparatus. The storach contents were pooled in a 50 ml Erlenmeyer flask with approximately 25 ml distilled water and sonicated for 30 min in a Bandlin brand sonic bath. Scanning electron microscopy revealed that this procedure left most sand grains free of attached diatoms. After shaking the flask vigorously to suspend the freed material, this material was washed from sand in the flask with three volumes of distilled water and concentrated in a graduated centrifuge tube by centrifugation. The volume of the resultant pellet was noted. The freed material was resuspended and transferred to a preweighed aluminum pan. The sand was washed from the flask into a second preweighed aluminum pan, and both materials were dried at 70°C to constant weight, cooled in a dessicator and weighed to 0.1 mg. The density of sand was determined as the weight of a sample of dry, sonicated terrace sand, divided by its displacement in a 10 ml graduated cylinder filled to the 5 ml mark with distilled water. The volume of sand in the pooled stomach sample was calculated as stomach sand weight divided by sand density.

When microscopic examination revealed striking differences in the concentration of diatoms relative to other algae and detritus in the gut content of different specimens, I decided these differences should be quantified. Ideally, it would have been best to separate the diatoms from the other components of a sample, and express diatom concentration as the weight of the diatom component per total sample weight. Methods of centrifugation through step and continous sucrose density gradients were applied to this problem, but neither was able to effect even a partial separation of diatoms and detritus. As an alternative, I have chosen to express the diatom concentration in a sample as the number of diatoms per sand free sample weight. Since all diatoms common in gut content samples were approximately the same size, this relative index was likely to reveal differences in diatom concentrations where they were great enough to be of importance. For two reasons, it was desirable to exclude sand from this analysis of gut samples. Firstly, sand appears to play no significant role in the nutrition of S. mossambicus. Unlike the mullet (Odum 1970), S. mossambicus does not use sand to crush diatom frustules in

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Figure 12. A light micrograph showing diatoms with and without visible cytoplasm. Approximately 225 times magnification. its stomach. Secondly, with a high density relative to other gut contents, the sand in a gut sample is likely to have an influence on the total sample weight disproportionate to its volume in that sample. Since the smount of sand in gut samples was variable, inclusion of sand in weighed stomach content would tend to obscure differences in diatom concentration relative to other algae and detritus in the gut contents, and it was these differences that were sought.

In the enumeration of diatoms, it is important to distinguish between frustules which contain cytoplasm and empty frustules that hold no nutritional value for a consumer (Fig. 12). As will be shown in Chapter III, no detectible digestion of diatoms takes place in the stomach so samples for diatom enumeration were taken only from stomach contents and only frustules with visible cytoplasm were counted.

To enumerate the diatoms in a sample of stomach content, the sample was put in a 50 ml volumetric flask with approximately 25 ml distilled water and was sonicated for 30 min as described above. Distilled water was added to the flask to a volume of 50 ml and after suspension by vigorous shaking, a subsample was diluted to produce a concentration of diatoms within the range 50 - 450 per ml. Required dilution factors ranged between 5 x 10^3 and 2 x 10^5 . Diatoms with visible cytoplasm in 1 ml of this diluted subsample were counted with a Zeiss inverted microscope using standard technique (Lund et al. 1958). Variation in counts of ten replicate The sample dilutions exhibited a Poisson distribution as reported by Lund. was then resuspended and poured into a preweighed aluminum pan with care that the sand, which settled out immediately, stayed in the flask. The sand free sample was dried to constant weight at 70°C, cooled in a dessicator and weighed to 0.1 mg. Diatom concentration in the sample was calculated as

Number of diatoms counted x Dilution factor

Weight of sand free gut content.

Throughout this work I have employed parametric rather than nonparametric statistics, because of their greater sensitivity. A statistical analysis was conducted only after establishing the validity of the necessary assumptions, usually normalcy and homogeneity of variance. Programs furnished with the Hewlett-Packard model 9810A calculator were employed for Analysis of Variance, Least Squares Regression Analysis,

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Table 2. Relative abundance of diatom species in pooled gut content of 10 juvenile <u>S</u>. <u>mossambicus</u> collected from the terrace in April, 1973. Number of diatoms counted = 778.

Diatom species	Percentage	
Mastogloia elliptica (Agardh) Cleve.	59.8	
<u>Stauroneis karstenii</u> (Zanon) Hustedt	9.6	
Melosira granulata (Ehrenberg) Ralfs	9.0	
Mastogloia smithii Thwaites	6.0	
Navicula pupula Kuntzing	5.3	
Navicula salinarium	3.7	

Total =

93.4



Figure 13. Typical gut content from juvenile S. mossambicus in Lake Sibaya. Scanning electron micrograph (SEM), 400 times magnification.



• Figure 14. <u>Mastogloia elliptica</u>, the most common diatom in the guts of juvenile <u>S. mossambicus</u> in Lake Sibaya. SEM, 2000 times magnification.

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Stident's t Test, Bartlett's Test for Homogeneity of Variance and estimates of skewness and kurtosis. All other statistical techniques were taken from Sokal and Rohlf (1969), except that for partial correlation analysis which was taken from Bernstein (196¹). The significance level of a test statistic is given as p, the probability that the null hypothesis is correct. Where it was appropriate to report an index of dispersion, I have reported the coefficient of variation (CV). This is simply the standard deviation expressed as a percentage of the sample mean. Unlike the standard deviation, variance and standard error, the coefficient of variation is independent of the value of the sample mean and thus coefficients of variation for different samples may be compared directly (Sokal and Rohlf 1969). I have reported the sample mean and number of observations with the coefficient of variation, so other indices of dispersion may readily be calculated.

Results

Described in general terms, the guts of all 1262 <u>S</u>. <u>mossambicus</u> examined contained a mixture of sand, detritus and algae. The presence of sand and detritus in these samples attests the benthic origin of the food. This amply confirms the conclusion shared by Minshull (1969) and Bruton (1973), that such material is the primary food resource consumed by <u>S</u>. <u>mossambicus</u> in Lake Sibaya. In contrast to the findings of these two authors, I rarely found other items in the gut content. The guts of only four juveniles and three adults contained small fish, and copepods were found in the guts of only four juveniles. No fresh macrophyte material or terrestrial insects were ever found.

Considered in more detail, the algal component of juvenile and adult gut contents differed markedly. Diatoms were predominant in the guts of juveniles collected from the terrace, other algae occuring very rarely (Fig. 13). One diatom species, <u>Mastogloia elliptica</u> (Fig. 14), represented 60% of all diatoms in the pooled sample of 10 gut contents examined by Professor Giffen (Table 2). Of the six most common diatom species in the sample, <u>Mastogloia elliptica</u> and <u>M. smithii</u> are both benthic and epiphytic, <u>Stauroneis karstenii</u> and <u>Navicula salinarium</u> are benthic while <u>Navicula</u> <u>pupula</u> and <u>Melosira granulata</u> are important in the plankton although they may also have a benthic phase. Diatom species composition in gut contents of juveniles appeared to be essentially constant throughout the study. In

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Figure 15. Typical gut content from adult <u>S. mossambicus</u> in Lake Sibaya. SEM, 800 times magnification.



Figure 16.

16. Cells of bacterial dimensions occasionally found attached to detritus in the guts of juvenile and adult <u>S. mossambicus</u> in Lake Sibaya. SEM, 10,000 times magnification.

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addition to these algae, a stalked periphytic diatom and filamentous algae were present in the guts of the few juveniles collected from a sheltered bay.

Algae in the guts of adults were primarily <u>Melosira grazulata</u>, the planktonic diatom <u>Synedra</u> sp. and the planktonic desmid <u>Closterium</u> sp. (Fig. 15). Small but variable numbers of green algae were also present. Those diatom species found in the guts of juveniles were uncommon in the guts of adults with the exception of one collection from the deep part of the terrace at site 7 (Fig. 11), and small adults caught on the terrace at high lake level during March and July, 1975. Material ingested by these fish appeared identical to that ingested by juveniles. Adults collected from the terrace habitat on other occasions contained <u>Melosira granulata</u>, <u>Synedra</u> sp. and <u>Closterium</u> sp. as described above.

A particularly significant sample was collected from the gradual slope during the cool season of 1975 (Fig. 11 site 12). Digestive tracts of 14 out of the 45 adult fish collected contained two to four alternating segments of dark olive green gut content and fluid, light green gut content. The others contained only the olive green material. Microscopic examination identified the dark olive material as the commonly observed mixture of sand, detritus and algae, but the light green material was primarily a mixture of <u>Closterium</u> sp., <u>Melosira granulata</u>, and <u>Synedra</u> sp. with very little detritus and no sand. This food appears to have been collected from suspension in the water column.

The possibility of phytoplankton feeding in <u>S</u>. <u>mossambicus</u> requires some comment. Although at least one other investigator has reported that <u>S</u>. <u>mossambicus</u> can feed on phytoplankton (Le Roux 1956), it is possible that this material was collected as sediment rather than from suspension. Because the method of plankton collection employed by cichlids is not clearly understood (Fryer and Iles 1972), the ability to collect suspended plankton cannot be deduced from anatomical structure. If this ability could be established experimentally, it would support inferences of phytoplankton feeding based on gut contents. A fortuitous bloom of suspended, spherical, unicellular algae that developed in an aquarium provided an opportunity to test <u>S</u>. <u>mossambicus</u> for the ability to collect suspended plankton.

The suspended algae was presented to juvenile and adult \underline{S} . <u>mossambicus</u> in an aquarium in which the possibility of substrate feeding had been

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eliminated. First the algae rich water was transferred to plastic buckets and the aquarium was scrubbed scrupulously clean with a large test tube brish. A panel of plastic webbing was fitted into the aquarium to prevent access of fish to the bottom and the algae rich water was returned to the These algae were dense enough to give the water a greenish cast aquarium. but did not affect the transparency noticably. Twelve S. mossambicus were removed from a clean aquarium where they had been allowed to evacuate their guts for several days. Three specimens were examined to find that their guts contained very small amounts of the common sand - detritus - algae mixture, while the other nine, 5.0 to 15.5 cm SL, were put in the algae After several hours the fish were sacrificed and their gut rich aquarium. contents were examined. The guts of three specimens remained empty, the guts of four specimens contained variable amounts of the unicellular algae, and two specimens, 5.0 and 10.1 cm SL, had intestines completely filled with the algae. I believe this demonstrates unequivocally the ability of S. mossambicus to collect phytoplankton from suspension.

The evidence indicates that both juvenile and adult S. mossambicus in Lake Sibaya consume a mixture of sand, detritus and algae from predominantly sand substrates throughout the year. In addition, some adults supplement their diet with phytoplankton during the cool season. Bruton's (1973) observations on seasonal distribution of S. mossambicus provide a valuable complement to these findings. As most juveniles are found close to the lake bottom throughout the year, they always have direct access to benthic In contrast, Bruton's echo sounding program indicates food resources. that during daylight hours in the cool season, the majority of adults are found distributed through the surface 5 m of the open water and well away Phytoplankton would be one of the few food resources from the lake bottom. available to these fish, a group I have been unable to sample. The adults that I did capture during the cool season appeared to migrate diurnally to and from the lake bottom. Despite good catches of other cichlids throughout the day, the bottom trawl caught S. mossambicus only after dark. Considering the alternating segments of benthic material and phytoplankton in the guts of some of the fish, the evidence suggests that these adults fed from the gradual slope floor at night and optionally from phytoplankton away from the gradual slope floor during the day. From the data available, it is not possible to establish the relative importance of plankton in the feeding of adults during the three month cool season.

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Table 3. Diatom concentrations in the stomach content of juvenile and adult <u>S</u>. mossambicus

A. Juveniles collected from the terrace

Date	No.	Diatoms per mg gut content	CV (%)
5 - 7 - 73	30	6.75 x 10 ⁴	81
27 - 12 - 73	21	6.29×10^4	66
Student	's t	= 0.328, p)0.75	

B. Juveniles from the terrace and adults from the gradual slope

Size	No.	Diatoms per mg gut content	CV (%)
Juveniles	21	6.29 x 10 ⁴	66
Adults	9	0.59×10^4	43

Student's t = 4.054, p(0.001

Whatever the cool season diet of adults may be, cool seasor feeding is less productive than feeding during other seasons. As described in Chapter I, Bruton (1973) observed two periods of relatively rapid growth for adult S. mossambicus in Lake Sibaya; one prior and one subsequent to migraticn to the open water for the cool season. During the warm season, energy and materials were devoted to reproduction, but neither reproduction nor significant growth were found for adults during the cool season. Bruton noted that the few adults he was able to collect during the cool season were in poor condition. My results confirm and extend this observation. The mean condition factor for 27 adults from one evening's trawl catches in July, 1975, was 3.58 (CV = 9.2), a value well below values for adults collected just prior to migration to open water in 1970 and 1971 Thus it appears that adult feeding during the cool season may (Fig. 8). be insufficient for even maintenance of condition. At other times, when S. mossambicus are growing or engaged in reproductive activities, adults are found in shoreward parts of the lake where their diet consists of algae and detritus from the lake bottom.

Diatom concentration in the guts of juveniles did not appear to vary with season (Table 3A). Expressed as the number of diatoms with visible cytoplasm per milligram of sand free gut content, diatom concentrations in the guts of two groups of juveniles collected from the terrace, one in the cool season and one in the warm season, had almost identical means. Striking differences were found in the diatom concentrations of juvenile and adult guts (Table 3E). Nine representative adults, three from site 6 and six from site 8 (Fig. 11), had a mean diatom concentration that was about one tenth that of juveniles.

Sand and detritus were less variable elements of the gut contents than were algae. Sand comprised a small proportion of the pooled sample of twenty stomach contents; 13% by weight and 0.6% by volume. Although the amount of sand in guts appears to be somewhat variable, I believe this sample is generally representative of both juvenile and adult gut contents collected throughout the study. Detritus from all specimens looked about the same. Most detritus was amorphous (Fig. 15) but plant fibers and bits of macrophyte debris were occasionally noted from adult guts. Small cells of bacterial dimensions were sometimes found in association with detritus (Fig. 16).

A review of the literature on materials digested by fish suggests that the difference in diatom concentrations of juvenile and adult diets may

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bespeak an important difference in their food values. Fryer and Iles (1972) stated that the solid cell walls of green and blue-green algae make them generally indigestible for cichlids, but diatoms, with poriforate cell walls, are readily digested. Fish (1955) reported that, of the many types of algae eaten by S. mossambicus, only diatoms are digested. Baier (1935) suggested that animals which feed on particulate organic matter including detritus digest only the associated microorganisms and the detritus itself is of little nutritional significance. Newell (1965) found that the gastropod Hydrobia ulvae in the Thames estuary was unable to digest the detritus in its diet and depended on the detrital microorganisms for its Findings that neither cellulose nor lignin are digested by nutrition. those invertebrates studied have been taken as further support for the belief that detritus is indigestible (Hargrave 1969, Calow 1975b). In a study of the feeding of the mullet, Mugil cephalus, which has a diet essentially identical to that of S. mossambicus in Lake Sibaya, Odum (1970) concluded that most of the mullet's nourishment was derived from the microorganisms in its diet, primarily algae, and detritus was of little nutritional significance. This view has also been emphasized by Darnell (1967), Madsen (1972), Olah (1972) and Seki (1972). Judging from the literature, we would expect the food value of a mixture of detritus and microorganisms to be directly proportional to the concentration of digestible micro-In Lake Sibaya, we would expect the food value of the diets organisms. of juvenile and adult S. mossambicus to be directly proportional to their diatom concentrations.

Contrary to what would be expected, preliminary observations found that diatoms frequently passed through the guts of <u>S. mossambicus</u> in Lake Sibaya undigested. Although large numbers of empty diatom frustules were occasionally found, diatoms from the posterior intestine of many specimens looked just like diatoms from their stomachs; full of cytoplasm and thus undigested. This meant that before conclusions could be drawn concerning the significance of diatom concentrations in the diet, it would be necessary to examine the conditions under which diatoms are digested. The examination of this problem is the subject of the next chapter.

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Figure 17. An adult <u>S. mossambicus</u> with part of the body wall removed to expose the viscera.



Figure 18. A dissection of the viscera of an adult <u>S</u>. <u>mossambicus</u> to display the digestive tract and the common bile duct.

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Chapter III. Digestion of diatoms and bacteria by Sarotherodor mossambicus

The digestive tract of <u>S</u>. <u>mossamplicus</u> is comprised of a stomach and an intestine which are separated by a well developed pyloric sphincter (Fig. 17 and Fig. 18). The stomach is of a cocal type (Smit 1968) with the entrance from the oesophagus and the exit to the intestine in close proximity. The enlarged anterior intestine receives a common bile duct on the intestinal side of the pyloric sphincter (Fig. 18) and is correctly termed the duodenum. A previous author has erroneously described this segment as the pyloric stomach (Kamal Pasha 1964). The remainder of the intestine is of uniformly small diameter and ends at the anal sphincter.

Very little information is available on the digestive physiology of <u>S</u>. <u>mossambicus</u>. Fish (1960) reported that the pH of intestinal juice in <u>S</u>. <u>mossambicus</u> remains relatively constant within the range pH 8.0 to 8.8, but the pH of stomach juice ranges from 7.5 to less than 2.0. Based on laboratory observations, this author concluded that both starvation and handling result in increased gastric pH. Digestive enzymes in tissues of the digestive tract of <u>S</u>. <u>mossambicus</u> have been described by Nagase (1964). Pepsin is present in stomach tissues, trypsin and amylase are present in intestinal tissues and lipase is present in both stomach and intestinal tissues. Maximum rates of enzymic activity were found at pH 2.8 for pepsin, pH 6.7 for trypsin, pH 8.0 - 8.2 for amylase and pH 7.2 for lipase. After a review of the literature, Nagase (<u>op cit</u>) concluded that <u>S</u>. <u>mossambicus</u> "appears to have an enzyme complement comparable to other fish".

Although this basic information provides a useful orientation, it does not suggest what specific physiological conditions would be required for digestion of diatoms. Therefore, it was necessary to begin the study of diatom digestion with a general survey of diatom digestion during a 24 hour cycle to determine 1) where in the fish's gut diatoms are digested and 2) if variations in the extent of diatom digestion are associated with a specific time of day or some discernible aspect of feeding activity. The results of this investigation drew attention to the possibility that variation in gastric pH might be responsible for variations in diatom digestion. To evaluate this possibility, changes in gastric pH of <u>S</u>. <u>mossambicus</u> in Lake Sibaya were examined in relation to feeding activity. The unexpectedly low pH values that were found suggested that bacteria as well as diatoms may be digested by <u>S</u>. <u>mcssambicus</u>. The effect of gastric

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acid concentration on digestion of both diatoms and bacteria was examined using radiotracer techniques in <u>in vitro</u> simulation of the digestion of <u>S</u>. <u>mossambicus</u>.

Methods

1. Investigation of diatom digestion in different segments of the guts of <u>S</u>. mossambicus collected at regular intervals over a 24 h period.

S. mossambicus were seined from the terrace at 2 h intervals over a period of 24 h starting 0900 h on December 15, 1973. All specimens caught were juveniles. Each collection was made from the same site approximately 200 m south of the research station (Fig. 35 site 1). At each appointed hour, the 15 m long seine net was positioned by pulling it end first to the deep edge of the terrace and then along the edge until it was parallel with the shore. The net was then pulled to shore by two persons in the water and the catch was landed on the beach. If more than 30 S. mossambicus were caught, 30 were arbitrarily chosen for analysis and unneeded specimens were quickly returned to the lake. If fewer than 30 were caught, all were kept. Fish from these collections were used for two purposes: for study of diatom digestion as reported in this chapter Specimens and for estimation of ingestion rates as reported in Chapter VI. used for study of diatom digestion were immediately fixed in formalin as described in Chapter II (page 21).

Diatom digestion in four specimens from each two hourly collection was examined as follows. The gut was removed and divided into six segments: stomach, duodenum, and first, second, third and fourth quarters of the remainder of the intestine. The content of each segment was put into a separate 25 ml volumetric flask, sonicated and the diatoms enumerated as described in Chapter II. In addition to counts of diatoms with visible cytoplasm, separate counts were made of empty diatom frustules in each sample. The proportion of all diatoms that did not contain cytoplasm was taken as an indication of the extent of diatom digestion. Results from specimens collected at 1900 h were unusually variable so two additional specimens were examined.

2. Measurement of changes in gastric pH during two 24 hour periods.

An increase in lake level of more than one meter occurred between November 1973 and March 1975. This made it necessary to modify the above



Figure 19. The seine net positioned at the deep edge of the terrace showing the ropes used to haul it to shore. March 7th, 1975.

method for collection of S. mossambicus from the terrace. Because the water's edge extended into marginal trees at the previous sampling site, samples were seined from the terrace immediately in front of the research station (Fig. 35 site 2) where trees had been cleared from the shore. Terrace waters were too deep to stand in at high lake level, so the seine net was positioned using an aluminum launch with an outboard motor. About 1.5 h before each collection, the seine was towed to the deep edge of the terrace where it was held in position by weights tied to both ends of the lead line (Fig. 19). At the appointed hour, the net was pulled to shore with ropes, one attached to each end of the net, and the catch was landed on the beach. Juvenile S. mossambicus were collected by this method at 2 h intervals for periods of 24 h beginning at 0800 h on March 4th and 0600 h on March 7th, 1975. Depending on the size of the catch, the gastric pH of eight or fewer specimens was measured with test papers designed to measure pH in the range pH 0.5 to 5.0 (Acilit, E. Merck, Darmstadt). The chyme was smeared onto one side of the test paper and the color that developed on the other side was compared to the manufacturer's color chart to estimate pH to 0.25 pH units. The accuracy of the color chart was confirmed by comparison of pH measured by test papers and by a standardized glass electrode pH meter for solutions of HCl that ranged from pH 1 to pH 5.

3. <u>In vitro</u> simulation of digestion of <u>S</u>. <u>mossambicus</u> to establish the role of gastric pH in digestion of diatoms and bacteria.

Experiments involving <u>in vitro</u> simulation of the digestion of <u>S</u>. <u>mossambicus</u> were conducted in Grahamstown using laboratory facilities of the Rhodes University Department of Zoology. Benthic floc to be used in these experiments was collected at Lake Sibaya from the terrace just south of the research station and immediately transported by road to Grahamstown, a journey that requires approximately 36 h. While in transit and prior to its use in experiments, the flocculent material was held in a 2 ℓ Erlenmeyer flask filled with lake water and kept in the shade under ambient temperature and light conditions. All experiments in which this material was used were completed within 6 days of its collection and no macro- or microscopic changes were observed during that 6 day period.

Since facilities required for separation and storage of stomach and intestinal juices were not available at Lake Sibaya, these materials were taken from <u>S. mossambicus</u> collected from a pond at the Amalinda Fish Hatchery, East London, South Africa. At the hatchery, stomach and



indestinal contents of 10 actively feeding adult <u>S</u>. <u>mossambicus</u> were pooled in two separate Erlenmeyer flasks and stored on ice while transported to Grahamstown, a trip that requires three hours. The two samples were then centrifuged in a refrigerated centrifuge at $3 - 5^{\circ}$ C and the clear supernatants were decanted and stored at -10° C until required.

Protease activity in gastric juice was determined by the standard method of Anson and Mirsky (1932). This method employs 2.5% hemoglobin as a substrate and detects pepsin concentrations as low as 2 μ g ml⁻¹.

Diatoms in one sample and bacteria in another sample of benthic floc were labeled with ¹⁴C so that experimental digestion of these cells could be assessed by monitoring release of the previously assimilated label. Diatoms in 20 ml of suspended floc were labeled by exposure to 50 µl $NaH^{14}CO_{z}$ solution (500 mCi ℓ^{-1}) for 10 h under incandescent (tungsten filament) illumination (Fig. 20). The sample was centrifuged and unassimilated label was removed by suspension of the sample in sterile water followed by centrifugation, this procedure being repeated until the supernatant contained negligible radioactivity. The floc was then resuspended in 30 ml sterile water and 3 ml of this labeled suspension was transferred to each of eight 15 ml centrifuge tubes. Bacteria in another 20 ml sample of suspended flocculent material were labeled by incubation with ¹⁴C-glucose at a concentration of 32.4 μ g glucose ℓ^{-1} (281 Ci M^{-1}) for 1 h at 25°C. Concentrations of glucose below 500 $\mu g \ell^{-1}$ are taken up by bacteria but are too low to be taken up by algae (Wright and Hobbie, 1965). Unassimilated label was removed as described above, the labeled floc was resuspended in 20 ml of sterile water and 3 ml of this suspension was transferred to each of six 15 ml centrifuge tubes.

Stomach conditions in <u>S</u>. <u>mossambicus</u> were simulated by addition of 10^{-1} M HCl to produce a range of pH values in six centrifuge tubes containing labeled diatoms and five centrifuge tubes containing labeled bacteria. Resultant pH values measured with a standardized pH meter were 1.5, 2.0, 2.0, 2.5, 3.0, and 3.5 for suspensions with labeled diatoms, and 1.3, 1.5, 1.75, 2.0, and 2.0 for suspensions with labeled bacteria. Two ml of stomach juice at pH 2.0 and 2 ml of boiled stomach juice were added to the 7th and 8th centrifuge tubes containing labeled diatoms, respectively (Fig. 20). To compare the effects of acid and lysozyme on bacteria, 2 mg of lysozyme (from egg white, Sigma Chem. Corp.) was added to the 6th suspension with labeled bacteria.

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Figure 21. Frequency distribution of the proportions of diatom frustules without cytoplasm in the stomachs (N = 32) and samples of intestinal contents (N = 209) of juvenile <u>S</u>. <u>mossambicus</u> collected from the terrace.

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and placed horizontally in a shaking water bath at 25° C. After 1 h, the estimated time that benthic floc is resident in the stomach of <u>S. mossambicus</u>, the suspensions were centrifuged and the radioactivity in 0.1 ml of the supernatant was counted by liquid scintillation. The remaining supernatant was discarded.

Intestinal digestion in <u>S. mossambicus</u> was simulated by resuspension of each pellet in 3 ml of the intestinal juice which had a pH of 8.6. Boiled intestinal juice was added to one centrifuge tube containing labeled diatoms and one centrifuge tube containing labeled bacteria. These suspensions served as controls. The release of previously assimilated ¹⁴C was monitored by periodically centrifuging the suspensions and counting the radioactivity in a 0.1 ml subsample of each supernatant. After subsampling, the pellets were quickly resuspended and returned to the water bath.

Results

The systematic investigation of diatoms in different segments of the digestive tract revealed that extensive digestion of diatoms does take place in the intestine. Proportions of diatom frustules without visible cytoplasm were consistantly much greater in intestines than in stomachs of specimens collected throughout the 24 h sampling period (Fig. 21), and this difference may most reasonably be attributed to removal of diatom cytoplasm by digestion.

Interpretation of the data with respect to digestion of diatoms in the stomach is less straightforward. For reasons discussed in Chapter V, proportions of diatoms without cytoplasm in the stomach content may not be directly comparable to proportions in floc collected from the lake bottom. In 10 samples of floc collected at the same site and within a few days of collection of the fish examined here, proportions of diatoms without cytoplasm ranged from 0.36 to 0.65 (Table 4): none were as low as 0.30, the mean proportion found in fish stomachs. Although it was not possible to determine the exact proportion of diatoms without cytoplasm in the floc ingested by these fish, the data do suggest that little if any cytoplasm is removed from diatom frustules while they are in stomachs.

In order to interpret the data further, it is helpful to know how the feeding activity of the population of juveniles sampled varied during the 24 h sampling period. Judging from the absence of food in stomachs (Fig. 22), we can conclude that juveniles did not feed at night between

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Figure 22. Proportions of diatom frustules without cytoplasm in six segments of the guts of S. mossambicus collected from the terrace. Segment 1 is stomach, 2 is duodenum, 3, 4, 5, and 6 are the first, second, third and fourth quarters of the remaining intestime, respectively. The population sampled was actively feeding from 9099 h to 1900 h (A - F) and 0700 h through 0900 h (L - M).

2100 and 0500 h. The presence of food in stomachs during daylight hours suggests that the rich were feeding at that time. Diurnal feeding periodicity of <u>S</u>. <u>sambicus</u> on terraces is examined in detail in Chapter VI and the conclusions are confirmed.

Comparison of proportions of diatoms without cytoplasm in the five intestinal segments reveals a curious pattern of variation. When the fish were feeding. the extent of diatom digestion within intestines decreased from the duodenum to the last intestinal segment (Fig. 22). As the feeding period progressed this pattern became less pronounced as seen by inspection of the raw data or as shown more clearly by regression analyses (Fig. 23). Careful scrutiny of this changing pattern and data for digested diatom proportions in the intestine at non-feeding hours suggests a cycle of diatom digestion related to feeding activity. It appears that diatoms in the food consumed at the start of a feeding period pass undigested through the stomach and anterior intestine to the posterior intestine where they remain undigested but mix with digested diatoms left from the previous day's food. Although diatoms in the duodenum are usually digested, diatoms from the duodenum of one specimen collected early in the feeding period on December 16, 1973, appeared to be totally undigested (Fig. 22L). It was the presence of these undigested diatoms in the posterior intestine which originally lead me to question digestion of diatoms by S. mossambicus. As feeding continued, digestion became effective and newly digested diatoms were mixed with undigested diatoms in Together with defecation of undigested diatoms, this the intestine. resulted in the observed gradual increase in proportions of diatoms without cytoplasm in the intestine (Fig. 23). Near the end of the period of feeding activity, the extent of digestion appears to be reduced since diatoms in the duodenums of specimens collected at 1900 h (Fig. 22F) were largely undigested. Most but not all diatoms that remained in intestines after feeding stopped were digested (Fig. 22G - K).

A review of the literature suggested that variation in gastric pH might be responsible for this cycle of diatom digestion. In a study of digestion of bluegreen algae by the cichlid <u>Tilapia nilotica</u>, Moriarty (1973) found that gastric acid was used to lyse blue-green cell walls; a process which allowed enzymes access to the algal cytoplasm when the food passed into the intestine. Gastric acid concentrations at pH values less than 2 were required for lysis. A number of fish species are known to

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all three regressions were very highly

ordinates were calculated from each regression equation, the coordinates were transformed back into proportions and then used to plot

Two pairs of co-

significant (p<0.001).

the lines in this figure.

secrete gastric acid only when food is present in the stomach (Barrington 1957, Smit 1968). In fish like T. nilotica and S. mossambicus that have one extended feeding period per day, this would be expected to result in a diurnal cycle of low gastric pH during periods of feeding activity and higher gastric pH during non-feeding periods. Moriarty (op cit) found such a cycle of gastric pH in T. nilotica and showed that it resulted in a cycle of digestion of blue-green algae virtually identical to the cycle of digestion of diatoms by juvenile S. mossambicus in Lake Sibaya. Bluegreen algae ingested early in the feeding period when gastric pH was relatively high passed undigested into the posterior intestine. As the feeding period progressed, digestion of blue-green algae became increasingly more efficient. This resulted in a pattern of decreasingly digested algae from the anterior to the posterior intestine. Moriarty (pers. comm. 1974) concluded that a similar cycle of digestion would not be expected for fish feeding on diatoms since he believed digestive enzymes should be sufficient to detroy diatom cell membranes. He suggested that this would allow access to diatom cytoplasm through pores in the silicon cell wall and lysis by gastric acid should not be necessary. However, the results reported above lead me to suspect that lysis by gastric acid is in fact required before diatoms are digested in the intestine of S. mossambicus.

To assess the validity of my hypothesis, it was necessary to examine firstly, variation in gastric pH in relation to feeding activity and secondly, the effect of different gastric acid concentrations on subsequent intestinal digestion of diatoms.

Both 24 h periods during which gastric pH was studied included a period of feeding activity by terrace fish. The time when feeding began was estimated as described in Chapter VI. All specimens collected in the first catch during both feeding periods had full stomachs with low gastric pH, frequently as low as 1.25 (Fig. 24). No differences were found in pH values measured for juvenilés'and adults' stomach contents. Low gastric pH persisted for several hours but rose to pH 4.5 and higher some time after most feeding ceased.

Unfortunately, these data give little indication of how gastric pH changes during the initial phase of feeding activity. No specimens were caught in the sampling just before either feeding period and specimens in the first collection from each feeding period had been feeding long enough to completely fill their guts. If gastric pH goes up at the end of one

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Gastric pH of juvenile and adult S. <u>mossambicus</u> collected from the terrace habitat during and after two periods of feeding activity, one on March 4th (0----0) and one on March 7th (X-X), 1975. Lines are plotted through means. Some points have been displaced horizontally for clarity.



Figure 25. Changes in gastric pH as the stomach fills at the beginning of a feeding period.

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feeding period it must come down for the next, but is gastric acid secreted in anticipation of feeding or only as the stomach fills? Data from one spot collection shed some light on this question. Adult S. mossambicus were collected from the gradual slope (Fig. 35 site 3) with a trawl early in a period of feeding activity on July 17, 1975 and the guts of 12 specimens were examined. Food was present in the stomachs of only five Stomachs of these specimens were first estimated to be 1/4, specimens. 1/2, 3/4, or completely full and then the gastric pH was measured (Fig. 25). These data argue that gastric pH is lowered only as the stomach is filled, but gastric pH values to which the first food ingested are exposed are still in question. Eleven of the 12 specimens had at least some food in their anterior intestines. Because recently ingested food is macroscopically indistinguishable from food that remains from the previous feeding period, it was not possible to determine how much food passed from the stomach into the intestine before low gastric pH values were reached. Moriarty (op cit) found that blue-green algae ingested early in a feeding period by T. nilotica were easily distinguished from those which remained from the previous feeding period; the former retained their green color while the latter were brown. As a result he was able to observe that early in the feeding period, when the stomach was small and contracted, some algae passed almost directly from the oesophagus into the intestine. As the stomach filled and distended and gastric pH dropped, algae were retained in the stomach for much longer periods. The stomachs of S. mossambicus behave in much the same manner; empty stomachs are small and contracted and only distend as they are filled with food. Therefore, it is likely that in S. mossambicus too some food passes into the intestine before gastric pH drops much below the values found in resting stomachs.

To my knowledge, pH 1.25 is the lowest pH ever recorded from the stomach of actively feeding fish. Higher values are quoted for a variety of fish species in reviews by Barrington (1957) and Smit (1968). Judging from results reported by Moriarty, the low pH measured in <u>S. mossambicus</u> would be expected to produce very efficient lysis of blue-green algae. The physical and chemical similarities between cell walls of blue-green algae and bacteria lead Moriarty to speculate that low gastric pH would also lyse bacteria. Although blue-green algae are rare in the diet of <u>S</u>. <u>mossambicus</u> in Lake Sibaya, bacteria are associated with the detritus ingested by both juveniles and adults. Because I suspected that bacteria may play a













Figure 28. The effect of simulated gastric pH on the amount of label released from bacteria and diatoms after approximately 3.5 h digestion by intestinal enzymes.



role in the nutrition of <u>S</u>. <u>mossambicus</u> in Lake Sibaya, the effect of gastric pH on digestion of bacteria as well as digestion of diatoms was investigated in <u>in vitro</u> experiments.

During treatment with HCl, no radioactive label was released from either diatoms or bacteria. However, after only 30 min subsequent treatment with digestive enzymes, large amounts of previously assimilated label were released from both diatoms and bacteria, relative to controls (Fig. 26 and Fig. 27). The release of label continued for about 3.5 h but no significant release was noted thereafter.

The pH of simulated gastric conditions had a marked effect on the subsequent amount of label released. Release from diatoms increased from pH 3.5 to pH 2.5 but releases from samples treated at pH 2.0 and pH 1.5 were not significantly greater (Fig. 26). In contrast, release of label from bacteria increased greatly below pH 2.5 such that more than twice the label released from the sample treated at pH 2.0 was released from the sample treated at pH 2.0. In lowering gastric pH below pH 2.0, <u>S. mossambicus</u> does not increase the efficiency of its digestion of diatoms but greatly increases the efficiency of its digestion of bacteria (Fig. 28). This important finding bespeaks the importance of bacteria in the nutrition of <u>S. mossambicus</u> in Lake Sibaya, a topic discussed in detail in Chapter V.

Lysis by gastric acid is necessary for digestion of diatoms. The fact that both the rate and the maximum amount of label released from diatoms was dependent on simulated gastric pH demonstrates that intestinal enzymes alone are not sufficient for digestion of these cells.

The sample of labeled bacteria treated with lysozyme released about the same amount of label after 3.5 h as the sample treated at pH 1.3 (Fig. 27). Since not all types of bacteria are lysed by lysozyme (Stanier <u>et al.1957</u>), this result can not be taken as proof of complete lysis of bacteria at pH 1.3. However, it does suggest that lysis is quite efficient at pH 1.3 and further confirms the conclusion that gastric acid does actually lyse bacterial cells.

Stomach juice had no effect on release of label from diatoms. Two samples treated at pH 2.0, one with 2 ml of stomach juice added, released almost identical amounts of label after 3.5 h. Although protease activity has been found in gastric tissues of <u>S. mossambicus</u> by Fish (1960) and Nagase (1964), no protease activity was detected in the stomach juice used

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here. Moriarty (op cit) found an identical condition in <u>T</u>. nilotica in Lake George: protease activity was present in the stomach wall, but could not be detected in the stomach juice. This is particularly interesting since, for both <u>T</u>. nilotica and <u>S</u>. mossambicus, maximum gastric protease activity is observed at pH values greater than pH 2.0 with greatly reduced activity at lower pH values. It appears that gastric proteases in these fish may not be secreted when gastric pH is unfavorable for their activity.

To summarize, results presented in this chapter reveal a daily cycle of diatom digestion related to feeding activity. Ingested diatoms pass through the stomach where they are lysed by gastric acid and into the duodenum The extent of lysis and subsequent digestion is where they are digested. inversely proportional to the gastric pH. As the stomach is filled at the beginning of a daily feeding period, gastric pH drops from values of 5 and above to values as 1cw as 1.25. Gastric pH remains low while the fish feed but rises to pH 5 and above some time after most feeding has ceased. The results indicate that diatoms ingested at the beginning of a feeding period pass into the intestine before gastric pH is low enough to affect lysis and thus these cells remain undigested in the posterior intestine. Less than two hours of feeding activity are required before stomachs are filled, gastric pH is quite low and digestion of diatoms becomes highly efficient. In vitro experiments indicate that gastric acid also lyses bacteria and allows them to be digested in the intestine. This is the first discovery of a mechanism for digestion of bacteria by a fish (Bowen 1976). Since several fish species, for example the carps, consume detritus and associated bacteria but lack true stomachs with gastric glands (Barrington 1957), it is possible that other mechanisms for digestion of bacteria await discovery.

Thus far it has been shown that juvenile and adult <u>S</u>. <u>mossambicus</u> in Lake Sibaya feed on benthic floc that consists primarily of detritus and diatoms, and that the floc consumed by juveniles is much richer in diatoms than that consumed by adults. It has been shown that the diatoms which are consumed are largely digested. Since it is believed that detritus has little or no food value, these results suggest that diatom concentration may play the principal role in determination of food quality in the diet of <u>S</u>. <u>mossambicus</u> in Lake Sibaya. For critical appraisal of this possibility, it was necessary to collect samples of juvenile and adult diets for quantitative comparison in terms of chemical and physical characteristics of known nutritional significance. Food collected from fish stomachs is not likely to be suitable for such comparisons. Apart from possible effects of gastric acid, post-mortem changes in the fish may significantly alter the chemical composition of stomach contents. Gill nets, the only reliable method for capture of adults that was available during most of the study, usually yielded specimens that had been dead for unknown periods. The possible importance of soft bodied organisms, for example protozoa, that would be digested almost immediately and therefore whose importance in the diet may not be reflected in stomach contents, further discouraged comparisons based on juvenile and adult stomach contents. A more suitable approach to this problem, and the approach used here, is to compare fresh samples of benthic floc from the feeding areas used by juvenile and adult Before such sampling can be undertaken it is necessary S. mossambicus. to identify the feeding areas of juveniles and adults: this was one of the goals of the observations reported in the next chapter.

Chapter IV. Observations on the Feeding Behavior of <u>Sarotherodon</u> mossambicus in Lake Sibaya

Three objectives were defined for the series of observations reported in this chapter. The first was to observe the foraging behavior of \underline{S} . <u>mossambicus</u> so a method for collection of fish could be selected that would be most likely to yield specimens representative of the entire foraging population. The second was to establish, through direct observation, how \underline{S} . <u>mossambicus</u> feed from benthic substrates so that a comparable method could be devised to collect benthic floc for analysis. The third objective was to identify the areas in Lake Sibaya where juvenile and adult \underline{S} . <u>mossambicus</u> feed.

Methods

During seven visits to lake Sibaya between January 1973 and August 1975, approximately 56 h were spent diving in the terrace habitat, 6 h on the steep slope and in deep water below 15 m, and 9 h in the gradual slope habitat using SCUBA and free diving techniques. Because of the particular danger represented by crocodiles in sheltered bay habitats, diving observations in these areas were limited to 1.5 h. Diving observations were made at chosen points and along transects that extended from shore into deep When transects covered more than 100 m, discrete rather than conwater. tinuous observations were usually made. Observations in sheltered bay habitats were limited to chosen points. Notes were made on the presence and behavior of S. mossambicus. Although it proved impossible to make sufficiently extensive direct observations of the feeding of S. mossambicus to establish feeding areas, small poc marks in the sand made by S. mossambicus feeding from the lake bottom provided an invaluable record of their feeding activity. Notes were made on the diameter and distribution of these feeding marks.

Approximately 30 h of observations were made on the terrace from a 3 x 1.5 m paddle board that could be poled several hundred meters in an hour. This proved to be a particularly useful way to examine the distribution of feeding marks and was most productive of results just after dawn when the lake was often calm and some of the suspended matter had settled out of the water. Observations were also made from shore and from the research station jetty.

The relationship between feeding mark diameter and the length of the

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feeding fish was determined in a 100 ℓ aquarium. Sand from the terrace was put in the aquarium and allowed to stand for two days. Seven to nine fish of one length group were then introduced, allowed to feed for several hours and the diameter of resultant feeding marks was measured. This procedure was repeated with fish of three different length groups.

Results

Juvenile <u>S. mossambicus</u> were seen frequently in the terrace habitat and occasionally in the gradual slope habitat, but adults were seen only in association with nests in the terrace habitat. Juveniles on the terrace were the only <u>S. mossambicus</u> whose feeding was observed. Although juveniles occasionally nibbled at the surfaces of submerged plants, they fed primarily from the terrace flocr.

The conspicuous presence of an observer appears to have interrupted the foraging behavior of juveniles on the terrace. When observations were made from the paddle board, from a boat or while snorkeling, juveniles were seen in shoals of as many as several hundred fish and little feeding was In contrast, when observed while lying on the jetty or motionless noted. among submerged vegetation, juveniles were seen to feed from the terrace substrate in small groups of approximately three to ten individuals. The distance between groups was generally greater than 0.5 m and each group appeared to move independently of the others. Individuals often left one group and joined another with the result that the number of fish in and the individual composition of each group changed frequently. As a group moved slowly along, one or two fish appeared to sample the substrate and occasionally several fish or the entire group would stop to feed. Thus, it appears that large shoals are formed in response to an observer or other disturbance and most foraging takes place in a dynamic network of small groups.

This result argues that collection of juvenile <u>S</u>. <u>mossambicus</u> from the terrace with a seine as described in the previous chapter would be expected to yield specimens representative of the population feeding on the terrace at the time of collection. Within the feeding population, each fish will have undergone a unique set of feeding activities for the feeding period sampled. Some will have started feeding earlier than others, some will have fed faster than others, each will have fed from different points on the terrace floor, etc. The crux of the problem of representative sampling is

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Figure 29.

9. A juvenile <u>S. mossambicus</u> about to bite into the sand. Photographed in an aquarium.



Figure 30. An area of dense feeding marks on rippled terrace sand at approximately 35 cm depth.



Figure 31. The relationship between fish standard length and the diameter of feeding marks produced. Lines show the range of experimental values and intersect at the means.

to collect a sample of fish that have themselves undergone a range of feeding activities representative of the range of activity experienced by the population as a whole. If <u>S. mossambicus</u> fed on the terrace in discrete shoals, each shoal would have undergone a different range of activity and it might have been necessary to collect several shoals to adequately sample the entire feeding population. But since they forage in small groups and the individual composition of each group is constantly changing, each feeding group would be expected to be roughly representative of the feeding population. This helps explain why examination of relatively few specimens was sufficient to reveal the cycles of gastric pH and diatom digestion described in the previous chapter. At hours when <u>S. mossambicus</u> are numerous on the terrace, the 15 m long seine net would be expected to capture several feeding groups. Shoal formation in response to seining would not alter the representative nature of the sample.

S. mossambicus employ two methods in feeding from the terrace floor. In the first, the fish faces the substrate at about 45° to horizontal and, in one short lunge, bites into the sand (Fig. 29). Still at 45° the fish moves a few cm from the substrate and mouths the material with several sharp downward movements of the lower jaw and the floor of the buccal cavity. During the last of these movements, sand is ejected through the opercular openings and finally, the fish spits out any large bits of macrophyte debris and the remaining sand. The second method of feeding is much like the first, but here the fish appears to peck rather than bite at the substrate surface, and several such pecks may be made before the fish stops to mouth the material and reject sand. This was observed on only a few occasions after several days of calm weather when settled material formed an unusually prominent flocculent layer several mm thick.

Feeding from sand substrates produces conical poc marks or depressions that persist for several days in the absence of heavy wave action (Fig. 30). Similar depressions in sand are produced by the feeding of a number of cichlid species (Fryer and Iles 1972) and mullet (Thompson 1966). In Lake Sibaya, only one other fish, the cichlid <u>Pseudocrenilabrus philander</u>, is known to feed from the bottom in such a way that poc marks may be produced. This species is abundant in deep water including areas below 15 m where <u>S</u>. <u>mossambicus</u> does not usually occur, but is uncommon on the terrace. Because very few feeding marks were found below 15 m, it has been assumed that most feeding marks found in the lake were produced by S. mossambicus.

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Figure 32. The distribution of feeding marks in the terrace habitat. Details in text.

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The proportion of feeding marks produced by <u>P. philander</u> is probably so small that it would have no significant effect on the considerations made here.

Aquarium experiments showed that the diameter of a feeding mark is directly proportional to the size of the fish that produced it (Fig. 31). Although the extent to which different substrates are stabilized may effect the depth to which a fish bites and thus the diameter of the resultant feeding mark, the general relationship found here can be expected to obtain in the lake.

At relatively low lake levels in June and December of 1973, feeding marks were commonly found on all parts of the terrace. Small marks were found close to shore and were more numerous than the larger marks that were found in deeper terrace waters away from shore. After a 0.52 m rise in lake level between December 1973 and July 1974, a somewhat different pattern of feeding mark distribution was found. This pattern was examined in detail. in February and March of 1975 and results are summarized diagrammatically in Fig. 32. For the purpose of illustration, the terrace has been divided into five zones. Areas shoreward of and among the flooded marginal vegetation (zone A) were heavily grazed and feeding marks were approximately 4 mm in diameter. This zone was so extensively utilized that few ungrazed areas were found. A second recognizable zone (B) extended several meters from the flooded vegetation toward the open water. Here dense patches of predominantly 6 mm diameter feeding marks were observed. Feeding marks that ranged from 7 - 13 mm in diameter were found in a third zone (C) still further from shore. These marks occurred at variable densities ranging from dense patches to scattered single marks. Still farther from shore, a zone (D) of sparcely distributed feeding marks 8 - 20 mm in diameter was found. On the deepest part of the terrace (zone E) very few feeding marks were found. These ranged from 13 - 45 mm in diameter, considerably larger than the largest feeding marks found on the terrace at low lake level, and were very sparcely distributed. This pattern was found on all terraces where feeding marks were present, including those of the eastern and western shores of the main basin and eastern shores of the south basin. Five months later, in July of 1975, the distribution of feeding marks was again examined in detail and the same pattern was found. These observations clearly demonstrate a directly proportional relationship between fish size and distance from shore or depth at which the fish feed.

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This conclusion is supported by the results of a fish trapping survey conducted in Lake Sibaya from September 1968 to May 1969 by Minshull (1970). Three traps were set on the terrace; one close to shore at 15 cm depth, one in the center of the terrace at 45 cm depth and one on the deep edge of the terrace at 1 m depth. In 85 such settings the mean standard length of <u>S</u>. <u>mossambicus</u> caught at 15 cm depth was 4.5 cm, at 45 cm depth it was 7.2 cm and at 1 m depth it was 8.2 cm. Welcome (1964) also found a strong positive correlation between fish length and water depth for four cichlid species along the shores of Lake Victoria. Comparison of observations at relatively high and relatively low lake levels suggests a constant relationship between fish size and the depth at which it feeds is maintained regardless of lake level. As lake level increased, the maximum size of marks found on the deepest part of the terrace increased. Feeding areas of the small fish followed the advance of the water's edge and thus maintained the same depth.

In a gradual slope habitat small feeding marks predominated close to shore, but at 5 m depth, 300 m from shore, most marks were greater than 20 mm in diameter and some were as large as 60 mm. Large feeding marks were found as deep as 6.5 m. In contrast to the terrace situation, large feeding marks were found in dense concentrations over extensive areas of substrate. This pattern was seen in both point observations and along a transect examined in July, 1975. Neither <u>S. mossambicus</u> nor significant numbers of feeding marks were seen in the steep slope or sheltered bay habitats, or at depths greater than 15 m.

Feeding mark distribution, size and density identify the terrace habitat as the principle feeding ground for juvenile <u>S</u>. <u>mossambicus</u> and gradual slope areas away from shore as the principle feeding ground for adults. The food value of benthic floc samples from these two habitats was investigated as reported in the next chapter. Chapter V. Quantitative comparisons of potential nutritional characteristics of benthic floc in terrace and gradual slope habitats in Lake Sibaya

The concensus from the literature on diets of fish suggests, as disin Chapter II, that the diatom concentration of floc from the cussed bottom of Lake Sibaya determines its food value for S. mossambicus. Operating on this assumption during the first part of the study reported in this chapter, comparisons of floc samples from different habitats in Lake Sibaya centered on comparisons of their diatom concentrations. There are, however, at least two drawbacks to assuming diatom concentration as the criterion of food quality. Firstly, the chemical composition of diatoms varies with growth conditions (Fogg 1965) so it is possible that diatoms from different depths, could differ in their nutritional value. Secondly, from the recent literature on detritus and its associated microorganisms it appears that these dietary components might also play significant roles in the nutrition of S. mossambicus. A second more critical approach to comparison of the focd quality of different floc samples was therefore required to confirm or refute conclusions drawn from comparison of diatom concentra-The second approach involved three steps. To begin with, samples tions. of floc were analysed in terms of chemical characteristics of potential nutritional significance. Total organic, caloric, total and soluble carbohydrate, nitrogen and protein contents were the characteristics chosen. Then utilization of these characteristics by S. mossambicus was assessed in aquarium experiments. Finally, quantities of utilizable chemical characters in different samples were compared within and among terrace and gradual slope habitats, and with the literature on animal nutrition to assess the significance of the results.

Part I. The distribution of benthic diatoms and benthic floc

Methods

Diatom concentration in benthic floc can be viewed as the product of two different simultaneous phenomena: the distribution of diatoms, and the distribution of the non-diatom components of the benthic floc. To determine how these two phenomena affect the diatom concentration of benthic floc in

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Core Length (mm)	Diatoms With Cyto. x 10 ⁵	Diatoms Without x 10 ⁶	Proportion Without	Floc Wt. (mg)	Diatoms Per mg Floc x 10 ⁵
Series I	n se ann ann ann ann an star ann ann ann ann ann ann ann ann ann a	alan an a			
5	1.51	1.31	0.42	11.6	1.30
10	1.89	1.38	0.42	13.3	1.42
15	1.91	2.30	0.54	16.6	1.15
20	2.11	2.79	0.57	17.5	1.21
25	1.54	2.85	0.65	19.8	0.77
Series II					
5	2.22	1.26	0.36	15.2	1.46
10 .	2.40	1.56	0.39	15.0	1.60
15	2.58	2.13	0.45	16.0	1.61
20	2.40	2.97	0.55	18.6	1.29
25	2.22	3.30	0.60	22.7	0.98

Table 4. Data from analysis of two series of five cores collected from the terrace.

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Lake Sibaya, a sampling method was developed for estimation of diatom numbers and mg floc per cm² benthic surface, as well as diatoms per mg of floc. The major factor to be considered in selection of a method for sampling benthic floc is the depth in the substrate to which the sample is collected. A1though viable benthic diatoms may be found several hundred mm deep in sand (Steele and Baird 1968), the vertical distribution of photosynthetic potential in diatom dominated benthic flora associations argues that these cells are usually most numerous in the top 1 cm with gradually reduced numbers at greater substrate depths (Hickman and Round 1970, Hunding 1971). - Direct counts of diatoms from core samples indicate that this is the case in Sibaya. Five glass cylinders of 5 mm inside diameter were marked at 5, 10, 15, 20, or To collect a core sample, a cylinder was pressed into 25 mm from one end. the sand so that the mark was level with the surface, the top of the cylinder was sealed with modeling clay, the cylinder was quickly inverted and the other end was similarly sealed. Two series of five cores were collected from two different areas a few cm square at about 60 cm depth on the terrace. Each core was transferred to a 50 ml jar and fixed with a few drops of 40 % The number of diatom frustules with and the number without formaldehyde. cytoplasm, and the weight of benthic floc was determined for each core as described for fish stomach samples. Although the weight of floc increased with increasing core length, no consistent trend in the numbers of diatoms with cytoplasm was observed (Table 4). This indicates that living diatoms were limited largely to the upper 1 cm of substrate. The same conclusion is suggested by comparison of vertical distributions of diatom shells with and without cytoplasm. Shells without cytoplasm are non-living structures with much the same distribution as the total floc, with the result that the proportion of diatom shells without cytoplasm gradually increases with increasing core length (Table 4). Thus, the length of a sample core can be expected to have a significant effect on the estimate of diatom concentration, longer cores yielding lower estimates (Table 4).

Assuming interstitial floc is evenly distributed in the top few cm of the sand as appears to be the case both from analysis of the terrace cores and from simple inspection of cores from other habitats, it is sufficient for comparison of samples within and among habitats that cores be of uniform length. However, if it is desirable to compare core samples with samples from fish stomachs, the length of the core should correspond to the depth to which the fish bites into the substrate.

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Figure 33. Glass cylinders 8 cm long by 2.6 cm inside diameter used to sample the benthic substrate and a 50 ml jar in which one sample has been stored.



Figure 34.

The 0.5 by 0.5 m grid through which sample cores were collected.

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But determination of the effective substrate depth to which the fish feed is far from a simple matter. To begin with, aquarium observations suggest that just as longer fish make larger feeding marks, longer fish bite to greater depths. There is also the possibility that as the fish bites it may partially or fully close its mouth with the result that most of the sand in the mouth is gathered from substrate depths much less than the maximum depth to which the fish actually penetrated. The extent to which the substrate is stabilized may also affect the depth to which a fish bites and further compound the problem.

To avoid these difficulties, I decided to collect cores 30 mm in length. Aquarium observations suggest it is extremely unlikely that even the largest <u>S</u>. <u>mossambicus</u> in Lake Sibaya penetrate 30 mm into the sand, so these cores would be expected to underestimate diatom concentration in benthic floc consumed by all sizes of <u>S</u>. <u>mossambicus</u>. Since larger fish are likely to bite deeper than small fish, 30 mm cores may possibly underestimate the diatom concentration of floc consumed by juveniles by a greater factor than they would underestimate diatom concentration in floc consumed by adults. Since it has been shown that the diatom concentration of floc from stomachs of juveniles is much greater than that in floc from stomachs of adults, the use of 30 mm long cores will yield conservative estimates of differences in diatom concentrations available to grazing juveniles and adults in different habitats.

Using 26 mm inside diameter glass cylinders (Fig. 33), ten 30 mm long cores were collected at each sampling site through a 0.5 x 0.5 m grid divided into 100 positions (Fig. 34). Each sample was collected as described above with the exception that rubber stoppers were used to seal the cylinders. Grid coordinates for each core were taken from a table of random numbers and marked on the grid with tape prior to sampling. No later than one hour after collection, each core was transferred to a 50 ml jar and preserved with 3 ml of 40 % formaldehyde. In the laboratory, benthic floc was freed from the sand in a core sample by shaking the jar vigorously for exactly 30 seconds and the suspended material was transferred to a 100 ml volumetric flask with a 25 ml volumetric pipette. This method of separation of floc from sand is roughly analogous to the method used by S. mossambicus as described in Chapter IV. Two additional 25 ml volumes of distilled water were used to wash the remaining floc from the sand and were similarly transferred to the Diatoms with cytoplasm were enumerated as described for 100 ml flask.

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Figure 35. Sites for collection of benthic floc samples and \underline{S} . <u>mossambicus</u> as described in the text. Letters denote transects.

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fish gut contents. Finally, floc from all ten cores at one sampling site was pooled in a separatory funnel, allowed to settle for at least 12 h, and then was drawn off, dried and weighed as described for gut contents.

On terraces, samples were taken at intervals along transects set perpendicular to shore. Intervals were chosen so that from eight to ten sites were sampled between the shore and the deep edge of the terrace. Distance from shore was measured with a rope marked at 4 or 10 m intervals. Depths of less than 2m were measured with a pole marked in cm while greater depths were measured with a la Spiro Technique brand diver's depth gauge. Samples were collected along one transect on each of five different terraces (Fig. 35), one in December 1973 and four in July 1974. In other habitats, samples were collected at arbitrarily chosen sites.

Results

Diatom densities in a total of 60 samples collected throughout the lake ranged from $1 - 60 \ge 10^4 \text{ cm}^{-2}$ with variable densities on terraces but consistently low densities in deep water (Fig. 36). Greatest diatom densities were found between 1.0 and 1.6 m depth. Along terrace transects, no relationship was found between diatom density and distance from shore (Fig. 39).

Similar results are reported by Moss and Round (1967) who found a range of $1 - 26 \times 10^4$ cm⁻² diatoms in 12 benthic samples taken at unspecified depths in the littoral of a freshwater lake in England, and by Smythe (1955) who reports a range of 1- 58×10^4 cm⁻² diatoms in 15 benthic samples collected from 2 - 20 m depth in a sea loch in Scotland. Smythe found a strong negative correlation between diatom numbers and depth. Round (1964) sampled benthic diatoms at 1 m intervals from 1 - 10 m depth and at other chosen points in Lake Windermere and Blelham Tarn. He found that diatom densities were low in the shallows, were greatest at about 1 m, and decreased markedly from 1-10 m depth; a distribution very similar to the one found in Lake Sibaya.

In contrast to planktonic algae, benthic algae are sessile cells whose distribution would be expected to indicate the zone in their environment that is most favorable for their reproduction; the greatest densities occurfing in the most favorable zone. From the distribution of diatoms in Lake Windermere and Blelham Tarn, Round concluded that, although scour and

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Figure 37. The distribution of benthic floc as a function of depth in terrace (0), gradual slope (X), and steep slope (x) habitats.



Figure 38. Diatom concentration in benthic floc in the terrace (0), gradual slope (X), and steep slope (x) habitats.

- 20 -







Figure 40. The frequency distribution of diatom concentrations in stomachs of 55 juveniles collected from the terrace and 49 terrace floc samples.

burial may cause local variations, light intensity was the major factor controlling the distribution of these cells. He suggested that photoinhibition, a well known phenomena in phytoplankton, limited production of benthic diatoms in the shallows, and below approximately 1 m light intensity was too low for optimal production. A more recent study by Hunding (1971) on the relationship between light intensity and primary production of benchic diatoms found that relatively low light intensities, 30 % or less of the surface illumination under the experimental conditions reported, were insufficient for light saturated photosynthesis. In Lake Sibaya, 30 % of the surface illumination is found at about 2.5 m depth (Allanson and Hart 1975) and diatom densities below that depth are consistently low. Hunding's results indicated that benthic diatoms are not normally subject to photoinhibition, so other factors such as physical disturbance by wave action may be important in limiting diatom densities in shallow waters.

The weight of benthic floc per cm² is variable on terraces but generally increases with depth throughout the lake (Fig. 37). This is not surprising since resuspension of sedimented floc in shallow areas and eventual transport to deep water would be expected to occur more readily than the reverse process. Together, the distributions of diatoms and benthic floc produce variable though generally high concentrations of diatoms on terraces, but consistently low diatom concentrations in other habitats (Fig. 38). In transects across terraces, no relationship was found between diatom concentration and distance from shore (Fig. 39).

These results fit well with data on diatom concentrations in the diets of juvenile and adult <u>S</u>. <u>mossambicus</u> in Lake Sibaya, and observations on their respective feeding areas. The diatom species associations in terrace and gradual slope samples appeared to be identical to those found in stomachs of juveniles and adults, respectively. Diatom concentrations in the stomachs of 55 juveniles collected from the terrace habitat and 49 terrace transect samples are essentially identical (Fig. 40). Diatom concentrations in the stomachs of nine adults, with a range of 0.11 to 0.85×10^4 diatoms per mg of floc, were very similar to those in 12 gradual slope samples that ranged from 0.16 to 0.39 x 10^4 diatoms per mg of floc. Taken together with data presented in previous chapters, these results demonstrate unequivocally that juvenile <u>S</u>. <u>mossambicus</u> in Lake Sibaya feed on diatom rich terrace floc while the adults feed on diatom poor





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floc in the deeper parts of the lake. The studies reported in the remainder of this chapter attempt to determine the significance that this fact holds for the nutrition of \underline{S} . <u>mossambicus</u>.

Part 2. Distribution of Chemical Characteristics and their utilization by <u>Sarotherodon mossambicus</u>

Before describing the methods used for collection and chemical analysis of benthic floc from Lake Sibaya, I would like to digress to précis what is currently known about the origin and chemical nature of detritus. Both are subjects of a relatively complicated recent literature that cuts across the fields of physics, organic and biochemistry and microbiology. Numerous theories concerning one aspect or another of detritus have been developed within these various disciplines but are either not generally accepted, or are of unestablished significance and have not been integrated into the general body of knowledge concerning detritus. In the following, I have attempted to précis the few generally accepted theories that I think will help explain my selection of analytical methods and provide a context for interpretation of the results.

As noted previously, detritus is readily divided into debris of animal and plant origin with recognizable cellular structure, or in the case of chitin, a recognizable form, and amorphous organic matter. Comparisons of the chemical structures of amorphous detritus and dissolved organic matter (DOM) have been used in combination with elegant in vitro radiotracer studies to show beyond reasonable question that amorphous detritus is derived from DOM. DOM in the seas is principally organic matter released from algae (Degens 1970) while in freshwater habitats terrigenous sources of DOM can be important (Wetzel et al. 1972). Both living and non-living agents may play roles in formation of amorphous detritus. Detritus is formed from DOM by adsorption to mineral surfaces and to other detritus (Riley 1970, Khailov and Finenko 1970, Degens 1970) and by precipitation (Povoledo 1972). Bacteria on the surface of detritus particles absorb dissolved compounds which subsequently become incorporated into the nonliving amorphous organic matrix (Paerl 1974). The relative contributions of each of these various types of formation is yet to be established for Small particles of detritus are bound together by any single ecosystem. bacteria (Paerl 1973, 1974) (Fig. 41) and form aggregates which frequently include large numbers of living algal cells, especially diatoms (Seki 1972).

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A



B

Figure 42. Detrital aggregates in benthic floc. A, photomicrograph, 300 times magnification. Although none is visible here, many such aggregates contain diatoms. E, diagrammatic representation to indicate spacial relationships.

Amorphous benthic detritus in Lake Sibaya is found in this aggregated form (Fig. 42).

Because microorganisms are always associated with detritus, and no technique has been developed for separating them, chemical studies have necessarily centered on naturally occurring aggregates. Studies of suspended particulate matter have centered on samples collected by filtration which also include some plankton. Carbohydrates in samples of particulate matter filtered from sea water have been characterized by N. Handa and coworkers. Water soluble and water insoluble fractions were distinguished. The water soluble fraction comprised 19 - 36 % of the total carbohydrate and consisted primarily of glucans, a class of carbohydrates thought to be storage products of diatoms which were the dominant phytoplankton in the waters studied (Handa and Yanagi 1969). The water insoluble fraction consisted of a mixture of polysaccharides having 1,2-or 1,4- linkages. The latter is a common constituent of algal cell walls and Handa (cited in Riley 1970) concluded that this fraction was a mixture of algal remains. Quantitatively, carbohydrates thought to be algal storage products or cell wall remains were the most important carbohydrates at all ocean depths sampled and were virtually the only ones in samples collected below 200 m. Handa and Mizuno (1973) report the presence of similar diatom derived carbohydrates in benthic floc from a freshwater lake but no quantitative data are given in the abstract seen. These results suggest that significant amounts of carbohydrate are present in both living and non-living components of detrital aggregates. In contrast, there is considerable evidence to suggest that protein in detrital aggregates is present primarily in the living components. Kaushik and Hynes (1968) found that elm leaves which fall into streams increase in protein content as they are colonized by microorganisms. Studies reviewed by Mann (1972b) indicate that during the early stages of macrophyte decomposition some protein is lost from the debris by leaching, but as the debris is colonized by increasing numbers of micro-organisms the protein content increases markedly. Calow (1975a) examined samples of benthic floc from three locations in a freshwater lake and reported that the relatively high protein concentration at one location was coincident with a large population of detrital bacteria. Gordon (cited in Riley 1970) treated aggregates of amorphous detritus with protein specific and carbohydrate specific stains and examined them microscopically. The amorphous matrix took up the carbohydrate stain but protein stain was taken up only by bacteria and by small unidentified inclusions that may also have been bacteria. The concensus from the

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literature is that, although detritus may contain some protein, most of the protein in detrital aggregate is in the form of microbial cells.

Lipids have been found to represent from less than 1% to about 10 % of the total organic carbon present in samples of particulate matter filtered from sea water (Parsons and Strickland 1962, Holm-Hansen 1972, Handa et al. 1972), but have not been studied in detail.

In addition to carbohydrate, protein and lipid, samples which include detritus contain "a chemically little known yet quantitatively very important portion" (Povoledo 1972). Several workers (Parsons and Strickland 1962, Holm-Hansen et al. 1966, Handa et al. 1972) have found that the sum of protein, carbohydrate and lipid frequently failed to account for one half or more of the total organic carbon present. This unaccounted for portion appears to be formed through extensive modification and combination of compounds in the DOM to form what has been termed "heteropolycondensates" (Degens 1970) or simply "combined forms" (Riley 1970). Such combined forms may be modified to the extent that they have little in common with their biogenic precursors. Although plant proteins are believed to be the original source of nitrogenous compounds in detritus (Degens 1970), Harrison and Mann (1975) found that as much as one half of the organic nitrogen in their samples containing detritus was soluble in TCA and thus clearly not Similarly, Parsons and Strickland (1962) found that a large protein. fraction of the organic nitrogen in detritus could not be identified as amino There is also evidence to suggest that a proportion of the acid nitrogen. combined forms is refractory to biological attack. Studies reviewed by Riley (1970) indicate that amino acids in detritus are combined in such a way that bacteria are unable to utilize them. Gordon (cited in Riley 1970) treated samples of particulate organic matter filtered from sea water with a mixture of trypsin, chymotrypsin and \propto amylase and determined the proportion of hydrolysed material. Commonly 40 - 70 % of the organic carbon in samples collected from the surface was not hydrolysed. Even less hydrolysis took place with deep water samples. Calow (1975a) reports that 60 - 70 % of the organic matter in 6 samples of benthic floc collected from a lake was "lignin", a term defined operationally as organic matter not rendered soluble by 12 h digestion with hot concentrated HCl. Whether or not this residuum is actually lignin is problematic, but its resistance to acid hydrolysis suggests that it is not likely to be broken down when ingested by animals. Furthermore, the simple fact that detritus accumulates in

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Figure 43. Apparatus used to collect benthic floc for chemical analysis. Details in text.

aquatic ecosystems attests its refractory property. These results emphasize that, particularly in the case of protein analysis, techniques for chemical analysis of detritus or materials including detritus must be chosen with the complex chemical nature of detritus in mind. Techniques commonly used for analysis of plant and animal tissues may not be appropriate for analysis of materials including detritus.

Methods

An apparatus was constructed to collect the top 1 cm of substrate from an area of 540 sq cm. The depth of 1 cm was chosen because aquarium observations suggested that this was about the depth to which large juveniles feed and thus represented an estimated mean feeding depth for the various sizes of <u>S</u>. <u>mossambicus</u> in Sibaya. Samples collected in this way would be expected to include all the floc consumed by smaller juveniles and most of the floc consumed by adults. Since no information is available on the vertical distribution of characteristics studied here, it was not possible to take a conservative approach similar to the one used in the study of diatom concentration. Substrate from an area of 540 sq cm yielded ample floc for analyses.

The sampling apparatus consisted of a metal guide and a plastic substrate collector as shown in Fig. 43. To collect a sample, the metal guide was pressed into the substrate so that it was level with the substrate surface and the plastic substrate collector was positioned at two marks on the guide (Fig. 43A). Wings on either side of the collector insured that the bottom leading edge was 1 cm below the substrate surface. As the collector was pushed forward along the guide, the top 1 cm of substrate was collected in the bag (Fig. 43B). When the collector reached the end of the guide, the forward end of the guide was lifted so that any substrate pushed in front of the collector also slid into the bag. The bag was removed from the collector frame and closed with a rubber band (Fig. 43C).

Because microscopic examinations reported in the previous section indicated that benthic floc did not differ from terrace to terrace, one terrace area (Fig. 35, site B) was chosen for intensive sampling. In February 1975, samples were collected at 2 and 4 m from shore and at 4 m intervals from 4 to 48 m from shore along each of three parallel transects which were set 10 m apart and perpendicular to the shore line. In addition, five spot samples were collected from the gradual slope habitat.

In the laboratory, a sample was put in a 2 ℓ Erlenmeyer flask and the flask was stoppered and shaken vigorously for exactly 30 sec. Freed floc was washed from sand with three volumes of water and poured through a 1.5 mm sq mesh sieve to remove any large bits of macrophyte debris, crustacea and molluscs that were occasionally present in samples. The floc was allowed to settle in a clean 2 & Erlenmeyer flask and after approximately 12 h the supernatent was drawn off by suction through a glass tube. A small sample of the settled floc was removed with a pipette and fixed in formalin for determination of diatom concentration as described in Chapter II. The remaining floc was then concentrated on Whatman No. 40 filter paper by vacuum filtration in a 11.5 cm Büchner funnel, scraped from the filter and dried in an aluminum pan at 70°C to constant weight. The dried sample was ground to a powder with a mortar and pestle and stored in a dessicator. This was chosen as the best method for sample preservation since deep freeze facilities were not available. Floc in this powdered form was used for all analyse ..

Caloric values were determined with a Phillipson type microbomb calorimeter (Gentry-Wiegert Instruments, Aiken, South Carolina, USA) calibrated with five samples of thermochemical standard benzoic acid supplied by British Chemical Standards. To produce complete combustion of floc samples it was necessary to add 1.0 μ l of mineral oil to each sample pellet; the caloric content of this oil was subtracted from the total calories measured to determine the caloric content of the sample. Caloric values determined for ten pellets prepared from one sample of terrace floc had a coefficient of variation of 7.9 %. Five pellets that had been ignited in the calorimeter were heated in a muffle furnace at 530° C for 3 h with no measurable weight loss (<2 %), so weight loss on ignition in the calorimeter was taken as the estimate of total organic content. Total organic content in ten pellets prepared from one sample of terrace detritus had a coefficient of variation of 2.4 %.

Soluble carbohydrate is thought to be non-structural carbohydrate that may be readily digested by consumers (Strickland and Parsons 1968). Soluble carbohydrate was extracted by Strickland and Parson's (<u>op cit</u>) crude fiber technique and quantified by their phenol-sulfuric acid technique. The coefficient of variation for seven replicates from the same

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sample was 4.1 %. Total carbohydrate was also determined by the phenolsulfuric acid technique and eight replicates from one floc sample gave a coefficient of variation of 6.9 %.

Total nitrogen was determined by the micro-Kjeldahl method (Scott and Hallet 1939).

Of the four methods that have been used to estimate the protein content of particulate organic matter including detritus, some are clearly more appropriate than others. Since not all nitrogen in detritus is protein, estimates of protein content calculated by several authors as elemental nitrogen times 6.25, protein being on average 1/6.25 nitrogen (Fruton and Simmons 1958), are certain to be overestimates. To avoid this source of error, Harrison and Mann (1975) estimated protein in samples of benthic detritus and associated microorganisms as elemental nitrogen in that fraction of the sample that was insoluble in a 5 % solution of trichloroacetic \ acid (TCA) times 6.25. These authors cited a review by Pirie (1955) as the reference for their technique, but I am unable to find any mention of such a technique in Pirie's review. Although it is accepted that proteins are insoluble in 5 % TCA, it does not necessarily follow that all non-protein nitrogenous compounds are soluble in 5 % TCA, and thus this method cannot be relied upon to accurately estimate protein in the presence of detritus. Parsons and Strickland (1962) estimated protein as the sum of amino acids. The relevance of such an estimate to trophic considerations is open to Since some amino acids in detritus are in combined forms that question. are resistant to microbial attack, it is likely that these amino acids are similarly indigestable for vertebrates such as S. mossambicus.

Two workers have estimated protein in samples containing detritus by direct colorimetric techniques. Kaushik and Hynes (1968) extracted protein from a dried and powdered sample with 10⁻¹ M _{NaOH} at ambient temperature for 24 h. To separate protein from alkali soluble pigments that interfere with colorimetry, protein was precipitated with 10 % TCA, centrifuged, and the supernatant was discarded. The protein pellet was resuspended in 10⁻¹ M NaOH and protein measured colorimetrically with the Folin reagent. Calow (1975a) used the protein method of Price (1965) which is very similar. I have chosen to use a modification of the method of Kaushik and Hynes because it clearly measures protein and only protein. Time required for protein extraction was determined by extracting ten replicates from one floc sample " at 25^oC for periods that ranged from 1 to 19 h. Extraction was complete

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after about 16 h (Fig. 44). To be reasonably confident of complete extraction from each sample analysed, an extraction time of 24 h was used. I have used the Biuret reagent (Bradshaw 1966) for colorimetric measurement of protein rather than the Folin reagent. Protein concentrations in floc samples were great enough that the high sensitivity of the Folin reagent was not required. In addition, the Folin reagent reacts with only specific amino acids and therefore develops slightly different color intensities with different proteins, while Biuret reagent reacts with peptide bonds and thus gives essentially the same color intensity per unit weight for all proteins (Fruton and Simmons 1958). For these reasons Biuret reagent would be expected to estimate protein with both greater precision and greater accuracy.

Aquarium experiments were conducted to determine the extent to which protein, total and soluble carbohydrate, organic content, and calories are assimilated from benthic floc by S. mossambicus. Juvenile S. mossambicus were collected from the terrace and allowed to empty their guts in a clean aquarium for two days. Food used in an experiment was taken from approximately 10 bags of substrate collected as described above from one area of a few square meters on the lake bottom. Floc was washed from the sand and sieved as described above, and allowed to settle overnight in a second clean aquarium filled with lake water. At about 0830 h the following morning, a sample of the floc was collected from the bottom of the aquarium with a 100 ml pipette and seven juveniles approximately 5 cm in standard length were then transferred into the aquarium. The aquarium was kept well shaded to minimize photosynthetic activity that could conceivably alter the chemical composition of the floc during the experiment. At about 1800 h the fish were transferred into a clean aquarium and their feces were collected at 2300 h. The observation that considerable amounts of feces were excreted in the feeding aquarium confirmed that any food present in the fishes guts at the start of the experiment was excreted in the feeding aquarium, and the feces collected at 2300 h were derived from the experimental food. Food and feces samples were dried within minutes of collection and their chemical compositions were determined as described above.

Assimilation of each component studied per g of food ingested was calculated by the method of Conover (1966). This method calculates assimilation by comparison of the amount of the component in food and in feces relative to food and feces ash contents and is reliable when the food contains a large proportion of indigestible ash as appears to be samples of benthic floc from Lake Sibaya.

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	Meters	Matana	Distore	ol.	Total Carbo.	Sol. Carbo.	Protein	Calories	Digestible P	Total
	from Shore	Depth	x 10 ⁴	Organic	as % Organic	as % Organic	as % Organic	ng Organic	Digestible KCal	Nitrogen
nsect 1	2	0.38	3.90	43.1	42.5	18.3	14.2	4.60	34-0	3.96
	4	0.42	1.77	41.4	76-1	13.3	12.1	4.41	30.2	3.67
	8	0.75	5.64	44.3	55.1	18-0	8-8	4-71	20.6	3-34
	12	1.05	3.16	39.3	62.3	16.5	5.1	3.42	15-4	2.62
	16	1.20	6.58	42.2	53.8	14.7	6.6	3.46	21.0	2.89
	20	1.33	3.14	45.7	38.7	13.6	5.7	3.42	18.4	2.74
	24	1.42	0.72	44.3	54.9	17.4	6.3	2.91	23.9	3-05
	28	1.53	1.54	39.5	43.4	15.2	4.4	3.00	16.2	3.37
ansect 2	2	0.63	5.55	48.9	61.4	14.3	7.8	4.69	18.3	2.58
	4	0.40	2.96	48.5	46.0	16.1	6.8	4.01	18.7	2.72
	8	0.66	1.13	44.3	50.3	12.9	6.3	4.77	14.6	3.07
	12	0.95	3.25	40.6	39-2	14.1	7.9	4.75	18.3	3.33
	16	1.16	3.20	41.6	41.8	15.2	4.8	3.48	15.2	2.91
	20	1.35	1.95	41.1	38.9	14.6	4.9	3.71	14.6	3.11
	24	1.40	2.01	43.8	42.7	17.5	4.1	4.11	11.0	3.08
	28	1.49	5.10	44.5	38.2	16.5	4.3	3.63	13.0	3.01
	32	1.55	1.75	41.8	44.5	17.0	5.7	4.02	.15.6	3.18
	36	1.61	2.27	50.3	34.0	14.2	4.0	3.16	14.0	2.92
	40	1.67	1.24	42.4	34.4	15.8	5-7	4.89	12.8	3.04
	44	1.72	2.20	44.8	42.6	20.9	4.7	4.16	12.5	3.30
	48	1.78	1.18	42-9	35-2	15.7	6.8	5.32	14.1	3.14
ansect 3	2	0.50	5.29	40.9	46.7	15.1	11.5	4.02	31.5	3.59
	4	0.60	3.19	43.4	37.3	14.3	8.8	4.18	23.2	3.16
	8	0.93	2.01	46.2	42.2	13.8	9.1	3.87	25.9	2.97
	12	1.12	3.45	42-7	44.7	14.6	8.9	3.76	26.1	3.19
	16	1.36	2.52	43.4	35.7	16.5	5.53	4.06	15.0	2.90
	20	1.58	3.04	41.9	38.6	13.5	4.15	2.68	17.1	3.13
	24	1.73	2-44	42.6	49.3	15.1	4.46	4.22	11.7	3.43
	28	1.79	2.35	39.6	46.0	18.7	4.8	4.31	12.3	3.43
	32	1.83	2.91	43.0	36.7	14.9	4.4	3.73	13.0	3.39
	36	1.85	1.46	38.9	53.7	18.5	5-1	4.69	12.0	3.91
	40	1.87	1.99	43.6	42.9	17.5	4.4	4.15	11.7	3.42
	44	1.92	1.66	41.4	47.6	15.8	5.7	4.36	14.4	3.70
	48	1.97	3.94	45.2	43.6	15.3	3.5	5.05	7.6	3-54
adual		2.5	0.12	47.8	40.1	14.9	3.6	5.09	7.8	2.91
lope		3.0	0.41	44.3	47.4	17.7	2.1	4.17	5.6	2.38
		3.0	0.28	42.6	48.6	16.2	3.1	4.00	8.5	2.41
		5.0	0.19	41.3	50.6	20.1	1.8	3.61	5.4	3.05
		5.0	0.16	39.0	42.7	16.0	1.8	4.24	4.7	3.36

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Table 6. Test statistics for comparison of total and soluble carbohydrate, organic and caloric contents of benthic floc samples.

ANOVA for comparison of three terrace transects Total carbohydrate Soluble carbohydrate Organic matter Calories

N=34	N=34	N=34	N=34
Mean = 45.1	Mean = 15.6	Mean = 43.4	Mean = 4.05
F=2.98	F=0.128	F=1.75	F=1.06
p<0.05	p<0.05	p40.05	p<0.05

Student's t test for comparison of terrace and gradual slope samples

Total carbohydrate Soluble carbohydrate Organic matter Calories

N=39	N=39	N=39	N=39
Mean = 45.7	Mean = 16.0	Mean = 43.0	Mean = 4.07
t=0.133	t=1.401	t=0.135	t=0.573
p<0.05	p<0.05	p<0.05	p<0.05

the case in Sibaya benthic floc. Any assimilation of materials that contribute to the ash component will result in underestimation of assimilation of the food components studied.

Two aquarium experiments were conducted in February, 1975, one using terrace floc as food and one using near shore gradual slope floc as food. These produced such interesting results that a trip was made to Sibaya in June, 1975, specifically to repeat and extend this approach to comparison of the food value of floc samples from different parts of the lake. Difficulties in handling and holding the fish encountered under cool season conditions in June prevented the successful completion of any of the attempted experiments and thus the results of only two experiments conducted in February are reported below.

Results

The organic content, total and soluble carbohydrate content and caloric content were similar in all samples regardless of habitat (Table 5). Mean values for each terrace transect were compared by Analysis of Variance and were found to be not significantly different (Table 6), so all terrace data were combined for comparison with data from gradual slope samples. Mean values for terrace and gradual slope samples were compared by Student's t test and were also found to be not significantly different (Table 6). Data for these characteristics of all 39 samples were therefore combined to describe benthic floc in both habitats. Distribution of protein is discussed later.

Organic material in the 39 samples ranged from 38.9 to 50.3 % with a mean of 43.0 %. Examined by scanning electron microscopy, most of the ash appeared to be diatom frustules. The largest identified fraction of the organic matter was carbohydrate. Total carbohydrate ranged widely from 35.2 to 76.1 % of the organic, with a mean of 45.7 %. Soluble carbohydrate was less variable with a range of 12.9 to 20.9 % and a mean of 16.0 %. Protein measured directly ranged from 1.8 to 14.2 % of the organic. When protein was estimated as total nitrogen times 6.25, estimates were on average 3.3 times the amount of protein measured directly. No statistically significant correlation was found between protein and total nitrogen (r = 0.235, N = 39). This result demonstrates that a large proportion of the nitrogen in Sibaya floc samples is not in the form of proteins and is in agreement with findings of other workers who have analyzed other types of particulate matter that included detritus.

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Table 7. Regression analysis of protein content as a function of m depth. F test statistic as recommended by Sokal and Rholf (1969). = p < 0.05, = p < 0.001.

N =	8	3	13		1	3
$\mathbf{F} = \frac{1}{2}$	36.	.1 ^{****}	6.6	6 *	63	•9 ^{****}
r = -	-0.	926	-0.6	12	-0	.924
Pool	ed	Gradual	Slope	and	Terrace	Data

r = -0.733

*

When, for each sample, the total carbohydrate and protein contents are summed and subtracted from the organic content, it is found that an average of 48.1 % (CV = 20.0 %) of the organic matter was unaccounted for. It is unlikely that lipid is a significant component of this unaccounted for fraction. The mean caloric value of the 39 samples was 4.07 cal per mg or just below 4.10, the mean caloric value of carbohydrate and well below the mean caloric values of protein, 5.10 and lipid, 9.50 (Cummins and Wuycheck 1971). Calow (1975a)reported that lipid was less than 1 %of the organic material in 5 out of 6 samples of benthic floc from the littoral of a freshwater lake. The sixth sample contained 2.5 % lipid to which Calow ascribed the unusually high caloric content of that sample, 4.72 cal per mg organic. The presence of a large organic component of Sibaya benthic floc that is not identifiable as carbohydrate, protein or lipid is consistent with the findings of other workers as discussed above.

The only published bjochemical data for benthic floc is that of Calow (op cit). This author reported a range of 10.5 to 63.7 % organic content for six littoral samples but noted that the lower values were probably artifacts caused by inclusion of small bits of stone in the samples. Values for the organic content of floc from Sibaya lie in the upper part of this range. Calow did not determine total carbohydrate and he used a very different method for determination of soluble carbohydrate, so no data are available for direct comparison. Using a technique essentially identical to the one used in this study, Calow found a very similar range of protein values, 0.0 to 8.6 % of organic. Caloric values reported for organic matter in surface sediment collected from the deepest point in 44 Polish lakes ranged widely from 1.20 to 6.78 cal per mg (Rybak 1969). Caloric values of Calow's six samples from the littoral of one lake ranged from 3.42 to 4.72 cal per mg organic. The range of caloric values for 39 samples from Sibaya, 2.19 to 5.32 cal per mg organic. lies within the range reported by Rybak and considering differences in the number of samples examined, is very similar to the range reported by Calow. All in all, there appears to be nothing unusual about the chemical composition of the benthic floc collected from terrace and gradual slope habitats in Lake Sibaya.

The most striking feature of the Sibaya floc is the variation of protein content with depth. Regression analyses of data for each of the transects taken separately and for all 39 samples taken together revealed

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Figure 45. The distribution of protein in benthic floc as a function of depth.



Figure 46. Dense concentrations of bacteria on benthic floc collected on a terrace at 38 cm depth 2 m from shore. Cells appear as collapsed rods due to air drying. Similar cells on detritus are identified by Paerl and Goldman (1972). SEM, 20,000 times magnification.

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highly significant trends of decreasing protein content with increasing depth (Table 7 and Fig. 45). As shown in Part 1 of this chapter, diatom concentration has a similar distribution in Sibaya. Can protein content be attributed to diatom concentration? Correlation analyses and partial correlation analyses (Table 8) show that even though protein content and diatom concentration are correlated, their correlation is entirely dependent on their common relationship to depth and no independent correlation exists between these two variables. Thus, the observed distribution of protein is clearly not a direct result of diatom concentration. As noted on page 77, there is considerable evidence to suggest that protein in particulate matter like the benthic floc in Sibaya is present primarily in bacterial cells. Dense concentrations of bacteria are found on benthic detritus from Sibaya (Fig. 46), and there is some circumstantial evidence to suggest that these cells are similarly responsible for a large proportion of the protein in the benthic floc. Five plastic bags containing substrate collected along the first transect from 32 to 48 m from shore were accidentally left for nearly 24 h before the floc was freed, concentrated and dried. When the bags were opened the samples smelled putrid. The protein contents of these samples were found to be nearly twice that of other samples collected at similar depths while soluble carbohydrate contents were considerably lower. The inference here is that confining the substrate in plastic bags stimulated bacterial growth which consumed some of the soluble carbohydrate and produced protein.

If bacteria are the primary source of protein in the benthic floc in Lake Sibaya, how can the observed distribution of protein be explained? Recently reported findings suggest an explanation that seems plausible in the Sibaya context. Although bacteria on amorphous detritus may utilize some of the detritus (Newell 1965), recent findings indicate that they are largely dependent on DOM for their nutrition (Riley 1970, Khailov and Finenko 1970, Olah 1972, Jannasch and Pritchard 1972, Paerl 1973 and 1974). Allen (1973) working <u>in situ</u> with plankton and Kleiber (1975) working <u>in</u> <u>vitro</u> with benthic algae in intact sediment cores both found that DOM released from algae is rapidly consumed by heterotrophs. Based on the rate at which ¹⁴C labelled photosynthate disappeared from the total DOM pool, Kleiber estimated the turnover time of newly fixed carbon in the DOM to be 0.30 h. Because released photosynthate is in solution for such a short time, it would seem reasonable to expect local effects in which

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Table 8. Correlation analysis of meters depth, diatom concentration, and protein as percent organic. Significance tested as recommended by Sokal and Rholf (1969) where the null hypothesis is that the coefficient equals zero.
²² = p<0.01, N = 39.</p>

		Partial			
Correla	tion coefficients	correlation coefficients			
	Depth Diatoms	Depth Diatoms			
Distons	-0 591 25	Distors -0 411**			

Protein -0.898** 0.022

Frotein -0.733 0.469**

Table 9. Correlation analysis of carbon fixation, percent release of photoassimilated carbon per 4 h incubation period, and depth using the data of Allen (1973). Significance tested as in Table 8, **= p<0.01, N=70.</p>

Correlation coefficients	Partial
	correlation coefficients

	Depth	% Release		Depth	% Release
% Release Carbon fixa- tion	-0.850 ^{***} -0.826 ^{***}	0.739***	% Release Carbon fixa- tion	-0.631 ^{***} -0.557 ^{***}	0.124

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bacterial density would be proportional to the availability of DOM. Allen (op cit) measured carbon fixation and release of photoassimilated carbon at 0.5 m intervals from the surface to 3 m depth in a small freshwater lake. Measurements were made on 10 occasions during the summer of 1971 to yield a total of 70 estimates of carbon fixation and DOM release. My correlation analysis (Table 9) of Allen's tabulated data reveals a highly significant negative correlation between release of photoassimilated carbon (expressed as a percentage of carbon fixation) and depth. This correlation is independent of the relationship between carbon fixation and depth. It is reasonable to assume that this result applies to the release of DOM from benthic algae in Lake Sibaya. Thus, all the information currently available suggests that the availability of DOM for bacterial mutrition would be expected to decrease with increasing depth in Lake Sibaya. This study has shown that diatom concentrations in the benthic floc generally decrease with depth, Hunding (1971) has shown that photosynthesis of benthic diatoms decreases with depth, and Allen's data indicate that release of DOM as a percentage of photosynthesis would be expected to decrease with depth. This may, in part, account for the distribution of bacteria implied by the distribution of protein found in benthic floc samples.

Temperature may also have an influence on bacterial populations in terrace floc. Bruton (1973) found that while the temperature of the open waters of Lake Sibaya remained essentially constant over a 24 h period, terrace water temperatures fluctuated, being warmer than the open water during the day and cooler at night. The degree of fluctuation increased from the deep edge to shore as the water became shallower. Kleiber (1975) found that the benthic algae he studied released DOM only when exposed to It appears that high temperatures on the terrace during the day light. would be coincident with the production of DOM and thus increase the rate at which bacteria could utilize DOM and reproduce. Lower night temperatures would reduce metabolism at a time when the availability of DOM in the benthic floc may be relatively low. Thus, diurnal temperature fluctuations in the terrace waters would be expected to result in a favorable relationship between DOM availability and metabolic scope for detrital bacteria.

Although the roles of bacterial density, DOM availability and temperature discussed above require confirmation, they seem plausible and I believe they offer the best possible explanation for the distribution of protein in benthic floc in Lake Sibaya that can be based on the current literature and





Table 10. Results of chemical analysis of stomach contents pooled according to fish standard length from one seine catch on the terrace in July, 1975.

Number of specimens	Size group cm standard length	Mg protein per mg soluble carbo- hydrate
13	5 - 6	0.728
6	6 - 7	0.651
6	7 - 8	0.641
4	8 - 9	0.407
3	9 - 10	0.337

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my own work.

It was surprising to find that diatom concentration is not significantly correlated with either total or soluble carbohydrate. This indicates that large proportions of both soluble and insoluble carbohydrate are in the non-diatom fraction of benthic floc. A simple calculation of the weight of diatoms in floc samples confirms that this is a realistic conclusion. Most diatoms in terrace floc are the size of <u>Mastogloia</u> <u>elliptica</u> (Fig. 14). The volume of this diatom can be estimated as 2/3 the volume of a rectangular solid of the same length, width and depth, or $40\mu \ge 7.6\mu \ge 7.6\mu \ge 2/3 = 180\mu^3$ per diatom.

Assuming a density of one, this is equivalent to 1.8×10^{-7} mg per diatom. The highest diatom concentration found in terrace flcc was 3×10^5 diatoms per mg of benthic floc, and this would be equivalent to 5.4 % diatoms in benthic floc by weight. The mean diatom concentration in the 34 terrace floc samples used for chemical analyses was 2.8×10^4 diatoms per mg of floc and would be equivalent to a mere 0.5 % diatoms by weight. The impression gained through microscopic analysis has been deceptive; diatoms actually represent a small fraction of the floc by weight. Since the amounts of soluble and insoluble carbohydrate are several times greater than the estimated weight of diatoms in floc samples, it is necessarily the case that most of these carbohydrates are associated with the non-diatom components of benthic floc.

If the observed relationships between protein content and depth, and the feeding areas of different sized fish and depth are accurate, an inverse relationship should exist between the protein content in floc from fish stomachs and fish length. This deduction was tested by collecting S. mossambicus from the terrace with a seine at 2130 h on July 6, 1975. Immediately after capture the fish were divided into 1 cm length groups, the stomach contents from each group were pooled, dried at 80°C and stored in a dessicator pending analysis. At the time when these samples were analysed, I thought the ratio of protein to soluble carbohydrate provided a valuable index of food quality in benthic floc so the stomach samples were analysed for these two variables. Soluble carbohydrate has a relatively even distribution in regard to depth with the result that ratios of protein to soluble carbohydrate for benthic floc show essentially the same distribution as does protein (Fig. 47). Protein - soluble carbohydrate ratios in the stomach samples (Table 10) were in extremely close agreement with the expected values based on the observed distributions of protein

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	sampled calculated by Conover's (1966) method. Amounts assimilated are given as mg or kcal per g food ingested.									
		Organic m	atter		Kcal					
_	Food, [*] mg per g	Feces,* mg per g	AE %	Amount Assimilated	Food, [*] kcal per g	Feces, [*] kcal per g	AE %	Amount Assimilated		
Terrace	421	330	32.3	136	1.65	1.18	38.8	6.40		
Gradual Slope	687	557	42.7	293	2.80	2.16	45.3	1.27		
_					· • • • • • • • • • • • • • • • • • • •					
	Protein				Total carbohydrate					
_	Food, ^x mg per g	Feces, ^x mg per g	AE %	Amount Assimilated	Food, mg per g	Feces, mg per g	AE %	Amount Assimilated		
Terrace	56.0	34.0	48.3	27.1	27.0	21.0	32:8	88.5		
Gradual Slope	42.5	34.0	44.3	18.8	34-5	30.5	37.5	_ 129.5		
_				•				• • • •		
	Soluble carbohydrate				Insoluble carbohydrate o "crude fiber"			e or		
	Food, mg per g	Feces, mg per g	AE %	Amount Assimilated	Food, mg per g	Feces, mg per g	AE %	Amount Assimilated		
Terrace	7.1	4.8	41.6	29.5	19.9	16.2	29.6	59.0		
Gradual Slope	14.0	13.6	31.4	43.9	20.5	16.9	41.8	85.6		

* mean of two determinations, * mean of three determinations

and feeding marks.

It is tempting to draw conclusions about the food quality of benthic floc found in different parts of Lake Sibaya based on protein content, but first the extent to which protein and other characteristics are utilized by <u>S. mossambicus</u> must be considered.

Estimated assimilation efficiencies and amounts assimilated for the various characteristics of floc samples collected at 0.5 m depth on the terrace (Fig. 35, site 2) and 2 m depth on the shoreward edge of the gradual slope (Fig. 35, site 4) are given in Table 11. Assimilation of protein and soluble carbohydrate was more efficient from the terrace food while assimilation of total carbohydrate, organic material and calories was more efficient from the gradual slope food. Although Conover (1966) and Hargrave (1969) report a high level of precision for the technique used here, the failure of attempts to replicate these experiments has made it impossible to determine if these differences in assimilation efficiencies are significant. In comparison to differences in the chemical compositions of the two samples used as food, differences in assimilation efficiencies are relatively small and had less effect on the chemical composition of the material assimilated than did the actual composition of the food.

Two additional features of these results are noteworthy. Firstly, in both experiments assimilated soluble carbohydrate accounted for only 33 % of the total carbohydrate assimilated. Contrary to what has been thought (Parsons and Strickland 1962, Riley 1970), the data clearly argue that insoluble carbohydrate, or what is sometimes called "crude fiber", is digestible. Secondly, when assimilated protein and carbohydrate are summed and subtracted from assimilated organic matter, it is found that 15 % of the assimilated terrace floc and 49 % of the assimilated gradual slope floc are unaccounted for as components that are likely to be part of living organisms. This result argues forcefully that, contrary to the opinions of several workers discussed in Chapter II, a large component of the material assimilated from detrital aggregate is actually detritus: it is not the case that only microorganisms are digested.

To summarize, chemical analysis of benthic floc collected from the areas in Lake Sibaya where <u>S. mossambicus</u> feed and of stomach contents demonstrate that as the fish grow larger, the protein content of the floc they ingest decreases relative to other dietary components. Assimilation experiments indicate that the chemical composition of the material assimi-

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lated is dependent to a large extent on that of the material ingested. The significance of these findings to the nutrition of <u>S</u>. <u>mossambicus</u> in Lake Sibaya becomes evident when considered in the context of the current knowledge of basic nutrition.

It is now generally understood that food consumption in animals under normal circumstances is regulated specifically to supply the energy needs of the consumer (Mayer 1967). The amount of digestible protein consumed depends directly on the ratio of digestible protein to digestible energy in the consumer's diet (Harper 1967). This has been demonstrated for a variety of animals including the fishes Ictalurus punctatus (Page and Andrews 1973), Chrysophrys aurata (Sabaut and Luquet 1973) and Carassius auratus (Rozin and Mayer 1961, 1964). As a result, the basic nutritional value of a given diet depends on its ratio of digestible protein to digestible energy (Boyd and Goodyear 1971). For a given species under a given set of environmental conditions, desirable protein to calorie ratios range from a minimum at which just enough protein is consumed for maintenance to a maximum at which growth is maximal. Below this range retarded growth, poor condition and other symptoms of protein deficiency become apparent. Above this range growth is reduced, presumably as a result of the high energetic cost associated with digestion and metabolism of protein (Boyd and Goodyear 1971). Within this range, growth is usually directly proportional to the protein content of the diet (Ogino and Saito 1970, Russell-Hunter 1970, Sabaut and Luquet 1973).

Using the C:N ratio as a rough indication of the protein to calorie ratio, Russell-Hunter (1970) generalizes that, with the exception of ruminants, animals at all trophic levels studied require diets with C:N ratios of 17:1 or less for maintenance. Diets with ratios greater than 20:1 result in conspicuous malnutrition. In diets comprised of only carbohydrate and protein, these ratios correspond to 22.0 and 17.9 mg of protein per kcal, respectively. Because studies of protein in the nutrition of fishes have been concerned with development of artificial diets for optimal growth in aquaculture (Cowey and Sargent 1972), no data on limiting dietary protein levels are available, but there is no reason to believe that Russell-Hunter's generalization would not be applicable.

Using mean assimilation efficiencies for protein and calories from the two aquarium experiments, I have calculated mg of digestible protein per digestible kcal for each of the 39 benthic floc samples in Table 5. These data are plotted in Fig. 49 as a function of depth, along with the

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A

B

Figure 48. Adult <u>S. mossambicus</u> collected A, from Inyamiti pan on the Pongolo river (Fig. 2) and B, from Lake Sibaya. The two Sibaya specimens were chosen as the thinnest (above) and the fattest from a trawl catch in June, 1975.



Figure 49. The ratio of digestible protein to digestible kcal in benthic floc as a function of depth. Curve fitted by parabolic regression. Expected nutritional significance of this ratio for fish feeding at different depths is indicated according to Russell-Hunter's (1970) criteria. line from a parabolic regression analysis of the relationship between these two variables. The parabolic regression model is more applicable to these data than the linear regression model since the latter would erroneously suggest that ratio values could be negative. Parabolic regression results were highly significant (R=0.839, p<0.01). The upward inflection of the regression line from four to five meters depth is a statistical artifact resulting from the small number of deepwater samples and has no biological significance.

For two reasons, the digestible protein - digestible kcal ratios in Fig. 49 may underestimate the food value of benthic floc. Firstly, the unidentified fraction of the assimilated organic matter may contain significant amounts of amino acids that could be, depending on the ratio of specific amino acids, as mutritionally valuable as assimilated protein compared on a weight for weight basis. This is a possibility that has not been examined. Secondly, relatively small errors in estimation of assimilation efficiency for either protein or kcal could have a significant effect on estimated digestible protein - digestible kcal ratios. Fish (1960) and Moriarty (1973) found that handling or other disturbance of S. mossambicus and Tilapia nilotica, respectively, resulted in increased gastric pH. Results reported in Chapter II have shown that the extent of lysis and digestion of bacteria, cells thought to be the principal source of dietary protein for S. mossambicus in Sibaya, is very sensitive to changes in gastric pH. Although every effort was made to minimize disturbance of the fish during assimilation experiments, the limited handling that was necessary may have resulted in estimates of protein assimilation that are less than assimilation under natural circumstances. These possible sources of error may have caused underestimation of the food value of benthic floc samples but would not be expected to seriously alter the estimated relationship between food value and depth.

In light of these results it is not surprising that adult <u>S. mossambicus</u> in Lake Sibaya are stunted and in poor condition (Fig. 48). By Russell-Hunter's criteria (Fig. 49), fish that feed consistently at depths greater than about 1.2 m would be expected to show conspicuous signs of malnutrition. Fish feeding at depths between 1.2 and 0.75 m would be expected to have enough dietary protein for maintenance and those that feed in water less than 0.75 m deep would be expected to have sufficient dietary protein for the good growth they exhibit (Bruton and Allanson 1974). Dietary protein deficiency is therefore identified as the cause of stunting and poor condition of adult <u>S. mossambicus</u> in Lake Sibaya.

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Figure 50. The general principle used to estimate ingestion.

Chapter VI. Diurnal feeding activity of juvenile <u>Sarotherodon</u> <u>mossambicus</u> in Lake Sibaya and estimation of daily energy assimilation

Work reported in previous chapters revealed the importance of feeding on the near shore part of terraces to the nutrition of juvenile <u>S</u>. <u>mossam-</u> <u>bicus</u> in Lake Sibaya. In this chapter, I describe the feeding strategy used by juveniles to gather their daily meal from the terrace habitat and how this strategy is modified in response to changes in the terrace environment.

Methods

The activities of juvenile <u>S</u>. <u>mossambicus</u> feeding on terrace floc were studied during four 24 h periods, two at low lake level in December 1973, and two at high lake level in March 1975. <u>Diurnal movements</u> to and from terrace waters were determined by seining at 2 h intervals as described in Chapter III. The number of fish caught and weather and wave conditions at the time of each collection were recorded.

Feeding activity of the juveniles on the terrace was examined by estimation of the amount of food ingested during each 2 h interval. Methods for estimation of ingestion by wild fish populations have been reviewed by Davis and Warren (1968) who distinguished four general methods. Three of these methods estimate ingestion based on comparisons of growth of fish under laboratory and field conditions and cannot be used to resolve differences in ingestion rates over relatively short time periods. The shortest time interval for which such methods can be used is the shortest time interval over which the growth of the wild population can be estimated, often a In contrast, the fourth method discussed by period of several months. Davis and Warren is based on direct examination of the amount of gut content representative samples of the wild population. This method can be in used to estimate ingestion for time intervals as short as the shortest interval for which significant changes in the amount of gut content can be Ingestion during a given time period (I) is estimated as the established. change in the amount of gut content during that time (ΔG) plus the amount of material defecated during that time (D) (Fig. 50). If the amount of food in the gut increased ($\Delta G>0$), ingestion will have been equal to that increase plus any material defecated. If the amount of food in the gut decreased

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Figure 51. One of the cages used to hold fish for determination of defecation rates.



Figure 52. One of the holding cages in position at the side of the research station jetty.

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$(\Delta G < 0)$ ingestion will have been equal to any amount by which the amount of material defecated exceeded that decrease. Ingestion is zero when the amount of material defecated is equal to a negative change in amount of gut content. This general method was used to estimate ingestion in juvenile <u>S. mossambicus</u>.

Juveniles used to estimate ingestion were taken from the seine catches made at 2 h intervals. If 30 or more juveniles were caught, 30 were arbitrarily selected for analysis and the rest were quickly returned to the lake. If fewer than 30 juveniles were caught, all were kept. Of those kept, half were fixed in formalin immediately and half were put in a plastic bucket of fresh lake water and transferred within five minutes of capture to one of two 1.0 x 0.75 m holding cages supported by 0.3 m long legs (Fig. 51). The cages were kept at the side of the research station jetty in 1 m of water (Fig. 52). This held the fish under wave, light and temperature conditions comparable to conditions in the area where they were collected and allowed defecation, but it prevented feeding from the terrace Exactly three hours after capture, these fish were fixed in formalin. floor. If there were fewer than 20 juveniles in a catch, this holding procedure was omitted and all specimens kept were fixed immediately.

In the laboratory, the gut content of a fish was carefully removed, dried at 70° C to constant weight, cooled in a dessicator and weighed to 0.1 mg. The fish, including intestinal tract, was dried to constant weight at 80° C in a vacuum oven and weighed to 0.01 g.

Amounts of gut content in different fish could not be compared on a weight of gut content per weight of fish basis because of the highly allometric relationship between gut capacity and fish size. Small juveniles with full guts contained much more food per g fish weight than large It was therefore necessary to convert the weight of gut content juveniles. for each specimen to weight of gut content per some arbitrarily chosen standard size fish. Three grams was chosen as the standard size since it is approximately the median fish weight in the samples under consideration. To determine the appropriate conversion, the relationship between maximum gut capacity and fish size was established by linear regression analysis of log weight of gut content as a function of log fish weight for fish caught and fixed immediately at 0900, 1500 and 1700 h on December 15, 1973 (Fig. 52). These fish were chosen because they appeared to be the fullest of the samples examined and would therefore define the approximate maximum gut





Measured wt. of gut content = 218.7 mg Sample fish dry wt. = 1.78 g Sample fish gut capacity = antilog (2.23 + 0.82 (log 1.78)) = 272.3 mg 218.7 Measured gut content as proportion of capacity = 0.803 = antilog (2.23 + 0.82 (log 3)) Gut capacity of standard 3 g fish = 418.1 mg = 418.1 × 0.803 Standardized weight of gut content = 335.7 mg

Figure 54. A sample calculation to show how measured weight of gut content was converted to standardized weight.

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capacity, at least within the context of the samples collected. Regression statistics show that the log - log relationship between these two variables is defined quite well by a straight line (r = 0.869, p<0.01). To convert the weight of gut content of a specimen to the equivalent weight of gut content for a standard 3 g fish, the weight of the sample fish is entered in the regression equation as x, and the weight of gut content that corresponds to maximum capacity for that size fish is calculated. The actual measured weight of gut content is then expressed as a proportion of the calculated maximum, and the weight of gut content corresponding to that proportion of maximum capacity in a 3 g fish is calculated. A sample transformation is given in Fig.54. Standardized weights of gut content were used for all calculations.

Defecation rate (D_t) , defined as mg of gut content defecated per hour, was calculated for each two hour interval starting at time t as

$$D_t = \frac{G_t - GH_t}{3}$$

where: G_t = mean weight of gut content for juveniles caught at time t and fixed immediately, and

GH_t = mean weight of gut content for juveniles caught at time t and held for 3 h.

The rate of change in weight of gut content (ΔG_t) over the 2 h interval. starting time t, expressed in mg per h, was calculated as

$$\Delta G_t = \frac{G_{t+2} - G_t}{2}$$

where: G_{t+2} = mean weight of gut content for juveniles caught at time t+2 h and fixed immediately.

The ingestion rate for the 2 h interval starting time t (I_t) , in mg per h, was calculated as

$$I_t = \Delta G_t + D_t.$$

Estimated ingestion rates were used to calculate daily meal and <u>daily</u> energy assimilation as discussed below.

Results

Diurnal Movements

The maximum number of <u>S</u>. <u>mossambicus</u> caught during different ?4 h periods showed considerable unexplained variation. Although maximum catches on March 4th and March 7th, 1975 were 64 and 34 specimens respectively (Fig. 57A and Fig. 58A), spot collections made on other dates in March usually produced larger catches, and on one occasion over 300 specimens were caught. Thus, daily movements of juvenile <u>S</u>. <u>mossambicus</u> were assessed by consideration of the relative abundance of fish in catches during each 24 h period studied.

Consistent with the findings of Minshull (1970) and Bruton and Boltt (1975) who examined diurnal movements of juvenile <u>S</u>. <u>mossambicus</u> in Lake Sibaya at low lake level, I found that these fish were common on the terrace only during daylight hours. In sharp contrast, at high lake levels I found that <u>S</u>. <u>mossambicus</u> were common on the terrace for only a few hours after dark (Fig. 57A and Fig. 58A). The latter finding is supported by numerous spot collections made in March and July, 1975 and by diving observations.

Daily movements similar to the ones observed in Lake Sibaya at low lake level have been reported for juvenile cichlids of other species in other systems (Welcomme 1964, Caulton 1975). Diurnal temperature fluctuation in the near shore waters appears to play an important role in these movements. As noted in Chapter V, temperature gradients develop on Sibaya terraces while the open water remains homothermal (Allanson and Van Wyk 1969, Bruton and Boltt 1975). During the day, terrace water temperatures are highest close to shore and decrease gradually with depth to the temperature of the open water at or just beyond the deep edge of the terrace. During the night the opposite situation is found with near shore terrace waters being much cooler than the open water. Both Caulton and Welcomme report the same sort of diurnally reversing temperature gradient for shallows visited by the species they studied. Caulton worked with juvenile Tilapia rendalli which he demonstrated has a thermal preferendum well above temperatures that normally obtain in the shallows of this species' environment. He therefore concluded that the tendency to seek the warmest temperature available naturally resulted in the

observed diurnal movements. Thermal preferenda for <u>S</u>. <u>mossambicus</u> have been found to range from $27 - 36.5^{\circ}C$ depending on fish size and acclimation temperature (Badenhuizen 1967, Donnelly 1969). Smaller fish and fish acclimated at higher temperatures showed higher preferenda. Bruton (1973) reports that maximum terrace temperatures in Sibaya are about $36^{\circ}C$ while maximum temperatures in open waters rarely exceed $28^{\circ}C$ (Hart and Allanson 1975), so Caulton's conclusion would appear to apply to diurnal movements of juvenile <u>S</u>. <u>mossambicus</u> in Lake Sibaya at low lake level.

At high lake level the situation appears to be more complicated. The absence of juvenile S. mossambicus on the terrace during daylight hours was so surprising that diving observations were made to confirm this fact. A few juveniles were found in mixed species shoals of 10 - 30 fish among the flooded marginal vegetation but none was present in the open exposed areas where they had been numerous at low lake levels. In addition, it was found that the barbel, Clarias gariepinus, was present on the terrace during daylight hours. At low lake levels this species ventures on to the terrace only at night. In a study of the feeding of C. gariepinus, Bruton (in prep.) has concluded that this species is the principal predator of S. mcssambicus in Lake Sibaya. It seems likely that visual recognition of the predator provides sufficient stimulus to override the tendency to seek warmer shallow waters during the day and thus defers movement onto the terrace until dark. It would be interesting to see if tethered or model Clarias would keep S. mossambicus off the terrace during daylight hours when the lake returns to a low level.

Diurnal movements of juvenile <u>S</u>. <u>mossambicus</u> were also altered in response to heavy wave action (Fig. 59). On Decembor 27, 1973, wave action was moderate and did not vary noticeably until after dark when the lake became calm. On this date, juveniles were most abundant on the terrace during the early afternoon (Fig. 56A). Bruton (1973) reports the same pattern of juvenile abundance for 163 seine net catches from the terrace at irregular time intervals during daylight hours between September 1970 to April 1971. On December 15, 1973, a strong onshore wind produced increasingly heavy wave conditions which reached peak intensity at about 1500 h, were significantly reduced by 1700 h and had subsided by 1900 h leaving the terrace almost calm. Catch data for this date (Fig. 55A) show that the number of fish on the terrace decreased as wave action increased, and as wave action decreased the fish returned to the terrace waters. This relationship between fish abundance and wave action is - 109 -





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Figure 56. Diurnal feeding activity of juvenile <u>S. moscambicus</u> on the terrace starting 0700 h on December 27th, 1973. Moderate wave action continued throughout the day until about 2000 h when the lake became calm. Confidence limits to mean mg gut content calculated as <u>+</u> t [0.05, N-1] times the standard error.







Figure 57. Diurnal feeding activity of juvenile <u>S. mossambicus</u> on the terrace starting 0800 h on March 4th, 1975. The lake was calm throughout the sampling period. Confidence limits to mean mg gut content calculated as <u>+</u> t [0.05, N-1] times standard error.

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confirmed by Bruton's (<u>pers. conm.</u>) observations. Since one of the goals of Bruton's seining program was to collect as many specimens as possible, he did not seine during periods of heavy wave action. Catches were too low to justify the effort.

A more quantitative approach to the study of wave action effects was planned for the March 1975 program, and a wave height recorder was constructed and installed. As fate would have it, both days in March chosen for 24 h sampling were unusually calm and no meaningful results were obtained.

Ingestion Rates

Variation in gut fullness will be considered first, then estimated defecation rates will be examined and finally ingestion rates estimated from these two variables will be considered.

The amount of food in guts varied over a similar range in the samples from each 24 h cycle studied (Fig. 55B, 56B, 57B and 58B). In collections A made at both high and low lake levels, the amount of food in guts was greatest at the time when fish were most numerous on the terrace. When variations in the amount of gut content in the specimens from each 2 hourly collection are compared, considerably greater variation is found in collections made during the period of heavy wave action (Fig. 55B) than at other times. This suggests either that some fish were more successful than others in feeding under heavy wave conditions or that fish in general feed more sporadically when subjected to strong waves. Considerable variation in the amount of gut content is also found in samples collected as the fish were moving onto the terrace at 1800 h on March 7th, 1975, and when most fish had left the terrace at 2100 h on December 29th, 1973. These collections appear to have been made at times when the fish were just beginning and just ending periods of feeding activity, respectively, and the results indicate that individual fish began and ended their feeding activities at different times.

There is some evidence to suggest that individual juveniles do not forage for long periods of time. Although the number of fish on the terrace varied markedly between 0700 - 1700 h on December 27, 1973, the amount of gut content in these fish increased at a remarkably regular rate (Fig. 56B). The data indicate that each individual in the sampled

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population fed at the same rate despite the fact that only part of the population was present on the terrace at any one time. Since all the fcod in the guts of these fish was terrace floc characterized by a high concentration of <u>Mastogloia</u> sp., this result indicates that individuals fed during several forays from the deep water rather than one extended foraging period.

Defecation rates were estimated for only the December, 1973 collections since too few fish were caught in collections made in March, 1975. Estimated defecation rates for the two dates in December, 1973 covered very similar ranges, 28 - 66 and 17 - 57 mg of gut content per hour, and had nearly identical means, 41.6 and 40.0 mg gut content per hour, respectively. Using Sokal and Rohlf's (1969) method for confidence limits to differences between sample means, 95 % confidence limits were calculated for each estimate of defecation rate (Fig. 55D, Fig. 56D). Estimates for December 27th had comparatively narrow confidence limits and some statistically significant differences were found (Fig. 5⁴). Greater variation in the amount of gut content found in samples collected on December 15th resulted in broad overlap in confidence limits to estimates for this date and no statistically significant differences were found.

One objection has frequently been raised against this method for estimation of defecation rates: unknown handling effects may significantly affect the rate of defecation of held specimens. Mann (1967) states that fish frequently pass copious feces soon after capture re-Overestimation does sulting in serious overestimation of defecation. not seem to be a problem in the present case. Only small amounts of feces were released in the plastic bucket while fish were transferred to the holding cage. During the time interval when terrace fish emptied their guts at the fastest rate for the day, between 1900 h and 2100 h on December 15th (Fig. 55C) and 2100 h and 2300 h on December 27th (Fig. 56C), estimated defecation rates failed to account for 56 % and 66 % of the change in gut contents, respectively. At all other times, defecation rate estimates were greater than negative changes in gut content. Thus, the data suggest that defecation estimates are more likely to be underestimates than overestimates. With this possible source of error in mind, estimated defecation rates have been used in calculation of ingestion as the best estimates available.

Ingestion rates calculated for December 15th and December 27th

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• Figure 60. Diagrammatic representation of diurnal movements of <u>S. mossambicus</u> in Lake Sibaya.

suggest very different patterns of feeding activity. Most ingestion on December 15th took place in a burst of feeding activity after heavy wave conditions subsided (Fig. 54E). Under moderate wave conditions on December 27th, feeding continued at a lower but comparatively constant intensity from mid-morning until after dark when the fish left the terrace (Fig. 55E). In light of the numerous sources of error involved, it is appropriate to ask whether these trends in ingestion rates are actually significant. If we adhere to the rigors of statistics, the answer is no. Ingestion rates are sums of estimated variables which, within themselves, have little statistically significant variation ... ith time. Nonetheless, the regularity and apparent meaningfulness of the data argues that the overall trends are biologically significant. Although the magnitude of individual estimates of ingestion rate may be subject to considerable error, the overall trends that are found appear to give a reasonable indication of feeding activity during the periods studied. A more detailed examination of errors associated with the method used here, especially of errors associated with estimates of defecation, would be necessary before the accuracy of the method can be defined in more precise statistical terms. At our present level of knowledge, all other techniques used for estimation of ingestion by wild fish populations are subject to similar uncertainties (Mann 1967, Davis and Warren 1968).

When catch data and estimated ingestion rates are compared for heavy wave conditions on December 15th and moderate wave conditions on December 27th, there is ample evidence to conclude that heavy wave action interferes with feeding of juvenile <u>S. mossambicus</u> on terraces in Lake Sibaya. This is not surprising since strong waves suspend some of the benthic floc and would make a fish's orientation to the substrate difficult, especially in shallow water. Even at 2 m depth, moderate wave action moves a diver enough to make bottom sampling awkward.

From results reported in this and previous chapters and from Bruton's (1973) observations discussed in Chapter I, the following emerges as a picture of the strategy employed by juvenile <u>S. mossambicus</u> to collect their daily meal from the terrace. Most juveniles spend non-feeding hours dispersed as individuals in deep water just off terrace areas (Fig. 60). This probably affords these fish protection from predation since they are so sparcely distributed that the probability of a <u>Clarias</u> encountering a juvenile <u>S. mossambicus</u> is quite low. Although these offshore juveniles

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can be captured with a large net, they are rarely seen by divers. When terrace conditions are suitable, the juveniles begin to visit terrace waters and feed. Small juveniles venture further toward the shore and thus into shallower water than do large juveniles. As the feeding period progresses the frequency of visits or length of visits or both increases to a maximum and then declines. Very few <u>S. mossambicus</u> are present on the terrace after most feeding activity is finished. Trawl catches discussed in Chapter II suggest similar diurnal movements for adults feeding in the gradual slope habitat (Fig. 60).

The tendency to seek the warmest waters available appears to be the fundamental factor regulating juvenile movements, but this may be altered in response to unsuitable wave conditions or the conspicuous presence of predators on the terrace. Since Lake Sibaya is subject to frequent periods of heavy wave action, disturbance by waves may be an important factor limiting utilization of terrace food resources. Deferring feeding until after dark to avoid predators may also seriously limit utilization of terrace food. Rates of feeding (Warren and Davis 1967) and digestion (Smit 1968) in several fish species are known to be directly proportional to water temperature. Data from 24 h studies at high lake level (Fig. 57 and 58) suggest that juveniles feed rapidly when they first enter terrace waters soon after dark, but feeding appears to be greatly reduced, if it continues at all, at 5 h after dark when terrace waters are several degrees cooler than the open waters (Bruton 1973). Neither temperature fluctuations nor wave action are likely to be important at 3 - 6 m depth on the gradual slopes, and here the tendency to avoid the predators which are present throughout the 24 h cycle is likely to limit feeding activities of adults to periods after dark, as has been observed. Thus, juvenile and adult S. mossambicus depend on food resources in habitats whose various features both contribute to and detract from population success, and it appears that their daily feeding strategies are chosen to minimize exposure to the undesirable features of their feeding habitats.

Daily Energy Assimilation

With the conservative assumption that ingestion is equal to any positive change in gut content for time periods when defecation could not be estimated, the daily meal of juveniles feeding on the terrace during the

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two 24 h survey periods in December, 1973 can be estimated as the mean hourly ingestion rate times 24 h. Estimates for days of heavy and moderate wave action are 722 and 814 mg of food ingested per day per standard fish of 3.0 gm dry weight, respectively. Together with estimates of the mean proportion of organic matter in the material ingested (26.0 %, N=37, (V=12.7 %), the mean caloric content of organic matter in the material ingested (4.07 kcalper mg) and the mean assimilation efficiency for organic matter (37.5 %), the daily energy assimilation of a juvenile <u>S. mossambicus</u> of 3 g dry weight may be calculated from the daily meal as:

Daily Meal = 722 - 814 mg benthic floc per day

- = 241 271 mg benthic floc per day per g fish dry weight
- = 63 71 mg organic per g fish dry weight
- = 253 286 cal per g fish dry weight

= 108 - 121 cal assimilated per g fish dry weight This estimate applies to warm season conditions and daily energy assimilation is probably less in the cool season. A comparable estimate may be derived from data published by Moriarty and Moriarty (1973a and b) for a similar cichlid, Tilapia nilotica, in tropical Lake George, Uganda. Daily meal was determined by a field method similar to the one used here and carbon assimilation was determined by radiotracer studies in aquaria. These authors report that a fish of 110 g wet weight assimilates 245 mg of carbon from a diet of Microcystis and Anabaena sp. per day or, assuming that the fish is 78 % water as is S. mossambicus (Pandian and Raghuraman 1972), 10.1 mg carbon per g dry fish per day. Assuming that the organic material assimilated is 50 % carbon (Riley 1970) and has an energy content of 5 cal per mg (Cummins and Wuycheck 1971 p. 48), this is equal to 101.0 cal per g fish dry weight per day; an estimate not significantly different from the one given here for S. mossambicus juveniles in Lake Sibaya.

The accuracy of the estimate of daily energy assimilation by juvenile <u>S. mossambicus</u> in Lake Sibaya is further supported by comparison with data published by Pandian and Raghuraman (1972). These workers fed different rations of <u>Tubifex tubifex</u> to 11 groups of small <u>S. mossambicus</u> juveniles held in aquaria at $25\pm1^{\circ}$ C and monitored the resultant growth. Daily energy assimilation from each of these rations was calculated using the caloric value of <u>Tubifex tubifex</u> given by these authors and the assumption that 85 % of the food consumed was assimilated (Mann 1967). The daily

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energy assimilation estimated for <u>S</u>. <u>mossambicus</u> juveniles in Lake Sibaya falls midway between the daily energy assimilation required for maintenance and that which produced maximum growth.

These two comparisons amply confirm the accuracy of estimates of daily meal and assimilation efficiency for dietary energy developed for <u>S</u>. <u>mossam-</u> <u>bicus</u> in this study. It is encouraging to discover that a variety of techniques currently used in ecological research can be combined to produce roughly comparable estimates of daily energy assimilation when applied to two different populations or to the same species under field and laboratory conditions.

Discussion

Several points raised in previous chapters warrant further discussion. We may begin with the question: is the relationship between food quality and depth that was found in Lake Sibaya likely to be found in other aquatic ecosystems? It seems reasonable to expect environmental factors that determine the food value of benthic floc in Lake Sibaya, ostensibly light penetration and temperature, to play similar roles in other ecosystems. If these roles are determinative, we can expect the feeding activities of fish that depend on benthic floc for their nutrition to be confined to shallow waters. To my knowledge, only three studies in the literature report on the distribution of fish with a diet of benthic floc. Dunn (cited in Moriarty et al. 1973) examined the diet of Tilapia leucosticta in Lake George, Uganda, to find that near shore fishes fed only on benthic floc while fishes in open water fed only on plankton. Fagade (1971) studied the diets and distributions of two sympatric Tilapia in the Lagos Lagoon, Nigeria. T. guineensis fed on a mixture of benthic floc and macrophytes and was found throughout the lagoon from the shore to 6 m T. melanotheron melanotheron fed exclusively on benthic floc and depth. did not occur at depths greater than 2 m. Finally, Odum (1970) has reported on the diet and feeding activity of Mugil cephalus at Sapelo Beach, U.S.A. The diet of these mullet consisted of benthic diatoms and detrital aggregate and appears to be identical to that of juvenile S. mossambicus in Lake Sibaya. Odum found that the fish browsed sporadically during most of the day but fed intensively at high tide when they moved into waters that had apparently been too shallow to enter at low tide. Thus all available data show that fish which depend on benthic floc for their nutrition exhibit a marked preference for floc in shallow waters despite its abundance throughout the environment. This finding offers strong support for the conclusion that the relationship between food quality of benthic floc and depth found in Sibaya can be expected in other ecosystems as well.

With this conclusion in mind, it is instructive to compare Lake Sibaya with aquatic ecosystems in Africa in which adult <u>S</u>. <u>mossambicus</u> are more prosperous. Adult <u>S</u>. <u>mossambicus</u> attain a large maximum size and excellent condition in culture ponds, rivers and their pans, and in Lake St. Lucia. Lake St. Lucia is a large brackish coastal lake 60 km south of Lake Sibaya and has a mazimum depth of 3 m (Hill 1975). All of these systems are quite

Adult S. mossambicus in deep coastal lakes and large irrigation stallow. incoundments are generally less successful in achieving a large maximum size and, judging from photographs and personal communications, do not attain the heavy bodied condition typical of adults in shallow-water systems. The population in Lake Sibaya is the most extreme example of the small maximum size attained by this species in deep-water systems. One notable exception is the extremely eutrophic Lake McIlwaine in Rhodesia. S. mossambicus in this deep-water impoundment have been found to reach the unusually great total length of 47.3 cm (Munro 1967). This exceptional growth may be due to a superabundance of food. Marshall and Falconer (1973) state that McIlwaine has a permanently high standing crop of phytoplankton which is subject to frequent blooms. Munro (1967) reports that juvenile S. mossambicus in McIlwaine feed predominantly on diatoms while adults feed on planktonic and periphytic algae, bits of macrophyte presumably ingested while feeding on periphyton, and zooplankton.

The McIlwaine exception suggests an explanation for the differences in maximum size and condition of adult S. mossambicus in shallow and deep systems, and the lack of success exhibited by adults in Lake Sibaya. As discussed in Chapter IV, S. mossambicus and several other cichlid species tend to inhabit deeper water as they increase in size. In shallow-water systems, this tendency can result in only small changes in the depth at which the fish feed and thus fish of all sizes will always have access to benthic floc in relatively shallow waters where its food quality is likely to be adequate for growth. In deep-water systems, fish that do not enter the shallows will be dependent on deep-water food resources. In highly eutrophic Lake McIlwaine, periphyton on sublittoral macrophytes and plankton are sufficiently abundant to support growth to a large maximum. No data are available for phytoplankton or periphyton abundance in the impoundments and deep coastal lakes where S. mossambicus achieves only moderate condition and maximum size, but it is unlikely that either of these potential food resources is nearly as abundant in these deep-water systems as it is in McIlwaine. The meager standing crop of plankton in Lake Sibaya was discussed in Chapter I. Although macrophytes are common to 6 m depth on the gradual slopes in Lake Sibaya, my observations indicate that the periphyton is sparce and comprised largely of adnate diatoms that would be more difficult for a grazer to remove than most other periphytic It appears that, in the absence of a suitable alternative, organisms.

adult <u>S</u>. <u>mossambicus</u> in Lake Sibaya are forced to utilize the energy rich, but protein poor benthic floc in the gradual slope habitat as their food resource. Thus the failure of these fish to attain a large maximum size and good condition can be traced through the low standing crops of plankton and periphyton to the overall low mutrient status of the Lake Sibaya ecosystem (Allanson and Van Wyk 1969, Allanson and Hart 1975).

Despite the shortcomings of adults, <u>S</u>. <u>mossambicus</u> appears to be the most numerous fish species in Lake Sibaya. As discussed in Chapter I, Bruton (1973) credited the general population success to precocious breeding which he argued results in greatly increased recruitment and thus allows the population to withstand heavy mortalities inflicted by predation and dessication of nursery pools. Bruton also pointed out that precocious breeding would shift the population size structure such that juveniles would represent a larger proportion of the population and this would favor greater utilization of terrace food resources.

In light of the facts assembled in the present study, I would like to offer a revised interpretation of the factors which make precocious breeding advantageous for S. mossambicus in Lake Sibaya. Gadgil and Bossert (1970) have made a detailed theoretical analysis of the reproductive strategies that would be expected to maximize population fitness under various environmental conditions by maximizing population reproductive out-Three important factors required consideration: generation time, put. survival to reproductive age and reproductive potential. These authors note that in fishes, as in many other animal groups, reproductive potential is directly proportional to body weight. Since assimilated energy not required for maintenance will be divided between reproduction and growth, energy expended for reproduction at one age will result in reduced growth and thus reduced reproductive potential at all subsequent ages. On the other hand, early reproduction contributes toward increased reproductive output by shortening both the generation time and the time during which individuals are exposed to possible sources of mortality before they reproduce. Thus, Gadgil and Bossert argue that in order to maximize population reproductive output, natural selection will favor the onset of maturity and reproduction at an age that strikes the optimal balance between the advantages of shortened generation time and reduced mortality conferred by early reproduction and the advantage of increased reproduction potential conferred by late reproduction. In the case of S. mossambicus



Figure 61. Percentage organic carbon and nitrogen in feces cultured at 18°C in sea water under a neon light, (A) before and (B) after feeding to <u>Hydrobia ulvae</u>. Each point represents the mean of two estimations. Photocopied from Newell (1965).

in Lake Sibaya, resource limitations in deep waters deny adults the growth and thus the increase in reproductive potential that is available to adults of this species in other systems. As a result, we would expect the reproductive output of this population to be greatly increased by the precocious breeding that has been observed. The precocious breeding of <u>S. mossambicus</u> in Lake Sibaya is therefore identified as an adaptation that allows the population to maximize reproductive output given the limited resources available to adults, and doubtless makes a major contribution to the population's success.

To more clearly understand the characteristics of benthic floc that determine its food value, it is necessary to re-examine the literature cited in Chapter II which has lead to the putitative conclusion that detritus is essentially indigestible and thus that the food value of benthic floc is dependent upon its concentration of digestible microorgan-Most authors have reached this conclusion through microscopic isms. examination of food and feces. They observed that algae were digested, but they could see no change in the detritus so they assumed that it was not digested. Examined critically, such observations have little value. Since detritus is highly degraded organic matter and often amorphous, there is little reason to expect a change in the appearance of detritus even if 99% of it is digested. Other workers have concluded that detritus is indigestible because the detritivores they studied did not assimilate either cellulose or lignin. These workers were apparently unaware of the varied and complex chemical composition of detritus as discussed in Chapter V.

The only experimental evidence that detritus is indigestible comes from the work of Newell (1965) (Fig. 61). Newell reports that nitrogen in the feces of <u>Hydrobia ulvae</u> increases from practically zero to 1.75 % of dry feces weight as the feces are colonized by microorganisms. At the same time carbon in the feces decreases from 10 to 8 % of dry feces weight, a change which Newell reasonably attributed to use of detrital carbon by the microbes. When these feces were fed to <u>H. ulvae</u> and passed through the gut for a second time, their nitrogen content dropped from 1.75 to less than 0.1 % but their carbon content was unalterred. Thus Newell concluded that the microbes were digested but the detritus was not. For two reasons this conclusion cannot be accepted. Firstly, the possibility exists that considerable detritus was digested from the food on its first

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passage through the animal to leave only indigestible detritus in the feces. Microorganisms that colonize the feces will be available for digestion when the feces are reingested, but the digestible portion of the detritus would not be replenished. The result reported by Newell could be expected regardless of whether detritus is digested or not. Secondly, Newell's data contain a serious inconsistency. If the nitrogen in feces is microbial protein, as Newell's results clearly indicate, this protein will include protein carbon. From the facts that protein is, on average, 16 % nitrogen and 52.5 % carbon by weight (Fruton and Simmons 1958), we can calculate that the feces which consisted of 1.75 % protein nitrogen prior to ingestion for the second time (Fig. 61A) also consisted of 5.8 % protein carbon. We would certainly expect that protein carbon and protein nitrogen would be digested in equal proportions, but Newell reports that none of the carbon in the feces was digested while nearly all the nitrogen was digested. This curious result makes it impossible to interpret Newell's data as supportive of his conclusion. All in all, I am unable to find in the scientific literature any unequivocal evidence to support the opinion that no significant proportion of detritus can be digested.

The opposite opinion is supported by the findings of Gordon (1970). As noted in Chapter V, Gordon has reported that commonly 30 - 60 % of the organic carbon in particulate matter filtered from seawater was hydrolysed by a mixture of trypsin, chymotrypsin and < amylase. In approximately one hundred deep sea samples collected from a maximum depth of 2000 m, the amount of organic carbon hydrolysed ranged from 19 - 26 %. Some difficulty arises in interpretation of these results because Gordon gives no indication of what proportion of the samples was detritus and what proportion was plankton. In a discussion of Gordon's results, Riley (1970) seems to assume that essentially all the material present was detritus, and although this may not have been true of samples from the upper 200 m (Degens 1970) it was almost certainly true of deep water samples. Thus Gordon's findings demonstrate that at least a proportion of detritus in sea water is digestible. Assimilation studies reported in Chapter V indicate that S. mossambicus in Lake Sibaya digests a significant amount of the detritus in its diet. Based on this finding and Gordon's (op cit) results, it is not unreasonable to expect that a proportion of detritus in most aquatic ecosystems can be digested by consumers.

In view of this conclusion, it would appear that the food value of benthic floc is limited not by the indigestible nature of the detritus but rather by the fact that detritus is <u>too</u> digestible. Since there is little or no protein in detritus (page 77), digestion of detritus will supply the consumer with only calories. Protein must be gained by digestion of the associated protein rich microorganisms, principally bacteria. Only that particulate organic matter which includes an amount of digestible protein sufficient to result in a nutritionally adequate ratio of digestible protein to digestible calories will be suitable as a food resource. This conclusion leads me to propose the following theory: The food quality of benthic floc for consumers is directly proportional to its concentration of digestible, protein rich microorganisms.

On the basis of this theory it is possible to predict that successful consumers of benthic floc would either have the ability to digest bacteria or supplement the calories gained from digestion of detritus with protein from foods such as invertebrates or fish. Although a great number of fishes are known to ingest small amounts of benthic floc, diets comprised entirely of benthic floc have been reported only for populations of Tilapia (Fagade 1971, Dunn cited in Moriarty et al. 1973, the present study) and mullet (Odum 1970). The ability of S. mossambicus to digest bacteria has been demonstrated (Bowen 1976) and it is reasonable to assume that all of the Tilapia possess this ability. Using fluorescence microscopy with acridine orange stain, Odum (op cit) observed that detrital aggregate consumed by Mugil cephalus was covered with bacteria and protozoa whose numbers appeared to be greatly reduced as the food passed through the digestive tract, Although this is not a quantitative observation, it strongly suggests that mullet can digest bacteria. In a study of the diets of over 80 animal species in a subtropical estuary, Odum (cited in Mann 1972b) concluded that invertebrates were the principal consumers of benthic This is not surpirzing since many invertebrates are known to digest floc. bacteria (Zobell and Feltham cited in Newell 1965, Hargrave 1970, Cálow 1975b).

The above theory may hold considerable significance for aquaculture. <u>S. mossambicus</u> and <u>Mugil cephalus</u> are both important aquaculture species. Two other fish that are commonly cultured, the carps and <u>Chanos chanos</u>, ingest large amounts of benthic floc and may have the ability to digest bacteria. Essentially all invertebrates that are cultured feed on detrital aggregate that is either suspended or sedimented. Simple, inexpensive procedures that can be used to encourage the development of a bacterial flora on the agricultural waste materials that are commonly used as supplementary food in aquaculture would be expected to encourage the rapid growth of these aquaculture organisms. This could possibly obviate expensive protein supplements, in the form of fish meal or other animal products, that are required in the culture of many invertebrates using current aquaculture techniques.

As with most research, the study reported here has revealed several topics worthy of further examination. The unknown role of combined amino acids in detritus is a major gap in our understanding of the nutritional significance of detritus and needs to be studied in detail. Feeding strategies employed by invertebrates to gain a suitable protein-calorie ratio in their diets are largely unstudied. Newell (1965) found that the abundance of Hydrobia ulvae and Malcoma balthica in the Thames estuary was directly proportional to the bacterial content of the detrital aggregate on which they fed, but Calow (1975b) has offered some evidence that at least one invertebrate, the gastropod Planorbis contortus, can separate bacteria from other components of detrital aggregate. This suggests that some invertebrates may be less dependent on the concentration of bacteria in benthic floc than are others, a possibility that may have an important influence on invertebrate distribution and is well worth investigation. The reason why several cichlid fishes inhabit progressively deeper waters as they grow larger remains a mystery worthy of examination.

In conclusion, the study presented here revealed biotic and abiotic features of the Lake Sibaya ecosystem that largely determine the success of the <u>S</u>. <u>mossambicus</u> population through their effects on the population's feeding. This population may be an extreme case. Environmental factors are known to determine the success of other animal populations through their effects on reproductive success and survivorship as well as feeding. Nonetheless, the fundamental significance of environmental influences on a population's efforts to gather the materials which nourish and fuel all population activities is underscored by the results of the present study, and it is felt that the study of feeding ecology holds considerable promise for attempts to understand the environmental factors that determine the success of many other populations.

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